

XVIII. International Plant Protection Congress

Mission possible: food for all
through appropriate plant protection



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ABSTRACTS



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Keynote Speakers

O KN 1

Achieving Food Security for all in the Foreseeable Future: What will it take?

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Large increases in cereal prices in 2007-08 raised questions about the ability of world agriculture to produce the food needed by future generation. Predictions about impending world famine and continued increases in food prices are plentiful but almost certain to be wrong. Today, the world is awash in cereals and prices have decreased rapidly during the last three years. Enlightened policies, appropriate investments in research and technological change and better utilization of the currently underutilized productive capacity, are likely to result in continued increases in global food production sufficient to sustain a long-term trend of falling but more volatile real food prices. The mission is certainly possible.

Increasing food production is necessary but not sufficient for food security. To be food secure, households must have access to the quantity and kinds of food needed for a healthy and productive life. Very large stocks of food currently coexist with widespread food insecurity. Appropriate policies along with public and private investments are needed to enhance low-income people's purchasing power or food production capacity. Considering both the supply and demand sides, this presentation will discuss what it will take to achieve food security for all in the foreseeable future.

O KN 2

Gene- and biotechnology-driven approaches to durable pathogen resistance in cereals

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Broad-spectrum, quantitative pathogen resistance is of high importance to plant breeders due to its durability. However, it is usually controlled by multiple quantitative trait loci and therefore, challenging to handle in breeding practice. Knowing about the underlying genes would allow its more targeted utilization by allele introgressions. With the available omics tools and data of barley and one of its major fungal pathogens, the powdery mildew fungus *Blumeria graminis* f.sp. *hordei*, at hand we are now enabled to functionally address genes for defense and attack on both sides of this plant-pathogen interaction at a genome-wide scale. To identify genes that mediate race-nonspecific resistance of barley to *B. graminis* we combined a functional-genomics approach based on genomewide transcript profiling and transient-induced gene silencing (TIGS, 1400 genes) with a genetic approach consisting of association- and Meta-QTL mapping plus analysis of copy-number variation. This guided us to a shortlist of approximately 50 candidates with converging evidence for an important role in race-nonspecific resistance of barley. We have started marker-assisted introgression of potentially valuable alleles of some of these candidate genes in barley, followed by assessment of multiple pathogen resistance. As a biotechnological approach, we use host-induced gene silencing (HIGS) in *B. graminis* as well as the *Fusarium* head blight fungus to address potentially important genes for fungal attack and accommodation. This is revealing another list of candidates for a better understanding of these host-pathogen interactions and for disease control.

O KN 3

Landscapes, genetically modified crops and climate change: Wither IPM?

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The three-letter acronym IPM has been around for over fifty years and now not only guides research and extension in pest management, markets pesticides and is claimed to be undertaken by many growers, it even resonates with public perceptions and politicians. Whether or not IPM programmes are sustainable in the longer term under the conflicting stresses and strains of the modern agricultural environment is debateable. For Australia as a whole over this period insecticide input costs per hectare have increased faster than the price index, reflecting a mixture of more costly insecticides, changed cropping mix and increasing cropping area with possible concomitant changes in pest abundance. I review two case studies of IPM development in Australia: *Helicoverpa* management in cotton and management of an introduced pest, *Plutella xylostella* in brassica vegetables. Many pest management practices have improved over time. In brassica and cotton, IPM is predominantly of the sample and spray variety, increasingly with less broad-spectrum insecticides and, in cotton, *Helicoverpa* management has moved to transgenic (GM) plants. The latter necessitates an area wide approach to insecticide resistance management (IRM) if the GM technology is to remain stable. Any pest crisis will ensure rapid changes in practice and adoption of technologies, which mitigate the short term financial stresses caused; however, regression to former practices tends to follow (e.g. in brassicas) once the crisis has passed. In

Keynote Speakers

most cases we cannot test objectively if changed management practices are responsible for changes in pest abundance, as would often be claimed, or if the latter is simply a consequence of the weather and related large-scale landscape features (e.g. area of host plants). Changing climate will impact on pest abundance and distribution and the effectiveness of biological control in complex ways. For many systems the future of pest management practice will require a change to landscape or area-wide approaches. Given how entrenched the three-letter acronym has become, it will all, most likely, be called IPM.

O KN 4

Social participation – Key factor for food security and rural development

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The world will have to feed 9 billion people by 2050. Considering that fact, Food Security and Nutrition of a growing world population is one of the highest priorities for international development cooperation. In addition to a better distribution of food, eliminating hunger will also require increasing agricultural production in an ecologically, economically and socially sustainable way. Including vulnerable groups is a crucial part in this process of transforming agriculture. Through its work in over 130 countries worldwide, the Deutsche Gesellschaft fuer Internationale Zusammenarbeit (GIZ) contributes to implementing such a development-oriented agriculture which fosters agricultural investments and provides an income for the rural poor. Drawing on practical examples from GIZ projects and programmes in relevant sectors and at all levels (national, regional and local), this keynote speech illustrates the complexity of Food Security and Nutrition challenges, as well as the principles that guide the GIZ in this work in order to find answers responding to these challenges. It covers issues such as promoting rural development, developing policies and standards for sustainability, the market-integration of smallholder farmers, context-specific sustainable agriculture and the empowerment of women and youth.

Oral Presentations

Challenges in Plant Protection I

O CPP I-1

The Importance of Regulatory Data Protection and other forms of Intellectual Property Rights in the Crop Protection Industry

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Different Types of Intellectual Property. Patents: These provide exclusive rights to inventors for a fixed period of time in exchange for the disclosure of the invention. Patents exclude others from making, using, selling, offering to sell or importing the patented invention for the term of the patent. The patent term is usually 20 years but in the crop protection industry the time to first sales can be long and the patent term eroded. Some countries like those of the European Union can give up to an extra 5 years to compensate for this erosion. Annual fees need to be paid to keep patent rights valid. Trade Secret: This is a formula, practise, process, design, instrument, pattern or compilation of information which is not generally known or reasonably ascertainable, by which a business can obtain an economic advantage over competitors or customers. An example of this in the crop protection industry is the technical specification of the active ingredient for any given pesticide product and the process by which that active ingredient is manufactured. Trade secrets are sometimes called Confidential Business Information and are protected forever but only as long as they remain a secret. By definition and unlike patents they are not disclosed but they must be treated with respect and the appropriate internal systems must be in place to show that they are treated as secret. No fees are required. Protection of Safety and Efficacy Data: In order to obtain a product registration which allows a company to sell a product it is sometimes necessary to demonstrate safety and efficacy. In the crop protection industry it is necessary to demonstrate that the active ingredient in a given product is efficacious in controlling pests and has no unacceptable side effects on human health or the environment. These tests are expensive and take a considerable amount of time thus eroding the patent life of the active ingredient. These studies can then be given a period of exclusive use in which no other company can cite the data. The safety and efficacy data for a pesticide product are usually, but not always, given a 10 year period of exclusive use in OECD countries. Conclusions: IP issues for the crop protection industry will become increasingly high profile as food security becomes a key global issue.

O CPP I-2

Organization for Economic Co-operation and Development (OECD) in support of global IPM: a goal to meet pesticide risk reduction challenges

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With the changing landscape of the agricultural value chain and increasing demand for sustainable crop production, a continuing theme for discussion has been the question of what it takes to increase adoption of IPM at the farm level. Unanimously, it requires an integration of support and efforts among all stakeholders to enable and advance uptake of IPM practices by growers. While research into new IPM techniques is an ongoing requirement, there are still enormous gains to be made via diffusion of established practices through networking and collaborations.

In October 2011, an international IPM workshop facilitated by the OECD aimed to identify strategies for boosting IPM adoption by growers and its impact in reducing pesticide risks. In response to the workshop's recommendations, an Expert Group on IPM (EGIPM) was established to address the need for global collaboration in meeting local challenges in IPM implementation. The findings and recommendations have been translated into an action plan to address recognized gaps resulted from numerous group discussions. The EGIPM coordinates contributions from OECD member countries in moving forward various action plan activities i) to further advance and facilitate coordination and information exchange about IPM via the OECD IPM Hub, ii) promote and develop policies in favour of IPM adoption and implementation, iii) develop indicators of IPM adoption and impact and iv) facilitate awareness raising about IPM among the public & food chain operators.

As a result to date the OECD IPM Hub, a platform for information sharing and cooperation between all stakeholders has been developed. The platform ties together the wealth of information in particular related to IPM policies, programmes, production guidelines and IPM case studies in OECD countries. Other ongoing work activities such as the discussion of policy instruments and incentives to stimulate the adoption and use of IPM-methods will be presented and what these mean on the ground will be discussed.

Oral Presentations

Challenges in Plant Protection I

O CPP I-3

Filling the gap – academic education in crop protection between basic and applied sciences

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Introduction: The demand for stable and increasing crop yields with regard to a growing world population represents a major global challenge. Innovative, effective and environmentally friendly plant health management becomes a key factor. The development of crop protection methods requires the education of highly skilled scientists and executives in sustainable crop protection, both in developed countries and in the developing world. Over the past decades, research and education have taken a sharp turn towards specific and basic approaches in many agricultural faculties. This has led to significant advances in fundamental techniques and knowledge, but has been associated with a loss in a system-related knowledge and expertise required to address the complex problems caused by detrimental organisms acting within cropping systems.

Objectives: Experts understanding plant pests and diseases, the risks they pose and the strategies how these risks can be managed are urgently needed, both on a practical and scientific level. As a consequence, the urgently needed strengthening of the technical and scientific basis in crop protection requires a broader and multi-disciplinary approach both in teaching and in applied crop protection research.

Methods: In 2010, an international master study programme 'Crop Protection' has been newly established at the University of Göttingen. The four-semester programme offers advanced study courses covering all aspects of plant health and crop protection, presented in lectures, seminars, laboratory and field courses, an internship of 6 weeks, and a master thesis project. The language of instruction is English.

Results: The crop protection study program cooperates with research institutions and the agrochemical industry at different levels (internship, lectures and practical courses, master thesis projects). Until present, students from 19 different countries have been joining the programme. Several graduates successfully continued their career with a PhD or finding positions.

Conclusions: The master study programme 'Crop Protection' is an unique opportunity to educate and train young academics in a key subject of crop sciences and provides a link between basic and applied agricultural research to meet future challenges in crop protection.

O CPP I-4

The Yield Gap: Why farmers don't achieve potential yields and what we can do about it

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In the battle against food insecurity plant breeding and crop protection have significantly increased crop yield potentials. However, despite the massive investment in new technologies since the Green Revolution, on-farm yields of major food crops have increased only incrementally, while pest and disease losses remain high. In this talk I will discuss why the gap between potential and actual yields has grown, and will propose that working more closely with farmers to identify research priorities that lead to relevant and cost-effective outcomes is required to improve food security.

Sustainable production depends on good farming practices that maintain and improve crop and soil health. In many cases farmers know what is required, but decide not to implement best practices because of socioeconomic constraints, or because they lack the technical or financial incentives to do so. Farmers may decide that their investments are more profitably placed elsewhere. I will use examples from our work with farmers in Southeast Asia and the Pacific to illustrate some of the reasons farmers may choose not to intensify production and increase productivity.

Traditional, top-down, extension approaches tend to alienate farmers, extension agents and researchers. We have found that early consultation with farmers and other stakeholders engages them in the research process and leads to better uptake of research findings. Farmers are more willing to try new technologies if they are presented as a graded series of options, rather than a set of fixed recommendations. Model farmers can be supported to implement and demonstrate each option to their peers. We have successfully applied this participatory approach to assist cocoa, pineapple, citrus, tomato, potato, rubber, black pepper, durian and jackfruit farmers in Papua New Guinea, Indonesia, Vietnam and the Philippines, and believe it can be used to reduce the yield gap in other countries.

Oral Presentations

Challenges in Plant Protection I

O CPP I-5

The multiple dimensions of food security and their challenges: How important are plant diseases as major causes of food insecurity?

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Food security (FS) encompasses food availability (production, imports and reserves), physical access (transport, logistics and supply chains), economic access to food (food pricing, economic status of population) and food utilization (food quality and safety), all underpinned by stability. Each dimension may be affected singly or interactively by any of a number of transient (e.g. disease outbreak in one season) or chronic (e.g. endemic diseases) factors. Plant pathogens directly affect production, imports, reserves, food quality and safety and are major risk factors that lead to food insecurity globally. Historical observations and current research aid in the understanding of the disease process and help with disease management planning and prevention. As a means of management, risk factors should be recognized from the onset and reduced as much as is reasonable through an integrated management system designed for known risk factors. This presentation will present the problem of food shortages globally, why they exist due to the influence of plant diseases and examples of epidemics that lead to food insecurity.

O CPP I-6

Legal obligations re Access & Benefit Sharing An industry perspective

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Introduction: Since the signature of the Nagoya Protocol on Access and Benefit Sharing end of 2010 ("Nagoya Protocol"), the topic of the use of genetic resources and access and benefit sharing ('ABS') regulations related thereto have grown rapidly in importance. This is especially the case since implementing (regional and national) laws are being discussed. Since October 2014 there is also an EU Regulation on Access and Benefit Sharing in force, which is one of the first regulations on ABS to come into existence and will most likely be looked at as an example by countries implementing the Nagoya Protocol.

This new regulatory framework on ABS might impact the activities of the crop protection industry. A detailed analysis of the use of genetic resources in the different steps of the research and developments process, and the possible rights and obligations on ABS related thereto, is therefore needed.

Objectives: The three main objectives of the session are 1) creating an understanding of the new legal framework related to ABS; 2) creating awareness on the relevance of the new rules for the crop protection sector through an analysis of the potential use of genetic resources in the research and development process; and 3) identifying some issues and formulating some recommendations.

Materials and methods: The session will start with a detailed presentation, including an overview of the relevant legal provisions and an analysis of the different steps in the research and development process, as well as the potential obligations related to ABS in each step. In addition, a few case studies will be analysed in an interactive discussion and grey zones and issues will be identified; and recommendations will be formulated.

The overview of the relevant legal provisions will be along the following structure:

- History and background of the Nagoya Protocol
- Key legal provisions of EU legal framework
- Scope
- User compliance
- Monitoring user compliance
- Best practices
- Implementing acts
- National laws re access

Results and conclusion: The presentation is aimed at providing the audience with a thorough understanding of the key legal provisions on ABS, and increased awareness about the potential obligations related to ABS while conducting research and development activities in the crop protection sector. The interactive discussion of the case studies is aimed at identifying key issues and formulating some recommendations.

Oral Presentations

Nematodes I

O NEM I-1

Identification and pathogenicity of South African *Meloidogyne* species

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Root-knot nematodes (RKN) attack and parasitise agri- and horticultural crops globally, causing yield and quality losses. *Meloidogyne arenaria*, *M. hapla*, *M. incognita* and *M. javanica* generally are the four economically most important species that globally cause damage to crops. In addition, others such as *M. chitwoodi*, *M. enterolobii*, *M. fallax* and *M. lopezi* are also important pests of various crops. The first aim of the study was to identify RKN species from roots/tubers/pods of crop plants received for diagnostic analyses and from research sites across South Africa using molecular and morphological identification. This enables construction of phylogenetic-tree and distribution maps. The second aim was determining the pathogenicity of RKN species and populations identified during the study in a greenhouse trial (randomised complete block design with six replicates). Deoxyribonucleic acid (DNA) was extracted from mature females obtained from roots of crop plants received and subjected to polymerase chain reaction (PCR) analyses. DNA of RKN species was compared to that of known species, representing the respective standards, to ensure accurate results. For the pathogenicity study, 1 000 eggs and second-stage juveniles (J2) of the respective RKN species/populations identified were inoculated on roots of a susceptible tomato cultivar (Rodade/Floradade). Nematode parameters assessed 56 days later included egg-laying female indices, egg and J2 numbers and reproduction factors/root system. The four economically most important RKN species as well as the emerging *M. enterolobii* (= *M. mayaguensis*) have been identified. Other unknown species have also been detected and are currently being identified. Pathogenicity of the various RKN populations differed substantially within and among species. Positive identification of *M. enterolobii*, which is easily confused with *M. incognita* in terms of its morphological identification, during this and previous initiatives will contribute towards research aimed at determining the distribution, life cycle and pathogenicity of this pest. The latter will also apply for other unknown RKN species that are currently being identified. This study is ongoing and knowledge generated will benefit the research fraternity as well as producers and ultimately consumers of produce.

O NEM I-2

Effects of amino acid treatments on nematodes

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Amino acids (aa) are naturally occurring substances that are important for all living organisms. The application of certain aa at different concentrations affects various life stages of a broad spectrum of nematode species. However, the mechanistic details for the observed effects remain elusive till now. To investigate this question, we analyzed the effects of methionine (Met), lysine (Lys), threonine (Thr), isoleucine (Iso), 2-ketobutyric acid (Ket), homoserine (Hom) and tryptophan (Try) on the free-living nematode *Caenorhabditis elegans* and the plant parasite *Heterodera schachtii*. The activity and development of *C. elegans* was decreased by Try applications. No aa had an effect on the activity of *H. schachtii*. Interestingly, soaking J2 stage nematodes in aa solutions for twenty-four hours, led to more female nematodes per plant for Lys, and less for Thr. The strongest effects were observed when aa were supplemented to the nutrient-medium in a monoxenic culture of the host plant, *Arabidopsis thaliana*. This approach reduced the number of female nematodes per plant for Iso, Met, Thr, and Ket. Additionally, slight negative effects could be detected on the adult female sizes. Interestingly, these effective aa all belong to a group of metabolites that are derivatives of Hom. In this study we were able to separate the direct effects of aa applications to pre-infective juveniles, from effects that may also involve the host plant.

Oral Presentations

Nematodes I

O NEM I-3

Neuropeptide Biology and Sociality Behaviours in plant Parasitic Nematodes

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Plant parasitic nematodes (PPNs) significantly reduce crop production such that their control is a very significant issue for global food security. Whilst much effort has focussed on understanding the interaction between parasitic-stage PPNS and their plant hosts, relatively little effort has centred on understanding the behaviour of pre-parasitic J2 stage PPNS in the soil prior to host infection.

Recent data on the chemical ecology of *C. elegans* illustrate a rich capacity for nematodes to influence the behaviour of conspecifics. We aim to better understand pre-parasitic J2 behaviour to help seed the development of novel control strategies that disrupt soil-based orientation-behaviours. We hypothesise that inter-J2 signalling modulates the behaviour of conspecifics and set out to investigate social behaviours (dispersal, aggregation) and determine the role of FMRF-amide like (*FLP*) neuropeptides in such behaviours.

The role of environmental factors, such as population density and CO₂, on the dispersal of J2's was examined using a pluronic gel-based dispersal bioassay. Results showed that worms derived from higher density populations dispersed significantly further and faster than worms derived from less dense populations, e.g. after 6h, 43% of *Globodera pallida* J2s derived from high density populations had dispersed compared to only 14% from low density populations; similar results were seen for *Meloidogyne incognita* J2s. CO₂ was also shown to be a considerable factor in dispersal as after 6h, 31% of a low density population of *G. pallida* had dispersed after incubation in 5% CO₂, compared to 11% incubated in atmospheric CO₂ (~0.05%). The effects of population density and CO₂ exposure on J2 dispersal were found to be reversible.

Further qPCR analysis showed profound *flp* diversity within PPNS. Those *flp* genes most highly expressed were *flps* 6, 7, 12, 14, 16 and 18. The encoded peptides, along with FLP-21 (impacts sociality in *C. elegans*), were investigated for their ability to alter PPN J2 dispersal behaviour.

In conclusion, our results show that the sensory ecology of PPN's involves a complex array of both environmental and genetic influences enabling successful orientation and dispersal of J2's to a suitable host. This provides a unique system to exploit for the development of plant nematode control strategies.

O NEM I-4

The germin-like protein BvGLP-2 from sugar beet (*Beta vulgaris* L.) is a superoxide dismutase and participates in *Hs1^{pro-1}*-mediated nematode (*Heterodera schachtii* Schm.) resistance

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Resistance against beet cyst nematode (*Heterodera schachtii*) in sugar beet (*Beta vulgaris*) is controlled by the *Hs1^{pro-1}* locus. Here, we report the identification and characterization of *BvGLP-2* from sugar beet and potential function of *BvGLP-2* in the nematode resistance. The gene was identified by a comparative transcriptome analysis between resistant and susceptible sugar beet roots after nematode infection. The putative open reading frame of *BvGLP-2* encodes a peptide of 225 amino acids sharing a high homology with plant germin-like proteins (GLPs). Expression of *BvGLP-2* is root-specific in both resistant and susceptible plants, but enhanced by nematode infection only in the resistant roots, suggesting its possible function in the *Hs1^{pro-1}*-mediated nematode resistance. To substantiate this, we tested transgenic sugar beet roots and *Arabidopsis thaliana* plants overexpressing *BvGLP-2* in response to nematode infection. In comparison, overexpression of *BvGLP-2* led to a significant reduction in the number of nematode females in both of transgenic beet roots and *Arabidopsis* plants, while knockout of the *BvGLP-2* ortholog in *Arabidopsis thaliana* 'Columbia', a partial nematode resistant ecotype significantly enhanced susceptibility to nematode infection. In-gel enzyme activity assays with recombinant proteins of *BvGLP-2* that was either transiently expressed in *Nicotiana benthamiana* or overexpressed in transgenic *A. thaliana* plants revealed that *BvGLP-2* is a H₂O₂ - generating superoxide dismutase (SOD). Furthermore, the *BvGLP-2* overexpression in *Arabidopsis* specifically activates a serine/threonine kinase (*OXI1*) and its related signaling pathways. We conclude that *BvGLP-2* represents a key regulator in establishment of the *Hs1^{pro-1}*-mediated nematode resistance, which follows an *OXI1*-specific signaling route. A possible function mode is discussed.

Oral Presentations

Nematodes I

O NEM I-5

Influence of *Brassica* cover crops on root knot nematodes, soil abiotic factors and plant data in tomato production

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Plant-parasitic nematodes cause severe damage to various crops. Brassicaceae cover and/or biofumigation crops have been investigated as an alternative strategy to control these pests. Factors that play an important role in determining efficacy of Brassicaceae control strategies are climate, soil conditions and plant density. *Brassica juncea* (cvs. Rocket Trio and Nemat), *Eruca sativa* (cvs. Caliente and Fumigreen) and *Raphanus sativus* (cvs. Doublet and Terranova) were evaluated in a field experiment in the Mpumalanga Province of South Africa against a mixed population of *Meloidogyne incognita* and *M. javanica*. Tomato seedlings (cv. Monica) were planted as a follow-up crop in these plots. Results showed a significantly higher yield ($P < 0.05$) in both *E. sativa* and *R. sativus*-treated plots compared with untreated control (UTC) and *B. juncea*-treated plots. Nematode control was also substantially better in the *E. sativa* and *R. sativus* treatments compared with UTC and *B. juncea* treatments. Statistical analysis showed that cover crops could explain 70% and 40% of the variation in nematode and plant data respectively, while correlation analysis confirmed the relationship between nematodes and plant data. The effect of cover crops and abiotic soil conditions on nematode variability was also investigated. Although changes in soil condition took place between time of incorporation of the organic material and at planting of the tomato crop, changes were similar for all treatments. When the effect of cover crop and soil abiotic factors on nematode variability was determined at termination of the trial, cover crops could explain 31% of the nematode variability while 13% was explained by soil abiotic factors. The effect of soil factors increased from 0.6% to 13% during the trial period and was probably the result of the changes occurring in the soil substrate during the decomposition period. Data obtained during this research demonstrated the benefit of using Brassicaceae crops for their cover- and biofumigation characteristics to reduce root-knot nematode, increase crop yield and contribute towards soil health.

O NEM I-6

Host suitability and response of different vegetable genotypes to *Meloidogyne incognita* race 2 and *Meloidogyne javanica* in South Africa

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Vegetables are an important component of the human daily diet and represent high value cash crops for small-scale- and commercial farmers. The host suitability of 10 locally available genotypes of *Amaranthus*, 20 genotypes of *Capsicum*, 10 genotypes of *Daucus carota*, 7 genotypes of *Beta vulgaris* and 3 genotypes of *Spinacea oleracea* were assessed in separate greenhouse studies for resistance to *Meloidogyne incognita* race 2 and *Meloidogyne javanica* respectively. Substantial variation existed amongst the vegetable genotypes in the greenhouse screenings with regard to their host status to the respective nematode species tested. However resistance to *M. incognita* race 2 was identified in *Amaranthus* genotype 'Local 33' and *Capsicum* genotype 'Tobasco', which was subsequently verified in a follow up trial using a range of initial population densities together with a susceptible *Amaranthus* genotype 'Bosbok Thepe' and susceptible *Capsicum* genotype 'Paprika'. Reproduction factors of the nematodes were used as the main criterion to evaluate resistance. In the microplot trial, *Amaranthus* genotype 'Local 33' showed resistance to all inoculation levels while *Capsicum* genotype 'Tobasco' showed resistance at the lower inoculation levels but not at the higher levels. *Amaranthus* genotype 'Local 33' can thus be regarded as resistant to the population of *M. incognita* race 2 used in this study. The need exists for more frequent and extensive screenings of the various vegetable genotypes in order to provide small-scale farmers with better options for improved and sustainable yields.

Oral Presentations

Viruses

O VIR 1

From local lesions, via an inhibitor of virus replication (IVR), isolation of a gene involved in resistance to TMV, that induces resistance to several plant pathogenic fungi in tomato - 45 years of research

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Cells inside a local lesion contain less particles (about 10^3) than cells in a systemic infection (6×10^7). Localization seems to be due to reduced multiplication and not due to barrier substances. In the peripheral cells of a starch lesion on cucumber cotyledons the number of TMV particles was about 1/10 compared with those in the central part of the starch lesion, with no ultrastructural changes at the border of the lesion. Localization of TMV in tobacco, containing the *N* gene, is associated with an antiviral protein - 'inhibitor of virus replication (IVR). IVR was released into the medium of TMV-infected protoplasts from Samsun NN tobacco. IVR inhibited virus replication in protoplasts from both resistant Samsun NN, exhibiting local lesions and susceptible *N. tabacum* cv. Samsun plants (nn). IVR inhibited TMV in protoplasts and leaf disk. IVR also inhibited PVX, PVY and CMV in leaf disks indicating that IVR is neither host nor virus specific. The 23 kDa protein from SDS-polyacrylamide gels yielded a molecule with antiviral properties. Antibodies against the IVR protein enabled detection of the 23 kDa protein.

Sequence analysis of clone NC330 indicated that the C-terminus of the deduced protein is rich in aspartic acid and glutamic acid, hydrophobic and with a helical structure. The NC330 protein is mainly a tetratricopeptide repeat (TPR) protein and leucine rich repeats (LRR). Transformation of Samsun nn with NC330, encoding an IVR-like protein, gave transgenic plants, expressing variable resistance to TMV and the fungal pathogens *Botrytis cinerea*. Transformation of tomato plants with the IVR gene resulted in partial resistance to *A. alternata*, *P. aphanidermatum* and *R. solani*. The finding that an R gene associated with virus localization also induces resistance to fungal diseases suggests that some R genes have a wider range of activity than has been assumed.

O VIR 2

Anthropocene: viral spread, evolution and diversity in ornamentals

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The term "anthropocene" coined by Paul J. Crutzen in 2002 (Geology of mankind: the anthropocene, Nature 415, 23) is referring to our current epoch and illustrates the manifold influences by human existence and actions on geology and evolution.

Ornamentals are plants solely produced to please the eye of the beholder. The EU Commission stated 2014 an increase in flower production and cultivation of ornamental plants. The collected data reveals that in the EU one of the world's highest densities of flower production per hectare exists that comprises 10% of total world area and 44% of world flower and pot-plant production. It is predicted that the market for ornamentals in the EU will further expand and grow to 37 billion Euros in 2016 (Swedish Chamber of Commerce, 2011).

Global production and trade pathways as well as the consumer's growing demand for new species and cultivars and their availability in shorter periods of time open gateways for viruses. The mass production of naturally cloned material displaying a uniform phenotype has an inherently high risk for multiplying and spreading viruses and/or viroids unwillingly, especially in case of asymptomatic (latent) infection. The latter is a common feature for carlaviruses. Synergy of electron microscopy and molecular biology tools were used to resolve these diagnostic challenges. In samples obtained in Germany in 2006 to 2012 comprising the families of *Alliaceae* and *Convallariaceae* as well as dicot host plants of the families of *Cactaceae*, *Ericaceae*, *Passifloraceae*, *Ranunculaceae*, *Scrophulariaceae* and *Solanaceae* 12 different carlaviruses have been identified. Further characterization revealed the presence of three different isolates of *Shalot latent virus* in *Allium sativum* and for at least three *Potato virus M* isolates in *Solanaceae*. In the last 14 years, with the exception of the years 2002 and 2003, 7 different tobamoviruses were detected in submitted plant samples out of the families of *Balsaminaceae*, *Berberidaceae*, *Brassicaceae*, *Cactaceae*, *Gentianaceae*, *Gesneriaceae*, *Goodeniaceae*, *Lamiaceae*, *Orchidaceae*, *Poaceae*, *Sarraceniaceae*, *Scrophulariaceae* and *Solanaceae*. The highest diversity of detected virus species was found in solanaceous plants. Interestingly, the occurrence of tobamoviruses in distinct years was variable. Tobamoviruses are transmitted by contact. Thus strict hygiene and repeated testing for a broad range of tobamoviruses during plant production and distribution is essential to avoid virus spread and to recognize the spectrum of infecting tobamoviruses early.

Oral Presentations

Viruses

O VIR 3

Emergence of plum pox virus, the most damaging viral pathogen of stone fruits, in Japan

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Sharka disease, caused by plum pox virus (PPV, genus *Potyvirus*), is the most serious viral disease of stone fruits (*Prunus* spp.). Among the eight known strains of the virus, PPV-D is the most important due to its recent spread from Europe to North and South America and Asia. In 2009, we found PPV (which belongs to the D-type strain) in Tokyo, Japan, for the first time. PPV was found in a new natural host, *Prunus mume* (Japanese apricot), which is one of the most popular fruit and flowering trees in East Asia, including Japan. For the control of PPV, we developed rapid test kits for the detection of PPV based on immunochromatography and reverse transcription-loop mediated isothermal amplification (RT-LAMP), which can detect PPV within 15 and 60 minutes, respectively. Using these kits, the plant protection station of Japan has conducted nationwide surveys for six years, and found more than 20,000 PPV-positive trees across 11 prefectures (Tokyo, Ibaraki, Saitama, Kanagawa, Aichi, Mie, Shiga, Nara, Wakayama, Osaka, and Hyogo). However, the viral transmission routes in Japan are poorly understood, which is hindering the eradication of PPV. Therefore, we collected geographically-diverse PPV isolates and performed a molecular epidemiological analysis. Despite the low genetic diversity, the phylogenetic tree based on the complete genome sequences enabled precise estimations of the transmission routes of the PPV-D strain at the national and international level.

O VIR 4

Infection of grasses and cereals by wheat dwarf virus and a diverse set of luteoviruses

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To understand the ecology of viruses infecting grasses and cereals, we have carried out virus screens and sequence analyses. Barley yellow dwarf virus (BYDV)/Cereal yellow dwarf virus (CYDV) constitute a group of viruses in the family Luteoviridae that infect grasses and cereals. They have a genome of single-stranded RNA and are classified into several species, which are transmitted by different aphids in a persistent manner. The different virus species can be difficult to discriminate using serological methods, and the high viral diversity is only now beginning to be revealed. In a survey of B/CYDVs in cereals and grasses from different regions of Sweden and Estonia, we have detected a high virus incidence and sequence analyses have revealed infection with BYDV-PAV, BYDV-MAV, BYDV-PAS, BYDV-OYV, BYDV-GPV and BYDV-RMV. The four latter species were found for the first time in Sweden and BYDV-GPV for the first time outside China. Several species of BYDV/CYDV could be found in the same field with up to three species in the same plant. Different viruses were detected in forage grasses and cereals growing next to each other, suggesting that the forage grass was not the virus source for the infection in cereals. BYDV-OYV has previously only been described as a single isolate from an oat plant in Latvia. We could now find isolates of BYDV-OYV in different parts of Sweden. The first complete genome sequencing of BYDV-OYV shows that it is related to viruses within the genus Luteovirus, but sequence comparisons reveal that it is distinct from the other species and should tentatively constitute a new species. Wheat dwarf virus (WDV; genus *Mastrevirus*; family *Geminiviridae*) is also infecting grasses and cereals. It has a genome of single-stranded circular DNA and is transmitted in a persistent manner by leafhoppers. In our studies to identify virus reservoirs for cereal-infecting viruses, WDV was detected at a low frequency in randomly sampled ryegrass plants. Nucleotide sequence analyses revealed that the ryegrass isolates were closely related to those from wheat. Infection tests with WDV isolates from wheat resulted only in low infection rates and low virus titers in different ryegrass species and cultivars, while wheat plants were very susceptible. The results suggest that WDV may persist at low levels in ryegrass and other grasses.

O VIR 5

Establishment of an *in-vitro* assay for functional characterization of the viral proteinase and processing of RNA2-encoded polyprotein P2 of *Cherry leaf roll virus* (CLRV)

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Question: The bipartite genome of *Cherry leaf roll virus* (CLRV, Genus *Nepovirus*, subgroup C, family *Secoviridae*) consists of two positively orientated single-stranded RNAs, which encode for two polyproteins (P1 and P2). P1 harbors characteristic domains for a proteinase-cofactor (PCo), a helicase (Hel), a genome-linked protein (VPg), a proteinase (Pro), and an RNA-depending

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polymerase (Pol). P2 encodes, besides a region at the 5'-end that has not been functionally assigned by now, the movement protein (MP) and the coat protein (CP). The polyproteins are processed into their functional units by the viral proteinase. *In-silico* analysis of the full-length sequence revealed putative processing sites similar to already proven sites of related nepoviruses. Prerequisite for the functional characterization of viral gene-products is the elucidation of their processing into the mature subunits. Aim of the project is therefore to establish an *in-vitro* assay to prove the proteolytic activity of the proteinase and to identify the cleavage sites of CLRV.

Methods: The polypeptide constituting the putative proteinase of CLRV was heterologously expressed in *E. coli* and purified under native conditions. Regions surrounding putative cleavage sites were cloned and expressed *in-vitro*, using biotinylated lysins added to the nascent protein as a label. Activity assays were performed by subjecting the *in vitro* translation products as substrates to the proteinase. By western blot and streptavidin-AP conjugates, processing at the putative cleavage sites was monitored.

Results: The proteinase of CLRV was successfully expressed in *E. coli* and was purified by affinity-chromatography. The *in-vitro* assay for the testing of the proteolytic activity of the proteinase was successfully established. Translation products comprising the putative processing-sites of P2 were subjected to the *in-vitro* assay for their experimental verification.

Conclusion: The established *in-vitro* assay is suitable for experimental confirmation of cleavage sites utilized by the proteinase of CLRV responsible for processing of P1 and P2.

O VIR 6

MicroRNA-like fragments from Turnip mosaic virus targets host gene *HVA22D*: a new opportunity to develop resistant crops

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Plant viruses are notorious for their rapid evolution, enabling them to overcome host resistance and making them highly successful. We focus on a specific Australian strain of *Turnip mosaic virus* (TuMV), first sequenced in our laboratory. Previous research found that this strain differed from other previously sequenced strains of TuMV and that its genome also coded for viral miRNA-like fragments which were shown to act in a similar manner to plant miRNA in regulating gene expression. The virus-derived miRNA-like fragments were found to target the *HVA22D* gene in *Arabidopsis*. The T-DNA mutant *hva22d* shows increased susceptibility to TuMV. By introducing silent mutations in the viral miRNA binding site of *HVA22D* we were able to interfere with miRNA-host gene binding. In addition, we have developed a decoy construct that specifically binds to TuMV miRNA. Both of these approaches provide new strategies to create TuMV-resistant plants.

To better understand the mechanism of TuMV-plant host gene interaction, we focus on studying the function of *HVA22D* in more detail. *HVA22D* is an abiotic stress inducible protein and is also thought to have a role in controlling autophagy. *HVA22D* is one of five homologs found *Arabidopsis*, and is most tightly regulated by abscisic acid. The gene is highly conserved with homologs identified in diverse eukaryotes; the *YOP1* gene in yeast as well as *TB2* and *DP1* in humans have a role in tubule-forming proteins. The yeast homolog and those found in *Arabidopsis* are also thought to regulate autophagy in a negative manner. *HVA22D* may be involved in changes of membrane composition due to environmental stresses. We hypothesise that the plant may alter its lipid and membrane composition in an attempt to prevent the spread of the virus via their plasmodesmata or to inhibit virus replication. By using *HVA22D*-overexpressing viral miRNA-resistant mutants we will determine whether the plant does alter the lipid composition of its membranes of the mutant and wild type plants both challenged with the virus. It is hoped that this work will provide new plant viral resistance strategies and hopefully generate more durably resistant crop varieties to increase food security for the future.

Oral Presentations

Soil-borne Pests and Pathogens

O SOIL 1

Solving the challenges presented by soil borne plant pathogens through the application of nucleic acid detection methods

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Soil-borne plant pathogens are a significant constraint on crop production worldwide. Despite their negative impact on crop production, relatively little is known about soil-borne plant pathogens compared to foliar pathogens. Hidden from view, they are difficult to study. Often the symptoms expressed above ground can also be attributed to other diseases, environmental pressures and nutritional deficiencies. Nucleic acid based methods offer quantifiable, specific and sensitive ways to detect plant pathogens. Such methods can be utilized in solutions to the challenges presented by soil borne plant pathogens in principally in three ways: 1. investigating the causal agent in disease complexes and the epidemiology of the pathogen; 2. determining the effectiveness of integrated disease management strategies such as crop rotation and 3. Used to screen soil for the presence of important pathogens as part of a diagnostic service for growers. At Fera, high throughput, bulk soil DNA extraction methods and real-time PCR assays have been developed for various plant pathogens on several crops and are used as both a tool for research and as part of a diagnostic service offered to growers. The development of such soil DNA extraction capable of extracting from up to 1 kg of soil will be presented along with the development of new, more sensitive real-time PCR assays, including ddPCR assays. Using case studies from *Rhizoctonia* and *Verticillium* in potato, cereal and soft fruit crops, how various diagnostic assays have been deployed to determine the causal agent, investigate the epidemiology of a soil borne disease and provide evidence for suitable control options will be discussed. Finally, how the methodology has been deployed as a diagnostic service to growers will be presented. In conclusion, nucleic acid detection methods for soil borne plant pathogens offer a powerful tool for the study of the group of pathogens and can offer a cost effective, rapid tool as part of service to growers.

O SOIL 2

Suppressive capacity of different decomposition levels of compost and its role on severity of *Pythium* and *Rhizoctonia* damping-off in tomato and on substrate microbial activity

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Use of compost as amendment can be effective to suppress several soil-borne diseases. Decomposition level of amendment plays a fundamental role in suppression, since immature composts or excessively decomposed organic amendments are conducive to diseases. The objective was evaluate the suppressive capacity of a compost with different decomposition levels on the damping-off caused by *Pythium ultimum* or *Rhizoctonia solani* in tomato plants and on the microbial activity measured as the rate of hydrolysis of fluorescein diacetate (FDA). Pots were filled with compost incubated for 0, 9 and 12 month and a conductive soil. The increase of decomposition level was corroborated by CPMAS ¹³C-NMR spectra analysis where O-alkyl and Carboxyl picks were decreased with the time of incubation. Four pre-germinated seeds were sown per pot. The pots were maintained on greenhouse conditions and were watered each two days to maintain soil moisture. The design used was randomized block complete with 10 replicas. Variables were analyzed with two way ANOVA and Tukey test was used to mean comparison Damping-off severity was rated with a scale on 1 to 4 at 12 days after sowing. And FDA activity was measured on a spectrophotometer set at a wavelength of 490 nm. The decomposition level had effect on severity of damping-off, because increasing incubation time of compost increased the disease caused by *P. ultimum*. None of treatments were suppressive to *R. solani* because damping-off severity caused by this pathogen did not have significant differences with the conductive soil that reached severity of 4. FDA activity was inversely correlated with incubation time and disease severity. The suppressive capacity of compost is lost when advancing compost decomposition. Microbial activity measured as FDA can be a useful indicator of disease suppression

O SOIL 3

***Verticillium longisporum* on oilseed rape - reviewing the state-of-the-art of a hidden pathogen with uncommon properties**

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Introduction: *Verticillium longisporum* (VL) is a soilborne vascular pathogen of oilseed rape (*Brassica napus*; OSR) which does not cause a wilt but induces premature ripening leading to potentially significant yield losses. The disease has been limited to European OSR production, but in 2014, VL has been detected for the first time in Manitoba, Canada. The pathogen is an

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amphihaploid hybrid form of *Verticillium* and host-specific on *Brassicaceae*. Three different hybrids forming lineages with distinct host specificity have been identified so far.

Objectives: The paper reviews the state-of-the-art on the disease and responses induced by VL in OSR.

Materials & Methods: The studies were conducted on a broad range of experimental levels, from *in vitro* to field applying molecular, biochemical, physiological and histological methods.

Results: VL survives with microsclerotia in the soil and colonizes the root parenchyma intra- and intercellularly. Plants are systemically colonized in a tri-phasic manner. After an initial biotrophic phase, for an extended period until plant maturity, the fungus remains xylem-limited after which it breaks through the stem parenchyma to start its final saprotrophic phase. Expression of cultivar resistance is restricted to the hypocotyl and consists of accumulation of cell-wall bound phenols and lignins, and vessel occlusions. Resistance is quantitative and specific to VL, and does not operate against other vascular pathogens. No soluble antifungal plant metabolites are detected in the xylem. VL does not affect plant water relations and resistance induced vascular occlusions do not compromise drought stress resistance. VL signaling in OSR involves salicylic acid (SA) dependent genes but not the jasmonate/ethylene dependent chain. VL induces elevated levels of SA/SAG in stem tissue, which correlate with disease severity, indicating fungus-induced redirection of the phenylalanine/cinnamate pool towards SA synthesis. In contrast, SA deficient *nahG* transformed OSR plants exhibit a high susceptibility to VL.

Conclusions: VL is an amphihaploid hybrid species, which is unable to induce wilting, switches from biotrophy to necrotrophy, and induces accumulation of SA, implying a dual role of SA in basal and cultivar-specific resistance.

O SOIL 4

Developing, Implementing and Evaluating Management Strategies for *Rhizoctonia solani* on Sugar Beet

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Rhizoctonia solani Kühn causes damping off, crown rot and root rot of sugar beet (*Beta vulgaris* L.), and is the most damaging pathogen for growers in Minnesota and North Dakota, who produce 60% of the US sugar beet crop. Most commercial sugar beet varieties are susceptible to or have only partial resistance to *R. solani*. Research was conducted at Hickson, North Dakota, USA, to evaluate fungicide treatments for controlling *R. solani*. The site was artificially inoculated with *R. solani* AG 2-2 IIIB grown on barley at 36 kg ha⁻¹ just prior to planting. Penthiopyrad (14 g kg⁻¹ seed), a succinate dehydrogenase inhibitors, was evaluated as a seed treatment alone, and with a post application of azoxystrobin (0.7 L ha⁻¹), a quinone outside inhibitor. Azoxystrobin was applied in-furrow at planting followed by a post application; and as a post application only. Planting was done on 20 May, 2010 into six-row plots where the innermost 4-rows were treated. Plant populations were recorded for several weeks early in the season and at harvest. Roots were harvested on October 4, weighed and analyzed to determine recoverable sucrose. Azoxystrobin applied in-furrow followed by a post application consistently resulted in significantly greater plant populations and recoverable sucrose compared to the non-treated control. Penthiopyrad provided early season control by protecting plant populations compared to the non-treated control, but was not effective during the latter part of the season since populations were reduced. Penthiopyrad followed by azoxystrobin resulted in higher populations and recoverable sucrose compared to the non-treated control. Extension specialist recommended the use of penthiopyrad as a seed treatment followed by azoxystrobin at the 4-6 leaf stage. In 2014, about 70% of growers at American Crystal Sugar Company, the largest sugar cooperative in the USA, adopted the use of penthiopyrad seed treatment the first year it became available. Seventy-three percent of survey respondents who used penthiopyrad were satisfied with its performance and 75% reported excellent or good control with azoxystrobin. The use of penthiopyrad as a seed treatment followed by azoxystrobin should provide effective disease control and serve as a fungicide resistance management strategy to prolong the usefulness of these products.

O SOIL 5

The role of pathogens in scarab pest outbreaks and management

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Scarab pests (Coleoptera: Scarabaeidae) occur worldwide and outbreaks are frequently associated with land use change or crop renovation. High pest densities after land modification or invasion of new areas suggest a release from control by natural enemies. Conversely, it has been found that chronic diseases are abundant among stable, lower density, established populations. In the recent, severe invasive outbreak of the coconut rhinoceros beetle (*Oryctes rhinoceros*) on the island of Guam, the beetle has been shown to be free of the biocontrol agent *Oryctes nudivirus*, which has successfully controlled the pest and persisted for more than 40 years after release across the insect's range in the Pacific Islands. Bacterial diseases

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(*Serratia* spp.) are a common controlling factor of the grass grub (*Costelytra zealandica*) in New Zealand pastures, but forestry conversion to pasture has created *Serratia*-free habitats and spectacular outbreaks of the pest. Disease is re-established in the outbreak populations by application of *Serratia entomophila* as a biopesticide. Drainage of swamps and conversion to pasture has created conditions for an explosion of the population of *Pyronota setosa*, previously unrecognised as a pest, on the New Zealand west coast. In time, without further disturbance, natural diseases build up and stabilise the populations. In all cases, land use changes or invasion have created pathogen-free environments where the pests can prosper. Remediation can be made through introduction of pathogens into the pest populations through inoculation, product application or land management.

O SOIL 6

Towards a technical attract-and-kill formulation within the project ATTRACT

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Several soil-borne herbivorous insect pests like wireworms, western corn rootworm and black vine weevil cause tremendous losses in different crops like potato, maize or strawberry. Due to the fact that the control of these pests with soil insecticides is severely restricted or was recently abandoned, there is a need for effective alternative formulation and treatment strategies. The joint project ATTRACT aims at developing innovative attract-and-kill formulations.

These formulations are based on biopolymer beads combining an attractive component with an insecticide. CO₂-releasing baker's yeast which has the potential to act as an effective attractive compound for various insect pests was used as attractant. Amylase was co-immobilized to achieve a slow-release of glucose from corn starch which is then converted into CO₂ by baker's yeast. For control of pest insects attracted to the formulation a neem extract, acting as the kill component, was co-formulated. Here we report on the encapsulation and drying of this novel attract-and-kill formulation on lab and technical scale.

The co-immobilization of baker's yeast, starch and amylase in Ca-alginate beads led to a significant CO₂ production in peat soil for up to six weeks. It was observed that CO₂ release was temperature-driven thus matching CO₂ release and attraction to the mobility of insects in soil. Data will be presented on the generation of CO₂ gradients in different soil types and various moisture contents. Beyond this we were able to co-encapsulate the bioinsecticide NeemAzal® technical with an encapsulation efficiency of about 95 %. Depending on the formulation, baker's yeast showed high survival up to 60 % at a water activity of 0,1 during drying of the beads.

For scale-up, high throughput encapsulation technologies like jet cutting and several drying processes were investigated. Moreover the stability of the optimized formulations was investigated in accelerated and real time storage tests. Process details and data confirmed that this formulation can be produced on large scale with high survival and good shelf life. Data on the efficacy of this novel attract-and-kill formulation under field conditions and the capability of reducing wireworm damage on potatoes will be presented by our partners from the group of S. Vidal.

Oral Presentations

Tuta absoluta

O TUT 1

Tuta absoluta Management Programs of IPM Innovation Lab

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The IPM Innovation Lab (IPM IL) first became involved in forecasting and management of the South American tomato leafminer, *Tuta absoluta* when it invaded Senegal in 2012. It organized a workshop in Dakar, Senegal in May 2013 to sensitize West and Central African countries on the impending danger of invasion of this pest. When this pest showed up in Ethiopia, again the IPM IL organized a workshop in Addis Ababa, Ethiopia for East African and South Asian countries in November 2013. At present, *T. absoluta* is known to occur in Senegal, the Gambia, Niger, Sudan, Ethiopia, Kenya, and Tanzania in Sub-Saharan Africa. We have also distributed pheromone traps to these countries for monitoring. In 2015, the IPM IL is planning on incorporating control tactics of this pest in the IPM packages for tomato developed in Ethiopia, Kenya and Tanzania. Additionally it is planning on developing a project for modeling future spread of this pest around the world.

O TUT 2

The tomato leaf miner, *Tuta absoluta* (Meyrick), pest status and its integrated pest control programs in the Arab region

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The spread and damage of transboundary plant pests and diseases have significantly increased worldwide during the last few decades. This spread increase is attributed mainly to the increase in trade exchange with plant and plant products and means of transportation of plant consignments that might be associated with pests and diseases. Among such pests is the tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae) which has recently invaded the Near East region. The pest is originated from South America and was recorded for the first time in Europe in Spain in 2006. It invaded the Near East through North African countries, where it has been recorded in 2008 in Morocco and subsequently in Algeria, Tunisia and Libya. Later, *T. absoluta* was identified in tomato fields in Egypt and Jordan in 2009, Lebanon, Syria and Iran in 2010, Iraq in 2011 and Yemen in 2012. The *T. absoluta* is one of the most destructive pests seriously affecting tomato yields and production in most countries of the region as the tomato is an important cash crop for farmers. The pest attacks almost all parts of the plant's vegetative system in greenhouses as well as in open fields. In case of severe infestation, losses could reach up to 80-100% of the crop. Moreover, other vegetable crops within the same family have also been reported as plant hosts: potato, eggplant, pepperetc. Infestation rate of *T. absoluta* across countries of the region increased faster than the ability to cope with it. Therefore, the governments in these countries initiated integrated pest management (IPM) programs for preventing its further spread. IPM programs, based on pest surveillance, use of sex and aggregation pheromones, biological control measures and adoption of phytosanitary measures were implemented, with different degrees of success have been achieved. Regional collaboration and coordination among countries of the region are still needed for national and regional sustainable strategy for *T. absoluta* management.

O TUT 3

***Tuta absoluta* (Lepidoptera: Gelechiidae) development on wild and cultivated plant species**

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Introduction: The tomato leafminer, *Tuta absoluta* (Lepidoptera: Gelechiidae), is a widespread invasive species damaging economically important solanaceous crop plants, including tomatoes and potatoes. Little is known about the ability of the microlepidoptera to encounter and develop on alternative wild and agricultural plant species. These plants could provide refuges and have to be identified for more efficient integrated management strategies.

Objectives: In the present study, we assessed under laboratory conditions the ability of *T. absoluta* to develop on such plant species referred as potential hosts in the literature, including Solanaceae, Chenopodiaceae, Convolvulaceae, Fabaceae, and Malvaceae.

Materials and methods: For each plant species, fitness tests were performed in Petri dishes by isolating single individuals with excised leaf. Two choice behavioral assays were performed in flying tunnels. Volatile organic compounds released by solanaceous plants were trapped using a dynamic collection system, and analyzed by GC-MS.

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Tuta absoluta

Results: We found that *Solanum* species allowed higher larval survivability and shorter development time (from egg to adult emergency) compared to the other plants. Non-solanaceous plants were not able to sustain *T. absoluta* larvae. Two choice behavioral assays revealed that adult distribution and female oviposition did not differ between *Solanum* species, which were preferred to other tested solanaceous plants. The hypothesis that female host plant choice is influenced by plant volatile organic compounds was tested. *Solanum* volatile profiles showed similarities, and were presenting quantitative and qualitative differences with the other tested solanaceous plants, providing some explanations in the observed behavioral discrimination. Further electrophysiological and behavioral assays are required to confirm the effect of specific chemicals on the choice of the oviposition site in *T. absoluta*.

Conclusion: It can be concluded that *Solanum* species are the more suitable hosts for *T. absoluta* development. Other solanaceous plant species could be opportunistically colonized with little incidence but care should be taken in these results as genetic variability in insects and plants, as well as plant physiological state, might have an impact on the pest survivability.

O TUT 4

Interaction between *Beauveria bassiana* and certain insecticides used for management of *Tuta absoluta*

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Introduction: Control of tomato leafminer is highly dependent on the use of conventional chemical insecticides. Synergistic combinations of biological and chemical insecticides might yield promising alternative for management of this invasive pest.

Objective: The study was done to determine the potential of the entomopathogenic fungus *Beauveria bassiana* and chemical insecticides chlorantraniliprole, dichlorvos, indoxacarb, and their combination for controlling *T. absoluta*.

Materials and methods: The effect of recommended doses of chlorantraniliprole, dichlorvos and metaflumizone on germination, vegetative growth and sporulation of one native and one commercial isolate of *B. bassiana* was investigated in PDA culture medium. In this study, the interactions between *B. bassiana* isolates and sublethal doses of the chemical insecticides in 2nd instar larvae of *T. absoluta* were also assessed. To test for the interactions, tomato leaves and the larvae on them were treated with LC₁₀ or LC₂₅ of the insecticides. Then treatment with *B. bassiana* (at LC₅₀ level) was followed 0, 12, 24 or 36 h after insecticide application. The mortality of the larvae was recorded 96 h after fungus treatment.

Results: Chlorantraniliprole and indoxacarb did not reduce germination, vegetative growth or sporulation of *B. bassiana*. However, these parameters of the fungus were significantly reduced following exposure to dichlorvos. The reactions were similar in both isolates of *B. bassiana*. Applying *B. bassiana* immediately after insecticide treatment (0 h), caused antagonistic effect in dichlorvos, and additive effects in chlorantraniliprole and indoxacarb treatments. Applying *B. bassiana* 12 and 24 h after treatment with LC₂₅ of chlorantraniliprole and indoxacarb resulted in synergism. But, synergism with LC₁₀ of chlorantraniliprole and indoxacarb was observed only after 12 h. Treating the larvae with *B. bassiana* 12 and 24 h after dichlorvos application, resulted in additive effects. Treating the *T. absoluta* larvae with the fungus 36 h after application of the insecticides resulted in additive effect in all treatments.

Conclusion: Based on the results obtained, simultaneous use of the chemical insecticides tested and *B. bassiana* can be recommended for *T. absoluta* control except for dichlorvos. The combination effect can even be improved by allowing appropriate time interval.

O TUT 5

Semiochemicals mediate the tritrophic interactions between tomato plant, the leafminer *Tuta absoluta* and the generalist predator *Macrolophus pygmaeus*

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The invasive species *Tuta absoluta* Meyrick (Lepidoptera, Gelechiidae) originating from South America is a key pest in tomato crops. Accidentally introduced in Spain, it has spread throughout Europe and North Africa within a few years. *Macrolophus pygmaeus* Rambur (Heteroptera: Miridae) is currently released in tomato greenhouses as a biocontrol control agent against this leafminer. The effectiveness of predators partly lies in their ability to find their preys most likely by using volatile compounds emitted by infested plants (HIPVs).

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Tuta absoluta

In the present study we evaluated the ability of *M. pygmaeus* to use HIPVs to locate its prey. First, we found that *M. pygmaeus* was able to discriminate non-infested versus infested plant in double choice bioassays and flight tunnel. Secondly, we collected the volatile molecules released by *T. absoluta*-infested tomato plants. A total of 35 compounds were identified by gas chromatography, including monoterpenes, sesquiterpenes and C6-compounds. Nineteen of these chemicals significantly varied in quantities with the infestation level, and were used in the subsequent electrophysiological (EAG) assays, to determine whether they were perceived by the olfactory apparatus of *M. pygmaeus*. Most of the *T. absoluta* induced-tomato volatiles (17 out of 19) elicited electrical depolarization from *M. pygmaeus* antennae. Finally, we evaluated the behavioural responses of *M. pygmaeus* to each EAG-active chemical in double choice olfactometer. We found that none of the behavioural responses were comparable to those observed with natural blend. This study is a step forward in the understanding of *M. pygmaeus* attraction to *T. absoluta* infested plants.

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Challenges in Plant Protection II

O CPP II-1

Citrus Black Spot: history, epidemiology and pathways

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Citrus Black Spot (CBS) is primarily a cosmetic disease causing a variety of small black spots on mature citrus fruit, but in severe cases in highly suitable climates can also lead to fruit drop. CBS has been researched over many years in South Africa, China, Australia, Brazil and USA. Research findings are in agreement regarding CBS epidemiology and control measures. Despite the fact that CBS has never been recorded to spread to new areas through movement of infected citrus fruit, and that CBS has never spread to any part of the world with a Mediterranean type, winter rainfall climate, the European Union (EU) still regards *Phyllosticta citricarpa*, the CBS pathogen, as an A1 quarantine pathogen and imposed a zero tolerance of CBS on fresh fruit imported from production regions where CBS occurs. This is contradictory to pest risk assessments (PRA) on CBS by SA and USA that concluded citrus fruit is not epidemiologically significant as a pathway for introduction of *P. citricarpa* into new areas. This study presents a global perspective on CBS occurrence, CBS epidemiology and pathways for spread and introduction. The findings are discussed in context with the recent PRA on CBS conducted by the European Food Safety Authority (EFSA), which was disputed by an international panel of CBS experts from Argentina, Australia, Brazil, SA and USA. Based on existing and new scientific evidence, as well as their collective experience of 545 years of CBS work, the CBS expert panel concluded that it was in agreement with earlier PRAs, conducted by SA and USA, in which it was concluded that fresh citrus fruit is not a realistic pathway for CBS to enter, establish, and spread within the EU to cause significant economic impact on their citrus industries.

O CPP II-2

Challenges and Successes in Biological Control of Nematodes

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Introduction: The phaseout of methyl bromide stimulated development of new biological products for nematode management.

Objective: Evaluate the effectiveness of new biological products for managing nematodes on annual and perennial crops.

Materials and methods: Replicated field trials were conducted in California, USA. Various products were evaluated pre-plant on annual crops, and post-plant on perennial crops. Products evaluated included: DiTera (Valent, toxins produced in fermentation by the fungus *Myrothecium verrucaria*), Nema-Q (Monterey AgResources, an extract of *Quillaja*, the soapbark tree), MeloCon (Certis USA, the fungus *Paecilomyces lilacinus*), abamectin seed treatments (Syngenta), and several products containing plant growth regulators and micronutrients (Stoller USA).

Results: Many of the products evaluated were effective, but testing revealed new challenges for implementation. For example, two products registered for post-plant use on perennial crops showed increases in yield, trunk circumference, and tree vigor, but did not always reduce populations of root lesion nematode (*Pratylenchus vulnus*).

Conclusion: Newly developed products have been shown to be effective for biological control of nematodes. However, challenges to implementation need to be addressed including developing application methods and timing, product storage and shelf life, cost benefit analysis, and developing grower confidence in biological control.

O CPP II-3

Challenges in insecticide discovery and development: a case study

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Sivanto™ (common name: flupyradifurone) is a novel innovative insecticide belonging to the new butenolide chemical class. The discovery of Sivanto™ was primarily inspired by the natural product stemofoline (isolated from the plant *Stemona japonica*). Its

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unique pharmacophore system represents a new bioactive scaffold selectively acting on the insect nAChR, one of the most important target sites for modern insecticides.

The new butenolide-based chemistry provides a favorable pharmacokinetic and ecotoxicological as well as mammalian safety profile. Due to its physicochemical properties allowing versatile application methods such as foliar, drench, drip and seed treatment, Sivanto™ offers excellent and fast efficacy against a broad spectrum of sucking pests such as whiteflies, aphids and psyllids in many agricultural and horticultural settings.

The ready-to-use SL-formulation (soluble liquid) provides excellent adhesion, spreading and penetration properties on leaves with improved translaminar efficacy and rain fastness. Due to its safety profile to honey bees and bumble bees as well as beneficial insects, Sivanto™ perfectly fits into IPM systems and will be a sustainable tool to control sucking pests.

Sivanto™ is active against resistant pests including cotton whiteflies and not metabolized by recombinantly expressed CYP6CM1, a cytochrome P450 enzyme conferring metabolic resistance to neonicotinoids and pymetrozine. Sivanto™ received an IRAC (Insecticide Resistance Action Committee) 4D subgroup classification, reflecting its unique chemical nature. Thus it is a new tool for resistance management purposes especially against resistant aphid and whitefly species.

O CPP II-4

Advanced IPM in greenhouses: a smart balance between ecological services and innovating high tech

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Nowadays, greenhouse crop production constitutes the ideal candidate for cutting age IPM approach.

Several decades of IPM implementation in greenhouse productions have given valuable insights into the strengths and weaknesses of these strategies. Due to their intensive nature, these systems allow the setting up of a wide range of control and monitoring tools relevant to precision horticulture. In particular, the goal of “pest-free greenhouses” through the development, use and integration of early diagnostics and precise application technology, is well adapted to high-tech, i.e. closed or semi-closed greenhouses. This issue has been addressed in the recently finished (2011- 2014) INTERREG Program ‘Healthy Greenhouse’, supported by the EU and the Dutch and German provinces close to their joint border.

Nevertheless, low-tech greenhouses, with limited barrier against pest and disease entry, represent the most common greenhouse profile nearly all over the world. In this case, owing to its median biotic complexity, this kind of agro-ecosystem is also the most suitable candidate for the in depth investigation into ecological foundation of biological control strategies. This issue has been tackled within the PURE project supported by the EU through the Seventh Framework Program. It has been shown that, as biological control is a real part of IPM in protected cultivation via inoculative, inundative releases or conservation, species biodiversity increases and induces many unexpected and even counter-intuitive direct and indirect interactions among the trophic levels in the cropping system. We assume that building robust IPM strategies must involve characterizing the major species functional properties as well as identifying complementary traits able to enhance ecosystem functioning.

The results of the INTERREG and PURE projects will be presented. Attention will be given to knowledge and technology transfer from one greenhouse system to the other. Low-tech greenhouses might take over critical high-tech tools, which probably need to be less advanced, and therefore less expensive to be incorporated in a low tech system. High-tech greenhouses might profit from the knowledge of a resilient ecological balance between crop, pathogens, beneficials and environmental factors, as found in the studies on low-tech greenhouses.

O CPP II-5

Moving towards a Non-Transgenic RNAi approach to control a chewing insect

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The discovery and use of RNA interference (RNAi) technology for insect pest management has been demonstrated under laboratory and field settings (Bauman, et al 2007, et al., Hunter et al., 2010, Hunter et al., 2012). This new technology opens to an environmentally-friendly control strategy, called “Highly Specific Pest Control’ (HiSPeC), which means, control a desired target specie, with no effects on non-target species. The Diaprepes root weevil (DRW), *Diaprepes abbreviatus* (Coleoptera), is a pest of citrus trees in that the larvae feed directly on the tree roots and the adults feed on the leaves. In order to develop RNAi based approaches to control CRW, we screened dsRNA molecules designed to several CRW transcripts (single or combined). Each dsRNA was tested in feeding bioassay designed for larvae or adult. For larvae, the dsRNA was mixed with the diet and

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individual larvae was let to feed on the diet/dsRNA for 10 days, when larvae mortality was scored. For adults, a dsRNA solution was sprayed over a leaf bouquet, and after it dries, was caged with 20 adults. The bouquet was replaced every 5 days. Insect mortality was recorded daily up to 15 days and compared with the controls (water and GFP dsRNA). RT-qPCR analysis was carried out to evaluate down-regulation of RNAi targets. Among all dsRNA molecules tested, dsRNAs "F" and "S" induced higher mortality to both life stages. Larvae mortality reach 60% with dsRNA "F" and 65% when we used a combination of dsRNA "F" and "S". It seems that the adults were more sensitive to dsRNA ingestion, as the mortality induced by a combined dsRNA "F" and "S" reaches 84%. RT-qPCR analysis corroborates with mortality data. Furthermore, the higher mortality in adults was correlated with a higher gene suppression compared to larvae. These results indicates that DRW is susceptible to orally ingested dsRNA, open a possibility to use non-transgenic RNAi strategies to control DRW.

Baum et al., 2007. Control of coleopteran insect pests through RNA interference. *Nat. Biotech.*, 25, 1322-1326.

Hunter et al. 2012. Advances in RNA interference: dsRNA treatment in trees and grapevines for insect pest population suppression. *Southwestern Entomol.* 37(1):85-87.

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O CPP II-6

Rodent damage, management and rodenticide use in Europe

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Rodent population outbreaks of common voles (*Microtus arvalis*), field voles (*M. agrestis*) and water voles (*Arvicola spec.*) can severely interfere with cropping in several agricultural and forestry sectors. During outbreaks, common voles infest millions of hectares of agricultural land in Europe and can cause pre-harvest damage >100 million € at the country level.

Often, rodenticides are applied at large scale during outbreaks to manage overabundant pest rodent populations despite environmental concerns. In the European Union (EU) only approved rodenticides are allowed be used in plant protection. During recent years, the availability of such rodenticides for plant protection has decreased considerably partially because of shifting of compounds and associated products to the biocide market. About 150 rodenticidal products are available for plant protection and more than 1,600 biocidal rodenticidal products are registered currently in the EU.

The decreasing availability of rodenticides and environmental risk that can be associated with rodenticide use call for minimizing the use of rodenticides and for suitable alternatives. Several non-chemical management techniques can be used to manage overabundant rodents. However, efficacy and environmental risk are rarely quantified and all methods seem to have disadvantages to some degree including conflict with conservation aims. This demonstrates the need to systematically assess existing techniques and to develop more suitable methods or combinations of methods to reduce the negative impact of pest rodents in cropping with an ecologically acceptable and economically feasible approach.

In this presentation damage will be quantified, the recent developments of registration of rodenticidal compounds and products in the EU outlined and alternatives to the use of rodenticides in plant protection presented.

Oral Presentations

Nematodes II

O NEM II-1

Mitigation of root knot nematode damage in carrot production by a seed-delivered nematicide

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California accounts for about 80% of the US fresh market carrot production. Root knot nematodes (*Meloidogyne* spp.) are the primary cause of plant disease problems expressed as root galling and forking. Resulting production loss has been conservatively estimated at 5-8% despite use of various soil fumigants. Currently no effective non-fumigant nematicide is registered in California and no root-knot nematode-resistant cultivar is commercially available. There is considerable interest by stakeholders and State agencies to reduce fumigant use because of their negative impact on air quality and potential exposure risk. In greenhouse studies, seed coating with the microbial-derived nematicide abamectin, provided by Syngenta Crop Protection, mitigated early root stunting by reducing root penetration of second-stage juveniles (J2) of the Southern root knot nematode *M. incognita*. Reduction in root galling followed a positive dose-response up to the highest tested application rate of 0.016 mg a.i./seed. Field trials with fresh market carrots (cv. Imperator 58) were conducted at two sandy loam test sites, both infested with *M. incognita* (average $P_{i\text{SITE }1} = 95 \text{ J2/100 cm}^3$; $P_{i\text{SITE }2} = 34 \text{ J2/100 cm}^3$). Each 3-month long trial was set up as a randomized complete block with 5 replications. Abamectin seed coating (0.016 mg/seed) reduced root galling by 54% and 32%, and increased marketable yield by 47% and 27%, respectively compared to the non-treated control. In summary, the seed-delivered biorational nematicide abamectin at approximately 40 g a.i./ha significantly mitigated root-knot nematode damage in carrots and increased marketable yield. Nematicidal seed coating promises economical and environmental benefits and is a major advancement for reduced risk farming.

O NEM II-2

BioAct™ DC Liquid - New Liquid Solution for Biological Control of Nematodes

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BioAct™ is a high-quality solution for nematode control available as WP (wetable powder) and WG (water-dispersible granules) formulations based on living spores of the fungus *Purpureocillium lilacinum* strain 251. Bayer CropScience is further improving BioAct™ by optimizing the fermentation process of the active fungus and by developing a liquid formulation of the spores. Tests have shown enhanced soil penetration, more convenient handling and improved shelf-life based on an enhanced temperature stability of BioAct™ DC Liquid.

O NEM II-3

Using bacterial antagonists of fungal pathogens for control of root-knot nematodes on tomato

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Introduction: Crop infestation by root-knot nematodes often facilitates infection by soil-borne fungal pathogens resulting in synergistic yield losses. Individual control of each pathogen is time consuming and expensive. Within this respect, control measures that target both pathogens at the same time would provide an ideal solution. Such option is seen in microbial antagonists with dual use against nematode and fungal pathogens.

Objectives: To study the potential of bacterial antagonists of fungal pathogens to control the root-knot nematode *Meloidogyne incognita* on tomato and describe the mode-of-action.

Materials and methods: Seven bacterial antagonists of *Rhizoctonia solani* and *Verticillium dahliae* applied as seed treatment were screened for their potential to control *M. incognita* on tomato. *Rhizobium etli* G12 served as positive control and *Escherichia coli* JM109 as negative control. The top isolates were further tested regarding their mode-of-action, i.e. nematicidal potential of bacterial supernatants, direct antagonism, repellence, and induced systemic resistance.

Results: Following seed application three *Bacillus subtilis* isolates, i.e. Sb4-23, Mc5-Re2 and Mc2-Re2, plus the positive control *R. etli* G12 significantly reduced the number of galls and egg masses. Best control was achieved by Sb4-23 and *R. etli* G12 with over 90% reduction in number of egg masses. A soil drench with bacterial supernatants significantly reduced egg masses produced by

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M. incognita on tomato by up to 62% compared with the non-treated control. In choice tests bacteria treated plants showed a tendency to be less attractive for *M. incognita* than non-treated plants. All tested bacterial antagonists induced systemic resistance towards *M. incognita* as indicated in a split-root test system. When induced systemic resistance was combined with direct antagonism overall control potential was not enhanced. Therefore, the plant mediated effect is seen as the main mode-of-action of the tested bacterial antagonists in controlling *M. incognita* on tomato.

Conclusions: Bacteria known for their antagonistic activity against soil-borne fungal pathogens also suppressed *M. incognita* on tomato. Such “multi-purpose” bacteria might provide new options controlling nematode-fungus disease complexes that cause synergistic yield losses.

O NEM II-4

Endemic *Pasteuria penetrans* Isolates for Root-knot nematode Control in Thailand

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Root-knot nematodes (*Meloidogyne* spp.) are the key plant parasitic nematodes which destroy several crops in Thailand each year. Severe damages occur in many crops such as potato, tomato, chili pepper, black pepper, and guava. In addition increasing problems have been reported in cassava. Root-knot nematodes control in Thailand depends on crop rotation and non-fumigant nematicides; however, few nematicides are available. Biological control is an alternative option for root-knot nematode control. *Pasteuria penetrans* (Sayre & Starr) Thorne is an obligate hyperparasite of root-knot nematodes which can destroy the fecundity of a female nematode by the propagation of bacterial spores within nematode body. Endemic isolates of *P. penetrans*, which may have higher efficacy for controlling local nematode populations, were collected and evaluated for their potential. Eight *Pasteuria* isolates, obtained from root-knot nematode populations infected on *Solanum tuberosum* cv. Atlantic, *Coleus parvifolius* and *Capsicum annuum* were evaluated in a greenhouse for the efficacy on *M. incognita* control. The experiment was arranged in a completely randomized design with 5 replicates. *P. penetrans* isolates were applied at 10^6 spores/200 g soil and compared with 0.1 g cadusafos 10 G, inoculated, and non-inoculated control. Each pot was inoculated with 600 second stage juveniles. Five *P. penetrans* isolates reduced eggmass production on tomato roots lower than inoculated control. The numbers of second stage juveniles in the soil were not significantly different from inoculated control. The percentage of spore-encumbered juveniles in all *P. penetrans* treatments were low, the highest was 22.6%. However, higher percentage of spore-encumbered juveniles is expected when spores produced in mature female nematodes embedded in the roots are released into the soil. Endemic *P. penetrans* isolates showed promising results in *M. incognita* control. Therefore, screening for high infectivity isolates against broad root-knot nematodes populations will benefit the development of these bacteria as biocontrol agents for root-knot nematodes control in Thailand.

Figure 1

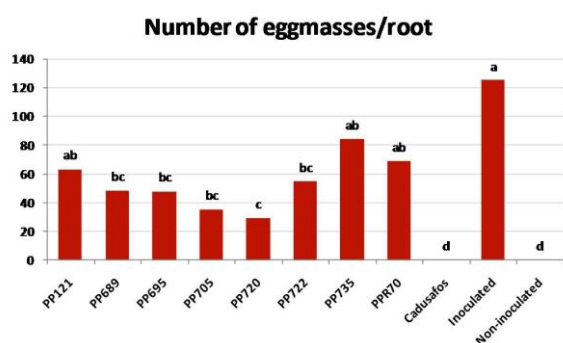
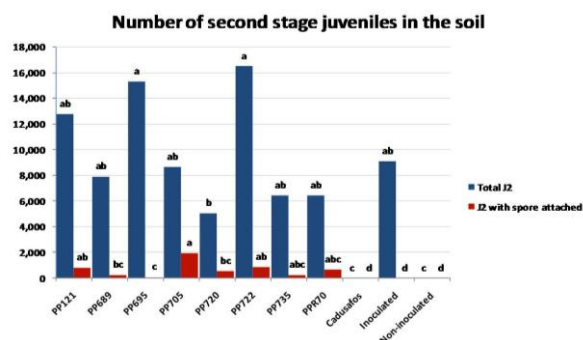


Figure 2



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Nematodes II

O NEM II-5

Biofumigation using mustard for nematode disease control – Canadian contributions

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Biofumigation *sensu stricto* refers to the suppression of pests, weeds, pathogens and nematodes by volatile biocidal compounds mainly isothiocyanates released from Brassicaceous plants when glucosinolates in their tissues are hydrolysed. Biofumigation for soil borne diseases, and pest control, results have not been consistent. In Canada, recent research using Canadian oriental mustard (*Brassica juncea* L.) has shown great potential for nematode control. Allyl isothiocyanate (AITC) is the most toxic isothiocyanates on several nematode pests such as root-lesion nematodes (*Pratylenchus* spp.), cyst nematodes (*Heterodera* spp.) and root-knot nematodes (*Meloidogyne* spp.), and the toxicities are species selective. Canadian variety Forge has the highest concentration of AITC, reaching up to 1% in its seeds. Different materials such as bran, seed meal and defatted seed meal have significant different concentrations of AITC, therefore were shown different nematicidal activities. Physical properties such as particle size; and environmental factors such as amount of water applied after application of materials also had effect on the efficacy.

O NEM II-6

Experiments on pathogenicity of *Pasteuria* spp. towards the Beet Cyst Nematode *Heterodera schachtii* as a novel biological control method in sugar beets

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The Beet Cyst Nematode (BCN) *Heterodera schachtii* is a major pest of sugar beets and is wide spread in German sugar beet production systems. Currently chemical or biological control methods are not available in this pathosystem. Unfavorable conditions for an appropriate cultivation of resistant catch crops drive the excessive use of tolerant sugar beet cultivars with partial resistance in intensive production systems. *Pasteuria* spp. is distributed worldwide and belongs to a group of endospore forming bacteria which parasitize on plant parasitic nematodes. The host range of *Pasteuria* spp. within genera level is not confined strictly between nematode species, hence isolates from other Cyst Nematodes are known as potential antagonists of BCN. Aiming at the development of new biological control method against BCN, greenhouse experiments with different *Pasteuria* isolates (*H. schachtii* and *H. glycines*) were conducted in co-operation with Syngenta Crop Protection. Using a susceptible and a tolerant sugar beet cultivar as test plants *Pasteuria* spp was inoculated as a seed treatment with different spore densities. As primary infection of BCN occurs in root penetrating second stage juveniles, 750 J2/100ml were inoculated as suspension in test plants 10-14 days after germination in small pots (400 ml). After 250 degree days (>8°C) penetration of BCN in roots of sugar beets was detected (staining with acid fuchsin). To find effects on the reproduction of BCN final population in cysts of the first generation was detected in the test substrate after 550 degree days. In comparison to the untreated control *Pasteuria* effectively reduced root penetration of BCN in susceptible sugar beet cultivars. Cyst density and reproduction of BCN was significantly reduced in both sugar beet cultivars already at low spore densities in comparison to the untreated control. Under field conditions *Pasteuria* possibly affects BCN reproduction not till a prolonged exposure time, since only a certain proportion of juveniles successively leave the cyst over the crop rotation.

O STO 1

New methodological approaches in stored product protection

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The Department of Stored Product Protection at the Julius Kühn-Institute Berlin is concerned with the prevention, early detection and control of pest organisms impairing the quality and quantity of stored plants and plant products. Our presentation will outline the progress of two projects currently under investigation in our laboratory and discuss the opportunities provided for stored product protection practice.

One methodological approach exploits the release of volatile organic substances (VOCs) by almost every organism on earth: Stored plants and plant products as well as pest insects and microorganisms associated with the insects and/or the stored products produce specific VOCs. We hypothesize that the cumulated odor profile of this tripartite interaction is characteristic for every stored product and pest species and likely to change over time, e. g. with temperature, humidity, and infestation level. Via headspace analysis we sampled the odor profile of different infestation levels and analyzed it by gas chromatography-mass spectrometry (GC-MS). On the basis of our findings, gas sensors tuned to recognize these odor patterns or individual marker substances can be built and installed in storage facilities allowing an early detection and estimation of insect and/or fungal infestations.

To take species-specific actions in case of an infestation, it is essential to identify the respective pest species. Thus, in a second approach, attenuated total reflectance Fourier transform infrared (ATR-FTIR) spectroscopy is used to characterize cuticles and surface extracts of different stored product pests. This technique allows fast chemotaxonomic discrimination of different insect species, whereas traditional morphological species diagnosis is often time-consuming, requires profound entomological skills, and can be unreliable in closely related species if only the phenotype is considered.

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O STO 2

Successful stored product protection with phosphine as an effective fumigant

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The most important active ingredient worldwide used for controlling stored product pests is phosphine gas. Through its positive properties with regard to eco-toxicity as well as its good penetration properties and the associated excellent effectiveness against stored product pests, this gas has become indispensable for successful stored product protection over decades.

Meanwhile, tests indicate that some insect species have developed increased tolerance to the agent. Time and again the question is raised whether and to what extent this agent can still be used successfully in stored product protection in future.

To resolve this issue, the goal of the described tests was to determine data on sufficient dosage and exposure time which would show the conditions under which successful fumigation can be performed and how further development of resistance in insects towards phosphine can be counteracted.

Laboratory experiments were conducted to determine the limiting concentrations in insect strains comprising grain weevil, flour beetle and indianmeal moth which result in the complete extermination of all insect development stages.

In subsequent fumigation tests under practical conditions, it was shown that insufficient dosages and short exposure times can frequently be regarded as the reason for unsuccessful fumigation and therefore contribute to the risk of the development of tolerance in insects to the gas.

Depending on the various parameters which play a role in fumigation –

- properties of the product or object to be fumigated
- sufficient sealing
- temperature and moisture conditions
- insect species and its tolerance status
- insect development state

– it is obvious how important dosage and exposure time are in order to cope with all of the uncertainties caused by the factors named above.

Recommendations for minimum dosage and exposure times are given.

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O STO 3

Influence of fumigants on sunflower seeds: Characteristics of fumigant desorption and alterations of volatile profiles

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Introduction: Fumigation of transport containers is common practice to protect stored products from pests in the shipping industry or to avoid the spread of alien species (ISPM 15). However, little is known on the effects caused by residual fumigation agents on human health and on the respective goods.

Objectives: To investigate absorption and desorption behaviour of fumigants by and from stored products, we fumigated peeled sunflower seeds (*Helianthus annuus*) either with phosphine (PH₃), methyl bromide (CH₃Br), or 1,2-dichloroethane (C₂H₄Cl₂). To analyse the interaction of PH₃ with the sunflower seed ingredients, we analysed volatile patterns of fumigated and non-fumigated seeds.

Materials and methods: Fumigations were conducted at 100 ppm for 72 h in 4 L fumigation chambers. After complete ventilation, the seeds were transferred to a desorption chamber (53 L). Chamber air was sampled on consecutive days, each time followed by complete ventilation of the chamber until the fumigant concentration attained the detection limit. Fumigant concentration in air samples were analysed by thermal desorption-2D-gas chromatography with coupled mass spectrometry and flame photometry detection (TD-2D-GC-MS/FPD). To investigate interactions of PH₃ with the ingredients of sunflower seeds, the volatile pattern of non-fumigated, fumigated and fumigated but outgassed seeds were sampled via solid-phase micro-extraction (SPME) headspace sampling and analysed by GC-MS.

Results: The total amount of desorbed fumigants depended strongly on the respective gas used. Compared to CH₃Br or C₂H₄Cl₂, significantly reduced amounts of PH₃ were desorbed by the seeds. Desorption time of several months was observed for C₂H₄Cl₂, whereas PH₃ and CH₃Br outgassed within several days. The volatile pattern of PH₃ fumigated and outgassed seeds was significantly different from non-fumigated seeds. Fumigated seeds (outgassed seeds) released around 5 (3) times more terpenoids than non-fumigated seeds.

Conclusion: Depending on the fumigant, desorption from the fumigated product can last several months and the fumigant is able to interact with the ingredients of the product. This interaction with the good is likely to affect food characteristics.

O STO 4

Comparison of toxicity between Ethanedinitrile (EDN) and Methyl bromide (MB) to five stored product insects

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Introduction: Methyl bromide (MB) has been used to control stored product insects however, it is listed as an ozone depleting substance under the Montreal protocol (UNEP, 1996). Ethanedinitrile (EDN) is a new fumigant that has been identified as an ozone safe alternative. It is highly toxic to stored product insects (Hooper *et al.*, 2003).

Objective: The aim of this study was to conduct bioassays and compare the toxicity between EDN and MB to five stored product insects.

Methods: Insects: Cigarette beetle, *Lasioderma serricorne*, Rice weevil, *Sitophilus oryzae* (L.), Warehouse beetle, *Trogoderma variabile* Ballion, Lesser grain borer, *Rhyzopertha dominica* (F.), Rust red flour beetle, *Tribolium castaneum* (Herbst). *L. serricorne*, *T. castaneum* and *T. variabile* were used. Fumigants: EDN and MB were sourced from BOC Gases Australia. Fumigation procedure: The fumigation was conducted in 250 mL Erlenmeyer flasks containing 50 g mixed-age cultures. The control was maintained in a sealed bottle without fumigant until completion of exposure. Each fumigant treatment was at 5-7 levels of fumigant and in 5 replicates ($n=5$) and 3 controls. At the end of the fumigation period of 24 hours, the treated and control bottles were opened for 1 hour of aeration. The adult insects counted and removed, and the remaining mixed-age cultures were counted weekly for a period of 5 weeks with live and dead adults being removed at each count.

Results: Comparison of toxicity between EDN and MB to five species of insects for 6 and 24 hours of exposure at 25°C and 65% RH is shown in Tables 1 and 2. Complete mortality was obtained in all mixed-age cultures of *T. castaneum*, *R. dominica*, *S. oryzae*, *T. variabile* and *L. serricorne* (Table 1 & 2). Both MB and EDN were effective for all the life stages of five tested insect species. However, the observed concentration x time (Ct) products of EDN, to completely kill all life stages of the five insect species, were substantially lower than that for MB for both 6 and 24 hours fumigation.

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Conclusions: The study shows that EDN is more toxic than MB to all the life stages of the five test stored product insects and can be used as a replacement of ozone depleting MB for stored product insects.

References: Hooper, J.L., Desmarchelier, J.M., Ren, Y.L and Allen, S.E (2003) Toxicity of cyanogen to insects of stored grain. *PestManagement Science* 59: 353-357

UNEP. Eighth Meeting of the Parties to the Montreal Protocol on Substances that Deplete the Ozone Layer, Vienna (1996)

Figure 1

Table 1. Insects emerging from media containing adults, pupae, larvae and eggs following no treatment (control), and exposure to EDN and MB for 6 hours.

Species	Treatment	Ct product (mg h/L) 6 hours	Before fumigation Live	After fumigation		Further live insects emerging after holding for (days)				
				Live	Dead	7	14	21	28	35
<i>Tribolium</i>	Control		2415	2410	0	576	348	672	425	6064
<i>castaneum</i>	EDN	18-22	3522	0	3522	0	0	0	0	0
	MB	80-96	3474	0	3474	0	0	0	0	0
<i>Rhyzopertha</i> <i>dominica</i>	Control		3286	3286	0	615	279	581	279	5418
	EDN	18-44	3847	0	3847	0	0	0	0	0
	MB	35-51	4015	1	4014	0	0	0	0	0
<i>Sitophilus</i> <i>oryzae</i>	Control		2018	2018	0	492	411	606	371	5104
	EDN	24-72	4102	0	4102	0	0	0	0	0
	MB	42-75	3916	0	3916	0	0	0	0	0
<i>Laxioderma</i> <i>senicorne</i>	Control		2176	2176	0	251	487	269	195	3126
	EDN	25-45	3573	0	3573	0	0	0	0	0
	MB	35-120	3721	0	3721	0	0	0	0	0
<i>Trogoderma</i> <i>variable</i>	Control		2485	2483	2	174	319	451	372	3026
	EDN	30-63	3816	0	3816	0	0	0	0	0
	MB	70-90	3923	0	3923	0	0	0	0	0

Figure 2

Table 2. Insects emerging from media containing adults, pupae, larvae and eggs following no treatment (control), and exposure to EDN and MB for 24 hours

Species	Treatment	Ct product (mg h/L) 24 hours	Before fumigation Live	After fumigation		Further live insects emerging after holding for (days)				
				Live	Dead	7	14	21	28	35
<i>Tribolium</i>	Control		3591	3591	0	309	356	239	521	4158
<i>castaneum</i>	EDN	18-24	4209	0	4209	0	0	0	0	0
	MB	75-92	4114	0	4114	0	0	0	0	0
<i>Rhyzopertha</i> <i>dominica</i>	Control		3708	3706	2	314	273	330	362	5025
	EDN	21-47	4150	0	4150	0	0	0	0	0
	MB	45-55	4283	1	4282	0	0	0	0	0
<i>Sitophilus</i> <i>oryzae</i>	Control		4011	4011	0	372	291	406	317	4519
	EDN	23-75	4112	1	4111	0	0	0	0	0
	MB	38-80	3961	0	3961	0	0	0	0	0
<i>Laxioderma</i> <i>senicorne</i>	Control		2516	2516	0	194	286	331	528	3207
	EDN	25-46	3024	2	3022	0	0	0	0	0
	MB	30-124	3218	1	3217	0	0	0	0	0
<i>Trogoderma</i> <i>variable</i>	Control		2193	2189	4	247	194	367	253	2196
	EDN	35-65	3118	4	3114	0	0	0	0	0
	MB	69-91	3261	3	3258	0	0	0	0	0

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O STO 5

Toxicity of *Newbouldia laevis* against the Angoumois grain moth, *Sitotroga cerealella* (Olivier) in paddy rice

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Toxicity of powders and extracts of *Newbouldia laevis* (Seem) against *Sitotroga cerealella* (Olivier) in paddy rice was carried out in the laboratory at temperature of 28±2°C and relative humidity of 75±5%. The powders of the leaf, stem and root were tested at 0.1, 0.2, 0.3, 0.4 and 0.5g per 20g of paddy rice and the extracts of same plant parts were assessed at 1, 2, 3, 4 and 5% oil concentrations. Results showed that all the plant powders caused high mortality, significantly reduced oviposition and adult emergence of *S. cerealella* and their effects were not significantly different ($p>0.05$) from each other but different from the control. Powder from root of *N. laevis* was able to achieve 100% mortality of *S. cerealella* at 0.5g concentration at 96hrs after treatment with LD₅₀ of 2.07. Seed weight loss in paddy was reduced by the powders. Extracts from all plant parts caused 100% mortality of *S. cerealella* at high concentrations of 4 and 5% oil after 96hrs. However, root bark oil extract appeared to be most effective since it was the only one to achieve 100% adult moth mortality within 72h of application at 4% concentration with LC₅₀ of 1.58. The extracts reduced adult emergence of the moth and there was no adult emergence at 4 and 5% oil concentrations. Similarly, there was no seed weight loss at 4 and 5% concentrations from all the plant parts. Water absorption capacity as well as viability of the treated paddy seeds were not affected by the oil extracts since results of treated seeds were not significantly different ($p>0.05$) from the controls. Root powders and extract of *N. laevis* appeared to be more effective than other plant parts. Results from fumigation activity showed that *N. laevis* was not a good fumigant since values from mortality, adult emergence and seed weight in treated seeds were not significantly different from that of control. Therefore, the use of *N. laevis* especially by small scale farmers may serve as an alternative to synthetic insecticides.

O STO 6

Effects of hermetic storage on insect pests, microbials, oxygen levels, moisture content, and stored product quality

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The best way to reduce losses and keep the quality of stored food or feed products is to prevent infestation. One can prevent immigration of stored product pests by hermetic enclosures. This is not yet common because low world-market prices prevented innovation in the past decades. Since 2008, however, the value of grain and other durables increased considerably. In a recent project on long-term grain storage in sealed warehouses we could prove that an insect-proof seal can prevent attack while grain quality is not affected. If we look at silobag storage or hermetic triple bags used for grain storage it is important to completely understand all changes produced inside a gastight enclosure. At high moisture contents germeability as an indicator of grain quality decreases. At moderate moisture contents and high temperatures respiration of grain, arthropods and micro-organisms may lead to a rapid reduction of oxygen. Some results indicate that under this type of storage mycotoxin levels can be kept at much lower levels than under conventional, aerated conditions. As a number of insects are potential penetrators of packaging materials it needs to be well understood when insects will be motivated to destroy the barrier film securing a gas-tight seal. In general, young adult beetles of the bostrychid and anobid families tend to leave an infested product to mate and oviposit. Drawing a vacuum or applying controlled atmospheres could possibly reduce the critical time when a plastic liner can be punctured from inside. A penetration by insects from outside is improbable as long as attractive, volatile organic compounds do not leak through the seal. In recent studies, penetrators destroyed only LDPE films of 25 µm but not those of 100 µm thickness. If chemotaxis triggers this penetration is not yet proven. Results will be discussed with other findings.

Oral Presentations

Fusarium

O FUS 1

Identification of different *Fusarium* spp. on *Allium* spp. in Germany

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The most important *Fusarium* species causing *Fusarium* basal rot of onion is *Fusarium oxysporum* in Germany. However, in Mediterranean countries like Israel the *Fusarium* salmon blotch caused by *Fusarium proliferatum* is more important than *F. oxysporum*. The aim of the survey was to evaluate different *Fusarium* spp. infecting onions and show the increasing importance of mycotoxin producing fungi like *F. proliferatum* in Germany. In 2013 *Allium cepa* bulbs from different fields in Northern and Southern Germany, seeds and sets from German onion breeders as well as different edible *Allium* spp. from local markets were detected for infestation with *Fusarium* sp.. Different *Fusarium* spp. were isolated and identified by morphological characterization. More than 20 different *Fusarium* spp. were identified. The examined *Allium* seeds were free of *Fusarium* infection, but *Fusarium* contamination on the surface were detected. The diversity of *Fusarium* spp. and the intensity of infestation was higher on edible bulbs compared to the younger sets. The most common species in onions from field are *F. oxysporum* and *F. solani*. On average 4 different *Fusarium* spp. per onion field and 2 different *Fusarium* spp. per onion were detected. The analyzed onions and other eatable *Allium* spp. from local markets showed also a high content of different *Fusarium* spp.. *Fusarium* sp. was identified in *Allium* sp. with visible symptoms and in healthy looking onions. The main identified *Fusarium* sp. in *Allium* sp. in Germany was *F. oxysporum*. *Fusarium proliferatum* could be detected in about half of the sampled onion fields and in approximately 10% of all analyzed onions. Also in the onion sets, on the surface of the seeds and in other eatable *Allium* spp. *F. proliferatum* were identified. Besides *F. proliferatum* further mycotoxin producing *Fusarium* spp. like *F. equiseti* or *F. tricinctum* were identified. Other *Fusarium* spp. like *F. sporotrichioides* and *F. poae* were first described in *Allium* sp. The two main *Fusarium* spp. are able to produce toxins like Enniatins, Fumonisin, Moniliformin and T-2 toxins. Further detected *Fusarium* sp. like *F. proliferatum*, *F. equiseti* and *F. tricinctum* are able to produce additional mycotoxins like Beauvericin, Zearalenone and Diacetoscirpenol. This broad spectrum of possible *Fusarium* mycotoxins could be a potential health risk for human beings and livestock.

O FUS 2

Cultivar selection and soil treatments to suppress *Fusarium* wilt in bunching spinach in Ontario, Canada

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Objective: To identify effective management practices to reduce *Fusarium* wilt losses in spinach through cultivar evaluation, and evaluation of seed and soil treatments.

Methods: Field trials were conducted in July and August of 2012-14 in naturally infested soils in Hamilton, Ontario, Canada. In 2012, 25 commercial cultivars were screened for susceptibility to *Fusarium* wilt, eight of which were evaluated again in 2013. Pre-plant soil fumigants and amendments were evaluated for control of *Fusarium* wilt in 2013-14. Treatments were: spent mushroom compost (75 t/ha), oriental mustard seed meal (Must Grow, 1120 kg/ha), calcium cyanamide (Perlka, 1000 kg/ha), and fumigants metham sodium (Busan, 730 kg/ha), dazomet (Basamid, 500 kg/ha) and chloropicrin (Pic Plus, 108 L/ha (2014 only)). A high nitrogen (200 kg/ha N) control was also included to reflect the additional N provided by Perlka and Must Grow.

Results: The cultivars Norgreen, Unipack 12 and Greyhound developed the most severe *Fusarium* wilt in both years: 28, 23 and 19% severity, respectively. In contrast, C2606, Carmel, Sardinia, POH-0438 and Imperial Green had <14% wilt severity in both years. Disease severity was low in 2013, but Busan, Basamid and high N soil treatments reduced wilt severity. In 2014, Basamid and Pic-Plus reduced disease severity to 3 and 1%, respectively, while Busan and high nitrogen reduced disease severity to 7 and 6%. Wilt severity was similar on spinach treated with Perlka (13%) and compost (15%) and the untreated control (14 %). Application of MustGrow increased wilt severity to 25%. The population of *F. oxysporum* in soil was reduced by pre-plant applications of Busan, Basamid, and Pic-Plus to 162, 75 and 0 CFU/g soil, respectively, vs. 9750 CFU/g in control plots.

Conclusions: *Fusarium* wilt of spinach can be managed by selecting less susceptible cultivars and preplant application of fumigants Pic Plus, Busan, Basamid or high nitrogen.

Oral Presentations

Fusarium

O FUS 3

Influence of nitrogen fertilization in barley on the epidemiology of selected *Fusarium* species

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Fusarium head blight (FHB) of barley is known as a destructive disease. The infection results in yield loss, quality reduction and mycotoxin contamination of grain. FHB is caused by a complex of several *Fusarium* species. Beside toxin spectra species vary in their epidemiology and respond differently to environmental and agronomic factors. In addition to soil cultivation methods, crop rotation and barley variety selection, other agronomical tools are necessary to prevent *Fusarium* infection and infestation, especially due to existing persistent lack of barley adapted and licensed fungicide strategies. Therefore, nitrogen application as a feasible preventative measure, including potential incalculable risks, is in the focus of the present study.

Nitrogen fertilization acts indirectly by changing canopy parameters like micro-climate conditions on the host-parasite-interaction as well as directly by influencing plant physiology.

Field trials were carried out to compare selected *Fusarium* species in response to different nitrogen fertilization levels. RT-qPCR measurements detecting *Fusarium* contamination on barley material were combined with collecting detailed information of canopy parameters. In doing so, distinct differences between single *Fusarium* species became apparent. Nitrogen fertilization yielded in supportive and reductive effects for specific *Fusarium* species at which parameters like canopy density, microclimate, degree of soil coverage, plant height and greenness might have played important roles. More detailed information about the influence of single factors will be revealed by correlation analysis.

Greenhouse experiments with inoculation treatments were conducted to investigate the effect of nitrogen fertilization on plant immune responses. For these purposes, barley plants were grown under greenhouse conditions and inoculated with *F. culmorum* at the time period of anthesis. Initially, gene expression studies were used to select relevant targets for future examination of nitrogen influence on *Fusarium* infection and infestation on barley plants.

First of all, generated information could support the choice of agronomical tools like nitrogen fertilization to prevent *Fusarium* infestation. Above all, a more detailed knowledge about *Fusarium* epidemiology and *Fusarium*-barley-interactions will contribute to future improvements and developments towards effective fungicide control strategies.

O FUS 4

Multitrophic interactions for the biocontrol of Fusarium Head Blight

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Introduction: Fusarium Head Blight (FHB) is one of the most important diseases of wheat, caused by a complex of species including *Fusarium graminearum* and *Fusarium culmorum* and competition plays an important role in biological strategies for its control.

Trichoderma gamsii 6085, whose genome was recently sequenced and annotated, is a known antagonist of *F. graminearum* and *F. culmorum*. *Fusarium oxysporum* is considered a competitor for cultural debris with a greater saprophytic ability than many FHB causal agents.

Objectives: Aim of the work is to investigate the possibility to combine *T. gamsii* 6085 and *F. oxysporum* 7121 as biocontrol agents (BCA) of FHB, with particular emphasis to the ecology of this interaction.

Materials and methods: The BCA *T. gamsii* 6085 (T6085) and *F. oxysporum* 7121 (Fox7121), this last here selected within a large population of isolates, were investigated for their nutrients utilization patterns (BIOLOG system) and compared with those of *F. graminearum* and *F. culmorum* in order to verify their co-existence or niche exclusion (NOI=Niche Overlapping Index). The two BCAs were also studied, alone and in combination, for their competitive ability on wheat debris against *F. graminearum*.

Results: BIOLOG analysis underlines the ability of Fox7121 to use the higher number of nutrients than T6085 and the pathogens. When nutrients utilization patterns were analysed at the end of the test, resulting NOI showed a high niche overlapping. Anyway, regression analysis of growth curves for each substrate showed a different slope, corresponding to significant differences in assimilation rate for each isolate, highlighting diverse nutritional dominances.

When tested for their competitive saprophytic ability, T6085 and Fox7121 were able to significantly reduce, alone and mostly in combination, wheat straw colonization by *F. graminearum*, whereas no competition between the BCAs was evident.

Conclusion: The co-existence of different isolates, confirmed by ecological studies, can help the choice of appropriate BCAs, mostly in a multitrophic perspective. Results here obtained show a potential exploitation of *F. oxysporum* in combination to *T. gamsii* 6085, thus opening a new scenario for the biocontrol of FHB.

Oral Presentations

Fusarium

O FUS 5

Compost and biochar alter root exudation and root morphology in *Fusarium oxysporum* infected tomato plants

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Soil amendments like compost and biochar are known to affect soil properties, plant growth as well as soil-borne plant pathogens. Complex interactions based on microbial activity and abiotic characteristics are supposed to be responsible for suppressive properties of certain substrates. However, the specific mechanisms of action are still widely unknown. Here we show the effect of compost and biochar towards the tomato pathogen *F. oxysporum* f. sp. *lycopersici* (*Fol*). We focused on disease development, the modification of tomato root morphology and changes in root exudates. Inoculated and mock-inoculated tomato plants were grown in different substrates (control, 20 % compost, wood biochar, green waste biochar). Root exudates were extracted, adjusted to the root fresh weight and studied in spore germination and mycelial growth assays. The root systems were scanned and analyzed using of the software WinRhizo® in order to identify their morphological traits. Our results showed that *F. oxysporum* f. sp. *lycopersici* clearly affected root lengths, root weight, root surface area and root volume. Moreover, an increase in fine root fraction and specific root length was observed in infected plants. We found, that compost and biochars altered root exudates differently. Microconidia germination was lowest in root exudates from tomato plants grown with the amendment of green waste biochar. To our knowledge this is the first report on the effectivity of biochar and compost in suppressing *Fol* through alteration in the root exudates.

O FUS 6

Mixed Actinomycetes as biocontrol agent against fusarium oxysporum TR4 in 'Cavendish' banana

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Banana is one of the major fruits in the Philippines in terms of volume of production and export earnings. The Philippines export of fresh Cavendish banana ranked No.1 with 22% share. One major threat to the industry is *Fusarium* wilt caused by *Fusarium oxysporum* f. sp. *cubense*. It tops as a major concern today affecting the Philippine banana industry since 2002 up to the present in Mindanao. Because of environmental and health issues concerning the use of chemical pesticides in the control of diseases, utilization of microorganisms has been significant in recent years as a promising alternative. This study aims to evaluate the potential of actinomycetes to control *Fusarium* wilt in Cavendish banana.

Actinomycetes were isolated from mangrove soils in areas in Quezon and Bataan, Philippines. A total of 199 actinomycetes were isolated and 82 actinomycetes showed activity against the local *Fusarium oxysporum* (*Foc*) by agar plug assay. Best isolates AQ6, AQ30 and AQ121 were selected inhibiting *Foc* by 21.0mm, 22.0mm and 20.5mm, respectively. The same actinomycetes inhibited well *Foc* Tropical Race 4 showing 24.6 mm, 20.2mm and 19.0 mm zones of inhibition by agar plug assay, respectively. Combinations of the three isolates yielded an inhibition of 13.5 mm by cup cylinder assay. These findings led to the formulation of the mixed actinomycetes as biocontrol agents against *Foc*.

A field experiment to evaluate the formulated mixed actinomycetes against *Foc* in a *Foc* infected field in Sto Tomas, Davao Del Norte, Philippines was conducted. Results showed that preventive method of application of the mixed actinomycetes against *Foc* showed promising results. A 56.66% mortality was observed in control set-up (no biocontrol agent added) compared to 33.33% mortality in preventive method. Further validation of the effectiveness of the mixed actinomycetes as biocontrol agent is presently being conducted in Asuncion, Davao Del Norte, Philippines.

Oral Presentations

Assessment of Invasive Species

O AIS 1

A new method to assess the impact of invasive alien species on ecosystem services and biodiversity

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Introduction: The risks by invasive alien species (IAS) increase due to increased global trade, transport, tourism and climate change. The assessment of impacts caused by such species on the environment is of increasing interest for decision-makers and risk managers. To prevent or reduce impacts on ecosystem services (ES) and biodiversity components (BC), it is essential to assess these as soon and as accurate as possible.

Objectives: A new framework is presented to enable the risk assessor to evaluate the overall environmental risk of a plant pest, which integrates the impacts on ES, as well as on BC and their probabilities of occurrence.

Materials and methods: The framework is based on the definition of a scenario for the assessment. It includes the consideration of the IAS abundance or prevalence, the temporal and a spatial scale, and one or several service providing unit(s) (SPU) i.e. environmental components or units responsible for the genesis and regulation of the ecosystem services. Factors reducing the impact of the IAS due to the ecosystem resistance and resilience or to the management measures applied against the IAS have to be considered. According to the species to be assessed the ES (e.g. food, air quality, erosion reduction) and the BC (e.g., genetic diversity, impact on rare species) that could be affected by the IAS are selected. The assessment is done by considering a rating system that can combine data modeling and expert knowledge elicitation. Quantitative measures are used to assess the uncertainty.

Results: The method has been applied to several species in different environments, as e.g. forests, urban and aquatic ecosystems. The impacts of the IAS on ES and BC are then evaluated regarding their magnitude and the degree of uncertainty is calculated. Thus, comparable, transparent and reproducible results for environmental risk assessment as a component of pest risk analysis are generated by a standardized approach. The method is implemented in a platform supporting a more efficient environmental risk assessment.

Conclusions: This new method efficiently supports the integrated assessment of the impacts of IAS on important components of the environment combining data modeling and expert judgments. This helps the risk manager to make decisions about the measures taken against the assessed IAS.

O AIS 2

Chances and constraints of risk assessment in plant health

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Introduction: Trade, travel, and transportation bear the risk to carry organisms harmful or potentially harmful to plants from one area to another. Several methods exist to assess the probability of entry, establishment, spread and the magnitude and likelihood of impacts, but often risks are identified when it is already too late and the organism has already entered the new area.

Objectives: The assessment of pest risks to plants faces several difficulties - e.g. lack of data, uncertainties about the organism's behavior and adaptation capacity, difficulties to predict spread and impacts over a long term. Risk assessment methods have to consider all of these and to give an evaluation whether phytosanitary measures are needed and which could be effective. The objective here is to critically review some of the available methods and to discuss aspects on how they would need to be improved to give more precise and timely advice.

Materials and methods: Current methods applied in the EU are mostly qualitative. Based on expert judgment, ratings are given to a set of questions for the different aspects of a risk assessment, also considering uncertainty. For some parts of the risk assessment, modeling approaches have been applied, which can help to fill data gaps. The introduction and spread of several pests in relation to the assessments that have been made for these pests were evaluated to identify the main constraints of the assessments.

Results: Qualitative methods are already quite advanced, but very long and detailed, needing a lot of time while the organism may already enter and spread. Quantitative methods could help to accelerate the assessment but they still need to be further developed.

Conclusions: An analysis of the different methods, both qualitative and quantitative, shows the need for accelerating the process and applying methods that help to compensate data gaps. With a more quantitative approach, it could be possible to give more timely warnings for emerging pests.

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O AIS 3

Invasive alien plants – impact, risk assessment and management options

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Introduction: Noxious organisms may be harmful for biodiversity, health and economic goals. They are consequently addressed by different sectoral policies such as Plant Health or Nature Conservation. Invasive alien plants are typically the subject of both environmental (e.g., the CBD) and plant health (e.g., the IPPC) conventions. In comparison to pests and diseases which are immediately harmful, invasive alien plants can become noxious after a lag phase of sometimes decades.

Objectives: In this presentation, the multitude and potential extent of impacts of the entry and establishment of invasive plant species, the potential of risk assessment schemes to predict damage before or at an early stage of an invasion, and the available measures that can prevent, mitigate or contain the negative consequences of an invasive plant species will be illustrated.

Material and methods: In order to demonstrate the objectives mentioned above, we will use the following examples, outlining in particular the invasion process, damage, and the assessment of damage:

- *Prunus serotina*, a North American forest tree that is a major problem in forestry in nature conservation in several European countries,
- *Ambrosia artemisiifolia*, a herb with negative impact on both human health and agriculture,
- *Ludwigia grandiflora*, an aquatic plant at an early invasion stage in some European countries.

Results: We argue that in addition to legal measures like the new EU Regulation on Invasive Alien Species, instruments of the phytosanitary sector may successfully be applied to decrease the risk posed by invasive plant species. This is in line with the International Plant Protection Convention that has recently published an annex to its standard on risk assessment dealing particularly with invasive plants.

Conclusions: The introduction and spread of invasive plants have had severe negative consequences for biodiversity and negatively affected ecosystems services. In order to prevent new invasions as far as possible, risk assessments are needed in order to describe the risks before a plant species is imported.

O AIS 4

When an insect incursion comes with a package: Management of the recently arrived tomato potato psyllid and the pathogen it vectors

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Since the tomato potato psyllid (TPP), *Bactericera cockerelli* (Šulc) (Hemiptera: Trioizidae), was first recorded in New Zealand in 2006 it has caused severe economic losses for the capsicum, tomato, potato and tamarillo industries. Only in 2008 was the full impact realized, when zebra chip (ZC) disease symptoms, similar to those observed in Mexico and the United States, were observed in South Auckland potato tubers. In the same year, researchers detected a bacterial plant pathogen, *Candidatus Liberibacter solanacearum* (CLso), in tubers showing ZC symptoms, other solanaceous crops and *B. cockerelli*. The first industry response was to control the vector using insecticides. Now, more fundamental understanding is sought of the vector-pathogen-plant host interaction, which is critical for effective and sustainable vector management. Our overall research aim is to provide sustainable TPP management strategies that complement ongoing Integrated Pest Management programmes for potato crops. Field trials were conducted to develop action thresholds based on the phenology of TPP, to use Degree Days to initiate a spray programme, to test the efficacy of reduced spray programmes, to evaluate the visual response of TPP to different coloured and sized sticky traps, to assess the optimum trapping height, and the role of non-crop host plants in the population ecology of TPP. In shadehouse trials, the impact of psyllid feeding on potato yield was assessed, and the impact of tuber-borne CLso on emergence, growth and yield of different cultivars. Reduced spray programmes and a threshold were successfully developed in the North Island. Non-crop host plants were found to be important in the psyllid's population ecology and as reservoirs of the pathogen in the absence of crops. The shadehouse trial showed tuber-borne CLso carried in volunteer potatoes may perpetuate disease pressure. Numbers of tubers decreased as the application stage of TPP on the plant became later, indicating greater consequences at certain developmental stages. Our knowledge of the vector-pathogen-plant host interactions has increased,

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providing guidelines that are being applied by North Island potato growers. Additional research on spray programmes and thresholds is needed for the South Island.

O AIS 5

'Candidatus Phytoplasma mali' - a molecular characterization of an emerging phytoplasma species in Croatia

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Introduction: A survey on the presence and diversity of phytoplasmas infecting fruit trees and their vectors in Croatia has been conducted since 2002. Although the presence of '*Candidatus Phytoplasma pyri*' and '*Ca. P. solani*' was sporadically associated with symptomatic apples, there was no direct evidence of the '*Ca. P. mali*', the main etiological agent of Apple proliferation disease (AP) in neither plant nor insect vector samples.

Objectives: The aim of this study was to reassess the presence of the '*Ca. P. mali*' in Croatian orchards as well as to investigate the variability of detected genotypes by multilocus sequence typing (MLST) analysis.

Materials and methods: Throughout 2011 and 2012, orchards from all fruit growing regions of Croatia were surveyed for AP symptoms and nearly 70 samples of different apple varieties were collected. Psyllid samples were collected in 2012 from two selected orchards in the north-western part of the country. Total nucleic acids were extracted and PCR/RFLP analysis of phytoplasma 16S rDNA was performed by using P1/P7 primers in a direct PCR assays followed by nested PCR using R16(X) F1/R1 primers. Amplicons from nested PCR were digested with *RsaI* and *SspI* to determine the affiliation to the '*Ca. P. mali*' species. To confirm the results, real-time PCR amplifying 16S rDNA was also performed. Furthermore, *pnp*, *secY*, *imp* and *aceF* genes were amplified using newly designed primers. All obtained amplicons were sequenced and phylogenetic analyses were performed.

Results: For the first time, in 2011, the occurrence of '*Ca. P. mali*' was detected in samples collected from seven locations in continental as well as Adriatic Croatian regions while the survey in the following year revealed one additional location with the '*Ca. P. mali*' occurrence. The analysis of psyllid vectors revealed one positive finding of '*Ca. P. mali*' in female specimen of *Cacopsylla picta* from an orchard in the north-western Croatia. PCR/RFLP results were consistent with the results obtained by real-time PCR. Phylogenetic analysis of *pnp*, *secY*, *imp* and *aceF* gene sequences revealed considerable diversity among Croatian AP strains with *pnp* being the most variable.

Conclusions: This is the first molecular detection as well as the MLST analysis and genotyping study of '*Ca. P. mali*' in Croatia, from both apple and insect vector species.

O AIS 6

Monitoring of European corn borer (*Ostrinia nubilalis* HÜBNER) with Trapview AURA

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European corn borer (ECB) is one of the most economically important pests worldwide. Unlike some other pests it cannot be reliably monitored using pheromone traps, while light traps are proven to be the most appropriate monitoring tool for this pest. Unfortunately, standard light trap require lots of infrastructure and maintenance. Therefore we were looking for alternatives from the emerging automated pest monitoring field in order to simplify ECB monitoring and make it more accessible for everyday use.

Monitoring of ECB on hops in Savinja valley is performed with light traps for the last 40 years. Different factors, like climate changes and placement of relevant crops require adoption of placement and quantity of traps. However limited access to adequate power sources in the fields, sensitivity to summer rains and lack of manpower are significant limitations which are very difficult to overcome.

To address these issues Trapview AURA (TVA) was introduced. It is a light trap with remote sensing capabilities. Its advantages are: complete power self-sufficiency, ease of use, providing data of catches in near-real time and high resistance to unfavourable weather events. The main objective of the experiment was to determine its efficiency in comparison with standard monitoring trap for ECB.

TVA is based on delta trap, enhanced with electronics, power supply and time controlled LED light source, which emits optimal light for ECB vision. The experiment was carried out on two locations in the hop fields area in Savinja valley in Slovenia in year 2014.

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TVA caught only 7-10% (depending upon location) of ECBs in comparison with catches from classical light trap, however the dynamics of catches was similar. In terms of energy efficiency, automated trap was nearly 30 times more efficient. It also proved significantly more reliable and easier to maintain.

In order to produce reliable forecasts for plant protection it would be necessary to spread the network of monitoring spots, but with the classical light traps this is usually not feasible. TVA can be a good substitute for standard light traps. In the future we will focus on increasing number of catches (compared to standard light traps) and on interpreting TVA catches.

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Plant Protection in a Changing Climate

O CHC 1

Climate Change Effects on Expression of Resistance to Insect Pests: Implications for Pest Management and Sustainable Crop Production

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Chemical composition of plants will change in direct response to global warming and climate change, affecting growth and development of insect pests. Moisture stress will lead to either an increase or decrease in insect damage. Water stressed plants, in general, are more susceptible to insect pests. Atmospheric humidity also interferes with insect-plant interactions as it influences detection of host plant odors, and thus, may influence host finding and antixenosis mechanism of resistance to insects. Increased levels of CO₂ will enhance plant growth, but may also increase the damage caused by some phytophagous insects. Increased CO₂ may result in a slight decrease in nitrogen-based defenses (e.g., alkaloids), but increase the carbon-based defenses (e.g., tannins). Environmental factors such as soil moisture, and atmospheric temperature and humidity have a strong influence on expression of *Bacillus thuringiensis* (*Bt*) toxin proteins in transgenic plants. Genetically modified cottons with *Bt* toxin genes have lower nitrogen, and higher carbon-nitrogen ratio, carbon defensive compounds, condensed tannins, and gossypol under elevated CO₂, which results in a significant decrease in *Bt* toxin expression. Decrease in relative humidity and increase in minimum and maximum temperatures increases the efficacy of transgenic cotton against the bollworms, but there is a significant decrease in biological activity once the minimum and maximum temperature are > 22 and > 38 °C, respectively. It is therefore important to understand the effects of climate change on the efficacy of host plant resistance to herbivores for pest management and sustainable crop production.

O CHC 2

Classical biological control in a changing climate

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Efforts to control invasive alien species with biological control often face a significant hurdle, in that the success of a natural enemy can be influenced by climatic factors. This is of particular importance in classical biological control. Climatic factors are of major importance in determining the outcome of biological control programmes. Climate change may alter existing biological control programs, but it may also provide new opportunities. Warming conditions may impact insect populations by extending the growing season, altering the timing of emergence from overwintering sites, increasing growth and development rates, shortening generation times, reducing overwintering mortality and changing their geographical distribution. Tritrophic interactions between plants, herbivorous insects and parasitoids are a result of a coevolutionary process that is related to specific, stable climatic conditions. Higher trophic levels are more likely to be affected by climate change since they require lower trophic levels to adapt to climate change. Climate change may affect host-parasitoid temporal or geographical synchronization. Though there have been reviews on the impact of climate change on biological control agents, there have been relatively few case studies on specific species. Here we present a case study assessing the impact of a changing climate on the response of the parasitoid *Peristenus digoneutis* (Hymenoptera: Braconidae), a classical biological control agent introduced into North America against *Lygus* species (Hemiptera: Miridae), to future climates for both spatial (continental and regional) and temporal (annual and weekly) scales through the application of two General Circulation Models for Europe and North America. Using this example, we further discuss the expected consequences for classical biological control programs in the future.

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O CHC 3

Diversification of current plant protection strategies to mitigate climate change effects

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Climate change is increasingly perceived as one of the major constraints that limit agricultural productivity. Crop losses due to climate change could be direct, such as damages through flooding or storms, or indirect such as altered distribution of crop pests. The real impact of climate change at global level is yet uncertain and likely variable from one region to another. Within this context, it is difficult to predict effects of climate change, particularly when long-term datasets from the past are missing to develop and test predictive models for the future. Nevertheless, our knowledge of plant-disease interactions, population genetics of pathogens as well as crops, and examples of overwhelming establishment of new diseases in a given region provides insights into how climate change may affect disease incidence and severity. Here we report examples of pest populations which have been established across regions previously considered detrimental for their survival and yield losses associated to these pests. Faced with the uncertainty regarding the effects of changing climate on crop protection, here we propose a number of action points that, to our opinion, may help improve current plant protection strategies. Given this uncertainty, policy, research, and extension should be prepared to promote resilience vis-à-vis pests which, at the biophysical level, entails uncovering what currently makes cropping systems resilient, while at the organizational level, the capacity to adapt needs to be recognized and strengthened (Lamichhane et al 2014). Such action points include increase in human resources, development of resilient cropping systems, more focus on crop-weed competition, anticipating of risks and international monitoring, and more effort on breeding for resistance, development of biological control strategies and pest risks analysis. This diversification could be achieved by improving current plant protection practices which might help mitigate the effect of climate change in future crop protection, particularly in the EU, but also at global level. The vision presented here is that of the ENDURE European Research Group, which brings together some of Europe's leading agricultural research, teaching, and extension institutes with a special interest in IPM.

References: Lamichhane JR, Barzman M, Booij K et al., 2014. Robust cropping systems to tackle pests under climate change: a review. *Agronomy for Sustainable Development*, DOI 10.1007/s13593-014-0275-9.

O CHC 4

Effect of Climate Change on Bioefficacy of IPM Technologies

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Host plant resistance, biopesticides, natural enemies and synthetic chemicals are some of the potential options for pest management, and the relative efficacy of many of these pest control measures is likely to change as a result of climate change. Global warming may result in breakdown of resistance to certain insect pests. Climatic change may also disrupt the balance between insect pests and their natural enemies. The natural enemy - insect host relationship is influenced by both temperature and relative humidity, which are critical for survival and development of the immature stages of the parasitoids. Postembryonic development of parasitoids is prolonged at cooler temperatures, and the mortality increases at temperature extremes. Conversely, the arthropod predators are directly exposed to the prevailing environmental conditions and experience direct effects of the changing climatic conditions on development and predation potential. Temperature, humidity, elevated atmospheric CO₂, and UV radiation also alter the rate of entomopathogenic fungi and nematodes entry into the host insect. Insect viruses are known to be inactivated by high temperatures, sunlight and ultraviolet rays. Sunlight, CO₂, and pH exhibit varied influences on the toxicity of synthetic pesticides. Rainfall reduces the insecticide toxicity, however, it varies with the intensity and amount of rain, insecticide formulation and the adjuvants used. Therefore, there is a need to have a concerted look at the likely effects of climate change on the efficacy of different crop protection technologies, and devise appropriate strategies to mitigate the adverse effects of climate change on the bioefficacy of IPM technologies.

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O CHC 5

Adaptation of crop protection to climatic changes - risk estimation for pests and diseases in four important arable crops in Lower Saxony, Germany

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Introduction: Winter wheat, maize, oilseed rape and sugar beet are the main arable crops in Lower Saxony, Northern Germany. Risks to these crops due to biotic stress is largely dependent on climate which is expected to change to warmer mean temperatures by 2-4°C and increased precipitation during this century. There is public interest to estimate future risks in crop protection based on current model predictions of climate change.

Objectives: To estimate the future risks of crop diseases and derive recommendations for future crop protection strategies.

Materials and methods: Meta-analyses were based on published knowledge. Empirical studies were performed in climate chambers and temperature controlled field plots. Disease and phenological forecasting models were linked to the regional climate model REMO (A1B) based on simulated weather data from 260 virtual weather stations (10 x 10 km grid) in 5 distinct agricultural production zones in Lower Saxony. Projections were made on a midterm (2021-2050) and long term (2071-2100) basis.

Results: Regional climate change calculation with REMO estimated an increase in temperature by 0.3-0.4°C and 2.7-3.0°C for the mid- and long term projection, respectively. Disease modeling revealed shifts in the prevalence of diseases due to extended crop growth periods and to conditions becoming more or less conducive for disease development. In oilseed rape and winter wheat, a pronounced change in the prevalence of major pathogens may occur without aggravating the overall threat to the crop. Diseases in maize and sugar beet may be increased in general potentially requiring stronger efforts in crop protection. Significant changes were only estimated for the long term perspective while until 2050 effects will be low.

Conclusions: Model based predictions do not suggest an overall aggravation of crop protection problems, instead, potential shifts in the relative prevalence of individual pathogens and pests may occur. Other factors such as cropping system, agrotechnical progress and market conditions are likely to have a much stronger impact on future challenges in crop protection. Current adaptability of farmers to annual changes in weather conditions imply that they will be able to cope with long term climate shifts as long as effective crop protection tools will be available.

O CHC 6

Climate change-caused changes in the distribution and abundance of potato pests and adaptation of IPM to cope with future pest risks

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Insect pest distribution and population growth potentials are mainly temperature-driven; hence a rise in temperature through global warming may either increase or decrease insect development rates and related crop damages in agroecosystems depending on the insect species' optimum temperature range. For better preparing policy makers and farmers and adapting Integrated Pest Management (IPM) to new pest situations, a better understanding is needed to predict potential changes in pest risks on global, regional and local scales. We use process-based climatic pest phenology models and apply three risk indices (establishment risk index, ERI; generation index, GI; and activity index, AI) in a geographic information system (GIS) to map and predict changes in risks for climate-change scenarios of the year 2050 based on downscaled climate-change data of the A1B scenario from the WorldClim database. All applications and simulations are made using the Insect Life Cycle Modeling (ILCYM) software developed by The International Potato Center (CIP), Lima, Peru (www.cipotato.org/ilcym). Potato (*Solanum tuberosum*) is severely constrained by many pest problems, which pests will be differently affected by climate change. The potato tuber moth, *Phthorimaea operculella*, invasive in >90 countries globally, will progressively increase its damage potential in all regions where the pest already prevails today, with an excessive increase in warmer cropping regions of the tropics and subtropics and a moderate range expansion into tropical highlands such as the Andean region. Predictions have been also made for the Guatemalan potato tuber moth, *Tecia solanivora*, the Andean potato tuber moth, *Symmetrischema tangolias*, the leafminer fly, *Liriomyza huidobrensis*, among others. In conclusion, farmers will be confronted with a shift in pest range and due to higher pest abundance with greater crop losses. The potential changes in pest risks call for creating better awareness and promote the inclusion of pest risks adaptation plans at country level. The capacity of national plant protection organizations (NPPO) needs to be improved to adequately incorporate pest risk mapping results in adaptation planning of IPM to manage future pest risks on regional and country level. Already under the current pest situation, farmers' main response to control pests in potato is to

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apply hazardous highly toxic insecticides; hence there is a strong need for strengthening the development and adaptation of IPM to support farmers adequately to manage future pest risks.

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Nematodes III

O NEM III-1

Velum[®] - A novel nematicide for efficient crop production

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Velum[®], common name fluopyram, is a novel nematicide from the chemical class of pyridinyl ethyl benzamides which is developed to control nematodes in a broad range of crops. Fluopyram is the first nematicide acting via Complex II (SQR) inhibition. Fluopyram selectively inhibits Complex II of the mitochondrial respiratory chain in nematodes which leads to a fast and severe depletion of the nematode's cellular energy (ATP). Moreover, fluopyram is characterized by a very favourable toxicological and ecotoxicological profile.

After the restriction or withdrawal of several nematicides, protection of crops from plant-parasitic nematodes has become a major challenge. Velum[®] exhibits an excellent level of efficacy at very low dose rates compared to currently available nematicides. Velum[®] has proven itself in a large number of trials conducted in many countries and crops to be a highly effective nematicide controlling different nematode species. The spectrum includes root knot, free living and cyst nematodes such as *Meloidogyne* spp., *Pratylenchus* spp., *Radopholus* spp. and *Globodera* spp.. Multiple field trials have demonstrated that Velum[®] provides an outstanding long-lasting nematode control also under high infestation pressure.

Velum[®] will be commercialised in different formulation types enabling it to be used in multiple application techniques such as granular spreading, drenching, in-furrow spraying and seed treatment. Target markets include fruit and vegetable crops as well as cotton, corn, tobacco and sugarcane.

O NEM III-2

Exploiting the integrity and effects of a soil-derived biological culture against Nematode Pests

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Research efforts are underway to exploit and identify novel, eco-friendly agents for the management of nematode pests, i.e. *Meloidogyne* spp., that damage a wide range of crops. SoilBioMuti (SBM) is such a potential agent that contains various biological organisms that is extracted from virgin soils. Identification of the microbial organisms contained in the extracted product were pursued by means of next generation sequencing (Illumina). In addition, the effects of filtered and non-filtered concentrations of SBM on the biology, physiology and reproduction of *M. javanica* and *M. incognita* in separate *in vitro* (laboratory) and *in vivo* (greenhouse) experiments were investigated. Various bacterial and fungal genera, including pathogens of plants, were identified. Furthermore, all SBM concentrations both filtered (F) and non-filtered (NF), reduced root-knot nematode second-stage juvenile (J2) motility significantly from 3 to 24 h after exposure compared to the tap-water control. No significant differences were evident in the number of egg masses/root system 30 days after J2 that were exposed to the SBM concentrations were inoculated on roots of susceptible tomato seedlings. However, *M. incognita* egg-mass production was reduced by 33% for the F and 90% for the NF treatments and that of *M. javanica* by 73% (F) and 45% (NF) 5% SBM concentration treatments. In addition, oxygen consumption of J2 of both root-knot nematode species suspended in 2.5% SBM F treatments for 24 h were significantly lower compared to those of the F and tap-water control treatments. The results thus far suggest that SBM has inhibiting effects on the motility and oxygen consumption of *Meloidogyne* spp. J2, although both species retained their ability to reproduce. Currently, two greenhouse studies are underway in which the effect of SBM soil applications are investigated and results will also be presented. Moreover, evaluation of the product after application in fields of three maize farmers is also underway. Field testing is envisioned to enable researchers to conclude about the potential of SBM as an agent to be used against nematode pests. Furthermore, follow-up studies will shed more light on the particular organisms/assemblages that are responsible for its adverse effects on root-knot nematodes. Also, identification of the mode of action of the causal agent(s) could assist in developing an alternative and environmentally safe product against nematode pests.

Oral Presentations

Nematodes III

O NEM III-3

Comparative analysis of nematode-tolerant sugar beet varieties

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The beet cyst nematode *Heterodera schachtii* (BCN) is a major problem in the sugar beet growing regions of Southwest Germany, causing yield reductions up to 30%.

Since 2010, numerous multi-annual field trials with three groups of sugar beet varieties (susceptible, tolerant and resistant) have been conducted in Rhineland-Palatinate and Baden-Wuerttemberg. Soil samples were taken from up to 30 field trials from two different soil depths (0-30 and 30-60 cm) after sowing and after harvesting sugar beet. Using hatching tests (Acetox) and nematode extraction in sieve trays the infestation rate at sowing time (Pi - population initial) and harvest time (Pf - population final) were determined. Numerous multi-annual field trials show that all 8 tested tolerant sugar beet varieties have an effective potential to reduce nematodes and can therefore designated as partial resistant.

In addition, it was tested if some variety-specific differences in root traits could explain the propagation rate observed in field trials. Five sugar beet varieties (1 susceptible, 3 tolerant, 1 resistant) with and without infestation of *H. schachtii* were analysed in 60 cm soil-filled rhizotrons. Pictures of growth and geometry of young sugar beet root systems were taken at different time points of every rhizotron and analysed by the software GROWSCREEN-Root. Root growth parameters like primary root length, secondary root length, total root length, convex hull, root system width and depth were determined. By comparing root traits of infested and non-infested plants variety-specific responses could be observed. First results suggest that the response of primary root toward nematode infection in three different lines (susceptible, tolerant and resistant) may therefore be an useful indicator.

O NEM III-4

Development of non-phytotoxic concentration in crude extracts used for management of plant-parasitic nematodes

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Most trials on the use of phytonematicides to manage population densities of nematodes do not go beyond the in vitro testing stage due to the inherent allelopathic effects of the materials to plants protected against nematode damage. The objective of this study was to develop an empirically-based procedure to avoid phytotoxicity in phytonematicides. The mean concentration stimulation range (MCSR) was established by subjecting test plants inoculated with nematodes to various concentrations of the phytonematicides for 56 days. Significantly affected plant variables were subjected to the Curve-fitting Allelochemical Response Dosage computer-based model to generate seven biological indices, where D_m and R_h indices were used to compute the non-phytotoxic concentration using the $MCSR = D_m + (R_h/2)$ relation. Second, the application interval (T) for the MCSR value was established using the concept of a "30-day-week-period", where T comprised 0, 1, 2, 3 and 4 application intervals. Significantly affected plant variables were further subjected to lines of the best fit, where quadratic curves ($bx^2 + bx + c$) were optimised using $-b/2b_2$ to generate the appropriate T for the empirically-based MCSR value. Using the proportion of T to the crop cycle (T_{cc}), the application frequency (T_{ca}) was derived ($T_{ca} = T/T_{cc}$) to allow for computation of the dosage ($D = MCSR \times T_{ca}$). In conclusion, the model has been successfully validated under diverse environments to manage *Meloidogyne* species without causing phytotoxicity to various crops.

Oral Presentations

Nematodes III

O NEM III-5

An on-line and updated list of plants associated with plant parasitic *Aphelenchoides* spp., with implications for host-parasite relations within the genus

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Within plant-parasitic nematodes (PPN), “foliar nematodes” *i.e.* *Aphelenchoides besseyi*, *A. fragariae* and *A. ritzemabosi* are well known in agricultural systems; and ten other *Aphelenchoides* spp. have been reported on a limited number of plant species. The number of plants associated with this genus has increased in recent years, above the already high number of described species, in combination with high intra-specific variation and limited number of established molecular information, have led to identification problems, consequently, to an ignorance of the actual diversity of this genus. To appraise the potential host range that a single or a specific combination of *Aphelenchoides* spp. could have, a compiled list of the plant species associated with parasitic *Aphelenchoides* was needed.

We compiled a comprehensive data set of the associated plants for these 13 plant-associated species, based on available literature and online databases. The data were plotted on an associated-plants' supertree, in combination with an *Aphelenchoides* consensus phylogeny.

The compiled list includes more than 1080 reports on 127 botanical families. Foliar nematodes represent more than 90% of the associations and around 83% and 16% of the total reports correspond to flowering plants and ferns, respectively. Most plant-parasitic *Aphelenchoides* have a remarkable broad diversity of associated plants (generalists), but only six plant species are associated with all the three foliar nematodes. Putative specialists are also present in the genus, reported only on one or two related species. The parasites' and the associated-plants' phylogenies do not show any relationship; furthermore, neither ecological patterns nor taxonomical groups are congruent with molecular phylogenies. Given the phylogeny of *Aphelenchoides* spp. and the ranges of their associated plants, the flexibility in their feeding behaviour denotes the ease with which this group can switch towards plant-parasitism. Moreover, even though the compiled list is long, it probably only represents a fraction of the potential range. Their flexibility and absence of more intimate interactions, as known for other PPN, highlights the primitive mode of parasitism in *Aphelenchoides*, making them potentially interesting in the study of the evolution of plant parasitism.

O NEM III-6

Nematode pests of Yam in Ekpoma, Edo State, Nigeria, and their response to Carbofuran and Yam variety

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White yam (*Dioscorea rotundata*) is a very popular root crop in sub-Saharan Africa. Its production and storage is hampered by nematode pests.

The abundance and prevalence of nematode pests of yam in Ekpoma, were investigated during the 2011 planting season to provide information on the species composition and potential pest status. The response of the nematodes to treatments with Carbofuran and yam variety (Alomaku, Alomaku golden and Ove) was investigated during the 2012 planting season to understand their relevance in the development of an integrated pest management option for the pests.

Soil samples were collected from around the developing yam tubers about 3 months after planting in 2011, while both soil samples and yam tubers were collected in 2012, for nematode extraction. Nematodes were extracted at the Nematology Laboratory of the International Institute of Tropical Agriculture, Ibadan, using the sieving method. Six genera of nematodes were identified from the soil samples in 2011.

These were *Helicotylenchus*, *Meloidogyne*, *Tylenchulus*, *Tylenchus*, *Pratylenchus* and *Hoplolaimus*. The prevalence of these nematodes per yam heap ranged from 10.00 % (in *Hoplolaimus*, *Pratylenchus* and *Tylenchus*) to 90.00 % in *Helicotylenchus*. The prevalence of *Tylenchulus* and *Meloidogyne* were 20.00 % and 40.00 %, respectively. *Helicotylenchus* was the most abundant (34.44 per 10ml), followed by *Meloidogyne* and *Pratylenchus* (15.00 per 10ml), *Tylenchulus* (12.50 per 10ml), *Hoplolaimus* (10.00 per 10ml) and *Tylenchus* (2.00 per 10ml). In 2012, only four genera (*Meloidogyne*, *Helicotylenchus*, *Tylenchus* and *Pratylenchus*) were found in the soil samples after the application of Carbofuran. However, the yam peel of the variety, Ove, contained *Scutellonema* and *Meloidogyne* species. The abundance of the four nematodes found in the soil, 7.92 per 10ml (*Meloidogyne*), 7.59 per 10ml (*Helicotylenchus*), 1.11 per 10ml (*Tylenchus*) and 0.04 per 10ml (*Pratylenchus*) was significantly ($P < 0.05$) lower in 2012 compared with 2011. In the peels of Ove, the abundance was 3.33 per 10ml (*Scutellonema*) and 6.67 per 10ml (*Meloidogyne*).

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It is concluded that nematodes are important pests of yam in Ekpoma. Carbofuran and yam variety can be exploited in the development of an integrated pest management option for nematode pests of yam in Ekpoma.

Figure 1

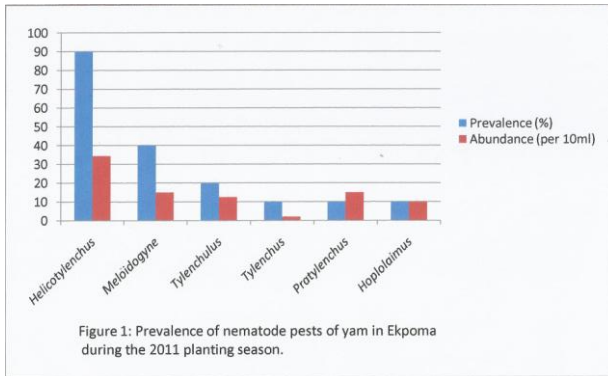
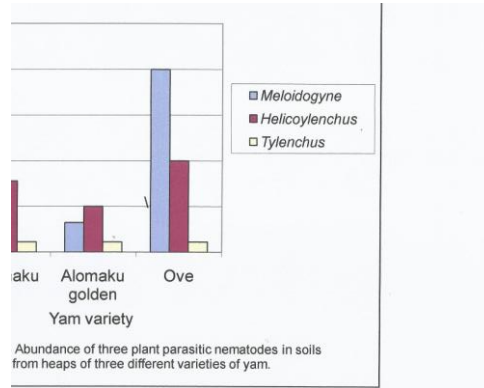


Figure 2



Oral Presentations

Pest and Diseases in Trees

O TREE 1

Upcoming diseases and pests threaten the urban green in Germany

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In urban green a variety of harmful organisms occurs, which may negatively affect the aesthetics and vitality of the plants and thus their ability to fulfill their functions. Due to the increasing globalization and climate change, new or so far unimportant pests move into focus.

Current major pests like the sycamore lace bug (*Corythucha ciliate*) and the box-tree pyralid (*Cydalima perspectalis*) and diseases like massaria disease (*Splanchnonema platani*) and bleeding canker of horse chestnut (*Pseudomonas syringae* pv. *aesculi*) in the urban green in Germany, their biology, diagnosis and ways to prevent and control will be presented.

O TREE 2

Bark damages on plane trees caused by drought and frost in urban stands

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Since some years stem fractures are recognized on plane trees (*Platanus acerifolia*) in urban stands in the winter season. They are located on the main stem, but on branches in the lower crown, too. Mainly older trees are damaged, more and more these fractures appear on young trees. The symptom is mostly in south-west direction, sometimes in others. Investigations showed that these damages are caused in a soft winter following a frost periode in a short distance. But the main reason is drought in the beginning, for example after a dry summer or on bad urban stands. So it is important in tree care to control the water situation of plane tree stands more and more and to understand what does it mean to prepare a new planting. The discussion is going on what happens in cities in the time of climate change with a special view to rain falls over the year.

O TREE 3

Fungal community of cork oak (*Quercus suber* L.) forest under drought scenario

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Mediterranean forests are one of the biodiversity "hotspots". The cork oak (*Quercus suber* L.) forest ("montado") is an unique and emblematic resource for Portugal, both social and economic manner, protected by EU (Habitats Directive 92/43/EEC). Portugal is the greatest cork producer with 50% of cork extraction worldwide. Nowadays, cork oak faces a severe global climate change and a reduction in water availability is expected for the near future, which is expected to decrease cork oak growth and productivity. Plant benefits from many symbiotic relations that occur between microorganisms and roots that are able to enhance nutrient and water supply. However, root colonization efficiency and fungal community structures are dependent on environmental conditions, such as water availability. The main goal of this work was to evaluate the relationship between fungal abundance and diversity conferred by different drought scenarios. The effect of drought in cork oak fungal ecosystem was accessed by studying cork oak field trees in 7 different Portuguese forests. Five different locations (Gerês, Macedo de Cavaleiros, Vimeiro, Grândola and Moura) were evaluated according to a gradient of water-availability. Samples from extreme conditions, the driest (Moura) and wettest (Gerês) places were sampled in two different sites. Soil samples (35) were analysed by ITS barcoding on ECM tips and metabarcoding using Illumina platform. The obtained results will help to understand not only the importance of fungi to drought tolerance in cork oak forest, but also which are the main fungal colonizers.

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Oral Presentations

Pest and Diseases in Trees

O TREE 4

The 'birch-leafroll disease' emerging in forests and urban parks in Fennoscandia - Viral agents associated with the disease

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Question: The 'birch-leafroll disease' has emerged the last decade to a serious problem in Fennoscandia. The extensive appearance of virus-infected birches was first reported in 2006 in Finland (Jalkanen *et al.*, 2007). Diseased trees exhibited foliar disorders including vein banding, leaf roll and chlorotic ringspots, the affected trees declining in vigor with time. *Cherry leaf roll virus* has been detected in symptomatic birches, it could however, not be directly correlated with the disease. A natural CLR_V population from birches is studied to improve understanding of the emergence of this viral disease. Additionally, the assumption that other viral pathogens are involved in the disease is investigated.

Methods: A natural population of CLR_V collected from infected *Betula* trees in Rovaniemi is analyzed and the population features are described. Detection trials targeting other viruses are performed. Samples from affected trees are analyzed with the next-generation sequencing (NGS) technology, allowing the whole microbial population present in each sample to be defined. Detection assays to confirm the presence of the viruses identified by NGS in the RNA samples isolated from the infected tissues are performed.

Results: The birch CLR_V population from Rovaniemi is characterised by high genetic variability; considerable haplotype diversity, long genetic distance among haplotypes and high within haplotype diversity are determined. Mixed infections of CLR_V strains in single *Betula* trees and recombination events in the CP region are demonstrated. The NGS analysis provided indication of more viruses being present in the birch samples.

Conclusions: CLR_V is strongly suggested to be associated with the "birch-leafroll disease" in Fennoscandia. We consider the increased genetic diversity and the coexistence of a complex of highly variable strains in the same host as a significant change in the pathogen population which could constitute a possible factor for the disease emergence. The beneficial effect for the CLR_V infection when trees are co-infected with another plant pathogenic virus will be discussed.

Jalkanen, R., Büttner, C. and von Bargaen, S. 2007. *Cherry leaf roll virus* CLR_V, abundant on *Betula pubescens* in Finland. *Silva Fennica* 41: 755-762.

O TREE 5

Emaraviruses infecting forest and urban deciduous tree species

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European mountain ash ringspot-associated virus (EMARaV) is the type member of the genus *Emaravirus* comprising plant viruses with a segmented ss(-)RNA genome mainly infecting woody hosts. EMARaV contains at least four monocistronic genome segments within the enveloped spherical particle, encoding the replicase (RNA1), a glycoprotein precursor (RNA2), the viral nucleocapsid protein (RNA3), and a putative movement protein (RNA4), while in other members of the genus up to eight genomic RNA molecules were found. Emaraviruses have a narrow host range usually restricted to few related species. Symptoms induced by EMARaV include chlorotic ringspot, mottle of leaves and decline which may lead to the death of *Sorbus aucuparia* (European mountain ash, syn. rowan). Other broad-leaved tree species are known for decades exhibiting similar virus-like symptoms, but no virus has been associated with the disease, yet.

Distribution and significance of emaraviruses as causal viral agents infecting important broad-leaved tree species of European forests and urban areas are addressed in this study.

Sorbus spp. were assessed for EMARaV infection by visual inspection and RT-PCR. Other deciduous tree species showing virus-like symptoms were investigated for plant virus infection utilizing next-generation RNA sequencing technologies.

Chlorotic ringspots, mottle and dieback occur frequently throughout the *S. aucuparia* population in several European countries including natural stands, managed forest and urban areas. EMARaV is closely associated with the observed disease as demonstrated by RT-PCR. The virus was also detectable in whitebeam species (*S. aria* and *S. intermedia*) with respective symptoms. Further, two previously unknown RNA viruses were identified in two different tree species showing chlorotic ringspots, line pattern and mottle of leaves. Sequence analyses revealed closest relationships to emaraviruses.

EMARaV is the main viral agent which affects rowans throughout Europe and is capable to infect other *Sorbus* species. Related plant viruses are able to infect other broad-leaved tree species in Europe. Thus, members of the genus *Emaravirus* have to be

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considered as serious threats to relevant forest and urban woody species and need to be included into health management strategies of deciduous trees.

O TREE 6

Earlier bud burst in Norway spruce causes shifts in the population dynamics of two eruptive sawfly species

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Lowland stands of pure Norway spruce (*Picea abies*) in Austria are highly prone to mass outbreaks of the small spruce sawfly, *Pristiphora abietina*. Until the mid-1990ies, large forest areas were heavily infested, but outbreaks disappeared thereafter. From 2011 onwards, significant feeding damages were reported from spruce plantations that had suffered severe and repeated defoliation by *P. abietina* in the past. The actual damage is caused by the mountain spruce sawfly, *Pachynematus montanus*, a species considered to be a minor pest with sporadic, local outbreaks in forests above 800 m a.s.l. Within an EU-funded project we investigated the population dynamics of the two sawfly species. We recorded the timing of adult sawfly emergence from the soil (photo-electors) and spruce budburst (stages) on five currently-infested lowland sites and two uninfested sites at higher elevations for 2-3 consecutive years. The data were coupled with air and soil temperature profiles and the results were compared with studies from the 1990ies. Despite year-to-year variations, the beginning of budburst in spruce advanced considerably more than adult wasp emergence. At the lowland sites, *P. abietina* catches were rare while *P. montanus* was the predominant species; both sawfly species were rare at the upland sites.

The phenological window for oviposition is very narrow for *P. abietina*, females accept exclusively the newly-expanding buds. The temporal window is wider for *P. montanus* wasps which lay their eggs on needles of expanding shoots up to 5 cm in length. Accordingly, tight synchrony between budburst and wasp emergence is a major determinant of variability in the abundance of *P. abietina*. We speculate that significant advances in the timing of spruce budburst due to climate warming in recent years outcompeted *P. abietina*, presumably due to the inability of the emerging wasps to find suitable, newly-burst buds for oviposition, but still allow *P. montanus* to colonize the buds.

The shift in population dynamics of the sawfly species has major impacts on forestry; feeding of *P. abietina* larvae is restricted to current-year needles, thus, even high needle loss does not kill the tree. By contrast, *P. montanus* larvae feed both current-year needles and old foliage, and thus predisposing heavily infested trees to attack by bark beetles.

Oral Presentations

Weeds

O WEE 1

Implications of climate change for invasive weeds

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This planet is facing changes in its environment since its advent. However, recent projection in CO₂ concentrations as a result of anthropogenic activities has seriously disturbed the equilibrium of global climate. In addition, increased international travels, change in land use, drought, global trade, nitrogen pollution and increasing atmospheric temperature are pertinent in the list of recent global climate changes. Invasive plant/weed species are the alien species which have negative implications for the economy, health and local vegetation. The climate change is supposed to enhance the invasiveness of invasive plants. We have discussed the X-factors which link the weed invasiveness with climate change. For example, high CO₂ concentration in the atmosphere can help the invasive weed species to germinate quickly, build high biomass and produce more seeds. Higher concentration of nitrogen resulting from nitrogen pollution can improve the nutrition of invasive weeds, and high atmospheric temperature can stimulate the early flowering and seed production in invasive weeds. Timely and properly management of invasive weeds would aid to avoid the ecological and economic damages caused by invasive weeds. The option of integrated management is desired to eradicate this noxious vegetation.

O WEE 2

Biological control of weeds in maize using seed predators

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Competitiveness between weeds and crops can lower yields for farmers. Weeds are hard to control. One of the reasons is that they produce large amounts of seeds that enter the seed bank. As a natural reservoir, the seedbank ensures the survival of weed populations in the long run. Post dispersal seed predators can reduce the input of new seeds into the seed bank by feeding on mature seeds on the soil surface. Weeds naturally occur in patches. If seed predators consume more seed in areas with high than in areas with low seed densities (direct density-dependent), they can reduce their spatial spread of weed patches.

The goal of this work was to quantify the response of seed predators to increasing density of artificially applied seeds of *Echinochloa crus-galli* (L.) P. Beauv. in maize fields. In the case of granivorous rodents, a directly density-dependent response was expected, because they are active all-year-round, and have a high metabolic rate. In the case of granivorous carabid beetles, an inverse density-dependent response was expected, because they are less mobility and have a lower metabolic rate. Depending on the dominant type of seed predator, the response can vary from directly to inversely density-dependent.

To examine the response of seed predators to increasing seed densities of *E. crus-galli*, an experiment was conducted on two maize fields, in North-Eastern Germany from August until October 2014. On 30 plots (1.5 m x 1.5 m) per field, *E. crus-galli* seeds were applied, at 0, 1200, 2400, 4800, 9600 seed m⁻², in six replications per density. The rate of seed predation was measured in so-called seed trays. In each plot, one 0.1 m² sized tray filled with seed free soil was buried flush with the soil surface and seeded with the appropriate seed density. After 4 and 6 weeks, trays were removed, seeds extracted from the soil with an elutriator and flotation technique with saturated NaCl and counted manually.

To estimate the degree of density-dependent seed predation in maize fields, the relationship between the initial number of seeds and the number of seeds retrieved after exposure will be determined. Results on the relationship between the seed density and predation rate will be presented and discussed.

O WEE 3

Current status of Parasitic Weeds in Sudan

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Parasitic flowering plants of the angiosperm includes 3000 plant species in 16 families, Characterized by forming haustoria linking them to the host tissues used as a morphological and physiological graft which taps water and nutrients from the host. Two main types of parasitic plants can be distinguished: stem parasites and root parasites. Stem parasites occurred in several families, and pathogenic members include some mistletoe and dodder (*Cuscuta*). Root parasites are more common and are found in diverse taxonomic groups. Most of the economically important root parasites are in the Orobanchaceae (*Phelipanchae*, *Orobanchae*, and *Striga*) (fig. 1). In Sudan *Striga* is considered the main biotic factor that limits sorghum production especially

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Weeds

under rainfed agriculture. Broomrapes are phanerogamic holoparasites that attack roots of many dicotyledonous crops. The most important species: *Phellibanchae ramosa* (attached Solanaceous crops), attacks mainly tomato, eggplant & potato in central Sudan. *Orobancae creneta* (Leguminous crops) attacks mainly faba bean in Northern Sudan and *O. cernua* parasitized sunflower. Mistletoe especially the genus *Tapinanthus* occurs in most citrus and gauva producing areas along the Blue Nile banks in central Sudan leading to losses in yield ranged from 50-100% leading to eradication of trees. Dodder (*Cuscuta* spp) is an annual obligate stem parasite. It produces a dense and shady barriers or canopy which drastically reduces growth and vigor of the host. Field dodder (*Cucuta campestris*) the most dominant species was noticed parasitizing 20 plant species belonging to 13 families. Among the most affected hosts; 6 vegetable crops and one fruit tree. Compared with non-parasitic weeds, the control of parasitic weeds has proved to be exceptionally difficult. Nevertheless different methods of control have tried and some of them adopted under field conditions to manage the parasitic weeds in Sudan.

Figure 1

The most important genera in Sudan



Orobanche



Striga



Tapinanthus



Cuscuta

O WEE 4

Practicality of suicidal germination for combating the invasive root parasitic weed *Striga hermonthica*

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Witchweeds (*Striga* spp.) have long been recognized as the greatest biological constraint to food production in sub-Saharan Africa. They are steadily increasing in distribution and intensity. Among the species, *S. hermonthica*, which parasitizes a wide range of economically important poaceous crops including maize, sorghum, millet, rice and sugarcane, is the most important. To germinate, *Striga* seeds require a pre-treatment in a warm moist environment and a subsequent exposure to a germination stimulant. In nature the germination stimulants, strigolactones (SLs), are ubiquitous in plants root exudates. Induction of germination in absence of host plants, suicidal germination, has become an attractive approach for *Striga* control and hence identification and synthesis of novel SLs and development of analogues with simpler structures, high activities and better stability in soil is imperative. In this study, novel SLs analogues comprising carbamates, phenylacrylonitriles, phenyliminoacetonitriles and phosphonates, were designed, synthesized and evaluated *in vitro* for potency as *S. hermonthica* germination stimulants. The carbamates, phenylacrylonitriles and phenyliminoacetonitriles analogues induced high germination while the phosphonates analogues were less effective. Based on high activity and ease of preparation a carbamate, designated as T-010 was selected, formulated (10% WP) and tested for effectiveness on *Striga* parasitism and sorghum growth. Pots filled with *Striga* infested soil were irrigated with tap water, treated with T-010 18 days later. Six days after treatment sorghum seeds were sown. The seedlings, thinned to 2/pot, were allowed to grow to maturity. T-010 decreased *Striga* emergence and increased sorghum shoot dry weight (DW) in a concentration dependent manner. No significant differences in shoot DW were observed among *Striga*-free treatments, irrespective of T-010 rate, thus implying that the stimulant had no obvious adverse effects on sorghum. The results were further validated in a field artificially infested with *Striga* seeds. Sorghum head DW was 69, 274, 326

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Weeds

and 280 g m⁻² in plots treated with T-010 at 0, 0.1, 1.0 and 10.0 kg ha⁻¹, respectively. The study demonstrated the practicality of suicidal germination in combating *Striga* and mitigating its adverse effects on sorghum.

O WEE 5

Environmental weed *Acacia longifolia* and their biological management by *Trichilogaster acaciaelongifoliae* in Australia

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Acacia longifolia subsp. *longifolia* (hereafter *A. longifolia*) is a significant environmental weed in parts of Victoria, South Australia and Western Australia and therefore its biological management is considered vital. Sustainable control of *A. longifolia* is not easy as the seeds of *A. longifolia* can live dormant in the soil for decades, which can germinate after temporary control of this species and can obstruct restoration program after some disturbance events (as for example fire). However biological control is more effective to manage *A. longifolia* for long term restoration of native ecosystems. *Trichilogaster acaciaelongifoliae* is a recognized agent to manage populations of *A. longifolia* in South Africa, its feeding behaviour and host specificity are little known. Moreover, the precise steps in gall development are unclear. Therefore, the aims of this study are (i) to explain the feeding behaviour and host specificity of the *T. acaciaelongifoliae*, and (ii) host plant response and community interactions in gall on *A. longifolia*. The gall materials on *A. longifolia* with *T. acaciaelongifoliae* and other associated insects will be collected during 2014-2016 from the Grampians National Park for SEM and TEM microscopy. Adults of *T. acaciaelongifoliae* will be captured from the field and released on glasshouse cultures of *A. longifolia* to monitor their feeding behaviour and process of gall development. The outcomes of this study will enable to understanding the environmentally control of weedy *Acacia*-s.

O WEE 6

Multiple introduction events of the invasive alien weed *Sicyos angulatus* in Japan and its spread by water systems

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Introduction: The Plant Protection Act of Japan does not include weeds among the injurious plants to be controlled. Since the 1990s, however, many alien weeds have caused serious problems in agricultural land. *Sicyos angulatus* is particularly problematic because of its aggressive vining habit in maize and soybean plantations. To develop effective procedures of alien weed control, it is crucial to understand their introduction dynamics and mechanisms of spread.

Objectives: The objectives of this study are (1) to elucidate the introduction dynamics of *S. angulatus* based on regional patterns of genetic variation and (2) to understand the spread pattern of *S. angulatus* through a case study of the Abukuma River basin, Japan.

Materials and methods: We used Inter-Simple Sequence Repeat (ISSR) genotyping to detect patterns of genetic variation in *S. angulatus* in central and northeastern regions of Japan. We surveyed the distribution of *S. angulatus* along the banks of the Abukuma River and searched for the seed source in the area surrounding the river's upper reaches. Chloroplast DNA haplotype composition was compared among populations.

Results: The analysis of molecular variance by ISSR genotyping revealed that 88.4% of genetic variation occurred within single areas, compared to only 1.1% between the two regions and 10.5% among areas within regions. The coefficient of gene differentiation was low between the two geographically distinct regions ($G_{ST} = 0.053$). We identified five multilocus haplotypes (A-E) along the Abukuma River. The surrounding area's population, located on nearby dairy farms, showed higher genetic diversity at the haplotype level than the riverbank population. Its haplotype composition was similar to that of the population in the upstream region.

Conclusion: These results suggest multiple introduction events of *S. angulatus* from the same gene pools into wide areas of Japan and the facilitation of its spread by water systems. Imported feed grains may be responsible for multiple introductions into dairy land. Thus, paddy areas connected with dairy lands through water systems may be at high risk of invasion. A nationwide alien weed management system including pre- and post-border controls will be necessary.

Oral Presentations

Management of Invasive Species

O MIS 1

Screening Date Palm Cultivars for Resistance to Red Palm Weevil, *Rhynchophorus ferrugineus* (Coleoptera: Curculionidae)

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Date palm, *Phoenix dactylifera* L. is the most important crop of the Arabian Peninsula. The Kingdom of Saudi Arabia is among the top three date producing countries of the world estimated to have over 400 date palm cultivars of which 25 are important that yield 1.3 million tons of dates annually. The red palm weevil (RPW) *Rhynchophorus ferrugineus* (Olivier) (Coleoptera: Curculionidae) is a key pest of date palm in the Middle East. We studied the mechanisms of resistance against RPW in seven major date palm cultivars of the Al-Ahsa oasis in Saudi Arabia viz. Khalas, Sheshi, Reziz, Khasab, Hatmi, Shahal and Gaar by determining the extent of attraction of female RPW adults to fresh palm volatiles emitted from date palm frond tissue through four-arm choice olfactometer assays. Further, we assessed the degree of antixenosis and antibiotic effects if any by evaluating the number of eggs laid (oviposition), percent egg hatch and larval tunneling in these cultivars. Results revealed that the popular date palm cultivar Khalas had the least antixenotic effect on female RPW adults where a high degree of attraction to palm tissue volatiles was recorded which was statistically similar to the cultivars Reziz, Sheshi and Hatmi. The cultivars Khasab, Shahal and Gaar exhibited high degree of non-preference (antixenosis). Further, Reziz registered the highest egg lay by RPW and was statistically at par with the cultivars Khalas and Sheshi. Similar and non-significant values for egg hatch and larval tunneling in the cultivars tested indicate no antibiotic effects against RPW in the seven date palm cultivars. Since over 50% of the area in the Al-Ahsa oasis is under the cultivar Khalas with several new plantations in the susceptible age of less than 20 years, RPW is likely to pose a major challenge to date farmers of the region in the years to come.

O MIS 2

Ecological impacts on native ant and ground-dwelling animal communities through the invasive Argentine ant management in Japan

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In the last 30 years some limited successes in alien ant control have been documented globally, and control programs remain challenging. Moreover, the potential non-target impacts of toxicants have not been well studied. The Argentine ant *Linepithema humile* is native to South America and is a highly invasive species that has become established worldwide. This species was first detected in Japan in 1993 and has since spread throughout the country. To prevent further range expansion of *L. humile* in Japan, early detection, rapid response systems, and control measures are required. Here, we assessed the efficacy and non-target effects of multiple products containing active compound fipronil, in the attempted control of two populations of *L. humile* in Tokyo, Japan. Three treatments were conducted: control, low-dose treatment (0.1 g/ha per treatment), and high-dose treatment (0.2 g/ha). Treatments were applied once per month for 11 months. The abundance of *L. humile* declined rapidly by up to 99.8% in treated areas, but remained at extremely high densities in the control area. The treatments had few negative non-target effects, with the abundances of native ant species and other ground-dwelling invertebrates except for cockroaches being greater in the treated areas after *L. humile* suppression. These results suggested that the ecological impact of *L. humile* must be much larger than that of insecticide to the native invertebrate community. Thus fipronil is an effective compound for controlling *L. humile* and can be used with minimal toxic effects on non-target organisms. The treatments cost approximately US\$ 575/ha in low-dose treatment and US\$ 1,250 / ha in high-dose treatment. Our research supports the creation of more ambitious invasive ant management projects.

O MIS 3

Monitoring of Exotic Fruit Fly Species in Bulgaria

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Question: The recent introduction of exotic fruit flies in North Africa and the Middle East poses a high threat to fruits and vegetable production of the Balkans and Eastern Mediterranean. In parts of the Balkans, the Medfly (*Ceratitis capitata*) causes major damage to fruit production. It has been detected several times in Bulgaria but records until now refer to interceptions or

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short-lived adventive populations only. It is still unclear how its impact in Bulgaria could increase with global warming. In the framework of IAEA TC regional projects RER5018/RER5020 a fruit fly trapping aiming at early detection of quarantine fruit flies and survey for presence and pest status of *C. capitata* in Bulgaria was set up.

Methods: The monitoring was conducted during the period 2013-2014 in Bulgaria following the Fruit fly trapping guidelines provided as Annex 1 to ISPM No. 26 or the FAO/IAEA trapping guidelines (2013). Three types of traps were used: Jackson traps with Methyl eugenol plug; Jackson traps with Trimedlure plug; Tephri traps with Biolure (3 components - trimethylamine, ammonium acetate, and putrescine) plus toxicant (DDVP red square). The monitoring were conducted in permanent plots as follows: 15 fruit markets, 14 orchards, 10 border checkpoints and 3 touristic areas with campings. The traps were located in the regions of the following towns: Blagoevgrad, Burgas, Varna, Vidin, Vratsa, Kyustendil, Kurdzhali, Pazardzhik, Pleven, Plovdiv, Ruse, Sliven, Sofia, Stara Zagora and Haskovo.

Results: Quarantine fruit flies were not detected during the survey in Bulgaria. *Ceratitidis capitata* was detected in fruit market of Sofia in September 2013 and 2014. In 2014 it was trapped in the field with Tephri traps with Biolure and Jackson traps with Trimedlure in region of Petrich (peach orchard, August), Blagoevgrad, (peach orchard, August, and September), Plovdiv (apple and peach orchards, September and October). No infested fruits were detected in the field.

In 2014 *Drosophila suzukii* was trapped with Tephri traps with Biolure in region of Blagoevgrad (cherry orchard, June; peach orchard, September), Kyustendil (plum orchard, September), Plovdiv (peach orchard, October). No infested fruits were detected in the field. Single specimens was trapped with Jackson traps with Trimedlure in fruit markets of Sofia and Purvenets in November.

Conclusions: *Ceratitidis capitata* was trapped in orchards in South Bulgaria but its establishment in the field was not confirmed. An initial stage of invasion of *Drosophila suzukii* in Bulgaria was observed. The recently launched project "East and South European Network for Invasive Alien Species - a tool to support the management of alien species in Bulgaria (ESENIA-TOOLS)" will facilitate the networking in the region what is crucial for early detection of IAS.

Acknowledgements: The participation in the congress has been supported by the ESENIA-TOOLS project.

O MIS 4

Development of rational management options for invasive pest, brown marmorated stink bug, *Halyomorpha halys* - lures, traps, barriers, and... .

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Brown marmorated stink bug (BMSB) *Halyomorpha halys* (Stål) (Heteroptera- Pentatomidae), an established exotic insect pest species introduced from Asia continues to challenge fruit growers in Eastern U.S. Currently BMSB is the number one pest of Pennsylvania pome and stone fruit orchards, responsible for severe economic losses. Initially, only frequent insecticide treatments applied at a set time interval provided satisfactory control of this stink bug. During last few season we attempted to develop an alternative, more Integrated Pest Management (IPM) friendly tactics aided to reduce the negative impact of insecticides used for BMSB management on orchard ecosystem.

During the 2013 and 2014 seasons multiple trials were conducted in commercial pome and stone fruit orchards to evaluate effectiveness of various BMSB management programs. Targeted insecticide applications based on stink bug monitoring as well as mechanical net barriers were evaluated for their effectiveness to reduce fruit injuries in orchards. Intercropping and crop barriers were also evaluated in field vegetable systems. Captures of BMSB adults and nymphs in commercially available BMSB monitoring traps were utilized as a provisional treatment threshold supporting insecticide applications.

Trap data based targeted BMSB treatments during the 2014 season resulted in over 40 percent reduction in the number of insecticide applications without a negative effect on the number of injured fruit. Mechanical net barriers and intercropping was also effective in reducing the number of BMSB adults observed in evaluated fields, however the results were more variable and appeared to depend more on the type of vegetation surrounding the crop. Netting used as mechanical barriers were more effective in preventing movement of BMSB instars from neighboring soybean or corn fields, but was not effective against BMSB migration from woods.

With no effective control provided by native beneficial organisms, the management options for this invasive pests still need to be based mainly on judicious use of insecticides, however mechanical barriers combined with targeted use of pesticides are promising options for effective, more environmentally friendly integrated pest management of brown marmorated stink bug populations in fruit orchards.

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O MIS 5

Developing management strategies for the invasive *Megacopta cribraria* in soybeans in the southeastern United States

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An exotic plataspid native to Asia, *Megacopta cribraria* (Fabricius 1978), was found in Georgia in October 2009. Insects were identified as *M. cribraria* using morphological characters which were corroborated by maternal DNA fingerprinting. Known as the kudzu bug or the bean plataspid, *M. cribraria* has now spread across the southeastern and southern United States where it can cause major yield loss to soybean. This presentation will provide an overview of our regional projects on management and ecology of this invasive pest in soybean. Our initial work in South Carolina and Georgia in 2010 and 2011 focusing on insecticide efficacy and timing of applications showed that pyrethroid insecticides are effective and the presence of nymphs may be a good indicator of the need to trigger applications. Additional studies on dispersal of *M. cribraria* from overwintering sites to soybean are helping to optimize timing of insecticide applications in soybean. To facilitate decision making, sequential sampling plans were developed using sweep net and beat cloth sampling methods. An early planting of soybean (April) can often help to avoid high infestations in later plantings (May-June). We have also documented *Beauveria bassiana* and a mermithid entomopathogenic nematode infecting *M. cribraria* in South Carolina, as components of biological control. Initial screening of soybean lines suggest that plant resistance has potential as an additional component of integrated pest management programs.

O MIS 6

Cultural control in Switzerland continues to be a sustainable strategy for *Diabrotica v. virgifera* containment

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Ever since 2000 Switzerland belongs to the 22 European countries where the quarantine pest *Diabrotica virgifera virgifera* LeConte, Western corn rootworm (WCR), has been detected. WCR is reported to be the most important maize pest worldwide with annual economic damages reaching 1.5 billion US\$. In Switzerland it is constantly present in the Canton south of the Alps while only few beetles are sporadically found in the north. Observations from 2000 up to 2014 support the hypothesis that populations in the southern part of the Alps are generated by yearly migrations from principal pest foci situated in neighbouring Italian areas of Lombardy. Neither the tight correlation between travel distance and time of first arrival at various points from South to North, nor the steady decline of population along the route can be explained otherwise.

Control measures enacted by Swiss authorities were principally based on a tightly enforced crop rotation scheme without chemical inputs as usually practiced in parts of the European Union.

The effectiveness of crop rotation has been tested in a 7 year field trial comparing a continuous maize cropping system with a crop rotation system and with a maximum of one year of maize within any two year period. Population density was measured using synthetic pheromone baited traps and observations of root damage. Results showed that no economically relevant population built up during this period in the *crop rotation* treatment, whereas in the statistical evaluation of *continuous maize cropping* root damages could be detected after 4 years already.

One to one (1:1) year crop rotations are a common practice, are mandatory since 2004 in Southern Switzerland, and are well accepted by farmers. Consequently, not a trace of pesticide has been employed against WCR in Switzerland up to now. The low level population density also helped to avoid the introduction of WCR populations into Swiss Cantons north of the Alps and thus prevented further spreading towards the state territories of northern neighbour states.

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Genetic Resources I

O GR I-1

Resistance reaction of elite black soybean lines to *Phakopsora pachyrhizi*

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Indonesian tropical climate is ideal for both of soybean growth and harmful disease development. Soybean rust, *Phakopsora pachyrhizi*, has been a serious disease in Indonesia and may have an impact on soybean production. Ten elite black soybean lines were evaluated for rust resistance in ILETRI's greenhouse in 2011. At 30 days after planting, plants were inoculated with spores by density of 10,000 spores per millimetre. Agronomic characters were evaluated based on research conducted in 16 soybean production centers in Indonesia. The rust severity was rated using IWGSR soybean rust rating system.

Reaction resistance to rust disease fluctuates over time. Period of 45-69 days after planting was the most critical time because plants were in the stage of pod setting and seed filling. Three elite lines showed resistant to moderately resistant reaction at the plant age of 45, 52 and 69 days, and two of them (Cikuray × W9837-171 and W9837 × 100H-236) consistently showed a moderately resistant at the age of 76 days.

Of the three best lines, Cikuray × W9837-171 produced the highest yield (2.72 t/ha), have days to maturity of 74 days, and the seed size reached 11.22 g/100 seeds. The reaction resistance of the Cikuray × W9837-171 to rust disease at the plant age 38 was resistant, and the next plant age of 45, 52, 69, and 76 days were entirely showed moderately resistant. Therefore, the elite black soybean line Cikuray × W9837-171 has a great opportunity to be adopted by farmers due to its resistance to rust disease and high potential yield.

O GR I-2

Identification and Safeguarding Novel Sources of Resistance to Biotic Stresses in Crop Diversity from Eastern Ghats

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Introduction: Broadening of diversity in the primary gene pool is fundamental to any crop improvement programme to draw genes of significance especially those imparting resistance to biotic stresses. The Eastern Ghats of Coastal India is a treasure trove for ethnic diversity in various Agri-horticultural crops. National Bureau of Plant Genetic Resources (NBPGR) undertook over 100 germplasm collection missions in Eastern Ghats for enriching the national gene pool.

Objectives: Identification of genotypes resistant to biotic stresses there by preventing the loss of land race diversity possessing traits of significance with special reference to biotic stresses. Enrichment of national gene pool with genotypes offering traits of resistance to biotic stresses.

Materials and methods: Screening of over 5,000 accessions against significant pests was undertaken based on natural incidence and artificial inoculations. Pest problems of eggplant (fruit borer), chilli pepper (thrips, mites, powdery mildew, anthracnose, *Peanut bud necrosis virus* and root-knot nematode), cowpea (*Blackeye cowpea mosaic virus*-BCMV), dolichos bean (anthracnose and aphids), sorghum (sugarcane aphid) and wild tomato (root-knot nematode) were investigated. The per cent disease incidence on each genotype was converted to rating scale developed based on the field reaction leading to identification of promising accessions.

Results: Results indicated significant variability for resistance among the genotypes of different crops to different biotic stresses. A system of soft protection of intellectual property rights available with Indian Council of Agricultural Research, was used for registering these promising genotypes. **Cowpea** (INGR 08084) resistant to BCMV, **Dolichos bean** (INGR 11031) to anthracnose and aphids, ***Lycopersicon peruvianum*** (INGR 08094 and 08096) to *Meloidogyne javanica*, **Sorghum** (INGR 02022) to sugarcane aphid, **Chilli peppers** to thrips and mites (INGR 08095), to anthracnose (INGR 14041), and to thrips and powdery mildew (INGR 08097) were found resistant.

Conclusions: Efforts of NBPGR for preventing the loss of crop biodiversity resulted in enrichment of national gene pool to the tune of 20,000 entries. Numbers of landraces possessing traits of resistance were identified, soft IPRs derived and deposited in National Gene Bank.

Oral Presentations

Genetic Resources I

O GR I-3

Virulence status of the brown planthopper to resistant rice varieties in Asia

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The brown planthopper (BPH) *Nilaparvata lugens* is the major pest on rice throughout Asia. After the mid-2000s, outbreaks of BPH have been occurred frequently in East Asia and Indochina. The major factors causing these outbreaks were 1) the shift of rice cultivars to susceptible ones and 2) the development of insecticide resistance against some neonicotinoids such as imidacloprid. The breeding for resistance to BPH became an important research subject for management of Asian BPH.

Twenty-eight rice genes for resistance to BPH have been detected and many resistant rice cultivars were released since 1973. Wide-scale monoculture of rice cultivars with monogenic resistance to BPH resulted in the development of virulence to resistant cultivars in BPH. Thus, the virulence to rice differential varieties was monitored using the BPH strains collected from wide areas of Asia from 2006 to 2011. The virulence test was conducted on the basis of the adult mortality rate and the presence or absence of a swollen abdomen 5 days after infestation.

All the BPH strains (East Asia, Vietnam, and Philippines) were highly virulent to Mudgo (carrying *Bph1*) and ASD7 (carrying *Bph2*), suggesting that these two genes have already been broken down in Asia. In contrast, the virulence to Babawee (carrying *bph4*) varied among strains: the BPH in Southern Vietnam were highly virulent and those in Philippines were partially virulent, but the BPH in East Asia and Northern Vietnam were avirulent on 2006. However, the virulence to Babawee in East Asia and Northern Vietnam strains have been developed and fluctuated simultaneously after 2007. The varieties Rathu Heenati (carrying *Bph3* and *Bph17*) and Balamawee (carrying at least two BPH-resistance genes) had a broad spectrum of resistance against all the Asian BPH strains tested from 2006 to 2009. However, the virulence to Rathu Heenati and Balamawee in Southern Vietnam strains had slightly developed after 2010.

In conclusion, the virulence status of Northern Vietnam (the source area of BPH migration to East Asia) and East Asia changed simultaneously, suggesting that virulence monitoring in Vietnam is important to estimate the change of the virulent status in East Asia. The results also showed that The BPH in Southern Vietnam developed virulence more rapidly than those in East Asia and Northern Vietnam.

O GR I-4

Exploiting wild relatives of grain legumes for developing insect-smart crops

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Productivity of grain legumes is quite low worldwide mainly due to the narrow genetic base of crop cultivars coupled with many abiotic/biotic stresses. Of these stresses, pod borer, *Helicoverpa armigera* is one of the most damaging pests worldwide. Losses due to this pest have been estimated to be US \$ 317 million in the semi-arid tropics (SAT), and over US\$ 2 billion on different crops worldwide. Only low to moderate levels of resistance to *H. armigera* have been detected in the cultivated genepool of most of the crops including the ICRISAT mandate grain legume crops chickpea, pigeonpea, and groundnut. In contrast, the wild relatives of crops which continue to evolve in nature, have defense mechanism to survive when challenged by the herbivores. To bridge the gap between the potential and realized yields of grain legumes on farmers' fields, there is a need to develop high yielding cultivars with resistance to pod borer. Wild relatives are a potential source for introgressing resistance genes against *H. armigera* into the cultigen. Several accessions of wild relatives of grain legumes with high levels, and different mechanisms of resistance to *H. armigera* (oviposition non-reference and antibiosis) have been identified, and these can be utilized as sources of resistance in breeding programs to introgress useful genes into cultivated background following wide hybridization. At ICRISAT, efforts are underway to introgress different components of pod borer resistance from different wild *Cajanus* species (*C. scarabaeoides* and *C. acutifolius*) into commercial pigeonpea cultivars, and from *Cicer reticulatum* and *C. echinospermum* into the cultivated chickpea for developing pod borer-resistant introgression lines for improvement of grain legumes. This will also lead to broadening the genetic base through wide hybridization.

Oral Presentations

Genetic Resources I

O GR I-5

Genetic resources of barley resistance to leaf blights and their rational using in Russia

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Net blotch caused by *Pyrenophora teres f. teres* and spot blotch caused by *Cochliobolus sativus* are two most damaging and widely distributed fungal pathogens of barley. Breeding for durable barley resistance is based on knowledge of micro evolutionary processes in pathogen populations and genetic diversity of resistance.

The objectives of studies were (i) screening on resistance to *P. teres f. teres* and *C. sativus* the barley germplasm collection of landraces and cultivars from N. I. Vavilov Research Institute of Plant Industry, (ii) to identify major genes and QTLs for resistance to *P. teres f. teres*, and *C. sativus* in some barley accessions, and (iii) to find the most effective combinations of resistance genes for barley breeding.

More than 10.000 barley accessions from different genetic centers of barley diversity and commercial cultivars were evaluated for resistance to both *P. teres* and *C. sativus* in laboratory, greenhouse and field conditions.

Mapping of QTLs controlled resistance to different *P. teres f. teres* and *C. sativus* isolates in two double haploid (DH) populations was carried out with set of 384 SNP markers.

As a result of long-term joint research projects of the All-Russian Research Institute of Plant Protection and the Institute for Resistance Research and Stress Tolerance of the Federal Research Center for Cultivated Plants, more than 10.000 barley accessions from different genetic centers of barley diversity and commercial cultivars were evaluated for resistance to both *P. teres* and *C. sativus*, and as a result about 450 accessions with different level of resistance were identified.

In two DH populations one major gene and four novel QTLs of resistance to *P. teres f. teres* was detected, also seven novel QTLs contributed resistance to nine *C. sativus* isolates.

35 combinations of resistance genes in barley accessions, to which isolates with complementary combination of virulence genes were not found in natural *P. teres f. teres* populations, were determined.

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O GR I-6

Disease control and yields in traditional durum wheat (*Triticum turgidum* L. subsp. *durum*) variety mixtures in Morocco

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Durum wheat (*Triticum turgidum* L. subsp. *durum*) is one of Morocco's most important cereal crops. Its production faces challenges in the form of climatic variability and disease damage. Septoria leaf blotch (caused by *Zygomoseptoria tritici*) and brown rust (*Puccinia recondita* f. sp. *tritici*) are prevalent and serious diseases affecting wheat in Morocco. Twenty-four different varietal mixtures combinations based on of one modern and five traditional varieties popular in the study area, were planted at two distinct experimental sites. Data on disease incidences and yields were collected. Analysed using generalized linear mixed models (GLMM) showed that increasing both the number of varieties and the proportion of resistant variety within a mix contributed significantly to lower levels of infestation for both diseases. Yield increased significantly with more varietal components -being less affected by the overall level of disease incidence. Most mixture treatments performed better in terms of disease than the mean of the pure stand of their best components as monocultures, but less than the best component. The majority of tested mixtures exhibited higher productivity than monocultures of their highest yielding component. The results suggest durum wheat varietal mixtures are a viable option for disease management and yield stability.

Oral Presentations

Fruit Flies

O FF 1

Risk ranking of importation pathways using fruit flies hierarchy - Reunion Island case study

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Question: Transfers of fresh fruits and vegetables between countries *via* passengers or commercial trade enables insects such as Tephritidae (Diptera commonly named 'fruit flies') to colonize new areas, causing crop losses as well as displacement of indigenous species. Islands are very sensitive areas to alien species introductions, making application of import regulations important to protect local agriculture. In Réunion Island (Indian Ocean), 309 import pathways of fruits and vegetables have been identified using data registered between 2007 and 2012. The question, raised by the French Ministry of Agriculture to Anses (Agency for Food, Environmental and Occupational Health & Safety), was to rank the pathways representing a potential infestation risk by some of the 224 fruit flies species considered as the most threatening.

Methods: Two methods were developed: an information system, gathering information collected in databases, websites and publications, helped to identify the potentially infested pathways. A decision support system enabled pathways ranking according to the fruit flies hierarchy established using Promethée multi-criteria method^a.

Results: 55 risky import pathways were ranked, linked with potential infestation by 16 fruit fly species belonging to the genera *Anastrepha*, *Bactrocera*, *Ceratitis*, *Dacus* and *Rhagoletis*. Because of their high probability of entry and establishment, the threatening species would be, in order of importance, *B. invadens*, *C. rosa* (the African strain), *B. dorsalis* (separated from *B. invadens*), *B. tryoni* and *D. vertebratus*. The most risky pathways would be, in order of importance, fresh fruits from the genus *Citrus*, *Prunus*, as well as *Cucumis melo* and *Cucumis sativus*, all coming from South Africa, then *Citrus* imported from Madagascar and peaches from Zambia^b.

Conclusion: The ranking of pathways allowed by our method enables the customs risk manager to better define border management measures and to adapt the control of each pathway according to the threat linked to the fruit flies potentially conveyed.

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O FF 2

The Opportunities and Challenges: Review of the Invasion, Prevention and Control of Tephritid Fruit Flies in China

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In the trends of economic globalization and integration, Tephritid fruit flies are spread more quickly and widely in the world, which are causing significant economic and biological losing. Therefore, China has attached great importance to Tephritid invasion, prevention and control. Continuing efforts have being made not only in the area of the management, but also scientific research and higher education in the last decade. On the base of summarizing the Tephritid invasion and official control, this paper reviewed the main research developments in China during the last 10 years, such as quantitative risk assessment, species molecular identification, quarantine treatment, fields control, national monitoring of fruit flies. With that, the potential opportunities and challenges are analyzed for Tephritid prevention and control in China against the backdrop of quickly inter-connecting of China's economy with the rest world. To properly deal with the challenges and seize the opportunities, it is proposed to further streamline the prevention and control system, to strengthen the researches of scientific theory problems and applied techniques, and to enhance the classified management of Tephritid fruit flies in China.

Oral Presentations

Fruit Flies

O FF 3

Report on the occurrence of the peach fruit fly, *Bactrocera zonata* (Saunders) (Tephritidae) in Sudan

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Introduction: The peach fruit fly *B. zonata* is widespread and was reported in many countries, e.g. **Asia:** Bangladesh, India, Iran (southern), Pakistan also in Oman,, Saudi Arabia, United Arab Emirates...etc, **africa:** Egypt, Mauritius, Réunion , **North America:** Trapped in USA (California). The peach fruit fly, *Bactrocera zonata* (Saunders) (Tephritidae) was first identified from Sudan through samples of fruit flies captured in Chempac bucket traps, containing methyl eugenol and an insecticide.

Materials and methods: In this study Chempac bucket traps were used to capture fruit fly species. Methyl eugenol was placed at the bottom of the trap as well as Dichlorvos tablets to kill any attracted flies. Three traps were mounted at three different locations in Wad Medani area at approximately 2 km apart. Two were mounted in Hantop and one in Gezirat Alfil.

Results: The results indicated that both species of fruit flies (*B. invadens* and *B. zonata*) were caught in all traps at the different periods surveyed in Wad Medani area. The percentage *B. zonata* of the total fruit fly catch ranged between 32-82, 34-68 and 4-34 for Hantop 1, Hantop 2 and Gaziret Alfil, respectively. In Singa area all traps in all periods contained *B. invadens* at levels ranging from 197-1296 flies/trap. *Bactrocera zonata* was caught on only two observation dates in two traps with a very small percentage of the total catch (1.4 and 0.2%). In Elkamlin area the catches of *B. invadens* ranged between 7-45 flies/trap. *Bactrocera zonata* was caught in only one trap on one date and the percentage of the species was 11.8% of the total catch per trap.

Conclusion: There is a need to develop a management program trying to limit the distribution of this species within Sudan and the possible containment of the species in the infested areas such as Gezira and possibilities to eradicate it.

O FF 4

Integrated Pest Management of Invasive *Bactrocera* Fruit Flies with Novel Biopesticide Approaches

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Introduction: The family Tephritidae, the true fruit flies, includes over 4000 species, many of which are among the most economically important pests attacking soft fruits worldwide. Specifically, many species within the genus *Bactrocera* are major pests of tropical fruits and have been rapidly spreading around the world. These new invasions can be attributed primarily to increased global trade of fruits and vegetables and movement of people. For example, *Bactrocera dorsalis* (Hendel), oriental fruit fly, *B. cucurbitae* (Coquillett), and *B. zonata* (Saunders), peach fruit fly, significant pests of a number of fruit commodities in Asia are now found throughout many areas of Africa. Carambola fruit fly, *B. carambolae* Drew and Hancock, a sibling species of oriental fruit fly, has invaded South America.

Objectives: These flies are typically difficult to control and conventional methods of control particularly in developing countries rely heavily on broad-spectrum insecticides applied either as cover sprays or incorporated into protein baits. The Hawaii Area-Wide Pest Management (AWPM) program through a 10-year research and development program promoted adoption of safer and more effective methods of detection, monitoring and control.

Materials and methods: Evaluations were conducted by researchers at the United States Department of Agriculture (USDA), Agricultural Research Service (ARS), Daniel K. Inouye, U.S. Pacific Basin Agricultural Research Center (MKIUSPBARC), Hilo, HI, USA in conjunction with researchers from the University of Hawaii, Manoa and the Hawaii Department of Agriculture through the Hawaii AWPM program.

Results: Over the past 15 years an arsenal of biopesticide treatments were developed and tested in Hawaii that include bait-sprays, male annihilation treatments, and soil drenches.

Conclusions: Results from field demonstration trials suggest that these approaches offer safer and more effective treatments with reduced-risk, environmentally friendly insecticides than conventional treatments against multiple species of fruit flies throughout the world. These approaches can be used in conjunction with other fruit fly management tactics (sanitation, mass trapping, parasitoids, and SIT) as components of integrated pest management (IPM) programs.

Oral Presentations

Fruit Flies

O FF 5

Prospects for the use of the brewery yeast waste as an alternative attractant and bait for monitoring and controlling fruits flies in Burkina Faso

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Question: Mango production ranks first in Burkina fruits production with more than 300 thousand tons produced annually, the third of which has been exported. Several constraints among of which, diseases and insects pests are of major concern. Fruits flies, with two main species (*Bactrocera dorsalis* and *Ceratitis cosyra*), represent the major biotic constraints with losses ranging from 40-80%. In addition, several mango containers have been often blocked at the borders of European countries because of fruit fly infestations, leading to their destruction at the owner risks. Three components of the fruits flies control strategy have been successfully used in Burkina Faso, based on spot bait sprays of GF120, MAT using Timaye (made of methyl eugenol and insecticide) and sanitation. However, even successful, the products used are not only expensive for mango growers but not always available in the local market. Therefore, there was a need to work out alternative ways.

Methods: The brewery yeast waste which is always available from the local Breweries was tested as an attractant at six locations using Mac phail traps compared to a reference product, the Torula yeast (*Torulospis utilis*). The process of obtaining the brewery yeast attractant includes heating to remove the alcohol and excess of water. Then an enzyme (papain) and a food grade preservative (potassium sorbate) were added. Brewery and Torula yeast replacement as well as trap catches counting were done at weekly basis.

Results: Result showed that the brewery yeast was more attractive to the fruits flies compared to the Torula yeast. The index of fruit flies catching ranged from 2 to 12 for the brewery yeast as compared from 0,2 to 1,8 for the Torula yeast over the six weeks of trapping.

Conclusions: These first results indicated that the brewery yeast could be used as attractant in monitoring as well as bait in controlling fruit flies. Prospects for its use in combination with other components (SIT, biological control, bio-pesticides) in the development of a sustainable area wide management of fruit flies in Burkina Faso and in West Africa are discussed.

O FF 6

Decis Trap - Platform Technology for the sustainable management of fruit fly and other diptera.

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Decis Trap is an innovative tool for monitoring, mass trapping as well as attracting and killing of insects. The unique trap design combined with insect specific attractants and the insecticide Decis ensure the highest number of fly catches. It is a closed-system device for maximum operator safety in a convenient ready-to-use concept with season long control and leaving no residues. Decis Trap is available for Mediterranean Fruit Fly (*Ceratitis capitata*) since 2009. The Decis Trap platform technology is under development for several further diptera species.

Oral Presentations

Mycotoxins

O MYC 1

Optimized infestation control and mycotoxin reduction strategies against *Fusarium* diseases of corn and wheat

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Epidemic and damage dynamics of 13 *Fusarium* species on wheat (3 different susceptible varieties, 2008-2014) and corn (4 different susceptible varieties, 2011-2014) were recorded at 9 locations in Germany. In addition to yield factors, disease severity were analysed by q-PCR and the *F.* toxins deoxynivalenol (DON) and zearalenone (ZEA) were analysed by LC/MS. The same *F.* spp. spectrum was detected in wheat and corn. Based on year-long national results, a head blight forecast model for the optimized control was derived for wheat. There was a close relationship between rainfall and temperature at flowering (infection parameters) on the one hand and *Fusarium* DNA ($R^2 = 0.78$), DON ($R^2 = 0.80$), and ZEA content ($R^2 = 0.76$) of the grain on the other. The correlation between mycotoxin contents and *Fusarium* DNA at harvest and predicted values which were calculated on temperature and precipitation at flowering document the high quality of the model (DON $R^2=0.85$, ZEA $R^2=0.86$, *Fusarium* DNA $R^2=0.83$). Both in susceptible and tolerant corn varieties, maximum values of > 26 000 micrograms DON and > 2000 micrograms ZEA per kg dry mass were measured in no-till cropping systems with monoculture. Plowing reduced DON content by 82 % and ZEA content by 65 %. The triazole fungicide combined with strobilurine reduced mycotoxin levels in wheat by ≥ 60 %, and in corn by up to 90 % when applied at flowering and panicle stage, respectively.

O MYC 2

The effect of maize plant density on fumonisin producing *Fusarium* spp. infection and fumonisin synthesis.

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Maize (*Zea mays* L.) is one of the most important crops planted worldwide, and is a host of many plant pathogens, such as *Fusarium verticillioides*. This fungus produces secondary metabolites, fumonisins, which cause mycotoxicoses in animals and humans when ingested. The aim of this study was to investigate the effect of plant density on *F. verticillioides* ear rot infection and possible fumonisin contamination under field conditions. Plant density field trials (2011-2014) were planted at the ARC-GCI experimental farm in Potchefstroom, South Africa. Soil analysis was conducted at individual experimental plots before planting. Plant densities comprised of 10 000, 20 000, 30 000, 40 000 and 50 000 plants/ha. These plant densities (treatments) and cultivars CRN3505 and PAN 6P-110 were planted in a complete randomized block design. The size of each plot was 12m X 8m. To obtain the desired plant densities, kernels were planted at 0.9 m (inter-row) x 15 cm (intra-row), 0.9 m x 30 cm, 1.0 m x 30 cm, 1.2 m x 30 cm and 2.0 m x 30 cm, respectively. After emergence, maize seedlings were counted in each respective block to determine actual plant densities. Grain from the individual trials were milled and subjected to qPCR (fungal biomass) and HPLC (fumonisin quantification). ANOVA indicated a highly significant interaction (treatment x season x cultivar) regarding fungal infection ($P < .001$). Target DNA was the highest at 10 000 plants/ha (180.14 pg/ μ g) and the lowest at 50 000 plants/ha (53.29 pg/ μ g). Target DNA increased from 2011 to 2014 with target DNA concentrations of 5.89 - 294.06 pg/ μ g respectively. CRN3505 had a lower mean target DNA of 83.90 compared to 134.50 pg/ μ g in PAN6P-110. A weak treatment x season interaction could be observed for fumonisin synthesis ($P=0.06$). Fumonisin synthesis was the highest at 10 000 plants/ha (1.53 ppm) and the lowest at 50 000 plants/ha (0.72 ppm). Fumonisin synthesis declined from 2.15 ppm to 0.79 ppm during 2011-2014. Plant density had an effect on fungal infection with a decrease of target DNA as plant densities increased. Similar to fungal biomass, fumonisins decreased as plant densities increased although target DNA increased over seasons and fumonisins decreased. Weather data, additional localities and genotypes will be included in future studies.

O MYC 3

Fusarioses complex on maize ears: disease severity and mycotoxin accumulation in infected kernels

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Maize (*Zea mays*) is an important cereal crop which provides staple food for human and livestock. Its cultivated surface area covers more than 180 million ha (FAO, 2013). Maize plants are threatened by numerous diseases, one of the most important of which is fusarioses, and caused by fungi of the *Fusarium* genus. Fusarioses is responsible for maize yield reduction and kernel contamination with fungal toxins (mycotoxins) which are harmful to human and livestock.

Oral Presentations

Mycotoxins

In nature maize plants are commonly infected simultaneously by more than one pathogenic *Fusarium* species. Virulence of the pathogen, host genetic background and environmental conditions might contribute to the dominance of a single species over the others. Which of these factors leads to this dominance is nevertheless unclear.

In our study, we investigated the interaction of *F. graminearum* and *F. verticillioides*, two species which are frequently isolated from maize fields and which produce distinct mycotoxins. Both species were inoculated on dwarf maize (var. Gaspé Flint) ears under greenhouse conditions. Specifically we used two chemotypes of *F. graminearum* which produced either deoxynivalenol (DON) or nivalenol (NIV) and one strain of *F. verticillioides* which produced fumonisins. Maize ears were either infected with a single *Fusarium* species or co-inoculated with two species. Disease severity, fungal biomass and mycotoxins accumulation was monitored on ears.

The individual inoculation experiment showed that *F. verticillioides* and *F. graminearum* NIV chemotype were the less aggressive strains while *F. graminearum* DON chemotype was the most aggressive one. Interestingly co-inoculation experiments with *F. verticillioides* inoculated prior to *F. graminearum* resulted in an inhibition of *F. graminearum* biomass but in an increase in biomass of *F. verticillioides* compared to single inoculations. Mycotoxin accumulation varied widely within samples. Overall our results suggest that mycotoxins might have a role in the competition among *Fusarium* species.

O MYC 4

Healthy & Safe - Cropping factors influencing the occurrence of dominant *Fusarium* species and mycotoxins in barley and oats from Swiss harvest samples

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Introduction: Small-grain cereals provide the major part of calorie intake of the Swiss population. Especially barley and oats can contain interesting levels of health promoting compounds (HPCs). Some of these HPCs (e.g. anthocyanins) possess antioxidant potential and thus can prevent various human diseases such as cancer and Parkinson's. Hence, cereal varieties with elevated contents of HPCs are desirable. However, cereals must be safe and therefore free of health threatening substances, such as *Fusarium* mycotoxins. For *Fusarium* head blight in cereals, *F. graminearum* (Schwabe) is the most prominent species worldwide. Still, cereal types differ in their susceptibility to different *Fusarium* species and various factors, such as weather and cropping measures.

Objectives: The main aim of the project is to reduce the contamination of small-grain cereals by *Fusarium* toxins while developing value added varieties containing higher levels of HPCs. Apart from assessing the most dominant *Fusarium* species in barley and oats, epidemiological studies will serve to reveal the most favourable infection conditions for these species. Moreover, different artificially infected barley, oat and wheat varieties with varying levels of HPCs will be investigated towards their *Fusarium* susceptibility.

Materials and methods: In a first step, barley and oat samples from all over Switzerland have been collected in 2013 and 2014, along with information on respective cropping factors. The incidence of different *Fusarium* species was obtained by using a seed health test and qPCR. The mycotoxins were quantified by LC-MS/MS.

Results: Based on the current results, the main occurring species and mycotoxins were *F. graminearum* and deoxynivalenol in barley and *F. poae* and T-2/HT-2 in oats, respectively. In depth analyses to reveal potential correlations between *Fusarium* species/mycotoxins and cropping factors are presently running.

Conclusion: Results from the monitoring and epidemiological studies will be used to extend the forecasting system FusaProg for wheat towards barley and oats. Thus, a system to reduce the application of fungicides and a tool for growers to decrease the infection risk will be developed. Several partners along the food chain will contribute to implement the results for an improved safety of healthy Swiss cereals.

Oral Presentations

Mycotoxins

O MYC 5

Integration of Biological Control, GMO Traits for Insect Control and Adapted Varieties for Management of Aflatoxin in Maize

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Aflatoxin contamination is a chronic problem in maize grown throughout most of Texas, USA, as well as in many developing countries that threatens food safety. In recent years, two non-aflatoxigenic strains of *Aspergillus flavus*, Afla-Guard and AF-36, have been commercially available in Texas for biological control of aflatoxin and represent one approach for its management, but there is no information on how these biocontrol agents interact with other control approaches, particularly adapted hybrids and insect-control traits in GMO hybrids. The objective of our field experiments was to evaluate such interactions in 32 hybrids grown in two different climates of Texas. Hybrids were planted in two-row, 5.5 m-long plots in a split-split plot design. Afla-Guard was applied at 11.2 kg/ha to the rows prior to flowering and all plots were inoculated with a toxigenic strain of *A. flavus* at flowering to increase aflatoxin pressure. Overall, the location in Corpus Christi had higher levels of aflatoxin (209 ppb average, range 0-1600 ppb) than College Station (27 ppb average, range 0-330 ppb). Hybrids differed significantly ($P=0.05$) in aflatoxin accumulation at both locations. The Afla-Guard treatment reduced aflatoxin in some hybrids, but not others. A hybrid expressing *Cry1Ab* and *Vip3Aa20* Bt proteins accumulated less aflatoxin than hybrids from the same parent that expressed only *Cry1Ab*, or no Bt proteins. Aflatoxin in a hybrid expressing *Cry1A.105* and *Cry2Ab2* was not less than the parent hybrid without these Bt proteins. Several non-GMO hybrids derived from public breeding lines that accumulate less aflatoxin had lower levels of aflatoxin than some of the GMO hybrids. The results at these locations suggest that Afla-Guard may perform better with some hybrids than others and the insect-control traits in some GMO hybrids will not reduce aflatoxin. Additionally, hybrid selection may be a significant component of aflatoxin management.

O MYC 6

Developing Value Chain Teams to Address Aflatoxin Management Strategies in Peanut Production and Processing in Africa

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Introduction: Aflatoxin is a major concern in peanut, maize, rice and cassava in semi-arid regions of the world. The management of aflatoxin requires a comprehensive approach across disciplines and must take into consideration the disciplines of agronomy and pest management, food science, health and nutrition, marketing and socioeconomics. This approach will provide incentives necessary to promote change and reduce aflatoxin levels throughout the value chain.

Objectives:

- Assemble multidisciplinary teams that focus on reducing aflatoxin contamination throughout the value chain.
- Develop and implement a cohesive research plan addressing aflatoxin contamination and socioeconomics benefits from planting the crop through harvest, drying and storage, processing, and entry into the market.

Materials and methods: The USAID Peanut Mycotoxin Innovation Labs funded two value chain projects in Africa in Ghana, Malawi, Mozambique, and Zambia in 2013. Projects include scientists in pest management, agronomy, food science, agricultural engineering, and socioeconomics involving 9 U.S. and African universities; 4 national and international research institutes in Africa; and NGOs and private partnerships. Research targets factors that reduce aflatoxin contamination within each discipline, but ultimately with a focus on the overall cumulative impact through the value chain and the appropriate marketing incentives that motivate farmers to adjust practices.

Results: Projects with these comprehensive teams are underway and coordinated by an overall leader to ensure synergies are developed amongst the projects. Annual meetings are held with collaborating scientists to adjust research objectives and maintain project focus.

Conclusions: The value chain projects are fully underway with numerous agricultural, processing, and food science projects initiated leading towards a comprehensive aflatoxin management program linked to socioeconomic village-level incentives to mitigate aflatoxin, improve human health, and increase incomes in rural villages.

Oral Presentations

Plant Pathogen Interactions I

O PPI I-1

A biochemical engineering approach to identify priming compounds

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Introduction: An alternative crop protection strategy is needed since the resistance of pathogens and pests to agrochemicals is increasing. Moreover, the extensive use of pesticides, fungicides. A promising alternative crop protection strategy is defense priming [1]. This way of defense exploits the immune system of plants, and thus enhances the stress tolerance to biotic and abiotic stress [1].

Objective: The demand for new compounds for an effective crop protection is increasing and so is the need for an effective screening system. Until now, the identification of priming compounds relied on invasive approaches [2], or required the detection of secreted furanocoumarin phytoalexins in parsley cell cultures [3] resulting in prolongation of the experiment. In this work, a simple, fast, and noninvasive technique is established for identifying new priming-inducing compounds for plant protection based on the oxygen consumption of parsley suspension cell cultures.

Materials and methods: The respiration activity monitoring system (RAMOS) [4] was used to on-line identify priming compounds. The impact and dose-dependency of well-known priming compounds and the impact of non-priming compounds on the oxygen transfer rate (OTR) was evaluated with the parsley model system. 2D-fluorescence measurements of the furanocoumarin phytoalexins [3] served as validation.

Results: Treatment of parsley suspension cells with the known priming compound salicylic acid (SA) resulted in a dose-dependent increase in OTR. The addition of putative priming-active and priming-inactive compounds confirmed that the presence of priming correlates with an increase in OTR. The results were underlined by the determination of the furanocoumarin phytoalexins via 2D-fluorescence spectroscopy.

Conclusion: The OTR was assessed to identify priming compounds immediately after addition of the compounds to parsley cell cultures. The online signal enabled a robust and fast determination of priming compounds and can be applied to other plant cell suspension cultures.

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O PPI I-2

Comparative analysis of *Acholeplasmataceae* genomes highlights the particular genetic repertoire of '*Candidatus* Phytoplasma' strains

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Introduction: *Acholeplasmataceae* comprises the genera *Acholeplasma* and '*Ca. Phytoplasma*'. *Acholeplasmas* are described as saprophytic bacteria in general, while phytoplasma strains are characterized as obligate intracellular parasites of the plant phloem associated to diseases in >1,000 plant species. Genome research enables the identification of effector proteins and the reconstruction of the metabolism. The complete genomes of 5 phytoplasma strains and 4 *Acholeplasma* spp. have been analysed (1,2).

Objectives: Comparative genome analyses provide insights into the evolutionary split of these two genera and the obligate parasitism of phytoplasmas in comparison to the acholeplasmas.

Methods: Different technologies ranging from clone-based Sanger sequencing, pyro-sequencing, sequencing by synthesis and single molecule real time sequencing were applied. Annotation included functional reconstruction and comparative analyses accomplished by gene expression studies.

Results: The conserved gene core of phytoplasmas is also encoded by the analysed 4 acholeplasmas in majority. Phytoplasmas are separated by a particular carboxylic acid metabolism, membrane proteins involved in host interaction and virulence factors.

Oral Presentations

Plant Pathogen Interactions I

Conclusion: Particularities of the phytoplasmas such as the symporter for the uptake of carboxylic acids and their conversion to pyruvate should be interpreted with respect to the Gram+ origin in contrast to genes encoding effectors, which may be derived from horizontal gene transfers.

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O PPI I-3

Pathogenicity of *Erwinia amylovora* on Host and Non-Host Plants

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Erwinia amylovora causes fire blight disease, a necrotic and invasive disease responsible for considerable economic losses in host plants and to elicit the HR in nonhost plants depends on the presence of a functional type III secretion system (TTSS) encoded by the *hrp* gene cluster. The TTSS effector DspA/E is an essential pathogenicity factor of *E. amylovora*. In the study, a wild-type and a TTSS mutant strains of *E. amylovora*, seeds of the *A. thaliana*, Col-5, Ler-0, Wseds, Ler-Fls2 ecotype and mutants, and two year-old 'Gala' apple seedlings, and eight week-old tobacco plants, *Nicotiana tabacum* cvs. Xanthi, Benthamiana and White Burley, were used. The leaves of *A. thaliana*, apple and tobacco seedlings were infiltrated with bacterial suspensions at a concentration of 10^8 CFU ml⁻¹ using a needleless syringe. Bacterial counting was performed at 24 hpi on NA medium. Symptom severity was scaled as described in our previously studies. Callose was stained with aniline blue and the leaf disks were examined by fluorescence microscopy. Enzyme extractions and measurements of peroxidase, catalase, glutathione-S-transferase activities and of total protein contents were assayed. We confirmed that DspA/E acts as a major cell-death inducer during disease and HR, because the *dspA/E* mutant is severely impaired in its ability to induce electrolyte leakage in apple, *A. thaliana* and tobacco leaves. Bacterial populations of the *dspA/E* mutant decreased immediately following inoculation. Wild type and mutant strains of *E. amylovora* activated the antioxidative enzymes and activation reached to maximum level after 24 h later. DspA/E repressed protein synthesis and triggered a defence response. Mutant *dspA/E* strain induced a HR in tobacco cvs. with different types and at 12-36 hours. In addition, wild-type strain led to a significant increase in electrolyte leakage in both host and nonhost plants. This study showed that *E. amylovora* wild type and mutant strains played role at different levels in the induction of cell death, activation of defense pathways, protein and ROS accumulation, the multiplication and survival in host and nonhosts, apple, *A. thaliana* and tobacco. Knowing of further details on the pathogenicity of *E. amylovora* and of defense mechanisms of the host and non host plants will be crucial for fire blight control strategies.

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O PPI I-4

Molecular characterization of the prehaustorial resistance against wheat leaf rust in Einkorn

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Leaf rust of wheat caused by *Puccinia triticina* f. sp. *tritici* causes yield losses worldwide. *Triticum monococcum* accessions are valuable sources for improving leaf rust resistance in wheat. In screening programs accession Pi272560 has been identified showing prehaustorial resistance (PHR). The race non-specific PHR prevents the infection by *P. triticina* prior to the formation of haustorial mother cells (HMC).

Goals of our studies were (i) to analyze the biochemical background of this resistance by microscopy, the detection of H₂O₂ and the peroxidase and chitinase activity in leaves and (ii) to determine the molecular background by genome wide expression studies using the massive analysis of cDNA ends (MACE) and a BlastX to identify gene ontology (Go) terms.

Microscopy was performed using a 1,3 Diaminobenzidine stain of H₂O₂ and Uvitex 2B for the specific stain of fungal cells. Concentration of H₂O₂ was measured by a xylenol orange assay, peroxidase and chitinase activity was determined using assays from Sigma-Aldrich (Taufkirchen). Within the first 24 hours after inoculation (hai) MACE was performed by the GenXpro GmbH (Frankfurt), for Go-term identification "Blast2Go" software package was used.

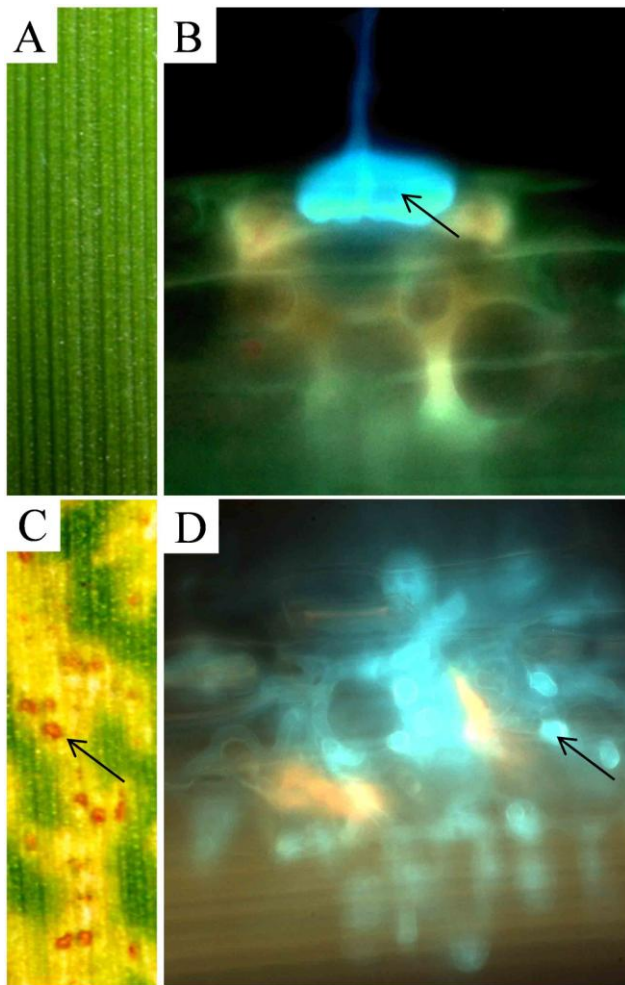
Microscopy showed an inhibition of fungal growth after the generation of very few HMC in Pi272560 accompanied by auto-fluorescence around infection sites and a reduced fluorescence of fungal cell walls. An increased concentration of H₂O₂ 24-48

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hours after the inoculation (hai) and an enhanced peroxidase and chitinase activity in Pi272560 was detected. By MACE 288 turned out to be differentially expressed between the resistant and susceptible accession 0-24hai. Go-terms related to membrane, oxidoreductase activity, metal ion binding and the oxidation-reduction process were identified. Genes matching this Go-terms were determined as increasingly expressed in Pi272560 and comprised a peroxidase 8 hai, different peroxidases and β -1,3-glucanases 8-16 hai and chitinases 16-24 hai.

Results give hint that PHR depends on early expressed peroxidases, β -1,3-glucanases and chitinases and an enhanced H_2O_2 production and peroxidase and chitinase activity which could be detected in enzyme assays.

Figure 1: Phenotype of resistant Pi272560 (A,B) and the susceptible accession (C,D). Arrows: Fungal structures. Bar 10 μ m



O PPI I-5

Towards revealing the allelic diversity of the HvGER4 gene cluster and its role in host defence against the barley powdery mildew

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The powdery mildew disease caused by the fungus *Blumeria graminis* f. sp. *hordei* (Bgh) serves as a model for studying PAMP (pathogen associated molecular pattern) triggered immunity (PTI) in barley. Pathogen-induced genes of barley germin-like protein 4 family (HvGER4) were found to be PTI components against Bgh. The encoded proteins with superoxide-dismutase activity are proposed to be targeted to the plant cell wall at the site of attempted penetration where they may catalyze production of H_2O_2 . This molecule has been shown to be a signaling molecule for a range of defense reactions, including cell death, and as a cofactor for cell wall reinforcement by cross-linking. A BAC clone containing a cluster of eight tandemly repeated paralogs of the HvGER4 gene in barley cv. Morex was identified and the genomic locus was physically localized near the distal end of chromosome 4HL of barley (Himmelbach et al. 2010).

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In frame of the project “BARLEY-fortress” 52 landraces of spring barley genotypes plus two cultivars and one wild barley introgression line carrying QTL for Bgh resistance were checked for HvGER4 expression levels by quantitative RT-PCR analysis. These genotypes were divided in 6 different groups: penetration resistant, late resistant, and susceptible, each of them subdivided into highly and lowly HvGER4 expressing. Eight candidate genotypes from these groups were chosen for construction of BAC libraries (in cooperation with INRA-CNRCV, Toulouse, France). Positive for HvGER4 signal BAC clones of each library were sequenced on Illumina HiSeq 2000 platform and all but one contained germin-like sequences. In addition a parallel sequencing approach using the Pacific Biosciences technology will be used in hope to resolve artefacts caused by the highly repetitive nature of genomic DNA in the HvGER4 locus, and to provide a valuable comparison between the sequencing technologies. Obtained sequence information will help to elucidate the organization and structure of the HvGER4 locus in different genotypes and its role in the interaction with Bgh.

The same BAC clones were transiently expressed via biolistic transformation of detached barley leaves followed by Bgh challenge. Preliminary results revealed enhanced resistance to Bgh in susceptible barley cultivars.

In a parallel approach 37 transgenic RNAi T1 events putatively silenced for HvGER4 genes were phenotyped regarding resistance to Bgh and HvGER4 expression. Five T2 RNAi events and one T2 azygous event were chosen for to generate stable RNAi events at T3 level for more detailed analysis of HvGER4 function.

O PPI I-6

Novel virulence factors in the vascular wilt pathogen *Verticillium dahliae*

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Verticillium dahliae is a soil-borne fungus causing severe wilt diseases in several hosts. Successful control measures of *Verticillium* wilts do not exist and research is focused on unraveling the molecular mechanisms of *V. dahliae* - host interaction. G Protein-Coupled Receptors (GPCRs) represent the largest family of transmembrane receptors. GPCRs are critical factors in regulating morphogenesis, mating, infection and virulence in various organisms. Seven different groups of GPCRs emerged from bioinformatics' analysis in *V. dahliae*, varying in sensing putative different environmental signals. Fungal secondary metabolites are compounds with various roles concerning toxin production, sporulation processes and biosynthesis of substances with biotechnological interest. *V. dahliae* produces phytotoxins and other molecules that induce programmed cell death or other forms of host resistance but the exact nature of these compounds remains unknown. In several species of *Aspergillus* spp. the gene *veA* encodes a protein that regulates fungal secondary metabolism, induce differentiation of fungal development in relation to light, and regulate reproduction and pathogenicity. Along with *VeA*, a second protein (*LaeA*) forms the nuclear Velvet complex. *LaeA* is a global regulator of secondary metabolism encoding a nuclear protein required for the expression of secondary metabolite genes while its presence is considered indispensable for mycotoxin, antibiotic and mycelial pigment biosynthesis. BLAST analysis of *V. dahliae* genome led to discovery of the homologous genes *VdSteA*, *VdLaeA* and *VdVeA*. *Agrobacterium* mediated targeted deletion of these three genes in *V. dahliae* were performed to study their role in virulence and physiology of the fungus. Pathogenicity experiments revealed that the $\Delta VdSteA$, $\Delta VdLaeA$, $\Delta VdVeA$ mutants displayed significant reduction in virulence in eggplants, tomatoes and *Arabidopsis thaliana* hosts. Deletion strains were also altered in conidiation ability, rate of germinating conidia, mycelial development, microsclerotia formation and programmed cell death. Gene regulation and expression data of *VdLaeA*, *VdVeA* and *VdSteA* *in vitro* and *in planta* will be also presented. In conclusion, pheromone responses and secondary metabolites are involved in virulence of *V. dahliae*.

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O NOC I-1

The impact of barley variety rotation, mixtures, and intercropping on leaf disease and silage production

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Question: Western Canadian barley silage producers, whether they are meeting on-farm needs or local market needs, will often look at continuous barley production, which leads to productivity issues related to leaf disease development. Although fungicides could be used, they represent an added input cost for silage producers. The objectives of the current study were to determine and contrast the effects of monocultures, mixtures, intercropping and rotational diversity on crop health, productivity, and quality in a cereal silage production system.

Methods: Three year rotational treatments were established in 2008 at two Alberta locations in Canada with comparisons made in 2010 and 2013. Treatments included: continuous barley, same variety; a mixture of the same three barley varieties each year; a mixture of three different barley varieties each year; an intercrop of barley, oat, and spring triticale with the same or different crop varieties each year; and an intercrop of barley, oat, and winter triticale with the same or different crop varieties each year. In 2010 and 2013, all treatments had the six-row barley variety Sundre.

Results: For both locations leaf disease, primarily net-form net blotch, was generally highest for continuous Sundre, and lowest for mixtures or intercrops with different varieties. At Lacombe in 2010 and 2013, silage yields were lowest for the continuous Sundre, highest for the intercropping treatments with the same or different varieties, and intermediate for barley mixtures. At Lethbridge in 2010 and 2013, continuous Sundre tended to have the lowest silage yield, although the intercrop treatments with winter triticale also had lower yields. Barley variety mixtures and intercropping with spring triticale tended to have higher, but similar yields.

Conclusions: Overall results indicate that the addition of diversity in terms of different crop types and perhaps barley genetics can help to reduce the level of leaf disease and improve silage productivity. The current study also demonstrated that high-quality silage can be generated for cereal silage produced from intercropping and that this approach could be a valuable asset in terms of avoiding monoculture production systems and the buildup of plant diseases and reduced productivity that are associated with this practice.

O NOC I-2

Evaluation of the potential of *Chaetomium* species as biocontrol agents

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Introduction: Species of the genus *Chaetomium* (Ascomycota) are widespread in the environment and have often been reported as endophytes in different plant species. The production of various metabolites suggests that these endophytes interact with their hosts and may affect the outcome of host-parasite interactions. Most of the literature in this respect refers to *C. globosum* and its effect on fungal plant pathogens.

Objectives: The objective of the study was to characterize different *Chaetomium* species regarding their ability to grow endophytically and to determine their antagonistic potential against fungal plant pathogens.

Materials and methods: The species studied were *C. aureum*, *C. cochlioides*, *C. elatum*, *C. globosum*, *C. indicum*, *C. nozdrenkoeae* and *C. piluliferum*. Dual cultures with different fungal plant pathogens were performed, and the latter were grown on media amended with filtrates from liquid cultures of the *Chaetomium* species. In addition, the pathogens were directly cultured in sterile culture filtrate. In greenhouse tests, potting substrate was amended with inocula of the *Chaetomium* species, and re-isolation from different plant parts was attempted by placement on agar media.

Results: Clear differences between the species regarding the effect of temperature on mycelial growth were observed. The results of the dual cultures and the experiments with culture filtrates indicated clear antifungal effects for most of the studied *Chaetomium* species. Adverse effects were especially observed against *Phytophthora infestans* and *Drechslera graminea*. Most of the species could be re-isolated from plants growing in inoculated soil. However, the frequency of re-isolation depended on the crop and the *Chaetomium* species, and the endophytes were generally isolated from only some parts of a given plant.

Conclusions: The study confirmed the antagonistic potential and ability to grow endophytically for *C. globosum* but also showed similar results for some of the other *Chaetomium*-species. While clear results were obtained regarding the production of antifungal compounds, the data do not allow final conclusions regarding ability to grow endophytically. Therefore, further studies in this respect involving different crops and fungal strains are required.

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O NOC I-4

Implementation of *Aureobasidium pullulans* pre-harvest application to control *Botrytis cinerea* decay in soft fruit production

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Botrytis gray mold decay in soft fruit, caused by the ubiquitous present fungus *Botrytis cinerea*, is known to occur frequently on different kind of crops and throughout all climatic zones. A gray mold infection is still one of the major concerns to the soft fruit producing industry worldwide. Research revealed that the fungus infect different plant sites, depending on the vegetative stage of the crop. Most infections take place during the flowering period, and continue until harvest, if climatic conditions stay favorable for disease development. Infections cause either the flowers to rot, or the pathogen can become dormant in floral tissue. Dormant infections resume activity later in the growing season any time before, or after harvest, when sugar levels increase in the ripening berry. Direct infection of berries and plant parts may also occur, if they are exposed during cooler temperatures to free water. However, all three infection stage options are, if an outbreak is serious, resulting in significant economic losses for growers.

To control *Botrytis cinerea* efficient and maintain a stable productivity, several plant protection treatments per season are mandatory. In the last decades extensively used chemical fungicides forced a *Botrytis* resistance development against several chemical active substances. A simple but effective measurement implements a biotechnological agent. *Aureobasidium pullulans*, formulated as Botector® inhibits the growth of the pathogen by antagonism for space and nutrients on plant surfaces such as blossoms, leaves, or micro scratches of the fruit skin.

Botector has been tested in several field trials in a number of situations in various countries and is demonstrating a high efficacy against *gray mold decay* on soft fruit.

Results of some trials, the mode of action of the agent and application recommendations are clearly represented.

Botector can be used as a significant efficient stand-alone application, and it is simple to implement in strategy spraying programs in alternation with other chemical fungicides. It provides therefore, an important tool for anti-resistance management and is an ideal partner for IPM programs, in line with the needs of a modern control strategy. Furthermore, it holds no pre-harvest intervals and leads to a reduction of chemical residues on the final crop and it is overall harmless to pollinators and beneficials.

O NOC I-5

Olive mill waste composts: A source of resistance for plants against vascular wilts

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Introduction: Biological control strategies based on the application of organic amendments have been explored intensively over the past decades. Among the different soil amendments that have been evaluated for their suppressive effect against plant pathogens are composts made from olive mill wastes (OMWs). Beyond the plant protecting effects of the OMW composts (OMWCs), it is conceivable that for the olive producing countries the management and recycling of the OMW is vital for the local agro-economies.

Objectives: The aims of this study were to investigate the efficacy and mode of action of three OMWCs against *Fusarium* and *Verticillium* wilt.

Materials and methods: Seeds of eggplant, cucumber and tomato were planted in pots containing soil amended with 20% of OMWC. At the third-leaf stage, eggplants, cucumber and tomato plants were transplanted to soil infested with *V. dahliae* microsclerotia or *Fusarium oxysporum* f. sp. *radicis-cucumerinum* or *Fusarium oxysporum* f. sp. *radicis-lycopersici* chlamydospores, respectively. Symptoms were recorded every 2 days after the onset of disease. The microbial nature of the OMWCs suppressive effect was investigated by autoclaving the composts and performing the previously mentioned bioassay. Potential antagonistic microorganisms against the pathogens were isolated from the rhizosphere of plants grown in soil amended with the OMWs and tested *in vitro* and *in planta* against the pathogens.

Results: It was observed the efficacy of the OMWCs to protect plants against *Verticillium* and *Fusarium* wilt in cucumber. The observed protection was mainly attributed to the microbial nature of the OMWCs and a number of microorganisms were isolated with *in vitro* and *in planta* pathogen suppressive activity.

Conclusion: The olive producing countries face the need to valorise the huge amounts of OMWs generated every year, while at the same time, their agriculture is challenged from destructive plant pathogens, like *V. dahliae* and *F. oxysporum*. In the present study it was shown the potential of the olive mill wastes as a compost amendment to control *V. dahliae* and *F. oxysporum* f. sp. *radicis-cucumerinum* and also the existence of microorganisms with significant plant protective effect.

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O NOC I-6

Incorporating *Jatropha curcas* Seed Waste into Soil for Controlling Root Knot Nematode *Meloidogyne* spp.

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Tomato is often attacked by the root knot nematode *Meloidogyne* spp.. Synthetic pesticides are usually used to control this nematode but they have several negative effects. This study aimed to investigate the potency of the physic nut *Jatropha curcas* seed waste in controlling *Meloidogyne* spp. in glass house. *J. curcas* seeds were ground into powder and pressed to release the oil. Certain amount of *J. curcas* deoiled seed cake (waste) was then incorporated into the growing medium (soil) of tomato. The result showed that the incorporation of *J. curcas* seed waste at doses of 10, 15 and 35 g/polybag significantly reduced the number of root galls and the population of *Meloidogyne* spp. in both soil and root. At a dose of 5 g/polybag, *J. curcas* seed waste also had a significant effect on the population of *Meloidogyne* spp. in both soil and root but it failed to significantly reduce the number of root galls. This result suggests that *J. curcas* seed waste have a potency to be developed as soil amendment for controlling *Meloidogyne* spp. on tomato.

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O GR II-1

Response of pure versus mixed banana varietal stands to banana weevil *Cosmopolites sordidus* (Germar) (Coleoptera: Curculionidae) infestation

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Use of banana varietal diversity is a cost-effective and environmentally-friendly technique that is being explored for managing the banana weevil, *Cosmopolites sordidus*, the most important insect pest of bananas in Uganda. Response of 14 banana varieties including East African Highland Banana (AAA-EAHB) and dessert bananas (AAA and AABB) to weevil attack was determined in an on-station trial. Additionally, varieties, Enzirabahima, M9, Grand Nain and Yangambi-Km5 were compared in mixtures and pure stands. Weevil damage varied significantly ($P \leq 0.05$) across varieties with higher levels on EAHB's than desserts. Total cross sectional damage (XT) was highest on Nakabululu (AAA-EA; 6.9) and lowest on Yangambi-Km5 (AAA; 0.1). Weevil damage on susceptible varieties, Enzirabahima (AAA-EA) and M9 (AAA-EA hybrid) was higher in pure than mixed banana varietal stands; but did not differ on resistant varieties, Grand Nain (AAA) and Yangambi-Km5 (AAA). This implies that mixtures reduce *C. sordidus* attack more on susceptible than resistant varieties. Most of the preferred and marketable varieties (AAA-EA) in Uganda happen to be susceptible. Thus, this strategy could be explored by our smallholder farmers who are already maintaining substantial amount of *Musa* spp. diversity in their plantations. However, the best-bet arrangements, combinations and ratios of resistant to susceptible varieties in mixtures are yet to be established. Our results further show that at plot level, adult weevil densities at each trapping date were higher in pure than in mixed varietal stand but not significantly different. However, weevil densities did not differ significantly across varieties at plant level. All growth and yield parameters assessed differed significantly ($P \leq 0.05$) across varieties but with no clear trend among clone sub-groups. However, most of these parameters were not significantly ($P \leq 0.05$) different in pure as compared to mixed stands. Multivariate analysis results show that bunch weight was directly related to number of clusters, number and length of fingers. These are major attributes contributing to yield and thus determining the market for bananas. In conclusion, use of varietal mixtures is a promising option which could be incorporated into the existing Integrated Pest Management (IPM) strategies for the banana weevils.

O GR II-3

Olive breeding for resistance to *Verticillium* wilt

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Introduction: *Verticillium* wilt (VW), a vascular disease caused by the soilborne fungus *Verticillium dahliae* Kleb., constitutes the major constraint in many olive (*Olea europaea* L.) growing areas such as Andalusia (Southern Spain). This situation is getting worse due to the rapid dispersion of highly virulent Defoliating pathotype (D) and the predominant use of cultivars highly susceptible to the disease such as 'Picual', the main olive cultivar in Spain.

Objectives: An olive breeding program was initiated aiming at obtaining new cultivars displaying both high levels of disease resistance and good agronomic characteristics. In this work, the resistance to VW was evaluated in new genotypes previously selected, from wider initial progenies populations, for several agronomic traits. In addition, the potential usefulness of wild olive trees as new sources of disease resistance has also been evaluated.

Materials and methods: VW resistance was evaluated in 146 genotypes from crosses, open pollination progenies and wild olive trees. Inoculation experiments were carried out under controlled conditions by dipping roots in a conidial suspension of a highly virulent D isolate of *V. dahliae*. A Relative Susceptibility Index (RSI) was developed to summarize information provided by different disease parameters and used for final classification of genotypes. Two cultivars, 'Picual' (susceptible, S) and 'Frantoio' (resistant, R) were used as controls.

Results: According to RSI values, 21.2 % of the genotypes were classified as R, being this percentage similar for the different genetic backgrounds evaluated (seedlings from crosses and wild genotypes). Similar vascular colonization by the fungus was found in some genotypes classified as R and S, which supports the assumption of tolerance for olive-*V. dahliae* pathosystem.

Conclusions: The results of the study have contributed to improve our knowledge about the inheritance of VW resistance in olive, the host-pathogen relationship and the implications to design efficient breeding strategies. Some interesting R genotypes

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were selected and future trials will be carried out to confirm the disease reaction of these genotypes under natural field conditions.

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O GR II-4

Exploring crosstalk between biotic and abiotic stress responses to identify targets for resistance breeding

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Research on plant stress responses in a globally changing environment has an important impact on agriculture. A thorough understanding will help to predict crop yield and quality as well as adopting plants to extreme climate conditions. In the future plants will be more often exposed to different stress combinations, such as pathogen pressure in combination with drought, salinity or elevated UV-B levels. When facing e.g. increased UV-B radiation in both intensity and duration, sessile plants produce UV-protective flavonol metabolites derived from the phenylpropanoid pathway. These secondary metabolites function not only as UV-sunscreens, but have also strong antioxidant capacity making them interesting nutraceuticals. The flavonol production involves activation of a set of genes, including chalcone synthase (CHS), the key-enzyme of the flavonol pathway. These genes are strongly suppressed when plants sense pathogen attack, resulting in decreased defense towards UV-B stress but increased defenses against pathogens, such as lignification and the production of antimicrobial secondary metabolites known as phytoalexins. In order to determine the underlying molecular mechanism we did ChIP-analysis of selected flavonol pathway genes (FPGs) and found that it involves chromatin remodeling and transcription factor interplay to fine-tune the plants secondary metabolism. Since several transcription factors are regulated by small interfering RNAs (siRNAs), the potential role of these molecules will be discussed and first insights into the complex regulatory network leading to pathogen-induced down-regulation of FPGs will be presented. New methods such as TALEN or CRISPR-Cas9 for targeted genome editing will allow the generation of new crop ideotypes with improved stress tolerance.

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O GR II-5

Marker saturation of the *Sbm1* locus in hexaploid wheat conferring resistance to SBCMV and SBWMV using the 90 K iSelect array and KASP technology

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Mosaic disease, caused by the closely related furoviruses *Soil-borne cereal mosaic virus* (SBCMV) and *Soil-borne wheat mosaic virus* (SBWMV), is a serious constraint of winter wheat production in parts of Europe, North America and Asia. Both viruses are transmitted by the plasmodiophorid *Polymyxa graminis*, a eukaryotic soil-borne microorganism. Chemical measures to control these viruses are not applicable due to economic and ecological reasons and crop rotation is not efficient due to the long-term survival of dormant *Polymyxa graminis* spores in the soil. Therefore, the only possibility of an efficient control of this disease on infested fields is the growing of resistant cultivars. In previous reports, based on field phenotyping experiments, a single gene controlling resistance to SBCMV and a major QTL controlling resistance to SBWMV, *Sbm1* was assigned to the long arm of chromosome 5D.

Objectives of this work were to (i) phenotype SBWMV infected plants in green house experiment in two segregating populations previously used for SBCMV mapping (ii) screen parental lines and bulks using the 90K iSelect SNP array, (iii) convert polymorphic iSelect single nucleotide polymorphism (SNPs) into Competitive allele specific PCR (KASP) markers (iv) genotype two doubled haploid (DH) populations and construct a consensus SBCMV/SBWMV resistance map.

An efficient method for SBWMV inoculation using NY isolate infected soil in the green house was established. Infection rate of susceptible plants was between 70 and 90%. A set of 19 polymorphic iSelect SNPs was converted into KASP markers and employed for map construction. Both resistances, SBCMV and SBWMV, co-segregated revealing that resistance to both viruses is most likely controlled by the same gene. Developed KASP markers can be efficiently employed in the transfer of resistance into elite wheat lines and the construction of a high resolution map towards the isolation of this resistance gene.

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Genetic Resources II

O GR II-6

Utilization of quantitative resistance to the fungal pathogen *Sclerotinia sclerotiorum* in canola (*Brassica napus*)

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Canola (*Brassica napus*) is produced in temperate regions of the world most extensively in Canada, China and Europe. Demand for canola oil has increased profitability resulting in tighter crop rotations thereby increasing the risk of yield loss from diseases. *Sclerotinia sclerotiorum* is a serious fungal disease of canola and many other broad leaved crops. Sources of resistance are rare but have been identified in a few *B. napus* germplasm lines originating from Pakistan, South Korea, Japan and China. Resistance is quantitative and conferred by several genes with additive effects.

Objective: To select germplasm resistant to the Canadian sclerotinia population, and develop molecular markers linked to quantitative trait loci (QTL) conferring resistance to be used by canola breeders for transfer into canola.

Methods: Sclerotinia isolates were collected in the major canola producing area of Canada. A sub-set of 129 isolates were genotyped using simple sequence repeat markers which revealed the presence of 17 clusters. One isolate from each cluster was tested for pathogenicity by inoculating six *B. napus* lines shown to have quantitative resistance. Mapping populations were developed from crosses with a susceptible line and lines with quantitative resistance, Zhongyou 821 (ZY821, China), PAK54 and PAK93 (Pakistan). Populations were genotyped with 6000 single nucleotide polymorphism markers and linkage maps were generated. Populations were phenotyped with a single virulent sclerotinia isolate and disease measurements were used for QTL analysis. Meta-analysis was used to align sclerotinia QTLs in each population as well as QTL from the literature.

Results and discussion: Germplasm from Pakistan had the highest level of resistance against the Canadian pathogen population. Five major QTLs were identified in PAK54 which were different from the five QTLs in PAK93. More QTLs were mapped in Zhongyou 821, but the level of resistance was lower. A meta-analysis showed that some QTLs reported in the literature mapped to our QTLs despite differences in disease phenotyping methods. Seed of sclerotinia resistant germplasm from Pakistan, Japan and South Korea is available for cultivar development from Plant Gene Resources of Canada via the corresponding author under a material transfer agreement.

Oral Presentations

Drosophila Suzuki I

O DSU I-1

Current status of the *Drosophila suzukii* management in Trentino, Italy, and research perspectives for sustainable control

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Unlike other *Drosophila* species, *Drosophila suzukii* is an economically damaging pest because the females have a serrated ovipositor enabling them to infest ripening fruit before harvest. In September 2009 in Trentino, Italy, for the first time in Europe both oviposition on wild hosts and economically important damage on soft fruits were reported. The increasing use of insecticide against this species augments the pesticide residues on the harvested fruits and jeopardises the results obtained with IPM on soft fruits. Development of alternative control methods is therefore urgent to ensure an economic future for the concerned fruit industry. Researchers and technicians of Fondazione Edmund Mach responded rapidly to this new threat. We considered that possible solutions would only arise from a coordinated and international network of diverse expertises, from molecular biology and neurophysiology to pest management techniques. Accordingly, we have determined the genome sequence of Italian *D. suzukii* in order to assist both basic and applied research and to provide information about genes involved in processes such as intra- and inter-specific communication and overwintering. Considerable efforts have been also made at the understanding of basic ecology of this pest. The population dynamics in different natural and agroecosystems have been followed and temperature-dependent fecundity and survival data were integrated into a matrix population model in order to forecast pest's phenology. Mass trapping and physical crop protection by using anti-insect nets are under experimental evaluation and seems the more promising alternative control strategies accessible in the near future. Improvement of the attraction efficiency of the available baits is hence one of our main objective. Several indigenous parasitoids of larvae and pupae were found in Trentino and are under investigation as possible biocontrol agents.

O DSU I-2

***Drosophila suzukii*: foraging, mating and host-finding in response to chemical signals**

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Introduction: The damage to fruit caused by the spotted-wing drosophila, *Drosophila suzukii* can be seen as a consequence of insect behaviour. Foraging, mating and host-finding behaviours are basic for survival and reproduction of the fly. Such behaviours are the output of chemosensory processes mediated or modulated by chemical signals.

Objectives: Our objective is to identify chemical signals of behavioural and ecological relevance for *D. suzukii*. In this context we are interested to identify sex specific differences in odour-mediated behaviours like host attraction. We furthermore want to know to which extent behavioural responses are modulated by internal factors like mating state of the flies. In addition we want to understand the meaning of chemical signals for *D. suzukii* reproductive behaviour.

Materials and methods: We applied wind tunnel tests and mating assays to study odour mediated behaviour of male and female *D. suzukii*. Fermentation odours and host odours were tested to study responses in the context of feeding and egg-laying. Mating assays were performed to study the role of social signals on fly reproductive behaviour. Antennal recordings in response to behavioural active odours and chemical analyses assisted the identification of chemical signals.

Results: We found distinct odour-mediated behavioural responses to fermentation compounds, fly odours and fruit cues. Mating state has an effect on fly behavioural responses. We could identify a number of antennal active fruit and fermentation compounds. The behavioural activity of synthetic blends of the identified odour compounds is under investigation. Flies were studied for emission of sex specific signals and behavioural response to fly pheromones. Analyses of cuticular hydrocarbons indicated that differences between sexes are small.

Conclusion: Chemical signals play important roles in the behavioural ecology of *Drosophila suzukii*. Behaviour-modifying chemicals are potential tools to attract or confuse insects in their natural environment. Tests on the attraction to fermentation products in the field are ongoing.

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Drosophila Suzukii I

O DSU I-3

Mating behaviour in spotted wing Drosophila species - example of coordination between visual and acoustic stimuli

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Understanding the mating behaviour of an insect pest is an important condition for developing successful control strategies. In *Drosophila suzukii*, like in most other *Drosophila*, males produce several types of acoustic signals to facilitate female's mating acceptance; some of these signals are substrate-borne and are produced by abdominal vibrations. Compared to the common model *D. melanogaster*, the *suzukii* subgroup, is further characterised by the emission of a specific vibration, the so called "toot" signals, which is characterized by an harmonic frequency structure. This peculiar signal is associated with wing movements and thus is likely produced in the thorax, rather than in the abdomen like all other acoustic signals. In this work we tested whether the "toot" signal is a specific characteristic of the *D. suzukii* group, and if it co-evolved with another key sexual character, the presence of spots on wings. To test this hypothesis we studied the courtship strategy and associated acoustic signals in several members of the *melanogaster* group, by carefully comparing the behaviour of spotted wings species (*D. suzukii*, *D. subpulchrella*, *D. biarmipes*, *D. elegans*) with the behaviour of unspotted species (*D. takahashi*, *D. melanogaster*).

Our results show that all species characterised by spotted wings, including *D. elegans* which does not belong to the *suzukii* subgroup, can produce a toot signal. Conversely, the "toot" signal was never recorded from unspotted wing species. During courtship, spotted wing males combine wing exposure with sound emission so that visual and acoustic cues work together to increase female acceptance. Only in one case (*D. biarmipes*), wing exposure and "toot" emission appeared completely unrelated.

This study advances our understanding of the toot signal in *Drosophila* and indicates that the "toot" signal is not a clade-specific feature, but rather an ancient *Drosophila* character associated with the presence of spots on wings.

O DSU I-4

Rapid spread of the invasive Spotted Wing Drosophila through Germany, its seasonal phenology and research approaches for managing the pest

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Introduction: Since its first record in Germany in 2011 the invasive Spotted Wing Drosophila (SWD), *Drosophila suzukii* (Diptera, Drosophilidae), spread rapidly through the country which resulted in high levels of fruit crop damage. As it oviposits in healthy, ripening and ripe fruits close to harvest, controlling the pest is extremely difficult.

Objectives: We tried to develop sustainable strategies to control the populations of SWD by identifying habitats during winter and spring, when the number of individuals is lowered. Research on food resources at this period of the year and on olfactory cues, used to orientate in the environment, are being explored to identify overwintering sites. This should lead to selective control strategies.

Material and methods: We determined the occurrence of SWD at landscape level and its re-immigration into fruit crops in spring by monitoring traps all year round. At locations with high captures at winter onset soil emergence traps were placed to check for survival rates. Moreover, we investigated food resources used during winter and spring by classical ecological and molecular methods. We tested, if odors of potential host plants or food resources were used for olfactory orientation by SWD in a Y-shaped olfactometer.

Results: In fall to early winter, trap captures in orchards decreased while they increased in forests and forest edges. Significantly higher numbers of SWD were caught in forest tree crowns compared to lower heights. In lab tests, flies survived several days when feeding on mistletoe berries. We succeeded in detecting chloroplast DNA using general plant primers in the digestive tract of field-caught SWD and started to establish olfactory studies.

Conclusion: Different trap captures in orchards, forests and forest edges during fall and early winter demonstrate the migration behavior of adult SWD searching for sheltered overwintering sites. During a mild winter, continuous captures in forest traps indicate the activity of SWD. We made progress in detecting food resources during winter and spring as well as in establishing olfactory studies. This knowledge will significantly contribute to the development of new control strategies.

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Drosophila Suzuki I

O DSU I-5

Reflections about the Pest Status of *Drosophila suzukii* (SWD) in German Viticulture

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SWD was first observed in Germany in 2011. Only in 2014 the first damages in viticulture were attributed to *D. suzukii*. Early red varieties were mainly attacked. Looking more into detail we often found damage that obviously had occurred before SWD-attack.

With regard to the weather conditions of 2014 it is obvious that this year was a sour rot year even without SWD. The year was characterized by a dry and hot summer and strong precipitations during veraison. As a consequence the berries swelled to an extent that the skin was mechanically damaged, frequently near the peduncle. This damage was an optimal substrate for both, *D. melanogaster* and sour rot.

We often observed that *D. suzukii* attacked single berries on clusters strongly smelling of vinegar, while the affected berries themselves didn't smell of vinegar. Maybe the SWD attack was not the reason but the consequence of SWD attack.

Checking berries under the stereomicroscope for egg incidence, we often found hidden powdery mildew attack. Most varieties which appeared to be highly susceptible to SWD in the field were not attractive for egg laying by SWD in laboratory trials as long as berries were not damaged. We know that there are susceptible varieties like Vernatsch and non-susceptible ones like Pinot Noir. For other German red varieties it is still unclear whether SWD is a primary or secondary pest.

O DSU I-6

Seasonal Dynamics of *Drosophila suzukii* on cultivated and non-cultivated areas of Blackberry (*Rubus* sp) and Preference of Wild fruits, in Michoacan, Mexico

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Introduction: Spotted wing *Drosophila* (SWD) was first reported in Mexico in November 2011. Since then this insect has been one of the most important pest of blackberry and other Berry crops in Mexico.

Objectives: The objectives of the research was to determine the seasonal dynamics of SWD in cultivated blackberry (*Rubus* sp.) and non-cultivated areas, and to define the preference of SWD for wild fruits growing in the blackberry producing region in Michoacan Mexico.

Materials and methods: From 2013 to 2014 the seasonal dynamic of SWD was determined in blackberry producing areas in Michoacan state using apple cider vinegar (ACV) in 1 L clear plastic containers with 200 ml. The ACV was replaced every 10 to 14 days. The traps were placed in blackberry commercial orchards, edges of the orchards, forest areas, and non-target wild fruits. In addition, to determine if these wild fruits were potential host of SWD, a series of choice and no choice experiments were conducted in laboratory at room temperature, on *Crataegus mexicana*, *Rubus adenotrichos*, *Spondias mombin*, *Eriobotrya japonica*, *Byrsonimia crassifolia*, *Psidium guajava*, *Prunus serotina* var *capuli* and *Vitis tiliifolia*.

Results: Results indicated that population of SWD varies throughout the blackberry growing season. Cultural practices such as pruning, defoliation, and host plants surrounding commercial plots influenced the population dynamics. Higher peaks were found at the edges of the orchards after the plantations were mowed down, in vegetative growth and green fruit stage of development. During the harvest season, the population increased from November to February. Also, highest peaks of the dynamic were obtained in the forest areas summer season when cultivated blackberry is not producing, but coinciding with wild fruits in ripening stage. Of the wild fruits tested in choice and no-choice experiments, *Rubus adenotrichos*, *Spondias mombin*, *Psidium guava*, and *Prunus serotina* var *capuli*, were the most susceptible to SWD infestation.

Conclusions: Our study indicated that seasonal dynamics in SWD, is not only influenced by wild plant species, as alternate host, but also by the cultural practices and vegetation surrounding the blackberry crop. *Spondias mombin* and *Prunus serotina* var *capuli* are new hosts not previously reported for SWD. Results will contribute to adjust an integrated management of this invasive pest in Berry crops.

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New and Emerging Pests and Diseases I

O NEW I-1

The need for coordinated European efforts to fight invasive crop pathogens

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Productivity in European agriculture can be severely hampered by plant disease. Research in the fields of resistance genetics of plants, host-pathogen interactions, and pathogen evolution has progressed enormously in the last decades, aided by technological and analytical advancements. However, the recent emergence of invasive rust fungi on wheat, *Puccinia spp*, potentially of non-European origin, has demonstrated the need for understanding pathogen diversity, spread and evolution at larger scales. Aggressive strains of yellow rust have resulted in escalating disease epidemics, so far peaking in 2014 where significant losses and additional fungicide sprays were observed in many European countries. The epidemics also spread to new areas in south and east Europe, where yellow rust was previously absent or scarce, and previous long-term effective host resistance have been overcome. We studied the origin and level of diversity of yellow rust in Europe using virulence phenotypic data of 2298 isolates sampled in seven countries between 2000 and 2013. A subset of 342 isolates was additionally investigated by microsatellite markers. At least four race groups of exotic origin (Warrior, Kranich, Triticale-non-aggressive and Triticale aggressive) were identified in the post-2010 populations, where the 'Warrior' race group was present in high frequencies in most of the West European countries and often associated with rust epidemics. Significant genetic divergence was estimated for 'Warrior' and 'Kranich' race groups from the pre-existing European population. It was concluded that they were of non-European origin being genetically related to sexual recombining populations from the pathogen centre of diversity in Asia. The triticale-aggressive race was genetically related to populations in the Middle East and Central Asia. The Europe-wide collaboration and compilation of data into a single dataset in a common database proved to be a major advance. It enabled us to identify the current invasion at an early stage, and to assess its implications at field level in many countries. Future collaborative efforts at the European scale should be continued and strengthened for timely early-warning of potential invasions of new variants of important crop pathogens with the capacity to spread very far within a very short period of time.

O NEW I-2

Identification of a New Whitefly-transmitted Ipomovirus of Cucurbits in the Imperial Valley of California

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A new and potentially damaging whitefly-transmitted virus of cucurbits was detected in the fall, 2014 in Imperial County, California, USA, in squash (*Cucurbita pepo* L.) and melon (*Cucumis melo* L.) plants showing virus-like symptoms including leaf yellowing and crumpling. The new virus is similar but not identical to *Squash vein yellowing virus* (SqVYV), a *Bemisia tabaci*-transmitted ipomovirus (family *Potyviridae*) that is present in Florida (Adkins et al. 2008). Sequence comparisons performed with the cylindrical inclusion (CI) and capsid protein (CP) encoding regions of this California ipomovirus revealed the highest identities, 83% and 98%, respectively, with the CI and CP genes of SqVYV (Batuman et al. 2015). Pumpkin and squash (*Cucurbita pepo* L.) plants inoculated with sap prepared from leaves of pumpkin plants in which the California ipomovirus was detected by RT-PCR, developed mild mottling, vein clearing and yellowing symptoms, similar to those reported for SqVYV (Fig.1); whereas inoculated watermelons (*Citrullus lanatus* Thunb. Matsum. & Nakai var. *lanatus*) became infected, and showed stunting, yellowing of the leaves, and approximately a month later, collapsed (Fig.2). Field and greenhouse studies are underway to further investigate the biology of the California ipomovirus and to ascertain the potential for the virus to impact cucurbit production in southern California.

References:

Adkins, S. et al. 2008 (Plant Dis. 92:1119,).

Batuman et al. 2015 (Plant Disease *in press*)

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Figure 1: Leaves of squash plant infected with California ipomovirus showing mild mottling, vein clearing and yellowing symptoms at 30 days-post-inoculation.



Figure 2: Watermelon plant infected with California ipomovirus showing vine collapse symptoms (left) and mock inoculated healthy watermelon (right) at 30 days-post-inoculation (White scale bar =10 cm).



O NEW I-3

Umbel browning and stem necrosis on carrot in France: isolation and characterization of the fungal pathogen

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Introduction: Since 2007, symptoms of umbel browning and stem necrosis have been regularly observed in carrot seed production areas located in France. The typical symptom is a triangular necrotic lesion on carrot umbel, compromising seed development. The loss in seed production was estimated at approximately 8% of the harvested carrot umbels during the cropping seasons of spring and summer 2007 and 2008 in France. The disease resembles the lesions on carrot umbels caused by *Phomopsis dauci* and described in the Netherlands in 1951.

Objectives: The disease epidemiology is not well known and the characteristics of the fungal pathogen have not yet been fully described. The aim of DIAPOCAR project is to constitute a reference isolate collection, to characterize these isolates, to study the epidemiology of the disease and to define possible control methods.

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Materials and methods: In our study, more than hundred strains were isolated from lesions developed on carrot or parsley from fields of seed production located in different French geographical areas. These isolates were characterized according to morphological criteria (mycelial growth, production of pycnidia and size of conidia).

In order to confirm the identification at the genus level and determine the species, molecular criteria were used (sequences in the ITS regions of the ribosomal DNA).

Results: Pycnidia produced alpha- and beta conidia that were typical of the genus *Diaporthe*. Results showed that 75 % of isolates belong to the *Diaporthe angelicae* species. Artificial contamination methods were developed for use both in field and in growth chamber on isolated umbels and Koch postulates were verified.

Conclusion: Tests are still under process and will contribute to a better understanding of the pathogen diversity and will improve the control methods against this re-emerging disease. The efficiency of 10 different fungicides will be tested in-vitro on 9 different strains. We will be able to determine the IC50 of each fungicide. Moreover, experiments are underway to determine if *D. angelicae* could be transmitted to the seeds.

O NEW I-4

Detection and epidemiology of *Tomato leaf curl New Delhi virus* in Spain

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Tomato leaf curl New Delhi virus (ToLCNDV) is member of the family *Geminiviridae* and genus *Begomovirus*. It was described for the first time in India in 1995 but gradually it has been detected in parts of Asia (Pakistan, Bangla Desh, Thailand, Indonesia, Taiwan) where it affects many species, both of the *Solanaceae* (tomatoes, peppers and potatoes) and *Cucurbitaceae* (cucumber, melon, watermelon, pumpkin, luffa and lagenaria). The presence of ToLCNDV in Spain was officially declared in crops of zucchini in the provinces of Almeria and Murcia during the summer of 2013. Afterwards it was detected in the provinces of Malaga, Seville and regions along of the South-East coast of Spain. Symptoms of ToLCNDV consisted of leaf curl and yellowing with deformation, cracking and skin roughness in fruits. As part of a study on the epidemiology of ToLCNDV in Spain, we evaluated the transmission of the virus to and from tomato and zucchini by its natural vector, the whitefly *Bemisia tabaci*. We also developed and compared techniques for its detection using molecular hybridization with non-radioactive probes, and convectional as well as real-time PCR. These techniques have been validated in samples from zucchini and tomato crops affected by ToLCNDV. During controlled transmission experiments using viruliferous whiteflies, symptoms of the virus consisting of chlorotic mottle and leaf curl, were first observed between 9 and 15 days post inoculation, depending on the crop species tested. The speed of virus detection varied between the host species and the detection technique used. The best technique for virus diagnosis was the one based on real-time PCR, followed by molecular hybridization. Using this latter method to compare the incidence of ToLCNDV in a number of naturally infected tomato cultivars, the results suggested that many of these cultivars have different susceptibilities.

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O NEW I-5

Investigating the genetic structure and diversity of the barley pathogen *Ramularia collo-cygni*

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Ramularia collo-cygni (*Rcc*) is the biotic factor responsible for the disease *Ramularia* leaf spot (RLS) of barley (*Hordeum vulgare*). The fungus is attracting interest in the scientific community as a result of the increasing number of economically damaging disease epidemics. Still essential parts of the pathogen biology, in particular the life cycle remain poorly understood.

To understand more about its epidemiology, the knowledge of its genetic structure and diversity is essential. Sequence analysis of four *Rcc* housekeeping genes, glyceraldehyde 3-phosphate dehydrogenase (GAPDH), β -tubulin (β Tub), E2 ubiquitin-conjugating protein (E2Ub) and a Thioesterase family protein (Thios) was used to further address the genetic diversity in *Rcc* isolates from a selection of geographically distinct isolates as well as isolates from hosts other than barley. Analysis of the

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sequence data indicated substantial genetic diversity between the isolates and a possible *Rcc* population size expansion, which might help explain the recent emergence.

Moreover, the genome of *Rcc* was sequenced completely and assembled using Allpaths-LG assembler. The finished assembled genome of *Rcc* is about 32 Mb and is currently to be found in 78 scaffold. The complete annotation of this genome is underway to generate consensus gene calls. The fungal RNA from 6 different conditions, especially one that mimics the plant environment was also sequenced to help in one hand the annotation and in other hand to uncover putative gene of interest that might be involved in the pathogenicity or the fungicide resistances for example.

To evaluate the true genetic diversity of this fungus, full genomes sequencing of *Rcc* isolates from multiple geographic locations and non-barley hosts are underway. We hope by this approach to provide valuable insights in to the genetic diversity of this organism and to address how this diversity has influenced the evolution of the fungus. The understanding of the diversity is essential to identify sustainable control in Integrated Pest Management.

O NEW I-6

Inventory and validation of pathogenic fungi occurring on maize leaves in Central Europe

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Introduction: In the last decade, the acreage of maize has increased continuously in Central Europe due to its high yield and diverse uses as food, feed and bio-energy crop. This has led to more intense maize cultivation in many regions with narrowed crop rotations and it is therefore likely that maize diseases will become more significant in the future. Nevertheless, the knowledge about the occurrence and significance of fungal pathogens and their epidemic development in maize fields in Central Europe is still scarce.

Objectives: The study aims at reviewing the phytosanitary state of maize crops in Central Europe, focusing on fungal leaf diseases and their epidemiology, particularly regarding less known or novel pathogenic species.

Methods: A qualitative monitoring of occurring species of potentially leaf infecting pathogens in maize was carried out in selected fields of Germany (28 locations), the Netherlands (five locations), the Czech Republic (six locations), Austria (three locations), France (two locations) and Poland (two locations) during the years 2012 and 2013. Here, leaf samples were collected and fungal isolates were isolated and analyzed morphologically. For a number of isolates, pathogenicity tests were conducted by inoculating healthy plants in the greenhouse with spore suspensions prepared from single-spore cultures in order to fulfill Koch's postulates.

Results: The studies revealed that seven isolates of *Kabatiella zea* (eyespot), five isolates of *Bipolaris zeicola* (Northern leaf spot), two unidentified isolates of *Bipolaris* spp., two isolates of *Colletotrichum graminicola* (Anthracnose) and six isolates of *Phoma* spp. (Phoma leaf spot disease) fulfilled Koch's postulates. The resulting disease symptoms were described in detail. For the *Phoma* isolates, which could not be clearly identified by morphological analysis, a taxonomical assignment based on molecular genetic analysis was conducted.

Conclusions: The results of this study suggest that a large number of newly emerging maize pathogens may potentially threaten maize cultivation across Central Europe. Due to the high frequency of detection of several pathogens, the importance of fungal maize diseases is likely to become a challenge in the future and suitable strategies of control should be taken into consideration.

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Plant Pathogen Interactions II

O PPI II-1

Evidence for suppression of plant immunity by microRNA targeting in the plant-*Verticillium* interaction

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Verticillium longisporum, a soil-borne pathogen, causes devastating vascular disease in the cultivation of oilseed rape (*Brassica napus*). Due to an extremely long and complex infection process, an intensive plant-fungus interaction is needed. Here report that plant-derived miRNAs play an indispensable role in the modulation of plant-*Verticillium* interaction.

We demonstrate that the fungus is able to suppress plant resistance response by targeting a series of TIR-NBS-LRR type resistance genes via miRNA1885, and by enhancing the gene silencing machinery via the modulation of miRNA168-AGO1 interaction.

Our data strongly support that the fungus *V. longisporum* has evolved a virulence mechanism by interference with plant miRNAs to reprogram host gene expression. A mode of action is discussed.

O PPI II-2

The plant pathogenic fungus *Verticillium longisporum* suppresses plant immunity by miRNA1885-mediated regulation of TIR-NBS-LRR resistance gene expression in oilseed rape (*Brassica napus*)

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The soil born pathogen *Verticillium longisporum* causes devastating vascular disease in the cultivation of oilseed rape (*Brassica napus*). We demonstrate that plant microRNAs (miRNAs) are involved in regulating plant-*V. longisporum* interaction. By deep-sequencing two small RNA libraries made from *V. longisporum* infected/noninfected roots, we identified 62 miRNAs, which were responsive to the *V. longisporum* infection. Strikingly, the majority of miRNAs were down-regulated, while a few miRNAs were up-regulated, of which one belongs to the miR1885 family. Genome analysis revealed that there are three loci of miR1885a,b,c, which are located in the chromosome A06, A09 and C07, respectively. The validated target of miR1885 belongs to the TIR-NBS-LRR resistance genes, which is one of the predicted donors of miR1885. Thus, understanding the expression regulation of miR1885 and its targets provides a deep insight into how the fungus suppresses plant R-gene-mediated resistance. Here, we present recent data for specific interactions and simultaneously reciprocal changes in the expression levels of miR1885 and their targets in infected roots. Furthermore, we demonstrate that miR1885 regulates the TIR-NBS-LRR resistance gene expression and consequently suppresses plant resistance response, which greatly benefits the fungus infection.

O PPI II-3

Transcriptome analysis revealed pathways controlling resistance against systemic colonization by *Verticillium longisporum* in *Arabidopsis thaliana*

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Verticillium longisporum is an economically important pathogen of oilseed rape. It infects the host via the root and colonizes the plant systemically through the xylem at the onset of flowering. QTL controlling quantitative resistance against systemic colonization by the fungus have been identified in *Brassica* spp. and also in *Arabidopsis thaliana*.

The objective was to identify genes and pathways controlling resistance against systemic colonization in *A. thaliana*. Alignment of candidate resistance genes to the *Brassica napus* genome was to show if the results can be transferred to the crop plant.

A major-effect QTL, the complex locus *vec1*, was fine-mapped in (BurxLer)-near-isogenic lines (NILs) and represented in a tailor-made NIL (tmNIL) that contained an introgression of the resistant parent Bur in *vec1*. To study the effect of *vec1* on gene expression and to prioritize candidate resistance genes within *vec1*, the transcriptome of the tmNIL was compared to a NIL that contained only alleles of the susceptible parent Ler in the *vec1*-region.

The tmNIL showed a strong induction of defence-related genes after infection with *V. longisporum* at the onset of flowering. The up-regulated genes were involved in the control of basal resistance, systemic acquired resistance, cell wall processes and secondary metabolites. In the susceptible NIL, only few genes were differentially expressed after infection. Genes differentially expressed between the lines within *vec1* were cloned as candidate resistance genes. Alignment of candidate gene sequences with the *B. napus* genome revealed that these sequences co-localized with known QTL for *V. longisporum* resistance in *Brassica*.

Oral Presentations

Plant Pathogen Interactions II

Resistance against systemic colonization by *V. longisporum* is controlled by a complex locus triggering various defence responses in *A. thaliana*. Co-localization of candidate genes with resistance QTL in *Brassica* suggests that comparable mechanisms are active in oilseed rape and could be exploited in resistance breeding.

O PPI II-4

Biotrophy-specific down-regulation of gene expression in the maize pathogen *Colletotrichum graminicola* is required for escaping PAMP-triggered immunity and for the establishment of compatibility

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Exposure of invariable molecules by plant pathogenic microorganisms, collectively known as pathogen-associated molecular patterns (PAMPs), triggers a broad spectrum of plant defense responses. Thus, in order to establish a compatible parasitic interaction with their hosts, pathogens may modify the exposure of PAMPs at specific stages of the infection process. This likely is particularly important for pathogens forming biotrophic infection structures, as these are growing in close vicinity of the plant plasma membrane, which contains pattern recognition receptors mediating a PAMP response.

We investigated synthesis and exposure of different defense-modifying molecules in the maize pathogen *Colletotrichum graminicola*. This fungus differentiates an infection cell called an appressorium, which invades the host primarily by force exertion (Bechinger et al. 1999). In the plant the fungus sequentially forms biotrophic and necrotrophic hyphae indicative of a hemibiotrophic lifestyle (Horbach and Deising 2013). Interestingly, we discovered synchronous infection structure-specific regulation of two components involved in cell wall biogenesis and iron uptake, both affecting plant defense responses. Genes encoding β -glucan and siderophore synthesizing enzymes are rigorously down-regulated during biotrophic development. Forced expression of β -glucan synthesis during biotrophy dramatically induced plant defense responses, as indicated by microscopy and RT-qPCR experiments. Maize leaves infiltrated with siderophores did not initiate defense responses unless *C. graminicola* had invaded the leaf and formed biotrophic hyphae (Albarouki et al. 2014). Our studies show that infection structure-specific regulation of genes involved in formation of defense-modulating compounds is indispensable for the establishment of a compatible parasitic interaction between *C. graminicola* and maize.

Literature:

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O PPI II-5

Proof of concept: Stacking of defense related genes in a marker-assisted backcrossing vs. a transgenic approach to improve disease resistance of barley elite cultivars

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Classical plant breeding and genetic engineering are both indispensable tools to equip crop plants with powerful traits against abiotic and biotic stresses. Many elite cultivars, however, are still highly susceptible to major pathogens because they lack favourable allele combinations for effective defence. The international research consortium BARLEY-fortress aimed at the utilization of basal defense genes to provide broad and durable pathogen resistance to barley with special focus on the improvement of fungal penetration resistance by re-enforcing attacked cell walls. Thereby, different strategies were followed: i) introgression of functionally validated genes from landraces and wild barley by marker-assisted backcrossing in a German elite variety and ii) a transgenic approach in which selected combinations of resistance associated genes are overexpressed or sets of susceptibility related genes are silenced by RNAi, respectively. Combinations of genes were chosen on the basis of preexisting knowledge within the consortium. Here, we report on success of the gene stacking approach validated by phenotypic and genotypic characterization of backcross and transgenic lines for resistance to cereal blast (*Magnaporthe oryzae*). Molecular and cytological data will provide insights into mechanisms accompanying this resistance.

O PPI II-6

Investigation of plant-pathogen interactions in the pathosystem *Solanum tuberosum* L. / *Rhizoctonia solani* Kühn

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Rhizoctonia solani Kühn is a world-wide occurring soil-borne fungal pathogen which infects a broad range of plants including important crops like potato (*Solanum tuberosum* L.). Various types of damage on infected underground potato organs lead to economically relevant yield losses. The increasing occurrence of this disease and the lack of efficient control strategies strengthen the necessity of new control mechanisms, e.g. the use of resistant cultivars. One of our future goals is the development of a resistance breeding method. Presumably, potato cultivars differ quantitatively in their susceptibility to the fungus. So far, little is known about the molecular background of these susceptibility differences. Therefore it seems to be reasonable to investigate the interactions between potato and *R. solani*, from initial attack through recognition of the fungus by the plant to an activation of plant defense mechanisms. The study of gene expression patterns is one way to get an insight into the responses of organisms. In the beginning, RT-qPCR analyses for several defense-related genes were run at different time points after an inoculation with *R. solani* to find the best sampling date for a subsequent RNA-Seq. Thus, we could detect an up-regulation of PR proteins like 1.3- β -glucanase and PR1 in inoculated plants at 10 dpi. These pre-tests were carried out to optimize the experimental methods in preparation of the RNA-Seq experiment. RNA-Seq is an excellent tool to simultaneously investigate the gene expression of plant and fungus. Our aim was to analyze the differential gene expression between *R. solani*-inoculated and non-inoculated potatoes. Hence, genes involved in the interactions between potato and *R. solani* are revealed and this knowledge can be used e. g. for breeding of less susceptible cultivars. The results will also give better insight into the plant-pathogen interaction.

Oral Presentations

Non-Chemical Control Options II

O NOC II-1

Does combining a wheat and pea mixture with methyl salicylate reduces aphid populations in both crops?

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Aphids are important pests of wheat and pea. Among the alternative methods to control them with less reliance on insecticides, crop associations already proved to be efficient. However, if increasing the chemical and structural complexity of vegetation can disrupt their host plants location, the searching efficiency of predators and parasitoids can also be reduced. Therefore, these beneficials may not always be more abundant in such systems. Combining crop associations with attractive semiochemicals for natural enemies can be interesting to solve this problem. In this research, we compared the effect of a wheat and pea pure stand, wheat and pea mixture, and wheat and pea mixture combined with methyl salicylate (MeSA) formulated in alginate gel beads, on the abundance and diversity of aphids and their natural enemies. These were weekly observed on plants during 2013 and 2014 growing seasons. Over these two years, significantly higher numbers of pea aphids (*Acyrtosiphon pisum* (H.)) were observed in the pure stand of pea compared with both mixtures (with and without MeSA). No significant differences were observed between treatments for wheat aphids (*Sitobion avenae* (F.), *Metopolophium dirhodum* (W.) and *Rhopalosiphum padi* (L.)), which were significantly less abundant than pea aphids. Aphid natural enemies were mainly observed on pea plants. Hoverfly larvae abundance was not significantly different between treatments during both years. The same phenomenon occurred with hoverfly pupae in 2013, while these were significantly more abundant in both mixtures compared with the pure stand in 2014. However, their number did not differ significantly between the mixture with and without MeSA. Few ladybirds and lacewings were observed. No significant differences were observed between treatments for parasitoid mummies in 2013. Their abundance was significantly higher in the pure stand of pea compared with both mixtures in 2014. Results from this study show that mixing wheat and pea is an efficient method to maintain aphid populations at a very low level on pea. The use of MeSA did not show significant effects on natural enemies. However, mixing these crops may be enough to reduce aphid populations under an acceptable threshold.

O NOC II-2

Effect of Powder Preparation of Clove, Ginger, Garad and Galangal on the Infestation of Sorghum Grains Caused by Khapra Beetle Larvae *Trogoderma granarium*

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Introduction: Sorghum is the most important cereal crops in the Sudan, used for both human and animal diet. Khapra beetle (*Trogoderma granarium*) is the major storage pest of Sorghum grains.

Objective: This study was conducted to reveal the efficacy of clove, *Syzygium aromaticum*, ginger *zingiber officinale*, Garad *Acacia nilotica* and galangal *Alppina officinarum* on Khapra beetle larva.

Materials and methods: The experiment was carried out in the laboratory to rear Khapra beetle larvae on Sorghum grains treated with powder of the above botanicals crops. Ten Khapra beetle larvae were added to each treatment. Five treatments replicated five times arranged in Complete Randomized Design. The treatments were consisted of sorghum treated with powder of clove, ginger, garad and galangal, and untreated sorghum grains taken as a control. Five parameters (weight losses of sorghum grain, Khapra beetle larvae mortality, adults emerged, seed germination and seed damage) were used to show the effect of these botanical crops on Khapra beetle larvae. Khapra beetle larvae mortality and weight losses were counted every week, adults counted after their emergency (after ten weeks), seed damage and seed viability were done at the end of the experiment.

Results: The result indicated that these botanical crops significantly ($p < 0.05$) reduced the damage level of Khapra beetle larvae on Sorghum grains. clove powder showed the lowest weight losses, highest mortality, highest seed germination, and lowest seed damage, followed by ginger, garad, galangal and untreated Sorghum grain.

Conclusion: From this study we can conclude that clove crop is the most important crop to control Khapra beetle larvae.

Oral Presentations
Non-Chemical Control Options II

O NOC II-3

Foliar Application of Microbial-Enriched Fermented Food 'Cheonggukjang' to Control Powdery Mildew in Organic Farming

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Powdery mildew is one of common diseases occurred on organically cultivated crops under field and greenhouse conditions. This study was conducted to evaluate control activity of 'Cheonggukjang' solution against powdery mildew caused by *Sphaerotheca fuliginea* in cucumber, tomato, lettuce and squash. 'Cheonggukjang' was naturally fermented soybean, Daepung with rice straw for two days. The population density of fermentation bacteria was adjusted to 10^{10-11} cfu/ml and they were applied as foliar-spray on the diseased cucumber, tomato, lettuce and squash leaves. Forty five days old seedlings of cucumber, tomato, lettuce and squash were used and occurrence of powdery mildew was dependent on natural infection with *S. fuliginea*. When the Cheonggukjang solutions were foliar-applied with the concentration of 6, 7.5, 10, 15 and 30%, they suppressed more than 72.7% incidence of powdery mildew in three crops for 14 days after their foliar application. When Cheonggukjang solutions contained with more than 6% of Cheonggukjang were applied, they showed strong control effect. Application of six percent of Cheonggukjang solution revealed more than 85.1% control value against powdery mildew of three crops. However, more than 15% Cheonggukjang solution showed the phytotoxicity on three crops leaves after four days of spray. These results indicated that foliar application of 6~10% Cheonggukjang solution could be used for controlling powdery mildews occurring on organically cultivated crops

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Figure 1: Population density of fermentation bacteria isolated from 'Daepung' cheonggukjang fermented with rice straw for two days.

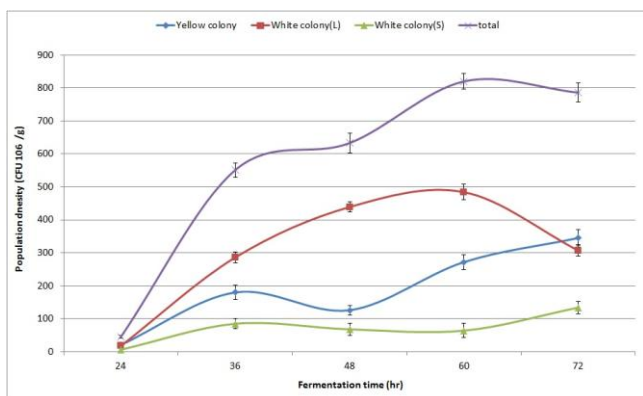
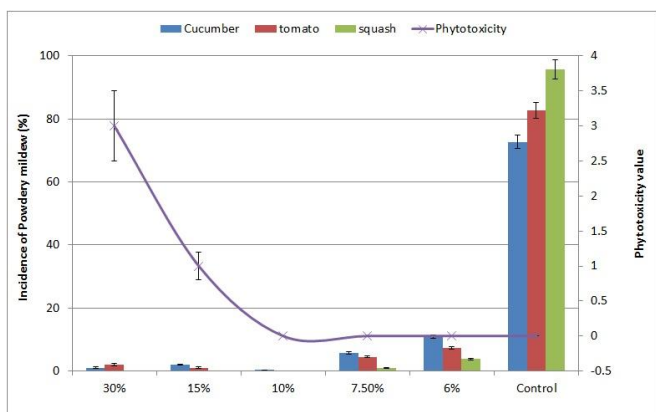


Figure 2: Control effect of Cheonggukjang solution on cucumber, tomato, and squash powdery mildew occurred with *Sphaerotheca fuliginea* in greenhouse



Oral Presentations

Non-Chemical Control Options II

O NOC II-4

Biological strategies for protecting maize from *Pratylenchus ZEA* and *Meloidogyne incognita*

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Pratylenchus zae causes serious root necrosis of maize and severe grain yield reduction. *Meloidogyne incognita* is a cosmopolitan pest of crops causing galling in infected roots. This research work involved the biological management of *P. zae* and *M. incognita* using an environmentally-friendly approach that could be adapted in organic crop production. A combination of host plant resistance and arbuscular mycorrhizal fungi (AMF) was investigated on the field with the nematode resistant maize variety Western Yellow, in two cropping seasons. A mixture of *Glomus mosseae* and *Glomus clarum* was used as the AMF inocula. Rugby nematicide was applied to the field used for this experiment at the rate of 65kg/hectare. The experiments were laid out in a split-plot design with nine treatments and four replications. Thirty plants were planted per plot. Treatments with nematode and AMF were concomitantly inoculated one week after germination. Five thousand nematodes were inoculated per plant, *P. zae* and *M. incognita* were inoculated singly, for plants having nematode inoculation. There were two levels of AMF spores inoculation, level one was 140 spores per plant and level two was 280 AMF spores per plant. Equal proportions of *G. mosseae* was used with *G. clarum*. The experiments were terminated sixteen weeks after planting. Data were collected on nematode density, reproductive factor (RF) percentage AMF colonization, grain yield, root length and root weight. Results obtained showed that there was no significant reduction in nematode density at $P \leq 0.05$. However, RF was limited to 0.95. Percentage AMF colonization reduced nematode infection but not significantly. There was 36.9% and 12.0% significant increase in grain yield respectively for *P. zae* and *M. incognita* treatments. AMF inoculation improved root length by 35.7% and 20.5% and root weight by 27.7% and 14.6% under *P. zae* and *M. incognita* inoculation respectively. The resistant maize genotype used limited the reproduction of the nematodes, thus the nematode density was kept within minimum threshold. The AMF enhanced root biomass production and root elongation, which reduced the effect of root pruning and root termination caused by nematodes. A combination of host plant resistance and AMF is a viable biological strategy in *P. zae* and *M. incognita* management and grain yield improvement.

O NOC II-5

Predator insects in organic and conventional production potato fields in Turkey

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Biological control of insect pests is more important in organic crop production than that of the conventional production. Allowable organic and inorganic chemicals or other natural materials are used for pest control to provide natural enemies in organic production. This can be accomplished through conserving and augmenting beneficial populations. In order to determine abundance and biodiversity of predator insect species in organic and conventional potato production fields, it was conducted a survey in Konya Province. Potato (*Solanum tuberosum* L.) is one of the most important field crops in Turkey, cultivated in 1.593 million ha with an annual yield of 4.397.305 tons. Konya was third province with production of 460 thousand tons potato in 2012. Konya is located at 1031 m (3383 ft) in altitude and in south-central Anatolia. It has a continental climate with cold, snowy winters and hot, dry summers. The insects were collected with sweep net method in the potato fields between June and October in 2013. To represent the district, two organic and two conventional potato fields were selected from each of three villages. At the end of the study, it was determined totally 33 predator species. The species number and their families; 6 syrphid, 16 coccinellid, 3 anthocorid, 3 mirid, 2 lygaeid, 1 nabid, 1 chrysopid and 1 reduviid species. Among the collected predator species; *Orius niger* and *Orius minutus* (1415 adults), *Hippodamia variegata* (454 adults), *Nabis pseudoferus* (169 adults), *Sphaerophoria scripta* (153 adults) were more abundant than the others. Interestingly, it was found only one sample from *Adalia decempunctata*, *Adalia bipunctata*, *Propylaea quatuordecimpunctata*, *Scymnus subvillosus*, *Subcoccinella vigintiquatuor punctata* (Coccinellidae: Col.), *Deraeocoris serenus*, *Deraeocoris ruber* (Miridae: Het.). *O. minutus*, *O. niger* and *N. pseudoferus* were effective on populations of psyllids, thripids and cicadellids which were collected on the potatoes. Coccinellid predators, especially *H. variegata*, were found often on the weeds, containing many aphids, around the fields. Consequently, it was not found remarkably differences between organic and conventional potato production fields in terms of abundance and biodiversity of the predators. This result must be analyzed with further studies in larger fields of the district.

Oral Presentations
Non-Chemical Control Options II

O NOC II-6

Olfactometer screening of repellent essential oils against the pollen beetle (*Meligethes spp.*)

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In organic farming, pollen beetles (*Meligethes spp.*) are difficult to control due to the ban of insecticides. Alternative methods are therefore needed. The beetles use olfactory cues to locate oilseed rape fields in early spring. Essential oils were shown to have an impact on host plant location behaviour. Lavender oil (*Lavendula angustifolia*) showed the highest repellency value in a laboratory study that compared five different essential oils (Mauchline et al., 2005). However, lavender oil is one of the most expensive essential oils - a fact that could seriously hamper on-farm implementation of this strategy.

The objective of our experiments was to find a cheaper essential oil with comparable efficacy to lavender oil. We compared the essential oils of *Mentha arvensis*, *Eucalyptus globulus*, *Melaleuca alternifolia*, *Citrus sinensis*, *Citrus paradisi*, *Citrus limon*, *Juniperus mexicana*, *Abies sibirica*, *Illicium verum*, *Gaultheria procumbens*, *Cymbopogon flexuosus*, *Syzygium aromaticum*, and *Litsea cubeba* using a Y-tube-olfactometer. Essential oils were diluted 1:10 in acetone and 40 µl of the dilution were applied on a filter paper. Filter papers were placed in the odour containers of the olfactometer together with a flower cluster of spring oilseed rape. Hungry pollen beetles were released individually into the olfactometer. The beetles' choices were recorded. Flowers and essential oils were changed between replicates. Six replicates with six beetles each were conducted.

Ten out of the 15 tested essential oils significantly repelled the pollen beetles; none of the tested essential oils was attractive for the pollen beetles. Highest repellency values were obtained for *Mentha arvensis* (100% repellency), *Cymbopogon flexuosus* (92% repellency), and *Litsea cubeba* (92% repellency). Lavender oil was less effective and repelled only 81% of the beetles.

With an average price of 17.5 and 18 € / kg, *Cymbopogon flexuosus* and *Litsea cubeba* oil are considerably less expensive than lavender oil (104 €/kg). *Mentha arvensis* oil has an average price of 31.50 € / kg. Based on the results of the experiments and on the prices of the essential oils, the development of a field application strategy will focus on *Cymbopogon flexuosus*, *Litsea cubeba* and *Mentha arvensis* oil.

Mauchline, et al. 2005: Entomol Exp Appl 114: 181-188.

Oral Presentations Biotechnology

O BT 1

RNAi-mediated Gene Silencing and Its Implications for Agriculture

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Given current trends in both food and energy demands of a growing population, as well as the changing climate, it will be necessary to greatly improve crop yields worldwide during the coming years. Meeting this challenge will require developing agronomic solutions that promote sustainable plant production systems despite conditions of increased biotic and abiotic stresses, while significantly reducing negative side effects on the environment. RNA interference (RNAi) has emerged as a powerful genetic tool for scientific research over the past several years. It has been utilized not only in fundamental research for the assessment of gene function, but also in various fields of applied research, such as human and veterinary medicine and agriculture. In plants, RNAi strategies have the potential to allow manipulation of various aspects of food quality and nutritional content. In addition, the demonstration that agricultural pests, such as insects and nematodes, can be killed by exogenously supplied RNAi targeting their essential genes has raised the possibility that plant predation can be controlled by lethal RNAi signals generated in planta. Indeed, recent evidence argues that this strategy, called host-induced gene silencing (HIGS), is effective against sucking insects (Abdellatef et al. 2015) and nematodes; it also has been shown to compromise the growth and development of pathogenic fungi (Koch et al. 2013), as well as bacteria and viruses, on their plant hosts (Koch and Kogel 2014). We present recent studies that reveal the enormous potential RNAi strategies hold for providing an environmentally friendly mechanism for plant protection.

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O BT 2

Interspecies gene transfer provides soybean resistance to Asian soybean rust, a major fungal pathogen

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Phakopsora pachyrhizi is the causative agent of the devastating fungal disease called Asian soybean rust (SBR) that poses a major threat to food security. This is mainly because no soybean variety with durable resistance to all *P. pachyrhizi* isolates are commercially available. Farmers need soybean varieties resisting SBR as these complement the use of fungicides for controlling SBR epidemics. Since plant nonhost resistance (NHR) is remarkably durable, the transfer of NHR-linked genes to otherwise susceptible soybean varieties is a promising strategy for genetically engineered SBR control. Here, we describe the identification of designated Arabidopsis *POSTINVASION-INDUCED NONHOST RESISTANCE GENES (PINGs)* by global transcriptome analysis of Arabidopsis genotypes with intact (wild type) and impaired NHR (*pen2*, *pen2 pad4 sag101*). We demonstrate that some of the identified genes indeed contribute to Arabidopsis postinvasion NHR. Furthermore, we provide evidence for functionality of identified Arabidopsis genes in soybean as expression of selected *PINGs* in soybean did confer enhanced resistance to SBR. Hence, interspecies NHR gene transfer presents a powerful strategy to complement current breeding programs for providing durable soybean rust resistance. We will also introduce a novel tool for controlling SBR and other plant diseases by functionalizing the plant surface for providing plant protection in the field.

Oral Presentations

Biotechnology

O BT 3

Development of molecular markers for breeding for disease resistant crops

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Rice blast disease caused by the filamentous ascomycetes fungus *Magnaporthe oryzae* (Fig. 1), and sheath blight disease caused by the soil borne fungus *Rhizocotonia solani* (Fig. 2) are the two major rice diseases that threaten stable rice production in the USA and worldwide. These two diseases have been managed with a combination of resistance (*R*) genes and fungicide application integrated into diverse culture practices worldwide. Application of fungicides adds additional production costs and excessive fungicide application brings increased environmental concern. Using *R* genes is the most economical and environmentally benign method for crop protection. In an effort to aid classical plant breeding for improving resistance to blast and sheath blight diseases, we have been developing DNA markers for use in marker assisted breeding. These molecular markers were derived from closely linked DNA markers to major or minor *R* genes, portions of cloned *R* genes by association analysis of disease reactions with DNA sequence variation of candidate genes, or cloned *R* genes in mapping populations and selected rice germplasm. Consequently, USA rice cultivars have been developed with enhanced disease resistance using these markers. Our current effort for DNA marker development using improved disease assays, and genotyping by sequencing (GBS) using next gen sequencing will be presented.

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Figure 1



Figure 2



O BT 4

Some like it hot: transgene expression and Bt toxin concentration in Bt maize under stressful environmental conditions

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Introduction and objectives: One of the two most widely used transgenic traits is insect resistance, conferred by insecticidal toxins from *Bacillus thuringiensis* (Bt). In most countries, cultivation of Bt crops has been approved on the condition of installing an insect resistance management (IRM) program. One of the prerequisites of IRM is that plants contain high and stable levels of Bt toxins that are lethal not only to susceptible target insects but also to heterozygous resistant target insects that carry one resistance allele. However, it was shown that the concentration of Bt toxin can be influenced by environmental conditions and

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may vary between different plant tissues. According to the IPCC report, the frequency of extreme climatic events, such as protracted droughts or heavy rains and floods, is likely to increase in the future. But it remains unknown whether such stressful environmental conditions influence transgene expression and the resulting Bt toxin concentration in Bt crops.

Materials and methods: Two Bt maize cultivars containing the same transgene cassette (MON810) were grown under optimal and stressful environmental conditions. Stress treatments included cold/wet conditions simulating water-logged conditions, and hot/dry conditions simulating water-limited conditions. Before and during stress, transgene expression and Bt toxin concentration in the leaves of Bt maize plants were quantified using RT-PCR and ELISA.

Results: Under optimal conditions, Bt toxin concentration differed significantly between the two Bt maize cultivars but there was no significant difference in the transgene expression. Transgene expression was correlated with Bt toxin concentration in only one of the cultivars. Under stressful environmental conditions, transgene expression was similar as under optimal conditions but Bt toxin concentration responded differently.

Conclusion: It is rather difficult to predict Bt toxin concentration in Bt maize under stressful environmental conditions. The concentration of Bt toxin does not seem to be only controlled by transgene expression but also by genetic background of the maize cultivar.

O BT 5

Mitigation of preharvest aflatoxin contamination in cottonseed and corn kernels by transgenic expression of a synthetic peptide or a heterologous α -amylase inhibitor

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Introduction: Successful conventional breeding of cotton or corn for resistance to aflatoxin-producing *Aspergillus flavus* is time consuming and is dependent on the availability of native resistant genes in germplasm. Corn has several antifungal proteins that can offer resistance to *A. flavus* but it is a quantitative trait involving several genes whereas cotton does not possess practical levels of natural resistance to aflatoxin producing fungi in its germplasm base. Enhancement of resistance in susceptible crops through safe expression of foreign genes provides an attractive means of addressing preharvest aflatoxin contamination. In this regard, we have identified two promising antifungal proteins/peptides in our laboratory: a) a synthetic lytic peptide D4E1, with broad-spectrum control of phytopathogens including *A. flavus* and b) a heterologous α -amylase inhibitor protein from *Lablab purpurea* (AILP).

Objective: Evaluate transgenic cottonseed expressing the peptide D4E1 and transgenic corn expressing the α -amylase inhibitor protein AILP for resistance to *A. flavus* infection and aflatoxin production.

Materials and methods: *Agrobacterium*-mediated transformation was applied to both cotton (var. Coker 312) hypocotyls and immature embryos of corn (var. Hi-II) to express the synthetic peptide D4E1 or AILP, respectively. Transgenic cottons were assayed for resistance to *A. flavus* under laboratory, greenhouse and field conditions. Kernel screening assays (KSA) were performed on transgenic corn lines and their segregation controls to evaluate resistance to *A. flavus* and aflatoxin contamination.

Results: Transgenic cottonseeds expressing the peptide D4E1 demonstrated significant control of *A. flavus* infection (50-75%) under laboratory and greenhouse conditions. Multiple field experiments are in progress. Transgenic corn kernels expressing AILP showed significant reduction in *A. flavus* growth (33%) and aflatoxin contamination (56%).

Conclusions: Transgenic expression of a synthetic peptide D4E1 in cottonseed or α -amylase inhibitor protein from *Lablab purpurea* (AILP) in maize kernels provided significant control of *A. flavus* growth and aflatoxin production.

O BT 6

Deoxynivalenol epimerization-A novel biotransformation with potential applications in improving crop resistance against *Fusarium* diseases

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Introduction: Deoxynivalenol (DON) plays a major role in the pathogenicity of *Fusarium*. Strains that produce DON efficiently are more virulent and invade plants more aggressively. This emerges from the ability of DON to bind to target-molecules, inhibit protein bio-synthesis, and suppress plant-defense mechanisms. The role of C-3 carbon in DON potency as plant immunomodulator is a well-documented. Targeting this group by enzymatic modifications, such as using *Fusarium* Tri101

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acetyltransferases for example, has been suggested as a strategy to enhance the plant's resistance against *Fusarium* invasion. Earlier trails showed mixed yet promising enhancement of plant resistance when Tri101 was introduced into transgenic plants.

Objectives: Our lab recently discovered a soil bacterium, *Devosia* sp. 17-2-E-8, that can epimerize the -OH group at C-3 within DON and reduce its associated toxicity. The aim of this study is to identify the genes/enzymes/metabolic pathways responsible for DON epimerization through genomic and transcriptomic analyses.

Materials and methods: Bacterial identification using various methods showed that the isolated strain belongs to the *Devosia* genus hence *Devosia riboflavina* IFO13584 was adapted as a control strain. Molecular techniques including *de novo* genome assemblies and entire transcriptomes profiling were utilized to highlight enzyme(s) and metabolic pathways that were up-regulated in the due course of DON epimerization. Overexpression strategies in exogenous hosts, such as *E. coli* BL21, are being perused to confirm the role of these enzymes. A recombinant Tri101 enzyme cloned from *F. graminearum* is also incorporated as a positive control in the enzyme-kinetics studies.

Results and conclusions: The efficiency of C-3 epimerization in reducing DON toxicity was demonstrated in different testing models. The epimerization process by *Devosia* sp. 17-2-E-8 is highly reproducible under wide-range of conditions with 100% efficiency. Furthermore, the obtained data have showed that the stereochemistry of DON influences its molecular interactions evident by the loss of 3-*epi*-DON ability to interact with Tri101 recombinant enzyme. The identification of such genes/enzymes not only will help the food/feed industry to reduce the adverse effects of DON contamination but will also provide additional opportunities for improving crop resistance against *Fusarium* diseases.

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Drosophila Suzuki II

O DSU II-1

Interactions of *Drosophila suzukii* with native parasitoids and *Drosophila* species

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Outside its native range, the exotic pest species *Drosophila suzukii* enters ecosystems that comprise already numerous native *Drosophila* species as well as their natural enemies. Interactions with these species may foster or impair the participating organisms and may have implications for the success of pest control strategies, such as the deliberate release of native or exotic parasitoids. To unravel the interactions of *D. suzukii* with native parasitoids we conducted a field survey in Switzerland. Sentinel larvae and pupae of the native *D. melanogaster* were exposed in the field at different sites, habitats (agricultural and natural) and times. Recollected samples were subsequently kept in the laboratory under controlled conditions until the emergence of parasitoids. Among the collected parasitoids a strain of *Pachycrepoideus vindemmiae* was able to parasitize *D. suzukii* at a high rate comparable to the parasitization of *D. melanogaster* when pupae of both species were directly exposed to the parasitoid under no-choice conditions. To investigate the interaction of *D. suzukii* with native Drosophilids rearing experiments were conducted. It was demonstrated that the native *D. melanogaster* and *D. subobscura* were able to utilize blueberry fruits after infestation with *D. suzukii*. Therefore it is possible that native *Drosophila* species could benefit from the ecological niche that is being created by the exotic invader. Taken together the results show that *D. suzukii* may influence native insect species on different trophic levels. While populations of native *Drosophila* could be enhanced, native species of parasitoids could play a role in suppressing populations of *D. suzukii*. These parasitoid species also have to be regarded for non-target effects when aiming for classical biological control with parasitoids from the area of origin of *D. suzukii*.

O DSU II-2

Biological control of the spotted wing drosophila - current status of research and perspectives for the future

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Introduction: *Drosophila suzukii* Matsumura is a fruit pest from Asia currently invading a wide range of habitats like woodland, hedgerows, but also gardens and commercial fruit plantations in Europe. Females deposit their eggs into healthy, ripe fruits which decay quickly due to larval development. The damaging risk for cultivated soft fruits and berries is extremely high, whereas the application of pesticides is difficult or even impossible due to the waiting period before harvest.

Objectives: This urgent demand for environmental friendly as well as residual free control methods initiated our research on invertebrate and microbial biological control agents since the last two years. We evaluated native parasitoid species and predators, but also examined samples of *D. suzukii* from the area of origin for the presence of pathogens.

Materials and methods: Bait traps with fruit-based diet were exposed or infested fruits of different plants were collected in various habitats to obtain parasitized *Drosophila*-specimen for subsequent cultures of parasitoids. Predatory species were obtained from lab rearings. Beneficials were exposed to different developmental stages of *D. suzukii* in a step-wise manner from petri-dish to "on fruit" bioassays to estimate host acceptance and control efficacy. Samples of larval and adult *D. suzukii*, collected by Dr. Nakai (Tokyo University of Agriculture) and shipped to JKI, were subjected to dissection. Squash preparations were investigated by phase contrast and some by electron microscopy.

Results and conclusions: Females of the parasitoid *Leptopilina heterotoma* readily oviposited into larvae of *D. suzukii*, but parasitization was not successful. Larvae of *Chrysoperla carnea* and adults of *Orius majusculus* preyed on eggs, larvae and puparia, directly exposed to them. However, developmental stages of *D. suzukii* within infested cherries and raspberries were not sufficiently attacked by the predators. Laboratory bioassays with isolated fungi belonging to the Entomophthorales and with selected bacteria against larvae of *D. suzukii* were not successful. Obviously, *D. suzukii* is still resistant against these natural enemies. Our investigations will continue with the aim to find more effective antagonists, both in the native range but also in the new invaded area.

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Drosophila Suzuki II

O DSU II-3

IPM turned upside down: response to *Drosophila suzukii* in eastern United States berry crops

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Introduction: Integrated management programs for insect pests in berry crops have been severely disrupted by the invasion of *Drosophila suzukii* in the eastern US. This required a rapid search for pest management tools to prevent crop loss. The immediate response has been to focus on pest ecology, design and development of trapping systems, investigation of cultural controls, evaluation of crop protectants, and the treatment of infested fruit post-harvest.

Objectives: To develop integrated programs for effective management of *Drosophila suzukii*.

Methods: Studies are underway to explore pest phenology, distribution, and the relationship between fly capture and infestation risk. This pest's exploitation of wild hosts has been monitored and efforts are underway to identify species with the potential to influence crop risk. Netting to enclose plantings has been tested in raspberry and blueberry plantings in New York and Michigan. In high tunnel settings, a fixed application system has been developed to allow rapid treatment of raspberries, providing high quality fruit with minimal infestation, and this was evaluated during 2013 and 2014. Laboratory and field studies were conducted to identify the most effective insecticides for use in berry crops, also providing insights into the use of additives to enhance efficacy. These results are being integrated with population modeling to explore different scenarios of chemical control for controlling *D. suzukii* in fruit crops.

Results: The phenology of *D. suzukii* is proving to be generally consistent relative to fruit maturation, but with annual and local fluctuations based on weather conditions. Traps are useful for initial detection of fly activity and for relative abundance across farms and habitats. Netting to exclude *D. suzukii* is effective and is being adopted at small scale farms. The use of a fixed spray system has allowed rapid treatment of plantings, with encouraging results for future optimization. Implementation of chemical control programs based on knowledge of efficacy and residual activity has greatly reduced the levels of fruit loss.

Conclusion: This presentation will review the post-invasion changes to IPM in berry crops in our region, and will discuss future directions to improve the sustainability of *D. suzukii* management.

O DSU II-4

Controlling *Drosophila suzukii* in Western North America sweet cherries

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Introduction: *Drosophila suzukii* Matsumura (Diptera: Drosophilidae) quickly became a serious pest of sweet cherries, *Prunus avium*, in Western North America after its detection in California in 2008. *D. suzukii* damage was first reported from Hollister, CA in March, 2009. Later that year, *D. suzukii* were found in Oregon, Washington and British Columbia, Canada. Since then, growers have used intensive insecticide programs to control *D. suzukii*.

Objectives: Monitoring and control options for *D. suzukii* in sweet cherry were investigated.

Materials and methods: Trap designs and attractants for monitoring adult *D. suzukii* were compared. Several cherry cultivars were evaluated for susceptibility when ripening. Cherry trees were treated with insecticides in the orchard, fruit and foliage was collected and brought back to the laboratory, and adult *D. suzukii* mortality was then assessed in bioassay arenas. The optimal timing of sprays to prevent *D. suzukii* damage to cherries was investigated. Studies are ongoing to relate *D. suzukii* emergence and population levels to the previous winter's temperatures.

Results: Studies demonstrated that red and yellow colored traps were the most effective for trapping adult *D. suzukii* in red-fruited crops. Commercially manufactured attractants are available in addition to user-mixed baits, but effectiveness varies by region and year. Cherries are susceptible to attack when they start to turn straw color. For three years successively, the early ripening cultivar 'Santina' escaped attack while the late ripening cultivar 'Staccato' was always attacked pre-harvest. Insecticides containing spinosad, spinetoram, OPs, carbamates, or pyrethroids are the most effective for controlling *D. suzukii*. Insecticides applied close to harvest are critical. Mild winters are associated with increased risk of *D. suzukii* damage the following growing season in cherry production regions north of California.

Discussion: Adult *D. suzukii* can be monitored but treatment thresholds are not currently available. Cherry growers are able to control this pest with insecticides but to the detriment of established integrated pest management (IPM) programs for other pests. Future research will focus on developing sustainable IPM programs for managing *D. suzukii*.

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Drosophila Suzuki II

O DSU II-5

Aiming to control *Drosophila suzukii* in the UK

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Introduction: The invasive pest, Spotted Winged Drosophila, *Drosophila suzukii* (Matsumura) has a wide host range, infesting many varieties of soft fruits. Much economic damage has been caused in various regions around the world where *D. suzukii* has become established. It has been recorded in the UK since the end of 2012.

Objective: To determine efficacy of a range of products, both chemical and biological, for the control of *D. suzukii*.

Materials and methods: Both direct and indirect exposure to chemical products was assessed. Direct effect of chemicals was carried out using an automatic-load Potter precision laboratory spray tower. Indirect effects were assessed via fruit dipping techniques. Invertebrate biocontrol agents were screened for efficacy against various life stages of *D. suzukii* using experimental Tashiro cages.

Results: Spinosad, chlorantraniliprole and an experimental product TA2674 showed excellent potential as control agents when used as either a pre or post-dipping treatment for blueberries with mortalities of 100, 93 and 98% mortality, respectively, being achieved following pre-treatment. Direct spray application of all products tested had limited impact upon adult flies. Highest mortality (68%) was achieved following direct application of TA2674. Entomopathogenic agents (nematodes and fungi) tested appeared to reduce fly population development (ranges of 34-44% mortality obtained) but would seem unable to control outbreaks. In regards to commercially available invertebrate predatory species *Anthocoris nemoralis* and *Orius laevigatus* produced the highest mortality rates of 44 and 36% respectively.

Conclusion: Several chemical products, under laboratory testing, offer excellent control of *D. suzukii*. The biological agents also offer much potential to be incorporated into integrated control strategies. The potential of developing integrated pest management strategies against *D. suzukii* is discussed.

O DSU II-6

Efficacy of insecticides against *Drosophila suzukii* on cherries

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Introduction: The Spotted Winged Drosophila, *Drosophila suzukii* (Matsumura) (Diptera: Drosophilidae) is an invasive, polyphagous and destructive fruit pest native to Southeast Asia, recently introduced into Europe. It is known to infest commercial soft and stone fruits causing extensive economic damage as opposed to most species of drosophilids which only infest overripe, fallen and rotting fruits. Development of integrated pest management strategies have become an urgent need for the control of this serious pest.

Objectives: The approach undertaken is included in the DROPSA European Project which has been designed to develop effective, innovative and practical strategies to protect major European fruit crops from *D. suzukii*.

Materials and methods: Considering the use of insecticides for the control of *D. suzukii* can be critical since they can damage ripening fruits. We tested the efficacy of products that have a favourable toxicological profile. Eighteen insecticides with various modes of action, belonging to different chemical groups, such as, organophosphates, pyrethroids, neonicotinoids, and biological were applied to cherries under laboratory conditions. Adult mortality, oviposition and larval activity reduction following application of the insecticides, was recorded over various time periods.

Results: In general, pyrethroid compounds showed good potential as control agents with mortalities of 79 and 94% respectively following 24 and 72 hours after application. The results following 72 hours after application proved that superior oviposition reduction was achieved by the treatment of lambda-cyhalothrin; giving a reduction of 99%. This was followed by deltamethrin (98%) and spinosad (91%). The highest percentage of cherry fruit damage was recorded following thiamethoxam and acetamiprid treatments.

Conclusion: It can be concluded that pyrethroids may have an important role in reducing infestations of *D. suzukii* in cherry orchards. The efficacy of neonicotinoids was not satisfactory. Further investigations under open field conditions are recommended.

Oral Presentations

New and Emerging Pests and Diseases II

O NEW II-1

Pathogenicity and population structure of *Magnaporthe oryzae* causing barley blast in Brazil

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Brazil is a hot spot for blast disease (*Magnaporthe oryzae*, anamorph *Pyricularia oryzae*) because severe epidemics occurred on important winter crops such as wheat, triticale, rye, barley and oat. They are still confined to South America but considered a global threat due to inefficacy of control and potential of spread to other continents. The first outbreak of barley blast occurred in 1998 and is currently distributed all over the Country. One particular feature of this disease is symptoms on all aboveground parts, distinguishing it from wheat blast, the first host among winter cereals. Due to little information, this work aimed to examine pathogenicity and population structure of the causal agent of barley blast in Brazil. For pathogenicity, 25 *M. oryzae* isolates from different tissues of barley were inoculated on five cultivars of barley, three of wheat, two of oat and rice, one of rye, corn, sorghum, and triticale, besides weeds: *Cenchrus echinatus*, *Setaria geniculata*, *Brachiaria plantaginea*, *Eleusine indica*. The genetic divergence of 41 blast isolates of barley was investigated through 15 RAPD primers. Also, five isolates of wheat were included as reference. The similarity matrix was obtained using the Jaccard coefficient and the clustering method applied was UPGMA, after converting the similarity in genetic distance. Pathogenic data showed that there was no resistant cultivar among winter crops. On the other hand, rice and weeds were immune to all barley isolates whereas reaction of corn and sorghum varied according to fungus isolate. The genetic divergence analysis identified four different groups, considering 0.5 as threshold. The largest group encompassed 37 barley isolates and three of wheat, the second cluster gathered three barley isolates, the third and fourth groups contained one single isolate each, from barley and wheat. Still, a simulation to examine if the number of employed markers was adequate indicated that the 50 loci generated by these primers provided sound results, once 50% of the estimated CV was below 25%. These results indicate high diversity among four groups, demonstrating that genetically different populations are causing blast disease either on wheat and barley in Brazil.

O NEW II-2

Banana Fusarium wilt: Resurgence of a catastrophic plant disease

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Half a century ago, one of the most destructive diseases in agricultural history was brought to an end. In Latin America, Fusarium wilt has destroyed Gros Michel bananas to the point where it had to be replaced by Cavendish cultivars in order to save the international export industry. Cavendish bananas now make up more than 40% of all bananas grown in the world to supply consumers of the sweet dessert fruit globally. In most tropical countries, however, local banana varieties are grown for food and income to small growers, thereby ensuring their livelihoods. The discovery of a new strain of the banana Fusarium wilt fungus, called *Fusarium oxysporum* f. sp. *cubense* (Foc) tropical race 4 (TR4) in Asia in the 1990s led to devastating losses of Cavendish bananas planted in monoculture primarily, but also to varieties planted by small producers. Foc has a larger host range than other races of Foc, and with a global replacement for Cavendish bananas not readily available, poses a significant threat to production worldwide. Foc TR4 has been discovered in the Middle East in 2012 and soon thereafter in Mozambique. The latter discovery was of significant concern, as banana serves as staple food to millions of people in east, central and western African. Research efforts in Asia in the past two decades have developed somaclones of Cavendish bananas tolerant to Foc TR4, and modified production systems in order to reduce the impact of the disease in small grower fields. It is, however, the intercontinental spread from Asia which moved the FAO to call for international support and develop a global programme to prevent a global epidemic. The objective is to manage Foc TR4 in areas where the disease is present, and to prevent its spread to unaffected areas by awareness raising, the introduction or legislation, and the development of capacity to deal with the disease.

O NEW II-3

Management of blackleg and slow wilt in seed potatoes: a supply chain perspective

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Introduction: Potato blackleg and slow wilt caused by bacterial species in the genera *Pectobacterium* and *Dickeya* threaten seed potato production worldwide, causing massive economic damage. Up to now, research on disease control has focused on field level. Yet, disease dynamics are multiannual and to a large extent determined by supply chain activities, which calls for a different approach.

Objectives: We aim to provide insight into blackleg dynamics and control in the Dutch seed potato supply chain, by means of a mathematical bio-economic model.

Materials and methods: The model follows a discrete-time state-transition approach commonly used in epidemiological modelling (Frid et al., 2013). It monitors the fraction of infected lots in six compartments representing successive quality classes, and calculates the direct costs of monitoring and control. It was parameterised in an iterative process with bacteriologists and field experts.

Results: Three scenarios were simulated: a default scenario (current situation), a Hygiene+ scenario (reducing transmission), and a Monitoring+ scenario (laboratory testing of S lots). Under the default scenario, the fraction of detected lots ranges from almost zero in the S classes to 28% in class E (Fig. 1). The alternative scenarios significantly reduce these fractions, causing a net decrease in costs (Table 1). However, producers of class S lots pay the price of achieving large savings in downstream classes. The costs of increased hygiene were not quantified as its implementation is flexible and farm-specific, but they may reach up to 181 euro per hectare to still be cost-effective.

Figure 1: Annual fraction of detected lots per class.

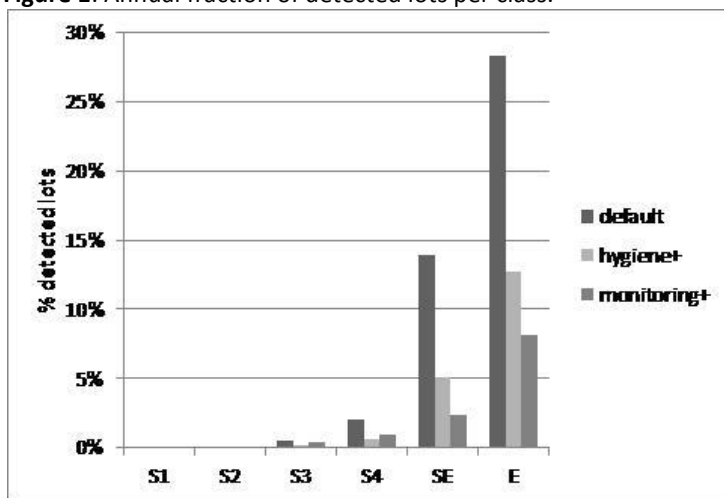


Table 1: Annual direct costs of blackleg prevalence (* 1,000 Euro).

Scenario	Quality classes						total
	S1	S2	S3	S4	SE	E	
Default	42	49	115	94	3,893	7,410	12,449
Hygiene+	42	49	93	49	1,710	3,867	6,248
Monitoring+	102	109	164	711	1,049	2,821	4,956

Conclusions: These results illustrate that blackleg control is cost-effective, but cannot always be justified from a private farmers' perspective. By providing transparency into cost-effectiveness of particular control measures, the model contributes to stakeholder support. Our approach is applicable to other diseases as well and facilitates a multi-disciplinary approach in plant disease management.

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New and Emerging Pests and Diseases II

O NEW II-4

Slugs and Snails - Pest Status, Control and Future Prospects

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While numerous entomologists are deployed throughout the world to develop IPM programmes for insect pests, one group of crop pests remains largely neglected by researchers. European slug species are frequent pests of a broad range of agricultural and horticultural crops. In arable crops most damage is done to emerging seedlings, whereas in vegetable crops, slugs cause severe economic damage throughout the cropping cycle. These European slugs, particularly the grey field slug *Deroceras reticulatum* have become serious invasive pests in North and South America, Australasia, and parts of Asia. Other invasive species of note include the South American golden apple snail that is a serious invasive pest of rice crops in many parts of South East Asia, and the Giant African Snail that has spread to much of Asia and the Pacific islands and parts of mainland USA. Traditionally molluscs have been controlled with bait pellets containing either metaldehyde or carbamates, but recently chelated iron phosphate compounds have developed. Much research has concentrated on improved formulations to increase palatability of bait pellets and persistence in the field, particularly under wet conditions when molluscs are most damaging. More novel approaches include use of nematode parasites as biological control agents, which are mass produced and sold in Europe, and the development of molluscicidal seed treatments to protect newly emerged seedlings. This paper will review current status and future prospects for pest control.

O NEW II-5

Emerging diseases associated with co-infection of phytoplasmas and other phloem limited pathogens in the state of Baja California Sur, Mexico

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In the Mexican state of Baja California Sur (BCS) during the last decade the phytoplasmas were revealed in different crops and wild plants with multiple symptoms of yellow-type diseases. A high incidence of emerging diseases with symptoms of strong foliar malformations, crinkled leaf veins and other diverse symptoms was noted in some tomato, pepper and citrus plantings, suggested a possible mixed infection with different vascular pathogens, known to cause the similar symptoms. Disease indexing, scanning electron microscopy (SEM) and molecular techniques were used to prove this hypothesis. Samples of foliar and floral parts and roots were collected from symptomatic and asymptomatic field grown plants and grafted test-plants and processed for SEM analysis. For molecular detection of phytoplasmas and begomovirus, the total DNA was extracted and nested PCR was performed with phytoplasma and begomovirus reported primer pairs. Selected PCR products were cloned and sequenced. Sequence analysis was made in GenBank database using BlastN and ClustalV methods. Phytoplasma cells ranging from 400 to 1800nm were observed in phloem tissue of all analyzed symptomatic and some asymptomatic samples. In tomato and pepper along with phytoplasmas groups of geminated particles characteristics of geminiviruses (*Geminiviridae*) were detected, as well as some rod shaped bacteria. In samples from diseased citrus trees phytoplasmas were detected together with large (2500 nm) rickettsia-like bacteria. A high content of crystals and starch granules were noted in diseased samples, a phenomenon reported in the case of phytoplasma and liberibacter infection. Analysis of samples using molecular techniques proved the phytoplasma infection and revealed a mixed infection with two begomoviruses in tomato and pepper along with phytoplasmas. Identified phytoplasmas associated with emergent diseases in BCS belong to ribosomal groups 16SrI and 16SrIII. The application of SEM together with molecular tools facilitate the diagnosis of some emerging diseases with a complex yellow-type symptoms, detecting mixed infection of phytoplasmas with viruses and fastidious phloem inhabiting bacteria. The work is in progress to prove by molecular techniques the cases of mixed infection with phytoplasmas and rod shaped bacteria in some citrus species.

O NEW II-6

Controlling potato cyst nematodes: new challenges ahead

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Potato cyst nematodes (*Globodera rostochiensis* and *Globodera pallida*) are highly damaging pests of potato. Despite the restricted host range, they are extremely difficult to control because of their survival strategy. Long crop rotations will limit their impact on the potato crop but in modern production systems this is economically not viable. Resistant cultivars play an important role in the control of these pests. Cultivation of *G. rostochiensis*-resistant potatoes has led to the selection of *G. pallida* in several regions and in some areas the latter species is the dominating species at present. Control of this species will become the main challenge in the coming years: Whereas potato cultivars with full resistance to pathotypes or virulence groups of *G. rostochiensis* are widely available for most production systems, this is not the case for *G. pallida*. Selection for virulent populations of *G. pallida* has been hypothesized for more than 30 years. We present strong evidence that such selection has occurred in production systems conducive to potato cyst nematode development.

Potato production is now challenged in a way that was not known for decades. Recent developments in selection processes under field conditions will be presented and potential solutions that may limit the impact of these events will be discussed.

Oral Presentations
Plant Pathogen Interactions III

O PPI III-1

Characterization of the infection stage-specific effector repertoire of the Asian soybean rust fungus *Phakopsora pachyrhizi*

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Asian soybean rust (*Phakopsora pachyrhizi*, ASR) is a major threat to soybean cultivation worldwide. It differs from other rust fungi by its broad host spectrum and a reduced life-cycle during which only urediniospores are formed. Soybean varieties with resistance to all isolates of the pathogen are not yet available and therefore costly fungicide treatments are the sole option to effectively control the disease. Our strategy to combat ASR is based on detailed knowledge obtained in our lab on ASR's Janus-faced infection process that starts with the induction of cell death of directly penetrated epidermal cells which is atypically for an intrinsic biotrophic pathogen. Later on, ASR establishes biotrophy which is accompanied by haustoria formation inside mesophyll cells. We hypothesise that ASR actively modulates plant responses in the early and late infection stages by secreting different effectors that lead to cell death induction and repression of defence responses, respectively.

Aiming at the identification of these effectors, and hindered by the fact that the genome of ASR is not yet sequenced, we *de-novo* assembled the transcriptome of axenically formed appressoria and compared it to the transcriptome of isolated haustoria. *In silico* screening for transcripts encoding putatively secreted proteins revealed distinct sets of ASR specific effector candidates in both infection stages. Mass spectrometry based investigation of the secretome of early infection structures led to the identification of proteins that are likely involved in host penetration. Data on the functional characterization of these candidate genes using transient expression and gene silencing assays will be presented.

O PPI III-2

Characterisation of sensor proteins important for infection structure differentiation and pathogenic growth in *Botrytis cinerea*

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Introduction: *B. cinerea* is an ubiquitous plant pathogen that infects a wide range of fruit, vegetable and flower crops. Germination and differentiation of infection structures are triggered by plant surface signals. For these differentiation processes, the BMP1 MAP kinase cascade is required. In several plant pathogenic fungi, the signaling mucin Msb2 has been shown to be involved in surface recognition and MAP kinase activation. Another plasma membrane sensor protein, Sho1, has also been described to be involved in stress adaptation and cell wall integrity as well as surface sensing and penetration.

Objectives: By using molecular genetic, cytological and genomic/ transcriptomic approaches, we are studying the role of signaling proteins in the infection process of *B. cinerea*.

Materials and methods: Mutants defective in the Msb2 and Sho1 sensors, as well as GFP-reporter strains were generated and characterised, using growth and infection tests, microscopy, phosphorylation assays, and transcriptomic studies.

Results: BMP1 and Msb2 regulate an overlapping set of genes encoding secreted proteins during germination. BMP1 phosphorylation levels during germination on hard surfaces were strongly reduced in the *B. cinerea* *msb2* mutant, compared to the increasing phosphorylation in the wild type. The *msb2* mutant showed abnormal germination and the germlings were almost unable to form appressoria or infection cushions. Later stages of infection occurred normally in the *msb2* mutant and infection tests only revealed a moderate delay in lesion formation. In contrast, the *B. cinerea* *sho1* mutants showed almost normal germination and penetration but usually failed to form expanding lesions. Increased phosphorylation of the stress response MAP kinase Sak1 in the *sho1* mutant, and enhanced H₂O₂ levels in the infected tissue indicated that the *sho1* mutant experiences increased stress during infection. First data suggest that the *sho1* mutants shows reduced acidification and altered secretion of carbon acids during infection.

Conclusions: While Msb2 functions as a hard surface sensor that triggers appressorium formation via activation of the BMP1 MAP kinase, Sho1 plays no obvious role in BMP1-MAPK cascade activation and seems to have a different function than in other plant pathogens.

O PPI III-3

Genetic and phenotypic diversity of *Botrytis cinerea* and related species

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Introduction: *Botrytis cinerea*, a ubiquitous necrotrophic broad host range pathogen of fruit and vegetable crops, represents a species complex, however, its genetic structure has remained elusive. *B. cinerea* is closely related to the host specific *B. fabae* and *B. calthae* and the host non-specific *B. pseudocinerea*. The molecular basis for their different host range is unknown.

Objectives: We have investigated the phylogenetic relationships of *B. cinerea* groups and related *Botrytis* species, to correlate them with differences in their phenotypic traits and infection behaviour.

Methods: Multilocus sequence typing (MLST) and genome sequencing was used for classification of strains from *B. cinerea*, *B. fabae*, *B. pseudocinerea* and *B. calthae*.

Results: *B. cinerea* isolates from a large scale sampling revealed the existence of distinct genotypes, referred to as groups N, S, B and I, based on genome sequencing and MLST. Although the groups are still interfertile, they have diverged from each other and show different degrees of host preference. *B. cinerea* group N is widely distributed and highly prevalent on grapes, group S is dominating in German strawberry fields, and group I is largely restricted to the monocot plant *Iris pseudacorus*. The dominance of *Botrytis* group S seems to be correlated with their increased accumulation of multiple fungicide resistance mutations in the fields, including a special efflux-mediated multidrug resistance phenotype, called MDR1h. Comparative genome analysis revealed the existence of group- and species-specific genes and toxin biosynthesis gene clusters, including those for botrydial and botcinic acid biosynthesis, in *B. cinerea* and *B. pseudocinerea*. Knock-out mutagenesis was performed with these genes to reveal their role in determining host preference.

Conclusions: The assessment of genetic diversity and comparative functional genomics are promising approaches to reveal the molecular basis of host specificity in *B. cinerea* and related *Botrytis* species.

O PPI III-4

Expression changes in *Hevea brasiliensis* roots in response to *Rigidoporus microporus* infection

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Question: *Hevea brasiliensis* is the most widely cultivated species for commercial production of natural rubber. One of the major threats to rubber plantations is the soil borne pathogen, *Rigidoporus microporus*. This fungus attacks root tissue causing rot and eventual tree death. 5-10% of rubber planted land is lost due to White Root Disease (WRD) every year.

Diseased rubber trees are difficult to diagnose and those displaying above ground symptoms are most often beyond treatment. Therefore, the ability to detect the pathogen infection at early stage is of crucial importance.

The aim of this study is to identify stress-related genes through the analysis of *Hevea* root transcriptomes infected by *Rigidoporus microporus*.

Methods: One year-old rubber plants were grown in poly-bags and infected with two different *Rigidoporus microporus* isolates. Roots from infected and control plants were harvested at 8, 12 and 15 days after infection resulting in 15 samples, biological replicates inclusive. Total RNA was extracted for sequencing using the Illumina HiSeq system.

Data obtained were mapped to the annotated rubber genome (generated by MRB); differentially expressed genes were identified using the Tuxedo protocol.

Results: Data identified 27,000 genes expressed in roots out of 45,000 annotated in the *Hevea brasiliensis* genome. In infected plants 1,660 genes were found to be down-regulated and 1,300 up-regulated.

Results showed that a number of key enzymes involved in cell wall, lignin and pectin biosynthesis were significantly down-regulated in the infected roots. The primary metabolism gene cluster such as PK (pyruvate kinase) and MDH (malate dehydrogenase) genes appeared to be down-regulated, while R-genes (Resistance genes) were up-regulated. Genes for RNA-mediated silencing components, ARGONAUTE and DICER, were up-regulated, suggesting pathogen-induced silencing of host genes. Similar trends were observed in protein expression profiles of the same samples.

Conclusions: This study provides a general view of gene expression profile changes in *Hevea brasiliensis* roots when infected by WRD. Our findings indicate a scenario where the host root is weakened at its primary defense (cell wall) 8 days after infection. This is followed by a reduction of metabolic capacity; as a result, the host becomes debilitated and therefore easier ground for colonisation.

O PPI III-5

Signaling pathways activated after exposure to phytoalexins: an Achilles'heel for necrotrophic fungi?

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Question: Due to their infection strategy, fungal necrotrophs are exposed to several antimicrobial defense metabolites during host infection. For successful colonization of host tissues, these fungal pathogens may have therefore developed efficient adaptation responses to overcome such chemical stress. Better knowledge on these protection mechanisms should allow the design of new strategies to control fungal diseases.

Methods: *Alternaria brassicicola*, the causal agent of the black-spot disease of *Brassicaceae*, was used as model fungal species. Transcriptomic and functional genomic analyses were performed to unravel the key response regulators of the fungus exposed to indolic phytoalexins. A toxicologic approach was used to interfere with some of these master regulators and identify chemical compounds that act synergistically with phytoalexin to inhibit fungal growth in vitro and in planta.

Results: Signaling pathways involved in the response to cell integrity (CWI) and unfolded protein (UPR) stresses were found activated in fungal cells upon exposure to indolic phytoalexins (Joubert et al., 2011a, b). Inhibitors of protein kinases C (CWI) were showed to act synergistically with phytoalexins to inhibit fungal growth in vitro (Simoneau et al., 2013). Pathogenicity assays conducted on cabbage in the presence or absence of these inhibitors revealed that they were very efficient to limit fungal progression in planta (Figure 1). Similar results were obtained with other tested pathosystems (*A. dauci* and *A. radicina* on carrot and *B. cinerea* on *Arabidopsis*)

Conclusions: Mixing inhibitors of fungal kinases involved in CWI and UPR pathways with plant defense elicitors might constitute a good strategy to improve plant protection against fungal diseases caused by fungal necrotrophs

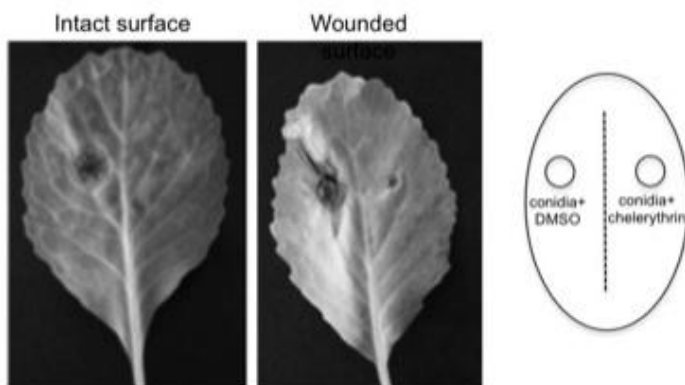
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Simoneau et al. (2013) *European Patent* PCT/EP2013/063574

Figure 1: Effect of a PKc inhibitor on the development of the black spot disease on cabbage leaves inoculated with a virulent *A. brassicicola* strain



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Plant Pathogen Interactions III

O PPI III-6

Breaking the spell - Are host hormone responses the key to parasitism of witchweeds and related parasitic plants?

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Introduction: Parasitic plants and in particular species of the Orobanchaceae family are serious pests of many crop plants. Among them witchweeds (*Striga spp.*) show persistent outbreaks in Sub-Saharan Africa. This often results in immense financial losses and severe socio-economic consequences for affected communities. Potential hosts include major staple crops such as sorghum, maize, pearl millet, rice or cowpeas. The interaction between the parasite and its host is complex and molecular events remain largely elusive.

Objectives: We aim to unravel infection and disease promoting strategies of parasitic plants by using state of the art methods of genome and transcriptome sequencing as well as non-invasive live cell imaging.

Material and methods: Our studies focus on *S. hermonthica*, *S. asiatica* and *Phtheirospermum japonicum*, a hemi-parasitic Orobanchaceae plant, which is native to Japan and accessible to genetic modifications. We generated a comprehensive library of genetic and transcriptomic resources and monitored host responses in the model plant *Arabidopsis thaliana*.

Results: Mutants in of the host auxin signalling module controlled by SOLITARY ROOT showed enhanced resistance to *S. hermonthica*, *S. asiatica* and *P. japonicum*. Furthermore, downstream components of this signalling pathway were activated during early stages of parasitism. Later stages of infections dramatically altered additional host hormone responses.

Conclusions: Our experiments provide insights in the complex interaction between host and parasitic plants. It highlights the importance of host hormone responses and may represent a common phenomenon in plant-plant parasitism. Monitoring and altering host hormone responses could promote development of early diagnosis methods and resistances.

Reference: Spallek T, Mutuku M, Shirasu K (2013). The genus *Striga*: a witch profile. *Molecular Plant Pathology* 14: 861-869 (Review).

Oral Presentations

Botanicals

O BOT 1

Biocontrol and botanical substances: Innovative methods for evaluation of essential oils on a triazoles resistant strain of *Venturia Inaequalis*

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Carried by the french Technical Institute of Organic Agriculture (ITAB) and financed by the Ministry of Agriculture, the CASDAR project entitled "Evaluation of the interest of using essential oils in crop protection" (2013-2015) proposes to study the biofungicide effectiveness *in vitro* and *in planta* of several essential oils on several biological models.

Here, the aim of the study is to test the efficacy of 7 essential oils on spores of *Venturia inaequalis*, the fungus that causes apple scab (70% yield loss). Two strains were used: a resistant strain to fungicides (triazoles) compared to a sensitive strain. A range of concentrations of seven essential oils and two references fungicides (metconazole and copper sulfate (CuSO₄)) are tested on two strains. The experiments were performed in a liquid medium in microplates and are carried out at least three times in independent way. The fungicidal effectiveness is modeled by the calculation of the IC₅₀. The IC₅₀ values of the tested products is compared by an F-test within a nonlinear regression approach. Among the 7 essential oils, the results show that some are significantly more effective than others on the two strains. The results also show that the resistant strain to metconazole is much more sensitive to copper than the sensitive strain. Similarly, the resistant strain seems sometimes to be more sensitive to essential oils than the sensitive strain. If metconazole remains the most effective fungicide molecule on the 2 strains, essential oils are as or more effective than copper on the sensitive strain. These results suggest that the behavior in the laboratory of the R and S strains triazoles is different not only to copper but also to the essential oils. Tests orchards will show the interest to essential oils in organic production methods (compared to copper) and in more conventional production methods.

O BOT 2

New natural bioactive products against *Fusarium sp* on Food legumes

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In Morocco, Food legumes are more important in traditional dishes and for basal alimentation as source of proteins. Unfortunately, abiotic and biotic stress are decreasing yield. The main stress is diseases especially due to fungus. During the prospection done in two principals regions of production of food legumes in Morocco (Marchouch and Ain Sbit), we observed that the wilt of chickpea and lens are due to two causal agents respectively *Fusarium oxysporum* f. sp. *Ciceris* and *Fusarium oxysporum* f. sp. *lentis*. Unfortunately resistant genes are not effectives in our country. In order to control this disease, we look for a new natural bioactive products acting against the *Fusarium* wilt of the two food legumes. They are four plants : *Artemisia herba alba*, *Lavandula stoechas*, *Daphne gnidium* and *Allium sativum*, used in traditional medicine that are evaluated for the first time *in vitro* towards these two forms of *Fusarium*.

The results obtained have shown that *F. oxysporum* f. sp. *ciceris* and *F. oxysporum* f. sp. *lentis* are sensitive to the four medicinal plants extracts. However, their inhibitory powers are different. Indeed, *Daphne gnidium* followed by *Artemisia herba alba* induced the higher inhibition percentage of radial growth. *Allium sativum* gave the lowest effect after *Lavandula stoechas*. These results show a good antifungal activity to limit and even stop the development of the pathogen.

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O BOT 3

Relationship *Parlatoria ziziphi*-Essential oil of *Citrus aurantium* leaves, its insecticidal activity and chemical characterization.

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The essential oil yield of four *C. aurantium* leaves samples, from March 17 to May 9, 2013 obtained by hydro-distillation. It varies depending on plant health status from 0.4% to 0.8% in healthy, during the infestation per *P. ziziphi* it is stable (0.008%). Jean Kuate *et al.*, (2003) mentioned that *Phaeoramularia angolensis* is the origin of yield loss. GC-MS Analysis showed a rat of essential oil components of healthy *C. aurantium* leave is (99.25%). Most of them are monoterpene hydrocarbons. Three chemotypes are probable characterized by differentiated levels, the Linalyl acetate (68.69%), Linalool (10.50%) and Limonene (6.20%) those represent 85.39% of the identified products. Three dilutions of this oil are used as treatment against the first instars' of *P. ziziphi* on lemon young glasshouse. The global rate of the first larval stage is reduced by D1 (0,4µl / ml) to 48.56%, by D2 dose (0,2µl / ml) to 69.3% and by D3 dose (0,1µl / ml) to 71.3% on the 3rd day. Dose and time contact of essential oil have a highly significant influence on the abundances variation of residual populations of *P. ziziphi* (p is signaled by Regnault-Roger, *et al.* (2008) on mosquito (*M. Domestica* L.) and by JUNG-Ok KONG, *et al.*, (2006) on nematode *B.s xylophilus* that mortality reached 85% at 24H.

References: Jung-Ok Kong, Sang-Myung Lee!,Yil-Seong Moon!, Sang-Gil Lee! and Young-JoonAhn, 2006. Nematicidal Activity of Plant Essential Oils gainst *Bursaphelenchus xylophilus* (Nematoda: Aphelenchoididae)School of Agricultural Biotechnology, Seoul National University; Seoul 151-921, Republic of Korea[Southern Forest Research Institute, Korea Forest Research Institute; Jinju 660-300, Republic of Korea J. Asia-Pacific Entomol. 9(2): 173-178.

O BOT 4

Protein hydrolysates as resistance inducers to Downy Mildew in Grapevine

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Downy mildew, caused by *Plasmopara viticola*, is one of the most important grape pathogen in Europe and North America. Although the control is traditionally performed with fungicides, the appearance of resistant pathogen populations and the possible adverse effects on human health and the environment are spurring the search for alternative strategies. In the present investigation, two protein hydrolysates of soybean (*soy*) and casein (*cas*) origin were successfully tested against *P. viticola*. On *Vitis vinifera* cv. Marselan plants, the application of *soy* and *cas* reduced the infected leaf surface by 76 and 63%, as compared to the untreated control, respectively. Since both hydrolysates seemed to trigger the plant immunity, we investigated their effect on selected grapevine defense responses. On treated grapevine cell suspensions, a different free cytosolic calcium signature was recorded for each hydrolysate, whereas a similar transient phosphorylation of two MAP kinases of 45 and 49 kDa was observed. These signalling events were followed by transcriptome reprogramming, including the up-regulation of genes encoding pathogenesis-related (PR) proteins and the enzyme stilbene synthase responsible for the biosynthesis of resveratrol, the main grapevine phytoalexin. Liquid chromatography analyses confirmed the production of resveratrol and its dimer metabolites, δ - and ϵ -viniferins. Overall, *soy* effect was more pronounced than *cas* one. Both hydrolysates proved to be able to enhance grapevine immunity against pathogen attack.

O BOT 5

Ovicidal and larvicidal activity of *Mentha longifolia* free and nanoencapsulated essential oils on *Tuta absoluta* (Meyrick) (Lepidoptera:Gelechiidae)

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Essential oil from *Mentha longifolia* was extracted by Clevenger-type water distillation, and analyzed using gas chromatography-mass spectrometry. Nano hollow silica spheres were synthesized by sol-gel method as a *Mentha longifolia* essential oil carrier, and functionalized with thiol groups. The success of *Mentha longifolia* essential oil encapsulation was clarified by Fourier

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transform infrared (FT-IR) spectroscopy, Energy Dispersive X-ray Microanalysis (EDX), ultraviolet-visible (UV-vis) spectrophotometry, thermal gravimetry analysis (TGA), and X-ray diffraction (XRD) techniques. In addition, the shape and size of the particles have been determined by scanning electron microscopy (SEM) and transmission electron microscopy (TEM). The fumigant toxicity of *Mentha longifolia* free and nanoencapsulated essential oils on *second instar Tuta absoluta* larvae and eggs under laboratory conditions of 25 ± 1 °C and $70 \pm 10\%$ R.H. and a photoperiod of 16:8 h (L:D) were investigated. Each treatment which consisted of five concentrations and a control was replicated at least six times with 20-25 larvae (or eggs) per replicate. Probit option of SPSS was used for analyzing concentration-mortality data and estimating lethal concentrations. Mortality was corrected based on control mortality using Abbott's formula. If control mortality was more than 20%, the results were discarded and the bioassay was repeated. LC50 values of *Mentha longifolia* free essential oil on *second instar Tuta absoluta* larvae and eggs under laboratory conditions were 3.793, and 6.172 $\mu\text{L L}^{-1}$ air after 24 h, respectively. Furthermore, LC50 values of the fumigant test of nanoencapsulated essential oil on *second instar Tuta absoluta* larvae and eggs under laboratory conditions were 1.68 and 3.679 $\mu\text{L L}^{-1}$ air after 24h, respectively. LC₅₀ values were judged as significantly different, if their 95% confidence intervals did not overlap.

O BOT 6

Natural substances as non-toxic baits for trapping common voles (*Microtus arvalis*)

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Common voles (*Microtus arvalis*) can cause severe damage in agriculture due to their regularly occurring mass eruptions. To manage populations, rodenticides are applied that pose the risk of affecting non-target species. Conventional and especially organic production need practicable methods to protect crops from pest rodents.¹

Here we investigate how Trap success can be increased by using natural non-toxic substances as an attractive bait. These agents could also improve the efficacy of rodenticide application because they may minimise the effects of active ingredient and alternative food supply on bait uptake by the target species.²

Substances attracting common voles could be oils and fatty compounds as well as sugars.^{3,4} Other observations indicate a preference of rodents for familiar food sources that occur in their environment. Especially protein rich plants are preferred to gramineous plants, the usual nutrition of common voles.⁵ A variety of these natural substances were screened in a T-maze offering one vole at a time the choice between accessing a trap at the endpoint with or without an attractant. The three most promising attractants are examined further under semi-natural conditions at population scale of 8 common voles.

Results of the T-maze and the enclosure experiments will be presented and the effect of natural substances on common voles discussed.

This project is funded by Federal Office of Agriculture and Food (BLE) in the context of the federal program "Ökologischer Landbau und andere Formen nachhaltiger Landwirtschaft".

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Oral Presentations

Workshop/Film Presentation • Highlights of Hidden Insect Worlds

O WS FILM 1

Film presentation 'Highlights of hidden insect worlds'

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The film (duration 62 minutes) documents the behaviour of various small insects at high magnification, thus providing a fascinating insight into a hidden world barely visible to the human eye.

Under the title 'from monsters to divas' the film first shows, how hoverfly and lacewing larvae turn into beautiful ladies after a ferocious and greedy aphid-devouring youth. Another example is presented by the antlion *Myrmeleon formicarius*. Subsequent sequences show how the gladiator or heel-walker *Mantophasma zephyrum*, endemic in South Africa and Namibia, catches prey with amazing skill. This kind of behaviour is then compared with that of a young praying mantid.

Especially impressive are the strategies evolved by parasitic wasps (parasitoids). It is shown how aphelinid and aphidiid wasps attack their aphid hosts and how the hyperparasitoid *Alloxysta vicrix* has to calm down aphids in order to climb onto their back for oviposition. The tiny egg-parasitic wasp *Trichogramma brassicae* recognises mated *Pieris brassicae* females and then climbs upon them to be carried to the oviposition site. Amazing host feeding strategies are used by the ectoparasitic wasp *Lariophagus distinguendus* when it attacks larvae of the granary weevil *Sitophilus granarius* inside wheat grains.

The caterpillars of the moths *Plutella xylostella* and *Lyonetia clerkella* behave like ingenious architects when they construct their pupation cocoons on the leaves of their host plants.

The hole in hazelnuts is well known but little is known about how it is formed. The film shows how the hazelnut weevil *Curculio nucum* deposits an egg into a young nut and how progeny develops until the massive, fully grown larva squeezes its way out of the nut by biting an exit hole through the hard shell. The film closes with an amusing story, showing the behaviour of two brothers of a parasitic wasp (*Nasonia virtipennis*) that compete for the favour of a sister. Finally the loser becomes the winner.

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Workshop • Implications of Insect Pest Movement and Behavior on Designing Insect Resistance Management Strategies for Transgenic Crops

O WS ECO 1

Introduction to Workshop: Implications of Insect Pest Movement and Behavior on Designing Insect Resistance Management Strategies for Transgenic Crops

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Genetically-engineered (GE) crops that express insecticidal proteins from *Bacillus thuringiensis* (Bt) have revolutionized the control of several lepidopteran and coleopteran crop pests (e.g. *Ostrinia nubilalis*, *Spodoptera frugiperda*, *Diabrotica* spp.). The concern that the high selection pressure applied by widespread adoption of GE crops will lead to the evolution of insect resistance has resulted in the design of several resistance management approaches that rely on the presence of refuge to delay the evolution of insect resistance to Bt proteins. Refuge configurations range from blocks of refuge plantings within a given distance to the GE crop, block refuge plantings adjacent to the GE crop, strips of refuge planted within the GE crop, mixtures of Bt and non-Bt seed in the bag (i.e., refuge in the bag), to reliance on natural refuge. Recent research, however, has indicated that as the target pest range of some GE crops expands, the species-specific behavior of some pests may favor one approach over the other, or compromise the existing refuge strategy currently in place for a given GE event. We intend to present current research on the movement and behavior of select lepidopteran and coleopteran pest species and discuss these findings with respect to resistance management strategy design. Also, research needs related to pest biology and behavior that affects resistance management design will be considered. This information will be useful to consider for improving IRM plans for existing GM crops and developing IRM approaches for future GM crops.

O WS ECO 2

A tale of two insects: challenges managing insect resistance to genetically-engineered crops

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Genetically-engineered (GE) plants with resistance to insect pests were first commercially sold in the U.S. in 1996. These maize hybrids were unique because they produced their own insecticide, a protein from the bacterium, *Bacillus thuringiensis* (Bt), that targeted the European corn borer, *Ostrinia nubilalis*. Since then several types of Bt crops have been produced that target important lepidopteran (moth) and coleopteran (beetle) pests. One of the primary benefits of these crops has been reduced use of synthetic chemical insecticides. However, insect resistance to Bt threatens sustainability of this technology, particularly in the tropics. Currently a high-dose refuge strategy has been used for insect resistance management (IRM). Yet, some insects have become resistant to Bt crops, while others have not: two distinctive types. In the U.S. efforts are underway to take a more integrated approach to managing insect resistance to Bt crops. In short, this means that Bt maize should be considered just one tool in the toolbox for managing this insect pest. This talk will provide an overview of lessons learned regarding insect resistance with a focus on the importance of larval movement, toxin dose and refuge, and current developments toward managing insect resistance in GE crops.

O WS ECO 3

Movement and Behavior of European Corn Borer Larvae on Non-Bt and Bt Corn

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European corn borer females deposit clusters of approximately 25 eggs on their host plants. The movement and behavior of neonates and later instars that result differs depending on whether the natal plant is non-Bt or Bt corn. These differences in outcome for developing larvae resulting from egg clusters may influence how we design refuges for this and perhaps other lepidopterans on crops with genetically incorporated insect protection.

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O WS ECO 4

Movement of Larval Western Corn Rootworm and Implications for Management of Resistance to Bt Corn

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In the United States and elsewhere, management of resistance to Bt crops by pest insects uses the refuge strategy, in which a non-Bt host plant serves as a refuge that enables the survival of Bt-susceptible genotypes. In recent years, the configuration of refuges within fields has moved from a stand-alone block (i.e., structured) refuge to an integrated refuge in which Bt and non-Bt plants are interspersed within a field. An integrated refuge increases compliance of farmers in planting of a refuge and places insects from refuge plants in closer spatial proximity to Bt-selected insects. Both of these factors are expected to delay the onset of resistance. However, movement of insects between refuge and Bt plants can increase the survival of heterozygous resistant insects and accelerate the rate of resistance evolution. In this talk, I will review data on the interaction between larval corn rootworm and Bt maize with respect to feeding preference, movement and survival. I will synthesize these results and discuss the implications for the use of integrated versus structured refuge in management of resistance to Bt corn by western corn rootworm.

O WS ECO 5

Effect of Cry1F corn on the behavior of susceptible and resistant fall armyworm and European corn borer

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Understanding the behavior of pests targeted with *Bacillus thuringiensis* (Bt) crops is important to define resistance management tactics. Particularly, the study of larval movement between plants is important to determine the feasibility of different refuge configurations. Studies suggest that exposure to Bt corn increases larval movement in several lepidopteran species. However, few studies have examined the potential for resistance to interact with behavioral responses to Bt toxins. Choice and no-choice experiments were conducted with *Spodoptera frugiperda* and *Ostrinia nubilalis* to determine if Cry1F resistance influences neonate movement. Leaf discs of Cry1F corn TC1507 and the corresponding isoline were used to characterize behavioral responses. In both experiments the location (on or off of plant tissues) and mortality of susceptible and Cry1F resistant neonates was recorded for five days. Analysis of larvae location was performed up to 7h after transfer to avoid mortality. Our results indicated that there is not a strong difference between resistant and susceptible phenotypes in *S. frugiperda* and *O. nubilalis*, although, a small percentage of susceptible neonates in both species abandoned corn tissue expressing Cry1F. However, significant behavioral differences were observed between species. *O. nubilalis* exhibited increased movement between leaf discs, while *S. frugiperda* selected plant tissue within the first 30 minutes and remained on the chosen plant regardless of the presence of Cry1F. *S. frugiperda* behavior observed in this experiment suggests that lack of larval movement may have implications to refuge configuration. This study represents the first step towards understanding the effects of Cry1F resistance on larval behavior. Information regarding behavioral differences between species could aid in developing better and more flexible resistance management strategies.

O WS ECO 6

The role of pest movement and behaviour in resistance development of the African maize stem borer (*Busseola fusca*) to Bt maize

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The African maize stem borer (*Busseola fusca*) developed resistance to Bt maize (Cry 1Ab) in South Africa. Several factors that may have contributed to resistance development have been identified. Although poor compliance to refuge requirements was blamed for resistance development, several other factors could also have contributed to the evolution of resistant populations, 7-8 years after release of Bt maize in the country. Resistance development highlighted huge gaps in knowledge of pest biology (for example migration patterns) and interactions in the wider agro-ecosystem (indigenous host plants) and resistance inheritance patterns. Seed mixture strategies that are being considered as IRM strategy have previously been considered not to be suitable for migrating pests. Results will be presented on migration patterns of *B. fusca* larvae inside different seed mixture

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treatments over time. Increased damage over time in seed mixtures is associated with migration of older larvae, indicating that the expressed dose does not kill larvae above a certain developmental stage. Migration of larger larvae late in the season may result in survival of individuals that developed on non-Bt plants and then migrated to Bt-plants, thereby contributing to survival of RS individuals and resistance development. IRM in Africa is faced by challenges unique to farming in subsistence and small scale systems where refuge requirements are unrealistic and difficult to manage and monitor. Furthermore, factors such as seed management practices, gene-flow to maize land races could be contributing factors to resistance development. Alternative IRM strategies such as seed mixtures may be more practical but have its own challenges and have not been proven successful yet.

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O WS FOOD 1

Description of a new predatory mite species of the genus *Agistemus* (*Agistemus burewalaensis*) Acari Stigmaeidae: Prostigmata as biocontrol agent from Pakistan.

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Mites comprise a large group of arthropods belonging to subclass acari of the class arachnida. They are biologically most diverse tiny creatures, microscopic in size and worldwide distributed. Mites of family stigmaeidae have widely been focused due to their predatory potential to regulate plant feeding mites and insect pest complex. A survey was conducted to explore the predatory mite fauna of Punjab. Permanent slides of collected specimen were prepared by using the Hoyer's medium and drawn with the help of phase-contrast microscope. The identification of species was done with the available keys & literature. The holotype of new species was collected from *Gossypium hirsutum* (Burewala) and has been described in this manuscript. Five (05) paratypes were collected from the same collection data & six (06) from the city Khanewal. All specimens were deposited in the Acarology Research Laboratory, Department of Entomology, University of Agriculture, Faisalabad, Pakistan.

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Integrated Pest Management I

O IPM I-1

IPM Components and Packages for Tropical Vegetable Crops

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The most common vegetable crops grown in the tropics are tomato, eggplant, and pepper in Solanaceae; cucumber, bitter melon, bottle gourd, pumpkin, and chayote in Cucurbitaceae; head cabbage, Chinese cabbage, broccoli, and radish in Brassicaceae; beans in Fabaceae; and okra in Malvaceae.

In the last decade, the IPM Innovation Lab has pioneered in developing IPM packages for these crops by stacking tactics developed for the pest problems of a crop from the time of planting the seeds to the harvest. Some of the components developed are: solarization of the seed bed, use of coconut pith and plastic trays for seedling production, treatment of seeds/seedlings with *Trichoderma* spp., *Pseudomonas fluorescens* and *Bacillus subtilis* for combating soil pathogens and inducement of defense, grafting seedlings of solanaceous crops on resistant rootstock to overcome bacterial wilt, use of pheromone traps for caterpillar pests, use of microbial and botanical pesticides, area wide management for fruit fly control, and adoption of conservation biological control in addition to augmentative and classical biological controls when needed.

IPM is crop, site and season specific and the components of the packages also need to be adjusted accordingly. It is dynamic and requires changes when pest scenarios change or a new pest is introduced.

O IPM I-2

A shift from Integrated Pest Management to Integrated Crop Management: a multilocation evaluation through farmers' participatory approach

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Rice is the staple food of 65% of the total population in India. India has the largest area under rice in the world and is grown in extremely diverse environments. Of the 45 million ha of rice area, about 28% is rain fed lowland, 46% irrigated, 12% rain fed upland, and 14% flood-prone. Biotic stresses are a major production constraint in rice causing large yield losses. The pest spectrum is dynamic and unique for different ecosystems. Pests are found infesting rice crop from nursery till harvest resulting in a yield loss of about 20 - 25%.

Integrated Pest Management (IPM), known since three decades was evolved from the concept of "Integrated Control" wherein only biological and chemical methods were employed for the pest control. The convergence of the concepts of integrated control and pest management, and the ultimate synthesis into integrated pest management, opened a new era in the protection of agricultural crops against the attack of arthropod pests, plant diseases and weeds. IPM is defined as a decision support system for the selection and use of pest control tactics, singly or harmoniously coordinated into a management strategy, based on cost/benefit analyses that take into account the interests of and impacts on producers, society, and the environment (Kogan, 1998). IPM strategy includes a combination of cultural, biological, physical and chemical methods in a compatible way that minimizes economic, health and environmental risks while managing pests. In any IPM, the emphasis is on creating hostile conditions for the pest growth and development while conserving natural resources. This includes crop management practices like tillage, crop rotation, trap crops, soil fertility and water management. Thus, IPM has become an integral component of integrated crop management (ICM). ICM is based on a good understanding of the interactions between biology, environment and land management systems. It is particularly appropriate for small farmers because it aims to minimize dependence on purchased inputs and to make the fullest possible use of indigenous technical knowledge and land use practices. IPM implementation at farmers level involves certain skills and knowledge that help in identification of pest and also their susceptible stages for effective management. As IPM involves a number of components, farmers must have capability of taking decisions and selecting IPM options accordingly for economical and long term management. Most of these options also need to be refined at individual farm level keeping in view the availability and feasibility of farmers. The present study was conducted under All India Coordinated Rice Improvement Program (AICRIP) at various locations across India in different rice ecosystems with an objective to validate IPM practices from a basket of options available and demonstrate to farmers the management of rice crop in a holistic way.

Experiments were carried out in farmer's fields with a local popular variety and in 4000 sq.m area of two blocks viz., IPM block and farmers' practices block. Each block was divided into five equal sized units, each representing a replication. Pest management practices were adopted depending on the prevailing pest problem and available options in IPM block and as per the farmers practice in another block. Observations on pest incidence were recorded on five randomly selected hills in each

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replication at weekly interval, starting from 15 days after transplanting. Thus, at each observation, data was recorded from 25 hills in IPM block and 25 hills in farmers practice block. The details of practices followed in both IPM and farmers practice plots were also documented. At harvest, yield was recorded from 5 x 5 m² area in each replication. Finally, cost involved for each practice taken starting from nursery to harvest in both the blocks was recorded to estimate the cost of cultivation and benefit cost (BC) ratio.

The study was conducted at 13 locations during 2012 wet season and at 10 locations during 2013 wet season (Fig.1a). Incidence of stem borer (*Scirpophaga incertulas*), leaf folder (*Cnaphalocrocis medinalis*), brown planthopper (*Nilaparvata lugens*), white backed planthopper (*Sogatella furcifera*), gall midge (*Orseolia oryzae*), whorl maggot (*Hydrellia philippina*), hispa (*Dicladisa armigera*), thrips (*Stenchaetothrips biformis*), blast (*Magnaporthe oryzae*), sheath blight (*Rhizoctonia solani*), bacterial leaf blight (*Xanthomonas oryzae*), brown spot (*Bipolaris oryzae*) and false smut (*Ustilagoideia virens*) grassy weeds like *Echinochloa* sp., *Digiteria sanguinalis*, *Paspalum distichum*, *Ichochaemum rugosum*, *Dactyloctenium* sp., sedges like *Cyperus* sp., *Fimbristylis miliaris*, *Scirpus maritimus* and broad leaved weeds like *Commelina benghalensis*, *Celosia argentea*, *Amaranthus* sp., *Ludwigia octovalvis*, *Eclipta* sp., *Ageratum conyzoides*, *Trianthema portulacastrum* were observed in both IPM and farmers practice blocks. Stem borer damage varied from 1.3 to 23.2% with maximum damage in Coimbatore followed by Ranchi (19.9%) in farmers practices block during 2012 while minimum damage was observed in Raipur and Jagdalpur during 2013 (Table 1). Leaf folder damage ranged between 1.6 and 24.86% in different locations. Brown planthopper (BPH) population fluctuated between 0 and 107 per hill while white backed planthopper (WBPH) population varied from 4 to 136 numbers per hill at different locations. Both these pests were found high in farmers' practices as compared to IPM block. Disease incidence was low during 2012 as compared to 2013 wet season. Both leaf and neck blast damage was high (233 & 138, respectively) at Gangavathi in farmers block while IPM block had high brown spot damage of 43.4 (Fig. 1b). Sheath blight (55), bacterial leaf blight (BLB-361), false smut (9) occurrence was high in farmers block at various locations. Maximum weed population of 300 numbers per sq. m was recorded in farmers practice block at Sakoli while minimum population of 4 numbers per sq. m was observed in IPM at two locations (Table 1). Similarly weed biomass was found high in farmers' practices. Grain yields were significantly higher in IPM plots compared to Farmers Practice blocks. The IPM block farmers reaped better net returns because increased yields along with reduced input costs resulted in higher benefit cost ratios ranging from 1.3 to 6.6 (Fig.1c).

The study created awareness among farmers about pests, symptoms of damage and stages observed in the field for easy identification. They were also apprised of the various management options available so that they can choose from them depending on the economic feasibility and accessibility. This paper also discusses about the lessons learnt during this experiential learning while working with farmers. About 90% farmers are adopting the management practices suggested during this study.

Figure 1

Table 1 Insect pest incidence and weed parameters at various locations

Treatments	% Dead hearts - 2012						% Dead hearts - 2013						% White ears - 2013		
	SKL	CBT	MVS	KJT	RMC	RPR	ADT	KJT	CHN	JDP	RPR	LDN	CHN	RPR	
IPM	2.8	6.6	7.2	3.2	7.3	18.6	5.8	4.7	4.2	1.3	1.3	3.5	3.5	7.4	
FP	6.6	23.2	13.6	6.0	19.9	7.3	18.4	7.2	17.2	8.8	14.1	6.7	11.4	6.4	
LSD (P=0.05)	0.4	1.5	0.4	0.7	1.0	0.6	NS	0.4	1.9	1.7	1.0	0.1	0.8	NS	
CV (%)	16.8	41.6	18.5	24.8	18.2	38.3		8.4	36.6	48.1	26.6	3.8	17.2		

Treatments	% leaf folder damaged leaves - 2012						% leaf folder damaged leaves - 2013		
	JDP	LDN	KUL	CBT	MVS	RMC	LDN	JDP	TTB
IPM	9.9	4.3	21.5	5.9	1.6	23.1	12.3	2.8	3.3
FP	18.4	9.6	24.2	12.0	3.7	76.0	24.9	8.0	5.4
LSD (P=0.05)	0.4	0.5	0.2	0.3	1.3	7.9	0.2	0.5	0.4
CV (%)	10.6	17.7	13.7	14.8	6.4	9.3	3.1	13.4	11.0

Treatment	BPH population - 2012 (Mean number/hill)				BPH population - 2013 (Mean number/hill)						WBPH population - 2012 (Mean number/hill)					WBPH population - 2013			
	JDP	SKL	GGV	MTU	GGV	MTU	LDN	CHN	JDP	RPR	DRR	LDN	SKL	KUL	GGV	MTU	GGV	LDN	CHN
IPM	2.1	11.8	37.3	33.0	46.6	0.0	75.8	0.4	12.2	4.0	1.0	4.4	15.3	113.0	31.3	37.5	69.8	38.6	6.4
FP	5.8	20.7	33.7	22.1	106.6	89.0	65.2	16.9	4.8	46.8	15.7	8.3	28.7	136.0	28.4	17.6	74.8	93.6	12.8
LSD (P=0.05)	0.6	0.9	NS	1.7	1.7	5.6	0.9	0.8	NS	0.2		1.1	1.3	1.7	NS	3.5	NS	0.9	NS
CV (%)	31.1	15.4	11.2	23.4	11.1	75.5	6.0	20.3		2.7		36.5	23.1	12.3	14.7	39.5		6.4	

Treatments	*Weed population (numbers / sq.m)						Weed biomass (g/ sq.m)				
	2012			2013			2012		2013		
	SKL	CHN	JDP	TTB	DRR		SKL	LDN	CHN	JDP	TTB
IPM	117.0	138.2	3.9	119.2	3.6		16.4	26.0	19.6	0.4	16.7
FP	300.0	227.0	19.3	178.4	22.2		42.4	49.0	30.4	6.2	60.8
LSD (P=0.05)	28.0	1.4	1.2	1.0	0.5		4.7	NS	4.9	0.4	12.3
CV (%)	7.7	7.0	22.1	5.9	11.3		9.2	114.5	13.5	22.5	21.7

ADT = Aduthurai; CBT = Coimbatore; CHN = Chinsurah; DRR = Directorate of Rice Research; GGV = Gangavathi; JDP = Jagdalpur; KJT = Karjat; KUL = Kaul; LDN = Ludhiana; MTU = Muteru; MVS = Navsari; RMC = Ranchi; RPR = Raipur; SKL = Sakoli; TTB = Talbar; NS = Not significant

* includes grassy weeds, sedges and broad leaved weeds

Figure 2

Fig 1a. IPM study locations across India under AICRIP



Fig. 1b Incidence of rice diseases in different treatments across locations

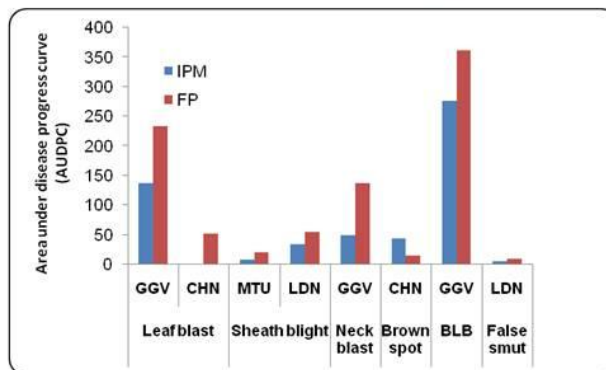
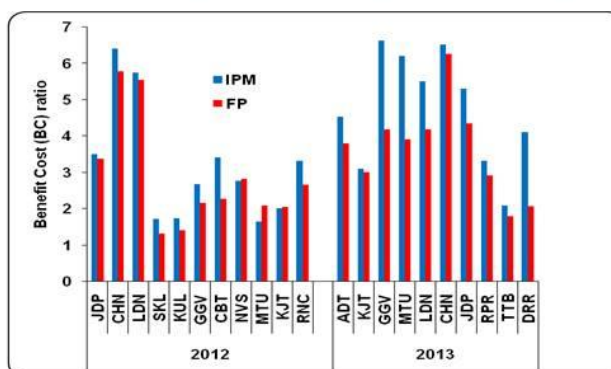


Fig. 1c Benefit cost ratio at different locations across treatments



O IPM I-3

Developing a sustainable pest management strategy for lowland brassica production systems in Asia

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Introduction and Objectives: Diamondback moth (*Plutella xylostella*) is the predominant pest in brassicas worldwide. However, it can be brought under reasonable control in highlands by a guild of parasitoids including *Cotesia plutellae*, *Diadegma semiclausum*, *Microplitis plutellae* and *Diadromus collaris* in Asia. Cabbage head caterpillar (*Crociodolomia binotalis*), cabbage web worm (*Hellula undalis*), common army worm (*Spodoptera litura*), imported cabbage worm (*Pieris rapae*) and striped flea beetle (*Phyllotreta striolata*) are pests of secondary importance in most vegetable brassica crops. These secondary pests often lack 'soft' control measures, thus vegetable growers rely on chemical insecticides for control. Indiscriminate pesticide use inadvertently kills the natural enemies of *P. xylostella*, which results in *P. xylostella* resurgence, especially in tropical lowlands. This has triggered a growing interest in alternative pest management techniques. Hence, the objective of the study was to test bio-pesticides and pheromones against secondary pests of brassicas.

Materials and methods: *Bacillus thuringiensis* δ -endotoxins and formulations were evaluated against secondary lepidopterans under laboratory and field conditions. Improved sex pheromone lures for monitoring DBM were evaluated in the field. Other approaches including trap cropping, host plant resistance and aggregation pheromones have been validated against *P. striolata*.

Results and Conclusion: The toxins Cry1A and Cry1C were equally toxic to *P. xylostella*. However, *C. binotalis* was susceptible to Cry1A toxins only, whereas *H. undalis* was highly susceptible to Cry1C only. Field trials with *B. thuringiensis* formulations also confirmed that secondary lepidoterans can be effectively controlled on vegetable brassicas. The number of *P. xylostella* adults attracted by pheromone lures which included host plant volatiles was the highest. This type of lure can be used as a monitoring tool to finalize the timing of bio-pesticide applications. Although other components are not as encouraging, aggregation pheromones show promise against *P. striolata*. Thus, bio-pesticides and pheromones combined with the natural enemies of *P. xylostella* could offer a sustainable pest management strategy against the major pests on brassicas in tropical lowlands.

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O IPM I-4

Field testing of IPM-based cropping systems: a diversity of experimental approaches in Europe

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Integrated Pest Management (IPM) emphasizes physical and biological regulation strategies to control pests while reducing the reliance on pesticides. It is often based on combinations of control measures, because each available alternative measure might have a moderate efficiency. Field experiments are required to analyse the interactions between control measures, and to evaluate the sustainability of IPM-based cropping systems (CS). A network of European agronomists managing field experiments at the CS level was set recently, aiming at sharing data and expertise to enhance our knowledge on IPM.

Comparing methodologies highlighted a diversity of approaches in CS design and experimental layouts. This diversity is partly related with the research context and objectives. Some experiments intend to explore really innovative strategies and gain scientific knowledge about how such innovative CS behave, while others aim at providing quickly adoptable solutions for local farmers. In some research programs, the experiment is part of the CS design process, and tested CS are regularly revised, while in other cases CS are kept stable across years so as to be able to evaluate cumulative long term effects. The concept of CS itself is viewed differently across scientists, and this affects protocols: some consider each CS as a sequence of techniques, which has to be similar across repetitions, others define a CS as a set of decision-making rules that allows a flexibility in the actual sequences of techniques. The main difference among experiments differentiates factorial layouts from systemic approaches: factorial experiments make it possible to quantify the effects of each IPM factor, and to analyse the interactions, without particular attention for the consistency among components constituting each CS. On the contrary, system approach focuses on the overall evaluation of CS designed with a great attention paid to their consistency, hence maximizing the chance to meet the system objectives (in the case of IPM, to use little amount of pesticide while maintaining the CS sustainability).

Such field experiments are costly, so preliminary reflections defining the experimental strategy have a critical importance. Networking at the European level may constitute a useful exchange platform with potential scientific added value.

O IPM I-5

Implementation of plant protection in vineyards of the Sarigöl District, Manisa, Turkey

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Question and methods: This presentation covers one out of four main components of a comprehensive survey study which was conducted using face to face discussions with randomly selected 373 grape producers from the Sarigöl District of the Manisa Province in Turkey out of 5800 enterprises in September-December 2014. Questions were included personal data such as age, gender, education level, and family size etc., enterprise characteristics such as land size, type of enterprise etc. as well as implementation of plant protection measures.

Oral Presentations

Integrated Pest Management I

Results: Diseases were considered the most important plant protection problem in vineyards, which was followed by entomological problems and weeds. The most prevalent diseases for enterprises is powdery mildew (64.1 %), mildew (16.4%), grey mould (15.0%), or esca diseases (4.6 %). Chemical control is the main control method although farmers apply some secondary methods removing diseased plant parts from the field, weed control and use of biological preparations respectively. The European grapevine moth is the main pest in 75.1 % of enterprises. Two-spotted red spider mite and aphids are the other main pests according to 20.9 % and 4 % of farmers, respectively. Biotechnical method for control grapevine moth is the main method by 61.9 % of producers. Glyphosate is the most applied herbicide (83.9 % of farmers prefer). The total number of pesticide application was over 10 times and reached to 30 in some fields. Mixing 3-4 pesticides is a common way although all farmers have stated that they prefer registered and less residual products. However, issues such as using pesticide at recommended rate and observing natural enemies have less attention.

Conclusion: There is a need for further extension, education and awareness raising activities for grape farmers in the region. In addition, their problems should be studied further and approaches should be developed.

O IPM I-6

The development of a protocol for non-target risk assessment studies with *Spodoptera exempta* and Bt maize

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The African Armyworm, *Spodoptera exempta*, is a sporadic but destructive pest of maize. It occurs over most of tropical Africa and, as a result of wind convergence, spreads seasonally into southern Africa. These polyphagous larvae feed on a wide range of plants and occur in a solitary and gregarious phase. Severe crop damage is caused during the gregarious phase, but it is not known if the density at which larvae occur has an effect on the quantity of food that individual larva consumes. In East African countries such as Kenya and Ethiopia preliminary trials with Bt maize have been conducted, all aimed at the maize stem borers complex, however, no varieties have yet been approved for release. A prerequisite for approval of a GM crop is an ecological risk assessment. The aim of this study was to develop a protocol for conducting non-target risk assessment studies with *S. exempta* on Bt maize. Larvae were divided into different densities, viz. 1, 2, 4, 8 and 12 larvae per container and provided with a predetermined mass of non-Bt maize leaves daily. Results indicated no significant difference in food consumption per larva at the respective densities. future risk assessment studies can therefore be done with one larva per container.

Oral Presentations

Microbiomes

O MIC 1

Needle microfungi from *Picea glauca* from a boreal treeline ecotone display different biodiversity patterns between a dense forest stand and nearby scattered trees above the timberline with potential responses to long-term growth dynamics of the trees

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The nature of plant-associated fungal communities in extreme and remote habitats is largely unknown in comparison to their biodiversity in more common environments, such as temperate forests. In the present study, Illumina-generated fungal ITS sequences of white spruce (*Picea glauca*) from the boreal treeline ecotone of the Central Alaskan Brooks Range were analysed to assess biodiversity patterns of needle-inhabiting microfungi. Fungal community patterns were correlated with various environmental parameters and host traits. Among others, the precise position of the trees, contrasting a forest plot with densely standing trees and a treeline plot above the timberline with comparatively unprotected scattered trees, was used to explain observed patterns of fungal communities. The influence of the trees' long-term responses to climatic warming was also analysed.

A highly significant positive correlation was observed for fungal community patterns and the geographical distance of their host trees. In general, neighbouring trees shared more fungal taxa with each other than with trees in farther distance. Moreover fungal composition of the entire forest trees was significantly different from that of the treeline samples.

When analysing fungal community patterns with respect to the above mentioned long-term tree responses, data interpretation was less straightforward. On the one hand, fungal species composition of positive and negative responders was almost identical for forest trees. On the other hand significant differences in fungal species composition were observed for the two responder groups in the treeline plot.

It is well known that secondary plant metabolites, such as condensed tannins directly influence richness and composition of needle-inhabiting microfungi. Whereas the biochemical constitution of needles was not studied here, the present results point towards long-term responses of fungal communities to their host trees, overlaid by environmental forces which act on narrow spatial and temporal scales.

O MIC 2

The possible use of AHL-priming in crop protection

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The communication between diverse organisms in the rhizosphere is based on a complex exchange and perception of molecules originating from the interacting organisms. In many Gram-negative bacteria, *N*-acyl homoserine lactones (AHLs) are the chemical base of quorum sensing (QS). Bacteria can detect AHLs and such detection influences gene expression and consequently the behavior of individual cells within a population. Not only bacteria, also plants perceive and react to AHLs with diverse responses.

Objectives: Our aim was to characterize the impact of long chain AHLs on plant immune system and to understand the physiological changes upon AHL-priming with pure molecules and AHL-producing bacteria.

Material and methods: We used a plethora of physiological and phytopathological assays to test the AHL-induced resistance, diverse plant mutants and bacterial strains.

Results: In plants, the *N*-3-oxo-tetradecanoyl-*L*-homoserine lactone (oxo-C14-HSL) reinforced the resistance towards biotrophic and hemibiotrophic pathogens. In plants treated with oxo-C14-HSL, secondary challenge with flg22 promoted stronger activation of mitogen-activated protein kinases, followed by increased expression of *WRKY* transcription factors as well as some defensin genes. The induced resistance was eventually reflected in modified composition of the cell wall. Similarly to pure molecules, AHL-producing bacteria, e.g. *Sinorhizobium meliloti*, were able to induce AHL-priming in model and crop plants.

Conclusions: AHL-priming may be of great importance for understanding the interplay between plants and bacteria, as well as for new applications based on induced resistance.

References:

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Oral Presentations

Microbiomes

O MIC 3

The endophytic community from several wheat accessions characterized by different longevity of their seeds

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Seed longevity is defined as seed viability after seed dry storage (storability). Seed longevity is of practical importance to preserve plant genetic resources for future agricultural crops in seed gene banks. The mechanisms that determine the longevity of the seeds are not known. Therefore four wheat accessions with different longevity stored either under room temperature or at -20°C were analyzed for the bacterial endophyte communities. Endophytes are microorganisms, which live part of their life time inside the plant and do not cause any symptoms of disease in the plant. In the last years there is growing evidence that endophytes play an important role in plant growth and health. The role of seed endophytes is still underestimated. It is likely that every plant species harbors a different set of endophytes, but like in maize a core microbiome exists across boundaries of evolution, ethnography and ecology. Seed associated bacteria have shown to be involved in plant performance like germination and seedling growth.

Using the cultivation-dependent approach, endophytes were isolated from all four accessions (figure 1) stored either at room temperature or -20°C. The isolates were characterized by 16S rRNA sequencing. From the wheat accessions 125 bacterial isolates were identified in total. Most isolates were obtained from the short living landraces under both storage conditions and from the short living cultivar stored under cold temperature (-20°C). The highest diversity of different bacterial genera was found in the short living cultivar stored under cold. 45 isolates were tested for the ability to improve the germination rate. Two isolates increased the germination rate significantly in different wheat genotypes. Additionally under non-sterilized conditions it was observed that the seeds inoculated with the endophytes showed significantly less fungal growth than the seeds without endophytes.

In the cultivation independent approach the microbiome of the four wheat accessions were analyzed at different germination stages by next generation sequencing. The DNA from the seeds were isolated from non-germinated seeds, seeds with a radicle of 1cm, seeds with a cotyledon length of 1cm and 5cm. The bacterial communities were compared between the accessions, storage conditions and developmental stage.

Figure 1

Figure 1: Spring wheat samples obtained from IPK Gatersleben (TS = total seedlings germination; NS = normal seedlings germination)

Genotype	Storage	Sample ID	Harvest year	Origin ¹	TS (%)	NS (%)
Long-living landrace	Room temperature	LLA	2003	ARM	80.5	64.0
Long-living landrace	-20°C	LLC	2003	ARM	92.5	76.5
Short-living landrace	Room temperature	KLA	2003	ARM	9.0	3.0
Short-living landrace	-20°C	KLA	2003	ARM	92.0	85.0
Long-living cultivar	Room temperature	LZA	1998	ARG	42.5	20.0
Long-living cultivar	-20°C	LZC	1998	ARG	86.5	80.0
Short-living cultivar	Room temperature	KZA	1998	MEX	21.5	3.5
Short-living cultivar	-20°C	KZC	1998	MEX	89.0	81.5

¹ ARG, Argentina; ARM, Armenia; MEX, Mexico

O MIC 4

Taxonomic analyses of microbial communities in stored sugar beets using high-throughput amplicon sequencing of different marker genes

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Introduction: Root rots are a serious problem during storage of sugar beets due to enhanced sugar losses. Storage trials conducted under controlled conditions revealed that the root rot severity is severely affected by the storage temperature, site of cultivation and genotype. Based on these results, it was hypothesized that these factors also influence the microbial community in stored beets.

Objectives: To prove this hypothesis, a high-throughput amplicon sequencing approach was applied to analyze the microbial communities of stored beets with particular focus on fungal, oomycetes and *Fusarium* species.

Oral Presentations

Microbiomes

Materials and methods: Sugar beet samples used in this study were obtained from a storage trial conducted under controlled conditions. PCR amplicons generated from different marker genes were sequenced using the MiSeq Illumina platform. High quality reads for analysis were imported in the CeBiTec in house amplicon analysis pipeline written in Perl that is based on the tools Usearch, RDP classifier and Krona.

Results: Rarefaction curves indicated nearly saturation by the plateau phase. Up to 150 OTUs were identified in freshly harvested beets. After storage, the number of OTUs showed a tendency to decrease, but this was strongly affected by the storage temperature. For fungi, the lowest number of OTUs was found after storage at 8°C (if the sample was highly infect by *Botrytis cinerea*), whereas the number reached the highest level when beets were stored at 20°C. The most frequently found OTU at 8°C could be assigned to *Botrytis cinerea*, a well known storage pathogen, whereas *Penicillium paneum* seems to be abundant in most of the 20°C samples. Apart from that, important mycotoxin producing *Fusarium* strains were also detected in stored beet samples.

Conclusion: Taken together, high-throughput amplicon sequencing has been demonstrated to represent a valuable tool for elucidation of the microbial community in stored sugar beets. Prevalent species found in this study are well known as generalists and ubiquitous spoilage fungi. This might explain the weak effects of environment and genotype on the species spectrum. In contrast, the storage temperature clearly affected the species composition. This shift is likely be explained by enhanced microbial growth accompanied by displacement effect.

O MIC 5

Diversity of olive tree fungal phyllosphere and their influence in tolerance to Olive leaf spot (OLS)

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The Olive leaf spot (OLS) is one of the most important olive diseases worldwide, including in the Northeast of Portugal. The yield losses caused by this disease in this region are variable according to the cultivar, being the cv. Cobrançosa more tolerant to OLS than Verdeal-Transmontana.

The present study aimed to clarify the effect of endo- and epiphytic fungal communities present in above-ground parts of olive tree in the mediation of host tolerance to OLS. .

Twigs and leaves were collected from seven olive trees of each cultivar Cobrançosa and Verdeal-Transmontana, located in Mirandela (Trás-os-Montes region).The plant material collected was used to isolate both endo- and epiphytic fungi in PDA media. The isolates obtained were further identified by sequencing the ITS region of rDNA. OLS incidence was assessed by evaluating the percentage of infected leaves per tree.

Statistical analyses and community ordinations revealed significant differences in endo- and epiphytic fungal community composition between cultivars, especially within endophytes (97% dissimilarity). The majority of differences associated with the tolerant cv. Cobrançosa were attributed to *Penicillium crysogenum*, *Coprinopsis gonophyla*, *Ulocladium dauci* and *Cladosporium iridis* within the epiphytic community, and *Phaeosphaeria avenaria*, *Penicillium crustosum*, *Alternaria* sp. and *Penicillium* sp. within the endophytic community. Verdeal-Transmontana was distinguished by the species *Prosthemia intermedium* and *Arcyria nigella* within the endophyte and epiphyte community, respectively. Disease incidence (% infected leaves) was significantly ($p < 0.001$) greater in cv. Verdeal-Transmontana (18%) than in cv. Cobrançosa (3%). A multivariate analysis between fungal diversity and disease incidence, over the two cultivars, allowed the identification of candidate's taxa that may influence the tolerance of cv. Cobrançosa to OLS.

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Oral Presentations Microbiomes

O MIC 6

Rhizobacterial community structure in Mahikeng rhizospheric soil and associated plant growth promoting potential

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Background: DGGE profiles of Mahikeng soil can indicate dominant soil bacterial types and Plant Growth Promoting Rhizobacteria (PGPR) can stimulate the growth of the host plant.

Methods: The aforementioned were examined in relation to nine rhizospheric soils. Rhizobacteria with PGPR traits were selected for use in pot experiments on tomato and spinach.

Results: The rhizobacterial isolates tested were found to produce ammonia; several of them produced indole acetic acid (IAA; 38%) and hydrogen cyanide (HCN; 38%). Also exhibited are 1-aminocyclopropane-1-carboxylate (ACC) deaminase activity (48%), phosphate solubilisation (48%) and antifungal activity (21%) against test pathogen *Fusarium solani*. All the HCN-producing bacteria belong to the genus *Bacillus*. *B. amyloliquefaciens* indicated high cyanogenic potential compared to other strains. The treatment of both crops with the bacterial inoculants promoted plant growth in terms of increased shoot length at PB. *B. amyloliquefaciens* MR16 had significantly higher growth at PB. *Bacillus* sp. (94%), *Rubrobacter* sp. (90%), Rhizobiales bacterium (95%), and soil bacterium (87%) besides the culturable *B. megaterium* (97%) and *Cohnella* sp. (84%).

Figure 1: Ethidium bromide stained agarose gel (2%) showing PCR amplification of 16S rRNA gene of bacterial isolates obtained from the farming sites in Mafikeng. M= DNA marker (500 bp); Lanes 1 to 9; = rhizosphere of lettuce, cabbage, tomato, green peas, beetroot, maize1, maize 2, onion and spinach.

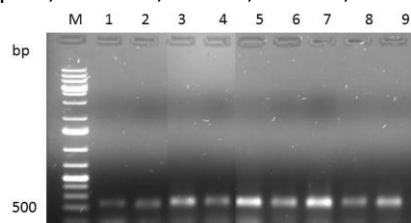


Figure 2: 16S rRNA gene PCR-DGGE patterns of 16S ribosomal DNA (rRNA) fragments profiles of rhizosphere bacteria on a polyacrylamide gel with a 40-60% denaturation gradient of Urea-Formamide DGGE patterns fragments from samples of rhizosphere plants collected at different crop plants. Lanes 1-9, represents bacterial community of the rhizosphere of maize1, spinach, tomato, cabbage, maize2, lettuce, onion, beetroot and green peas respectively and numbers 1-8 indicate the excised bands sent for sequencing

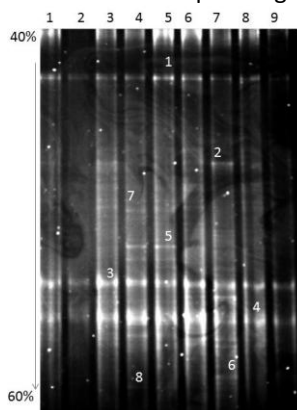


Figure 3: Amplified 200 bp ribosomal gene product from excised bands on the DGGE profile, Lanes 1-8; =PCR amplification of the fragments from isolates excised from DGGE gel.

No attachment submitted

Conclusions: PGPR can be used to make reliable and accessible products such as biofertilizers for farmers. Metagenomics holds the promise to reveal several important questions regarding the unculturable fraction of the rhizosphere community.

Oral Presentations

Plant Diseases and Irrigation

O IRR 1

Building Crop Health into Water Recycling Systems

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Introduction: Plant pathogens in irrigation water present a growing threat to crop health as agriculture increasingly depends upon recycled water for irrigation. A variety of decontamination technologies have been adapted from municipal water treatments to mitigate this risk, but their technical and economic performance is often compromised in agricultural settings due to greater turbidity, organic and inorganic content in recycled water. There is an urgent need for long-term solutions to this emerging crop health issue of global significance.

Objectives: The ultimate goal of our studies was to help farmers build science-based water recycling systems that capture and reuse agricultural runoff without recycling pathogens. Specific objectives included:

- Investigating pathogen distribution in water recycling systems using *Phytophthora* species as an example
- Developing a better understanding of recycled water quality dynamics
- Elucidating zoosporic responses to major water quality stresses in a simulated aquatic system.

Materials and methods: Pathogen dynamics were tracked by baiting irrigation reservoirs with rhododendron leaves, followed by plating onto selective media, and identification of resultant cultures by DNA fingerprinting and sequencing, plus morphological characters. Water temperature, pH, dissolved oxygen, oxidation-reduction potential, electrical conductivity, salinity, total dissolved solids, turbidity, chlorophyll a, blue-green algae were continuously monitored at multiple reservoirs in different U.S. states. Using these field data as a framework, zoosporic responses to major water quality stresses were assessed for a number of *Phytophthora* species including *P. ramorum*, *P. kernoviae*, *P. alni*.

Results: Pathogen populations declined along water path from runoff entrance to outlet in the reservoirs. Water quality changed diurnally and during the growing season. Most *Phytophthora* species assessed are intolerant of water quality stresses.

Conclusions: Many pathogens including some *Phytophthora* species, perceived as “water molds” for the past 71 years, are not well adapted to agricultural water environments. Crop health risk associated with irrigation water may be effectively managed by extending the runoff water path and increasing turnover time in recycling irrigation systems.

O IRR 2

Permanence in artificial soils and transmission through irrigation of *Cucumber green mottle mosaic virus* in cucumber crops

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Cucumber green mottle mosaic virus (CGMMV) is a tobamovirus that was detected in Spain first in the 1990ies in cucumber grown in the province of Almeria. During the following years, several small outbreaks took place which jeopardized greenhouse crops. The high infectivity of the virus and its transmission by contact urged the need to investigate following two aspects of the epidemiology of CGMMV: a) the permanence of CGMMV in different growth substrates, and b) the possibility of virus transmission by irrigation water. The former issue was addressed during the fall of 2013 (October-January 2014), when cucumber cv. Estrada plants were grown in 5 different substrates: perlite, rockwool, coconut fiber, peat, and sand. Plants were inoculated manually and grown until the end of the cultivation period. The substrates were preserved in their original containers and used to plant a new crop at the start of the spring campaign (March-June 2014) in order to investigate the persistence of virus in the soil. To evaluate the transmission of the virus by potentially infected water, drainage liquid was collected during fall and spring campaigns from infected crops and used for irrigation on healthy cucumber plants. The collected drainage was analyzed by ELISA for CGMMV. Preliminary results suggested that, under conditions corresponding to the autumn campaign in Almeria, there was no risk of CGMMV contagion through irrigation water for cucumber. As for the persistence in soils, the percentage of plants infected during the spring campaign reached 100% regardless of the type of soil substrate that was previously infected.

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O IRR 3

Potential of electrolytic disinfection of nutrient solution to hamper dispersal of plant pathogens

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Introduction: Closed irrigation systems conserve resources and minimize production costs, but they increase the risk of root diseases owing to the dispersal of waterborne plant pathogens by recirculation of the nutrient solution. A considerable number of pathogens is of significant concern as those are stable, difficult to combat and cause economic losses. Different physical and chemical techniques have been described to decontaminate irrigation water and nutrient solution. Beside cost effectiveness and ecological concerns none is suitable to inactivate the multitude of relevant plant pathogens, in particular viruses.

Objective: The potential of a sensor based disinfection procedure to inactivate fungal, bacterial or viral plant pathogens in hydroponic systems in greenhouse production was determined and evaluated.

Materials and methods: An electrolytic disinfectant (newtec Umwelttechnik GmbH, Germany), especially developed for disinfection of irrigation water in greenhouses was used. It produces low concentrated potassium hypochlorite (0.6-0.8%) by electrolysis of a potassium chloride solution. The efficacy of the disinfectant to inactivate selected plant pathogens was tested *in vitro* according to the standard (OEPP/EPPO, 2008). First trials under practical conditions were initiated focusing on the potential of the disinfection procedure to prevent the spread of PepMV by recirculating nutrient solution in tomato.

Results: Dose-effect relations were calculated for different plant pathogens. As expected, contact time and dose required to eradicate pathogens varies with pathogen species and life stage. A sensor based disinfection procedure was successfully established in tomato cultivated in NFT (nutrient film technique). Although the dispersal of plant viruses was hampered in all experimental approaches plants showed a phytotoxic reaction to chlorid dependant on injection intervals.

Conclusion: The sensor based injection of a disinfectant gained by electrolytic oxidation has shown its potential to suppress the dispersal of plant viruses by recirculating nutrient solution in diverse experimental set-ups. Its efficiency and suitability has to be tested and verified in large scale horticultural production sites.

OEPP/EPPO, 2008: Disinfection in plant production. EPPO Bulletin 38, 311-315. doi: 10.1111/j.1365-2338.2008.01235

O IRR 4

Water disinfectants interacting with nutrient solutions and substrates

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Chemical disinfectants are commonly used in greenhouse operations to control plant pathogens in irrigation. The effective dose to target plant pathogens in water is typically determined with clean water and under controlled environmental conditions. However, in commercial operations irrigation water can contain chemical and physical variables which may interact with disinfectants. With increasing recirculation of water, it is fundamental to understand how water disinfectants interact with other water quality parameters. The objectives of this research were to quantify the persistence of chlorine, chlorine dioxide, activated peroxide, copper, and quaternary ammonium chloride in the presence of peat; quantify the persistence of free and total chlorine in the presence of water soluble fertilizers; and evaluate the efficacy of chlorine to control *Phytophthora nicotianae* in the presence of peat and nitrogen in the water. The persistence of chemical disinfectants in the presence of peat was evaluated by preparing solutions with 50 mg·L⁻¹ peat and then measuring the concentration of active ingredients after 2 and 10 min contact time. The persistence of chlorine in the presence of fertilizers was evaluated by preparing solutions with 200 mg·L⁻¹ nitrogen with 11 commercial fertilizers and then measuring free and total chlorine after 2 and 60 min contact time. Efficacy of chlorine to control *P. nicotianae* in the presence of peat or nitrogen was evaluated by mixing five solutions with peat from 0 to 80 mg·L⁻¹ or 50 mg·L⁻¹ nitrogen and then combining with 0, 2, or 4 mg·L⁻¹ chlorine. Zoospore mortality and infectivity were measured after 10 min and 24 h contact time. Peat in the solution resulted in a rapid decline of chlorine and chlorine dioxide, whereas less effect was observed on copper, activated peroxygen and quaternary ammonium. Free chlorine decreased rapidly in the presence of fertilizers containing any concentration of ammonium-N. Efficacy of chlorine to control *P. nicotianae* was sustained despite the presence of peat or nitrogen in the solution. The concentration of some chemical disinfectants in solutions may significantly and rapidly decrease with common water contaminants. Therefore, a multiple barrier approach should be implemented to reduce the risk of waterborne pathogens in irrigation.

Oral Presentations

Plant Diseases and Irrigation

O IRR 5

Practical improvements to slow sand filtration for cleaning recycled irrigation water

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Introduction: Slow sand filtration (SSF) is a successful biofiltration method used since the early 1990s by horticultural nurseries to remove pathogens, especially oomycetes, from irrigation water. Uptake of SSF has been hindered by two important factors; 1) slow flow rates and 2) the frequent filter clean-ups needed if treating dirty water. Several commercial SSF constructed in the UK Westcountry utilised china clay waste sand (CCS). This sub-angular sand, derived from degraded granite, tends to retain an open structure, unlike the compaction normally seen with rounded river-sand grains, and also appeared able to maintain efficacy at faster flow rates than normal.

Objectives: A CCS of coarser grade than normally used for SSF ($ES_{10} =$, marketed as 'Horticultural Grit'), was assessed to:

- Investigate potential increases in SSF flow rate, including assessing the attributes of CCS.
- Increase SSF run-times by reducing clogging- either by deploying the sand throughout a filter column or as a protective top layer on established SSF
- Test sand cleaning by disruption of, and backwashing the protective top layer

Results: The flow rate of a medium sized SSF was increased to >0.5 m/h without loss of efficacy against oomycetes. This filter ran continuously for three years before being cleaned.

Microbial activity per unit sand surface area was significantly greater in CCS than a conventional quartz SSF sand.

A layer of CCS placed on top of a large commercial SSF successfully reduced the number of clean-ups required from once every 2-3 weeks to once per year. A novel cleaning protocol, involving disruption and backwashing significantly reduced the staff time required for cleaning and eliminated sand lost to scraping - efficacy was restored 24h post clean-up.

Conclusions: Coarse CCS is promising as a SSF medium. Work is needed to determine whether the structural properties (sub angular grains) or the mineralogy of CCS contribute to its efficacy.

O IRR 6

Interactions between organic and inorganic water quality parameters and *Pythium ultimum* in greenhouse irrigation systems

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Introduction: Oomycetes are ubiquitous in soilless cropping systems and display a considerable threat to hydroponically grown crops in closed cropping systems. Carbon is a driving factor for growth of heterotrophic organisms, and readily available in the rhizosphere. However, numbers of heterotrophic microorganisms oscillate both in the rhizosphere and in the nutrient solution of hydroponically grown crops. In order to stabilize closed cropping systems with respect to the resident and transient microbiota, the impact of other nutritional factors has to be investigated. This is of particular interest as the nutrient solution of closed hydroponics is a eutrophic environment.

Objective: The general goal of our studies is to increase root health of crops grown in closed hydroponic greenhouse systems. The specific goal was to

- Study the dynamics of total (TOC) and dissolved (DOC) organic carbon in the nutrient solution in a closed hydroponic cropping system with tomato from small plant stage to fruit-bearing plants and to
- Investigate the impact of oscillating organic and inorganic nutrient levels on the growth of heterotrophic microorganisms.

Materials and methods: Tomato was grown in closed liquid hydroponic systems under controlled conditions either with or without artificial infection with a kanamycin resistant isolate of *Pythium ultimum* (PU). During a 14-week-period, the heterotrophic microbiota inhabiting the nutrient solution was assessed by viable count using semi-selective media (R2A: heterotrophic culturable bacteria; 0.5x MA: culturable fungi; King B Agar: fluorescent pseudomonads). PU was reisolated in CMA supplemented with kanamycin ($200 \mu\text{g ml}^{-1}$) and rifampicin ($50 \mu\text{g ml}^{-1}$). In parallel, the nutrient solution was analyzed with respect to TOC and DOC as well as macro- and micronutrients.

Results: Irrespective treatment, C/N and C/N/P ratios as well as numbers of all assessed microbial groups rose with increasing crop age and Log TOC explained variations of all assessed groups. However, its impact was lower in the presence of PU. Manganese explained 54.5%, 77% and 60% of the variation of assessed microbial groups in the nutrient solution of healthy plants. Considerable interactions between most monitored mineral elements and fluorescent pseudomonads were stated in systems inoculated with and without PU.

Conclusions: Nutritional interactions in closed hydroponic greenhouse systems need further attention to favor root health.

Oral Presentations

Precision Farming

O FARM 1

Intelligent spray system development and evaluation in Oregon Nursery Production

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Question: A team of scientists from USDA/ARS, Oregon State University, The Ohio State University, and University of Tennessee developed and evaluated two different types of intelligent variable-rate spray systems in nursery production with a goal of determining whether these sprayers increase pesticide application efficiency and minimize environmental impact in field nursery production sites.

Methods: In Oregon evaluations were conducted for the two prototype spray systems in commercial nurseries from 2011-2014. The first prototype, a modified hydraulic vertical boom system, utilizes ultrasonic sensors to detect the size and volume of plants, and the second prototype is an air-assisted system utilizing a laser scanning sensor to measure plant structure and foliage density. This technology allows the sprayers to match nozzle outputs to the crop structures and to avoid the pesticide waste that occurs when a constant volume of pesticide is applied.

Results: In nine separate efficacy trials in commercial shade tree nurseries in four years, reductions in spray volume of the variable rate spray applications compared to the constant rate applications ranged from 34% to 76.8% while maintaining equivalent control of the insects and diseases sampled. Economic analyses of two trials in 2014 showed the use of the intelligent sprayer resulted in reductions of chemical costs, in a range from 32.5% to 53.2% to control insects and by 58.6% to control diseases, when compared with a conventional air-blast sprayer using best management practices.

Conclusions: The new spray systems significantly advanced the technology for efficient pesticide spray applications to increase growers' production, profitability, worker safety and environmental stewardship while maintaining tree quality.

O FARM 2

Influence of different spraying parameters on the spray liquid distribution of sprayers in vertical crops

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Question: Streaking can be a problem during the application of pesticides if the nozzle formation comes too close to the target area. This problem is mainly known in vineyards, if the sprayer which is used is too large compared to the narrow rows or if there are problems with the adjustment of the sprayer (Knewitz 2009). We were interested to find out what spraying parameters are influencing the occurrence of streaking and which combination of these parameters can be used to optimize the adjustment of the sprayers.

Method: Therefore, we considered five different parameters (distance from nozzle to target, distance in between the nozzles, application pressure, type of nozzles and air-assisted spraying) and analyzed their influence onto the quality of the vertical distribution using a vertical patternator to measure the variation coefficient of the spray liquid distribution.

Results: We came up with the result, that the quality of the vertical distribution is influenced by all these parameters mentioned, but that the impact of the specific parameters can be quite different. The distance from nozzle to target, the type of nozzles used and if the application was air-assisted or not had the greatest impact on the quality of the vertical distribution. Furthermore, the direction of these impacts are mostly in the same way. Also the distance in between the nozzles and the application pressure had an impact on the quality of the vertical cross distribution, but the direction of that impact was not clearly visible in all cases.

Conclusions: The tentative experiments demonstrated, that it could be very useful to investigate these and maybe some more parameters in order to optimize sprayer adjustment in future. Therefore, a lot of different parameter combinations have to be analyzed in further experiments.

Figure 1: Streaking after the application in vineyards (Knewitz 2015).



Figure 2: Experimental set-up in front of the vertical patterner.



Literature:

Knewitz, H. (2009): Die richtige Einstellung [The right adjustment]. Das deutsche Weinmagazin, Issue 11 (30. Mai), pp. 25-27.
Knewitz, H. (2015): Picture from a personal communication via e-mail (5.01.2015).

O FARM 3

An optimized gap detection and switching system (GDS) to reduce the amount of plant protection products (PPP) in orchards

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Introduction: Plant protection products (PPP) in orchards help to maintain the crop healthy and ensure a good product quality. To reduce unnecessary environmental pollution a reduction of the amount of PPP and drift minimization are important social and political goals.

Objectives: Therefore, the main objective of this study in orchards is to evaluate the reduction potential of plant protection products by the use of a high precise application technique. Another goal is to evaluate the influence on the drift potential.

Materials and methods: For this purpose, the system of sensor controlled application had been optimized with a strong focus on the needs of practical fruit cultivation. A sprayer (NH 63) with a radial fan was equipped with improved optical infrared sensors. The number of sensors was, compared to commercially available products, increased and the optical scanning has been improved. By the optimized sensor system, target surfaces and gaps can be detected more precisely compared to sprayers in

Oral Presentations

Precision Farming

use. Consequently, the associated nozzles can be switched off. Field experiments in Jork (Altes Land) were conducted to determine the saving potential of applied PPP, when using the GDS system compared to a deactivated GDS system. The two different sets of trials were conducted in different development stages of orchards.

Results: In young plants with sharp-edged application [precisely with no overlay: +/- 0 cm] a saving of almost 70% could be achieved. In older plantations and dense foliage stocks or with a strategy of advanced safety application [overlay: +/- 20 cm] at least a PPP saving of approx. 40% could be achieved.

Conclusion: The application with a GDS system can help to save PPP und accordingly protect the environment. In young orchard plantations with large gaps in the foliage, the saving potential is the highest. It can be suggested, that a reduced PPP output reduces the drift of PPP onto non target areas.

O FARM 4

Pesticide application and dewfall - from basic research towards a practical approach

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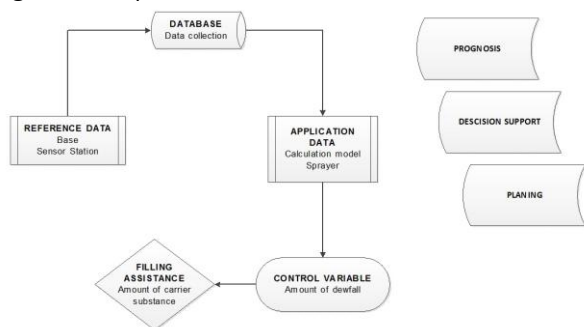
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Introduction: Due to limitation of pesticide use, further interest on the optimisation of the application process, via adjustment methods of the carrier substance considering the amount of dew, is induced.

Objectives: Based on a related study [1] regarding the feasibility of pesticide application during the nightly period of dewfall, the demand for a more practical approach, using standar machinery, is examined. Previously, a model based control system, aiming at an online adjustment of carrier substance, depending on the current amount of dewfall on plants, was developed [1]. Thus, the current main objective is to adapt the existing model control towards a state-of-the-art offline scheme, focusing on assistance and documentation.

Material and methods: First, the system is altered to meet the new requirements. Then, the functional model is tested in vivo. The measuring scheme, regarding temperature, humidity and leaf surface temperature, and the internal data logic [1] could be transported to the standard sprayer. The novel filling assistance comprises a sensor station, providing reference data for the related dew algorithm. A corresponding sensor scheme is mounted on top of the standard sprayer, providing actual field data as well as emending the reference data to present in vivo conditions.

Figure 1: Simplified scheme



Results and conclusions: During testing, the new system was found to be easily applicable and valid during spraying. Further advances have to be made on a possible integration into a sprayer terminal or further assistance by means of measured information, or daytime applications. Further research on biological effectiveness, pesticide agents and other crops is required. Site specific data of humidity leaf temperature and wetness offer additional data on the microclimate and is seen as a guide for farmers or advisors to an enhanced understanding of fungal diseases and the success of applications. Presuming further research on special applications knowledge concerning the application during dewfall could be constituent.

Reference:

[1] Fröschle, H.K. (2015). Tausensor - Entwicklung von Sensorsystem und Algorithmik zur Steuerung frei regelbarer Applikationsgeräte anhand des Klimaparameters Tau im Pflanzenschutz. Dissertation zur Erlangung des Grades eines Doktors der Agrarwissenschaften der Universität Hohenheim. *Not published.*

O FARM 5

Development and field test of a direct injection system without delay times for site-specific pesticide application

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Weed populations, as well as animal and fungal pathogens form spatial distribution pattern within agricultural land. To this effect areas are characterized by more or less heterogeneity. Precision farming makes it possible to carry out plant protection site-specifically according to this heterogeneity. But up to now in agricultural practice in Germany, it is common practice to apply plant protection products (PPP) on the entire field as single product or as a tank mix to save passes. With the existing farm equipment available it is impossible to apply different PPP site-specifically in a single operation. The solution for this problem can be a field sprayer with direct injection technology. In this way PPP can be sprayed individually where it is needed. This was the reason why the company Herbert Dammann GmbH and the Julius Kühn-Institute have started a joint research project. In this project, a sprayer with direct injection without delay time has been developed and tested in field trials. The direct injection without delay times is realized through a new direct injection system with three parallel nozzle lines at the boom. A great advantage of the direct injection system is that this technique allows an individual application of PPP according to varying field situations. Thus it is achievable to use different PPP with different necessary application rates in a single pass. In the sense of: as much as necessary, as less as possible.

O FARM 6

Research and Development of Precision Plant Protection in China

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Question: Plant diseases, pests and weeds are major problems in agricultural production, where they lead to significant losses of yield and quality. Attention to the pesticides used to control these problems has been increased, as they can have detrimental effects on the product quality safety and environment sustainability. Therefore, an optimal solution that would lead to a reduction in the use of pesticides is desirable but requires real time data and detailed knowledge of the potential occurrence and precision distribution of diseases, pests and weeds in the field.

Methods: Internet of Things would be a kind of solution, which are developing so fast and changing the agricultural system including plant protection.

Results: We think the most important issues for precision plant protection are following: First, Monitoring the pest tetrahedron, including plant, pest, environment and cultivation. In practice, the precise, low-cost and robust equipment for collecting, identifying, counting and transferring the information of pathogens plus pests are fewer, which may need intelligent analysis of hyperspectral image, microscope image, and flow cytometry, etc. Also with the extreme weather due to the climate change, and rapid development of facility agriculture, the plant production environment has changed a lot. So the digital models that describe quantified relationship of plant-environment-pest are essential for understanding the emergency of diseases and pests. This point also leads to the second issue: Model establishment. Although there were so many models for predicting the diseases, pests and weeds in different areas, their accuracy could not satisfied the users while extending to other areas. One of the reasons may be the lack of validation with huge data from different time and space scale. Big data simulation would be a possible solution with the characteristics of high volume, high velocity, and high variety information. Third, Decision and Conducting. The mobile internet has changed our life, and also marvelous app would contribute the decision support service for more and more farmers. The different platform, such as fixed-wing aircraft, unmanned aerial vehicle, ground equipment, which can provide conducting machine for precision crop protection.

Conclusions: Finally, we introduce the recent research and application of precision plant protection in NERCITA. These achievements could contribute to the precision plant protection.

Oral Presentations

Herbicide Resistance

O HR 1

Herbicide-resistant weeds - a global threat to sustainable farming

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Herbicide-resistant weeds are now widely spread all over the world due to misuse of herbicides in annual and perennial crops. Lack of crop and herbicide rotations combined with reduce tillage and over reliance on chemical control results in evolution of many weed species virtually resistant to all herbicidal mode of actions. Lack of a novel mode of action of herbicides worsens the situation. Weeds resistant to triazines, ALS and ACCase inhibiting herbicides have evolved in all continents threatening the sustainability of crop production. Altered target site (TS)' caused by point mutation in the binding site sequence and a non-target site (NTS) mechanisms may render weeds resistant to herbicides. The evolution of glyphosate-resistant (GR) weeds was inevitable due to the strong selection pressure employed by the repeated use of the herbicide. The mechanism of GR in most plants is associated with sequestration of the herbicide away from the target site in the chloroplasts whereas in some weeds such as *A. palmeri* the resistance is based on over expression of the target enzyme - EPSPS. Glyphosate resistance is observed wherever repeated application of high doses of glyphosate is practiced; including nurseries, perennial crops such as orchards and plantations, roadsides and chemical fallow. The fact that GR has evolved in countries where GM crops are not grown indicates that the resistance is not crop-dependent but rather a direct result of misuse of the herbicide by the farmer. The dimension of the damage caused by aggressive, herbicide resistant weeds such as *Lolium rigidum*, *Alopecurus myosuroides* in cereal crops, *Conyza* spp., *Ambrosia* spp. *Amaranthus palmeri* and *Sorghum halepense* in soybeans, maize and cotton, seriously threatens the sustainability of these crops. Similarly, The situation will be more complicated when the new GM crops stacked with three (or more) herbicide resistance traits (e.g., glyphosate, HPPD, 2,4-D, dicamba, glufosinate) will be offered to the farmer. Misuse of these important traits may increase the selection pressure on the weed populations and may enhance the evolution of multiple resistant weeds. Integration of non-chemical weed control methods into the management practices is crucial in order to slow down or prevent the harmful evolution of more herbicide-resistant weeds.

O HR 2

Influence of tillage systems and herbicide regimes on population dynamics and resistance evolution of blackgrass (*Alopecurus myosuroides*). - Presentation of a long term field trial II

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In autumn 2011 a long term field trial was started for about minimum of 6 years in Erwitte-Anröchte with the aim to assess the population development and resistance evolution of blackgrass. The site was chosen while it represents a typical German growing region with enough blackgrass infestation.

The trial is divided in two crop rotations. One with growing of winter oilseed rape - winter wheat - winter wheat and the other with corn - winter wheat - winter wheat. For each crop rotation there are two blocks sized 36 to 84 m. In each of these blocks three different tillage systems are used: One with ploughing, one with deep tillage without ploughing and one as shallow tillage system. In the oilseed crop rotation the blocks differentiate also in two sowing timings early and late for winter wheat. Further on there are different herbicide regimes practised which range from reduced usage of herbicides not changing the mode of action to intensive herbicide use on different mode of actions.

In each of the 120 plots the density and the efficacy on blackgrass are assessed. If the efficacy against blackgrass is not complete for each plot the metabolic and target-site resistance status will be researched.

Oral Presentations

Herbicide Resistance

O HR 3

Resistance of *Ammannia arenaria* to bensulfuron-methyl and its competition with paddy rice

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Ammannia arenaria has been becoming one of the most harmful weeds in paddy rice field in China in recent years, the object was to detect the sensitivity of the weed to mainly used bensulfuron-methyl(BSM), and to compare the competition ability between resistant biotype(RB, NB143) and susceptible biotype(SB, HZ001) to rice. For the 140 biotypes tested, 96.4% of them were resistant to BSM, the average resistance index of biotypes from Zhejiang, Jiangsu, and Anhui provinces and Shanghai city were 31.3, 20.7, 6.9 and 16.8, respectively. The seeds of the two biotypes were sowed simultaneously with rice in a basin in field, respectively, and were thinned to 58 plants/m² after emergence. The emergence dynamic of RB seeds were similar to SB with two peaks in 2-6 and 10-12 days after seeding (DAS). The weed grew slowly within 15 DAS but tremendously fast in the following time. Both the two biotypes were lower than rice plant before 45 DAS but much higher than it after 55 DAS. The RB plant was 14.7% lower than SB finally, and the rice yield was decreased by 50.8% under competition with RB and 73.1% for SB compared to the control, respectively.

The results indicated that the resistance of *A. arenaria* to BSM was first confirmed and widely distributed in China, and that the interfering of the resistant weed to rice reduced to some extent because of its fitness cost in height, even though it is still very noxious. It is helpful for the resistant weed risk evaluation and its management strategies making (The work was funded by National Natural Science Foundation of China (No.31171863), and Special Fund for Agro-scientific Research in the Public Interest (201303031, 201303022)).

O HR 4

Next Generation Sequencing Based Approach to Reveal Non-Target-site Herbicide Resistance Mechanisms.

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Weed-herbicide-resistance is a worldwide problem impacting crop-yield. Herbicide-resistance due to enhanced-herbicide-metabolism (EMR) in weeds is a threat which can confer broad spectrum herbicide resistance. EMR is not genetically well characterized. An RNA-Seq transcriptome analysis was used to identify genes conferring EMR in a population (R) of a major global weed (*Lolium rigidum*), in which herbicide-resistance to diclofop-methyl was experimentally evolved through recurrent selection from a susceptible (S) progenitor population. A reference transcriptome of 19,623 contigs was assembled (454 and Mysequ sequencing). Transcriptomic-level gene-expression was measured using Illumina 100 bp reads. In a forward genetics validation experiment, nine contigs, found overexpressed in R vs S plants, co-segregated with the resistance phenotype in an F₂ population, including 3 CytP450, 3 GST, and 1 GT. In a physiological validation experiment where 2, 4-D induced diclofop-methyl protection in S individuals due to increased metabolism, seven of the nine genetically-validated contigs were significantly induced. Finally 4 of these genes were found over-expressed in resistant populations collected in fields. Further studies aiming to characterize the physiological and biochemical function of these gene products will be discussed.

Oral Presentations

Herbicide Resistance

O HR 5

A genome approach for understanding herbicide resistance and developing new herbicides

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Question: Long time and mass utility of herbicides has resulted in the widespread herbicide resistance in weed populations. A large number of resistant biotypes in the rice weeds have been detected in at least 30 different species, including several species of the genus *Echinochloa*, which are major weeds in different rice cropping systems worldwide. Since, beside of target-site resistance, non-target-site herbicide resistance and multiple-herbicide resistance have been reported in many cases, it is difficult to detect the genetic basis for such resistance only using the target gene-based approach.

Methods: Recently, we sequenced/analyzed for the first *Echinochloa* genome and compared with rice and other cereals.

Results: The *Echinochloa* genome analysis provided us a series of novel insights into the herbicides resistance mechanisms, e.g. we found extremely high copy numbers of Cytochrome P450 monooxygenase (CYP450) and glutathione S-transferase (GST) in the genome, which are two key enzymes for the detoxification of synthetic herbicides or allelopathic compounds and therefore probably responsible for the increasing non-target-site herbicide resistance in weeds. Furthermore, the comparative genome analysis identified more than 10,000 *Echinochloa*-specific genes, which present a unique and unrivaled resource for target screening to develop new generation of herbicides.

Conclusion: Our *Echinochloa* genome and genome analysis illustrate the potential use for the rice weed management in the future.

O HR 6

Integrated weed management in the northern grain region of Australia

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Weeds are a major biotic constraint for growers and advisors in the northern grain region (NGR) of Australia, resulting in reduced crop production. The increased use of herbicides has resulted in the evolution of herbicide resistance in weeds, weed species shifts, and environmental pollution. These issues have led weed scientists around the globe to develop weed management strategies based on knowledge of weed ecology and biology, and integrating chemical, non-chemical and agronomic tactics. Some of the tactics are the use of weed-competitive cultivars with early vigour, the use of an optimum crop row orientation, the use of narrow crop row spacing and high crop seeding rate, the use of harvest weed seed control practices, and integration of herbicides with cultural practices to improve the sustainable use of herbicides. In addition, improved weed management strategies during fallow periods can substantially reduce weed problems in the subsequent crop. The use of cover crops before planting of the main crop, for example, can help in managing weed populations, especially herbicide-resistant. The improved weed management approaches should aim to reduce the weed seed bank before crop sowing and reduce weed emergence and weed growth in crops. Improved weed management techniques should focus on shifting the crop-weed balance in the favour of crop by integrating possible weed management tools with judicious use of herbicides. Together, these approaches may comprise the component of future integrated package to slow down the evolution of new weed problems in the NGR.

Oral Presentations

Integrated Pest Management II

O IPM II-1

Managing wild ungulates in forest ecosystems: goals and methods

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Wildlife management, especially large ungulates management, is a challenge but also a continuous source of conflicts between different groups of stakeholders, such as forest estate owners, foresters, wildlife managers, hunters, tourists and others. The reasons for that phenomenon are manifold, but in many cases they are resulting from the fact that wildlife itself underlies certain utilization and/or conservation interests beside the silvicultural goals. Thus, the success or failure of silvicultural management in many cases critically depends on the question, as to how the large ungulate management question will be solved and if the wildlife management concept is well established and integrated into the general concept of forest management. The present paper gives a survey of the actual status of ungulate management concepts in forest ecosystems and it deals with the question, how actual wildlife management concepts including monitoring systems are to be developed for different goals of cultivated as well as protected forest areas, how the different approaches will fit into the forest management concepts and what instruments such as participatory processes are to be engaged to accomplish acceptance in the public as well as from the different stakeholders.

O IPM II-2

Production of vegetables on rice straw bales in Egypt saving in soil pesticides, water and fertilizers

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Farmers in Egypt are facing water shortage, increased prices of pesticides, fertilizers and high paid labor power. Besides, vast areas of Lower Egypt suffer from seasonal serious air pollution when the farmers dispose the rice straw by burning. Moreover, traditionally desert land reclamation consumed large amounts of water, fertilizers and pesticides in addition to expensive labor power. In the present study, compacted rice straw bales (CRSBs) are used successfully for the first time in Egypt in production of vegetable crops, e.g. cucumber, tomato, pepper, squash, salad, onion, potato and strawberry under dripping irrigation system. It is a new approach in reclaiming the new desert land for agriculture. Compared to traditional agricultural practices, this approach saved 40-50% of water, 50% of fertilizers, completely avoiding the use of three groups of soil pesticides, i.e. fungicides, nematicides and herbicides, with short capital return cycle; and thus contributing to the production of economic healthy agricultural products either for local consumption or for export with fair prices. This approach could be also used for rehabilitation of vegetable production in the heavily infested soil with soilborn diseases, weeds and nematodes in the old land in the valley and Nile delta. When producing vegetables on CRSBs, the return per each 1 Egyptian pound (L.E.) reached 3.0 for producing squash, 10.2 for salad and 5.08 for strawberry compared to 1.3, 7.8 and 1.1 L.E. for the same crops produced traditionally in the soil with flooding irrigation, respectively.

O IPM II-3

Integrated cropping systems approach to arthropod pest management in Texas cotton

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Texas High Plains is the home of the largest contiguous cotton patch in the world, with 4% of the world cotton produced in this region, encompassing 41 counties ($\approx 150,000 \text{ km}^2$). The High Plains cotton production consists of both irrigated (40%) and dryland (60%) cotton. Irrigation practices include center pivot (70%), furrow (25%), and subsurface drip (5%) irrigation systems. An integrated cropping system approach has been used to address pest management issues in the Texas High Plains, consisting of agronomic, cultural, biological, chemical, plant physiological, and spatial (landscape) methods. This presentation will highlight the spectrum of current knowledge, both fundamental and applied, of biological and ecological crop pest management approaches, in cotton as a model system. *Lygus hesperus*, an emerging plant bug pest, is used to illustrate the integrated cropping systems approach to arthropod pest management in cotton.

O IPM II-4

The incidence of wheat crown rot depending on soil tillage and crop rotation

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Reduced soil tillage and wheat monoculture have become widespread in Latvia over the last years, but development of diseases, especially soil and plant residue born, can become an important risk factor. The aim of this study was to estimate the development of wheat crown rot depending on soil tillage and crop rotation and to identify the main causal agents of the disease. Two-factor experiments were carried out in 2009-2014: 1) crop rotation, and 2) soil management. The incidence of the complex of wheat crown rot was determined after wheat harvesting. Causal agents were determined according to morphological features, and the results were confirmed by sequencing of ITS region and comparison of acquired sequences to the ones available at NCBI nucleotide databases. The average incidence of wheat crown rot was around 40%. The most important factor influencing the development of this disease was year ($p < 0.001$) - incidence fluctuated from 14 to 56%. Soil tillage method significantly affected the level of the disease. Under reduced soil tillage, the incidence of crown rot was 45%; if conditional ploughing was performed, the incidence was lower - 41% ($p < 0.05$). The disease incidence was higher in continuous wheat sowings (47%) compared to fields with other pre-crops, where the average incidence was only 40% ($p < 0.5$). In the fields where wheat was sown without interruption, the disease incidence was 47% under conventional tillage, and 54% if reduced soil tillage was conducted. The disease symptoms were unspecific, and isolation of pathogens was necessary to identify the pathogens. The most important causal agents were different species from *Fusarium*, mainly *F. avenaceum*, but also *F. culmorum*, *F. graminearum*, *F. oxysporum* and *Microdochium nivale* were found. Another important group was *Oculimacula* spp., and co-existence of *O. yallundae* and *O. aciformis* was confirmed. Other pathogens, *Gaeumannomyces graminis*, *Cochliobolus sativus* and *Rhizoctonia* spp., were found only occasionally. The influence of soil tillage and crop rotation on the spectrum of pathogens was not clarified; the results obtained were inconsistent. The results confirmed the importance of soil tillage method and crop rotation on the development of wheat crown rot - continuous wheat sowings and reduced soil tillage increase the level of this disease.

O IPM II-5

Cultivar resistance and fungicide application in German winter wheat in the network "Reference farms plant protection" (2007-2013)

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Introduction: Winter wheat is the most cultivated crop in Germany with an area of 3,2 million hectares in 2014. Plant protection measures are an important tool to ensure yield stability and product quality in agricultural production by reducing yield losses caused by pathogens. In accordance to the strategy of integrated plant protection the application of pesticides should be limited to the necessary minimum of pesticide use. Therefore the cultivation of resistant cultivars becomes increasingly important.

Objectives: The network "Reference farms for plant protection", a joint project of the Federal Ministry of Food and Agriculture, the plant protection services of the German States and the Julius Kühn-Institute has been operating since 2007. The underlying objective of the network is the annual analyses of data on the intensity and application schemes of pesticides (treatment frequency index TFI) in major crops and regions. In combination with other representative, practice-oriented data the TFI is evaluated by experts with regard to the necessary minimum of pesticide use. The combination of retrospective information about weather conditions, infection pressure and TFI allow pointing out links between cultivar resistance and fungicide application in practice.

Materials and methods: The plant protection measures on wheat fields of 89 reference farms were documented by the growers in detailed field records and assessed by local experts. For further analysis the information was transferred in an Oracle database and linked to regularly updated quality, yield and resistance data of European cultivars.

Results: The number of grown cultivars ranged between 49 and 52 cultivars. In all years, at most 7 cultivars with a high quality but only a medium resistance level dominated on 50% of the cultivated area. Grain quality is an important criteria for the cultivar choice for farmers. The fungicide TFI ranged between 1,8 (2011) and 2,3 (2013) depending on the annual infection pressure. The percentage of resistant cultivars varied in the years between 15 and 19%.

Conclusion: The assumption that resistant cultivars show a lower fungicide TFI than susceptible ones was not confirmed. The reasons have not yet been completely clarified but will be processed in further analysis.

Oral Presentations
Integrated Pest Management II

O IPM II-6

Pest management and precision agriculture in irrigated crops in the USA

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Precision management of center pivot irrigated fields requires a knowledge of spatial variation within the field related to nutrient management, insects, disease and weeds presence. Mapping of soil properties, using site specific weed management, remote sensing, correlation between potato yields and physical and chemical soil properties, insect and disease distribution, are a few of the general concepts that are being evaluated at the Hermiston Agricultural Research and Extension Center (HAREC), in Hermiston, Oregon. The HAREC serves over 500,000 acres of irrigated agriculture in Oregon and Washington's Columbia Basin. The center concentrates on research, teaching, extension and outreach, implementing new technology based on basic and applied research. The objective of this presentation is to discuss precision agriculture related to pest management in irrigated potatoes in western USA.

Oral Presentations

Endophytes I

O END I-1

Assessment of endophytic bacteria diversity in olive tree: a search for biocontrol agents against *Verticillium* wilt

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Endophytic microorganisms, living in inner tissues of plants, are recognized to confer positive effects to its host, including increase resistance to diseases caused by phytopathogens. This feature has encouraged the scientific community to search and explore these microorganisms as biological control agents against an array of phytopathogens. *Verticillium* wilt, a vascular disease caused by the soil-borne fungus *Verticillium dahliae*, is one of the major constraints for olive cultivation worldwide, for which there is no cure. To the best of our knowledge, bacterial endophytic community associated to olive tree was never studied, and its exploitation as biological control agents was never investigated. Before commencing the search for biological control agents it is firstly need to know the diversity of native endophytes inhabiting olive trees. Therefore, the aim of this work is evaluate endophytic bacteria associated to cv. Picual and its distribution through the organs (leaves, twigs and roots) of the host tree.

Endophyte bacterial were isolated from roots, twigs and leaves of 21 trees from 3 olive orchards (*Olea europaea* cv. Picual) located in Granada, Spain. Samples were collected in autumn 2013 and spring 2014, and isolation was performed in PDA and PCA culture medium. Pure bacteria cultures were identified morphological- and molecularly through sequencing of V1 to V4 regions from 16S rDNA.

From a total of 630 roots, twigs and leaves segments analyzed, was identified 35 species belonging to 14 genus. The species *Serratia plymuthica* and *Alcaligenes faecalis* were the most frequent, each one representing 9,5% of the isolates. Only 3% of the species were found in twigs and 1% in leaves (fitting in *Alcaligenes* and *Bacillus* genus). The remain 96% were isolated from roots, being the species *Alcaligenes faecalis* the most frequently isolated. Similarly, the frequency of bacteria colonization was greater on roots (43%) followed by twigs (5%) and leaves (2%). The greatest diversity of bacteria endophytes in roots opens new perspectives for the exploitation of these microorganisms as biological control agents of *V. dahliae*. Further work will include the evaluation of the antagonist capacity of the isolates obtained against this phytopathogen.

This work is funded by FEDER through the Operational Competitiveness Program - COMPETE - and by national funds through the Foundation for Science and Technology - FCT - in the scope of the project PTDC/AGR-PRO/4354/2012

O END I-2

Persistent fungal root endophytes isolated from a wild barley species suppress seed-borne infections in a barley cultivar

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Introduction: Barley is subject to many pathogenic infections which cause significant economic losses. Growers have used an ever-changing arsenal of chemicals in an effort to control these pathogens. As well as being economically costly, chemical crop treatments for pathogens can have severe and long-lasting negative effects on the environment and reduce biodiversity. While these chemicals can be effective in controlling pathogens in a single cropping season, infections which are transmitted vertically offer more of a challenge. Alternative control measures using biological organisms may provide a more environmentally-friendly and long lasting solution.

Material and methods: Fungal root endophytes were isolated from wild populations of *Hordeum murinum* ssp. *murinum* L. and inoculated onto untreated seeds of a barley cultivar using five artificial and one soil-based growth media.

Results: A co-inoculant of ten isolates as well as two individual isolates successfully suppressed the development of seed-borne fungal infections on germinated and ungerminated seed. The two most successful isolates were also the most persistent as re-emergents and may provide real potential for development as crop inoculants. All isolates were more persistent in barley exposed to light after germination. The soil-based compost was associated with the greatest degree of seed-borne infection suppression, and the most successful artificial medium for suppressing seed-borne infections was also the medium with the most similar pH to the soil at the sampling sites. The endophyte isolate with the greatest suppression of seed-borne infections also appeared to retard the growth of the serious barley disease 'take-all', which is normally transmitted through the soil.

Conclusion: The results are important because the seed-borne infections that emerge from control seeds with no inoculant are some of the most devastating pathogens of barley, and suggest a direct antagonistic effect of the endophyte(s) on seed-borne pathogens without the induction of plant defences, such as systemic acquired resistance (SAR). To our knowledge, this is the first time that fungal root endophytes isolated from roots of *any* wild *Hordeum* species have been shown to control vertically transmitted infections in a barley cultivar.

Oral Presentations

Endophytes I

O END I-3

Below and above ground beneficial effects of a root associated *Fusarium oxysporum* endophyte.

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Fusarium oxysporum is a well-known global soil inhabiting fungus. Although being saprophytically competent and infamous for its plant-pathogenic activity on some plants, the majority are non-pathogenic, living on soil organic matter while other isolates colonize the endorhiza of plants without causing disease symptoms. Some of these endorhiza colonizing endophytic isolates were reported to be beneficial for plants. We have focussed on a highly active isolate, which induces resistance towards both sedentary and burrowing nematodes in various plant species, like tomato and banana. Besides the potential direct effects by its presence, split-root experiments and synchronized infection studies have shown that the isolate initiates certain systemic plant defense responses that affect both penetration and overall development of nematodes. Although colonization of the endophyte is restricted to the root system, the beneficial effect of the same endophyte can also be found in the leaves by negatively affecting herbivorous and omnivorous insects. The results of these diverse pest-beneficial interrelationships will be present and their importance for IPM discussed.

O END I-4

Screening of wheat endophytes against *Fusarium* head blight

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Introduction: *Fusarium* head blight (FHB) is one of the most important diseases on wheat crops worldwide caused by phytopathogenic fungi, *Fusarium culmorum* and *Fusarium graminearum* being the most common and most pathogenic species. In the present context of pesticides' use reduction, biological control is promising against diseases such as FHB but requires finding new effective microorganisms as biological control agents (BCA). Microorganisms naturally occurring within plants, without causing any damage to their host, can be good candidates as BCA. Indeed such microorganisms, called endophytes, are well adapted to their host and are considered as good producers of secondary metabolites, required for their survival facing host defense responses.

Objectives: The aim of the present study was to isolate fungi and bacteria from inner tissues of wheat plants and screen them for their ability to protect wheat against FHB.

Materials and methods: Endophytes have been isolated from roots and aerial organs of wheat plants. Isolates were identified by DNA sequencing. One hundred strains were selected and screened *in vitro* for their ability to inhibit the growth of *Fusarium* spp. Then, some strains have been selected to conduct *in planta* screening tests to study their ability to control FHB in controlled conditions. Quantitative PCR was developed to quantify the pathogen. Wheat defense genes expression was also studied.

Results: Many isolates have shown *in vitro* promising potential to control *Fusarium* spp. Two of the selected strains demonstrated high protection rate *in planta* against *F. graminearum*.

Conclusion: The screening of wheat endophytes in order to find new BCA against FHB might be promising. Our results suggest that *in planta* screening is more relevant than *in vitro* tests to find new BCA.

O END I-5

Evaluation of biological control strategies against soilborne pathogens: from lab experimentation to farming industry application

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Introduction: Vascular wilt diseases caused by soil borne pathogens like *Verticillium dahliae* and *Fusarium oxysporum*, result to billions of dollars in crop losses worldwide. Management of *Fusarium* and *Verticillium* wilt is mainly through chemical soil fumigation and use of resistant cultivars. However, the biocides used to fumigate soil are environmentally damaging and resistance appears to be genetically complex. Therefore, the development of alternative control methods, such as the use of biocontrol agents (BCAs) seem an appealing management strategy for the farming industry.

Oral Presentations

Endophytes I

Objectives: The aims of this study were to investigate the efficacy of two different BCA release strategies: seed coating and incorporation in the transplant soil plug, of the already known BCAs *Paenibacillus alvei* strain K165 and *Fusarium oxysporum* strain F2 against Fusarium and Verticillium wilt, along with the molecular aspects of the interaction with the host plant.

Materials and methods: Seeds of eggplant and cucumber were either coated with a powder formulation of K165 or F2 or planted in pots containing soil amended with powder formulation of each BCA at a rate of 5 and 10% (v/v). At the third-leaf stage, eggplant and cucumber plants were transplanted to soil infested with *V. dahliae* microsclerotia or *Fusarium oxysporum* f. sp. *radicis-cucumerinum* chlamydospores, respectively. Symptoms were recorded every 2 days after the onset of the disease. K165 and F2 rhizosphere colonisation was followed at 20, 27 and 34 days after sowing. RNA was isolated from the stem tissues of the various treatments at 5 and 10 dpi to monitor the expression of *PR* genes.

Results: K165 and F2 colonised efficiently the root system of plants and reduced significantly Verticillium and Fusarium wilt symptoms. In the case of Verticillium, the most suppressive treatment of both BCAs was the incorporation of the BCA in the transplant soil plug; while in the case of Fusarium, the two BCA release strategies were similarly effective. Both BCAs upregulated *PR* expression upon pathogen inoculation.

Conclusion: It was revealed that applying a bacterial and fungal BCA strain as a soil amendment of the transplant plug or seed coating agent confers an adequate BCA population size in the rhizosphere and primes plant defence resulting in reduced Verticillium and Fusarium wilt symptoms.

O END I-6

Attempts to biologically control Fusarium Head Blight of wheat and the associated trichothecenes

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Fusarium graminearum is regarded as the primary causal agent of Fusarium Head blight (FHB) of wheat. In addition to the extensive yield losses, kernels can also be qualitatively affected by contamination with trichothecenes which exhibit acute toxicity for humans and livestock. In the present study, the antagonistic potential of seven strains of *Trichoderma* (2 x *T. harzianum*, 3 x *T. hamatum*, *T. virens* and *Trichoderma* sp.) against the mycotoxigenic strain FG260 of *F. graminearum* were investigated both *in vitro* and under greenhouse conditions. All antagonistic strains were able to substantially inhibit mycelial growth of *F. graminearum* in dual culture, volatile and cellophane membrane assays. Two strains (T16 of *T. harzianum* and T23 of *Trichoderma* sp.) proved to be highly effective in the different assays used. For instance, in the presence of volatile metabolites emitted by T23 or T16, mycelial growth of the pathogen was retarded by 89.3% or 76.4%, respectively. Similar suppression of mycelial growth were detected when *F. graminearum* was inoculated on agar containing metabolites secreted by both *Trichoderma* strains. Formulations of all *Trichoderma* strains were prepared and investigated for their potency to minimize incidence of FHB and trichothecenes accumulation in wheat kernels under greenhouse conditions. The results revealed that FHB incidence was reduced when T23 or T16 were applied during anthesis by 78.6 or 66.4%, respectively. Moreover, thousand kernel weight (TKW) was negatively correlated with FHB incidence. An increase in TKW was observed in kernels treated with T16 or T23. However, all other strains showed no distinct increase in TKW compared to the infected control where *Trichoderma* strains were absent. Furthermore, trichothecenes analysis in the harvested kernels confirmed that some *Trichoderma* strains were able to degrade or inhibit the synthesis of trichothecenes. In the presence of the strain T23, deoxynivalenol (DON), 15-A-DON and 3-A-DON accumulation in the kernels were reduced by 48.2%, 45.9%, and 88.2%, respectively. All other antagonistic strains however, distributed between either moderate or slight trichothecenes inhibitors.

Oral Presentations

Insecticides I

O INS I-1

Towards production of active bioinsecticides with plant cell cultures and endophytes from *Azadirachta indica*

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All parts of *Azadirachta indica* show a broad spectrum efficacy against insect pests including insecticidal, anti-feedant or insect repellent activities. The commercial products are obtained only in low concentrations via complex extraction procedure. In light of the increasing reports on endophytes it can be hypothesized that some of these compounds like Azadirachtins are either directly produced by endophytic microorganisms or their production is linked to the plant metabolism. Further on, there is increasing evidence that plant cell cultures can produce Azadirachtin. Both strategies allow to by-pass the cost intensive transport and extraction of plant metabolites.

Consequently, the overall aim of a BMBF-supported project is to develop a competitive process to produce high amounts of bioinsecticidal compounds with Neem plant cell cultures and endophytes. Here, we present data on isolation of endophytes and induction of callus as well as first results of our screening approach.

First, we induced plant cell cultures from various plant tissues with a medium that allows a callus proliferation in more than 50 % of cultures. Besides, in total 303 endophytes (107 bacteria and 196 fungi) were isolated from plant material of different origins. Furthermore we developed a method to bind and stabilize more than 85% of the produced Azadirachtin in liquid medium as well as a high throughput bioassay in 96 well plates based on *Spodoptera frugiperda* (Sf9) cell cultures. Furthermore, we report first cultivation data obtained with the prototype of a novel automated pipetting and screening system (RoboLector[®] with an integrated BioLector[®] Pro from m2p-labs GmbH). Besides, we will present results on classification of selected endophytes as well as induced cell lines, submerged cultivation and classification of metabolites via UHPLC-DAD-MS/MS. Finally, we will show effects of extracted metabolites on Sf9 cell cultures.

O INS I-2

Evaluation of a botanical insecticide for the control of the major cocoa insect pest, *Sahlbergella singularis* HAGL (1895) (Hemiptera:Miridae) around Ondo Town, Nigeria

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Mirids, especially *Sahlbergella singularis*, are the most economically important insect pests of cocoa in West Africa. Their effective control relies on synthetic insecticides with the attendant challenges including elimination of beneficial natural enemies and pollinators, development of resistance and unacceptably high pesticide residues in cocoa. An effective and environmentally friendly control measure is needful. This study was carried out to investigate the efficacy of a new bio-insecticide BioneemEC in controlling mirids.

Phytotoxicity tests were conducted bimonthly by spraying cocoa seedlings and mature trees with the bio-insecticide at 1L/ha. Laboratory bioassays included direct and indirect (impregnated filter paper) contact toxicity and antifeedant (paired choice test) tests, using 0.5, 1.0 and 2.0 % concentrations. Pyrinex48EC (an organophosphate standard miridicide) and distilled water served as positive and negative control respectively. Small and large scale field trials of the bio-insecticide were conducted for two mirid seasons. Residue analyses of cocoa beans after harvesting were determined by HPLC. Descriptive statistics, ANOVA and regression were used for data analysis.

BioneemEC did not induce any adverse phytotoxicity on cocoa seedlings and mature trees. Total mirid mortality by direct contact toxicity test was recorded in four minutes at 1.0 and 2.0 % concentrations. This occurred in three minutes for Pyrinex48EC. Total mortality for indirect contact toxicity test at 2.0 % concentration was achieved in 7 hr. 20 min. Total mortality at 1.0 % concentration of Pyrinex48EC occurred in 3 hours. Negative control recorded no mortality. BioneemEC treated pods, on exposure to mirids yielded an average of 8 lesions compared to 17 lesions for Pyrinex48EC and 93 lesions in the negative control for the antifeedant test. In the small scale field trial, 100 % reduction in pod infection was recorded in 14 and 56 days for BioneemEC and Pyrinex48EC respectively. BioneemEC was significantly different (p < 0.05). BioneemEC can successfully replace Pyrinex48EC for mirid control.

Oral Presentations

Insecticides I

O INS I-3

Utilization of Salicylic Acid for the Control of Pod Sucking Bug, *Clavigralla tomentosicollis* Stal. (Hemiptera: Coreidae) on Four Pre-hardened Cowpea cultivars

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The experiment was conducted at the Agric Research farm Bayero University Kano (Lat 11° 58' N, Long 8° 25' E and 457m above sea level) from September-November, 2014 to evaluate the potential of SA-treatment in the control of Pod sucking bug. The mean annual rainfall was within the range of 865-1250mm with mean annual temperature of about 22-38°C and relative humidity of 65-90mmHg. Four different cowpea varieties (IT97K-1069-6, IT98K-205-8, IT89KD-288 and Dan'ila) pre-hardened with Salicylic acid were established in various replicated field cages in completely randomized pattern. Five-pairs each of fresh pre-mated bugs were introduced into the various cages. The different cowpea varieties screened showed variable response to the bug attack (P

O INS I-4

Toxicity of *Eucalyptus globulus* and *Achillea millefolium* essential oils and their nano-formulations on tomato leaf miner, *Tuta absoluta* (Meyrick)

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Tomato leaf miner moth, *Tuta absoluta* (Meyrick) (Lep: Gelechiidae) is one of the most dangerous pest of tomato in Iran and in many other countries. Essential oils of some plants can be used as an alternative to conventional insecticides due to their insecticidal properties and the required safety for humans and environment. Essential oils from *Achillea millefolium* and *Eucalyptus globulus* can be used as insect pest control agent due to consisting secondary metabolites with wide range of suitable properties including killing, repellency effects on insects and low toxicity to humans. Fumigant toxicity of *E. globulus* and *A. millefolium* essential oils and their Nano-formulations were determined against the second instar larvae of *T. absoluta* under laboratory conditions at 25 ± 1 °C, $65 \pm 5\%$ RH and a photoperiod of 16L:8D with 24, 48, and 72 h exposure time. The essential oils were extracted from dried leaves of *E. globulus* and flowers of *A. millefolium* by using Clevenger apparatus. Nano-formulations were prepared by synthesis SBA-15 loaded with different concentrations of essential oils. The results showed that by increasing the concentration of essential oil and Nano-formulations, the mortality was increased at all exposure times. Based on Probit analysis, the LC_{50} values of essential oils for *E. globulus* and *A. millefolium* were 0.34 and 0.53 $\mu\text{L/L}$ air, respectively. The LC_{50} values for nano-formulations of *E. globulus* and *A. millefolium* were 1.05 and 1.33 $\mu\text{L/L}$ air. After 72 h exposure, the obtained LC_{50} values for essential oils of *E. globulus*, and *A. millefolium* were 0.47, 0.80 $\mu\text{L/L}$ air and 0.35, 0.49 $\mu\text{L/L}$ air for their nano-formulations, respectively. The results showed that the pure essential oils present more efficiency at short time (24<h) compared to their nano-formulation and their effectiveness gradually reduced. But nano-formulations of the oils preserve their lethality for longer time (72 h or more) because of slow releasing of active compound. Overall, it seems that preparing nano-formulations of the essential oils may improve the efficacy of botanical insecticides and can be used as alternative for conventional pesticides.

O INS I-5

Insecticidal Joint Action of Mixtures of *Piper aduncum* Fruit and *Tephrosia vogelii* Leaf Extracts against the Cabbage Head Caterpillar, *Crociodolomia pavonana*

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One of the attempts to improve the performance of botanical insecticides is by mixing a certain botanical insecticide with other botanical insecticides containing compounds that can inhibit the activity of insecticide detoxifying enzymes (Scott *et al.* 2008). This study was conducted to assess the insecticidal joint action of *P. aduncum* fruit and *Tephrosia vogelii* (Leguminosae) leaf extract mixtures on the cabbage head caterpillar, *Crociodolomia pavonana*.

P. aduncum fruit and *T. vogelii* leaf powder was extracted with ethyl acetate (1:8 w/v) four and three times, respectively, by immersion method. The number of immersion was determined based on extract yield and insecticidal activity among the extracts obtained with two to six times immersion of plant materials. *P. aduncum* and *T. vogelii* extracts were tested separately and in mixtures at three concentration ratios (1:5, 1:1, and 5:1) by a leaf-feeding method against second-instar larvae C.

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Insecticides I

pavonana. Larval mortality data at 72 hours post-treatment were analyzed by the probit method. The combination index of the test extract mixtures, calculated based on the independent joint action model (Chou & Talalay 1984), was used as the basis for determining the type of joint action of the mixtures.

Based on LC₅₀ 72 hours after treatment (HAT), *P. aduncum* and *T. vogelii* extract mixtures at 1:1, 5:1, and 1:1 concentration ratios (w/w) were 3.28, 3.13, and 4.55 times, respectively, more toxic than *P. aduncum* extract alone and 2.51, 2.40, and 3.48 times more toxic than *T. vogelii* extract alone (Table 1). Based their combinations index, *P. aduncum* and *T. vogelii* extract mixtures at the three concentration ratios were strongly synergistic on *C. pavonana* larvae, at both LC₅₀ and LC₉₅ levels. Thus, the use of synergistic *P. aduncum* and *T. vogelii* extract mixtures will be more efficient than *P. aduncum* or *T. vogelii* extract applied separately.

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Figure 1: Toxicity of *P. aduncum* and *T. vogelii* extracts and their mixtures against *C. pavonana* larvae at 72 hours after treatment

Extract	<i>b</i> ± SE ^a	LC ₅₀ (95% CI) ^b (%)	LC ₉₅ (95% CI) ^b (%)
<i>P. aduncum</i> (Pa)	4.68 ± 0.38	0.141 (0.110–0.173)	0.317 (0.235–0.742)
<i>T. vogelii</i> (Tv)	3.95 ± 0.33	0.111 (0.072–0.163)	0.290 (0.186–2.014)
Pa + Tv 1:5	5.19 ± 0.42	0.047 (0.037–0.057)	0.097 (0.075–0.190)
Pa + Tv 1:1	4.47 ± 0.32	0.045 (0.029–0.059)	0.104 (0.075–0.232)
Pa + Tv 5:1	4.49 ± 0.37	0.033 (0.021–0.043)	0.077 (0.057–0.162)

^a*b*: intercept of probit regression line, SE: standard error, ^bCI: confidence limit.

Figure 2: Type of joint action of *P. aduncum* and *T. vogelii* extract mixtures against *C. pavonana* larvae at 72 hours after treatment

Extract ^a	Combination index		Type of joint action	
	LC ₅₀	LC ₉₅	LC ₅₀	LC ₉₅
Pa + Tv 1:5	0.43	0.34	Strongly synergistic	Strongly synergistic
Pa + Tv 1:1	0.39	0.37	Strongly synergistic	Strongly synergistic
Pa + Tv 5:1	0.25	0.26	Strongly synergistic	Strongly synergistic

^aPa: *Piper aduncum*, Tv: *Tephrosia vogelii*.

O INS I-6

Efforts to use soft Pesticides for the Control of cotton Insect Pests in Sudan

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Introduction: In the last decades during the post-war period, the agriculture has developed towards methods that are more intensive. Among these is increased use of agrochemicals. In the Sudan Gezira, as an example, cotton spraying started as early as season 1945/46 when only 1% of the cotton area was sprayed once. By 1978/79 the problem caused by the cotton insect pests, particularly the cotton whitefly (*Bemisia tabaci*) flared up. The number of sprays per season went up, reaching 9.25 sprays in season 1978/79, which might be attributed partly to the rapid resurgence of insects' pests as a result of the use of non-selective insecticides, which badly affected the natural enemies of these pests.

Objective: The joint use of natural enemies and selective pesticides might attribute to environmentally combating insect pest problems.

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Materials and methods: Studying the side effects of pesticides is of prime importance to save natural population and encourage their role as biological control agents. This paper discuss the various methods which can be used to study the side effects on natural enemies and the results of some studies carried on the side effects of some insecticides on natural enemies both at small and large scale levels in Sudan. The study includes testing the side effects of some insecticides and their impact on bio-safety (Talstar, Polo, Metasystox, Marshal and the mixture Reldan + Endosulfan) on two Predators at small-scale level at the Gezira Research Farm, Wad medani. The Impact of Polo (diafenthiuron) on natural enemies in the cotton-based ecosystem of the Gezira Scheme (Large Scale) was tested in the Study.

Results: The results indicated that Polo was relatively safe both at small scale and large-scale level to the natural enemies observed during the study.

Conclusion: This study can be considered as a begin of regional testing program in Africa with collaboration of international organization interested on conserving bio-agent such as the international biological control organization and united nations agencies.

Oral Presentations

Disease Monitoring

O DIS 1

Sensing of plant diseases - potential and limitations

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Automatable detection, identification and quantification of diseases on a small scale are the prerequisites for a site-specific application of fungicides, adequate to disease incidence and precise in space and time. Innovative sensor technologies in combination with informatics and modern application technologies may enable disease control where and when actually needed. Sensors have to spot specific symptoms at early stages of epidemics in order to enable the operator to initiate effective disease control. Sensor technologies tested for their suitability and reliability in disease sensing include mechanical, optical (spectral reflectance, thermography, chlorophyll fluorescence), and (bio-)chemical (e.g. electronic nose) sensors. Independent of the pros and cons for the technical systems, the biological systems limit sensor use in (decision making for) disease control. As early symptoms are often inconspicuous, spatial resolution of sensors should be high at the plant level. Pathogens may produce first symptoms near the ground and subsequently spread to canopy top in relevant intensities. Sensors have to inspect the relevant lower plant parts, also in case upper parts interfere with the system. Polycyclic diseases caused by airborne pathogens require other sensing intervals than diseases from stationary pathogens. Pathogen spread and latent infections have to be considered by using additional safety distances in disease control. The huge amount of data produced by imaging systems has to be reduced to the essential features in order to facilitate rapid processing. These requirements affect the sensor type and the way it may be used (e.g. offline vs. online). In case the action threshold for disease control is zero, precision crop protection technologies may be used for more accurate disease forecasting and the sensing of inoculum (e.g. molecular diagnosis of airborne inoculum).

O DIS 2

Hyperspectral imaging for the detection of plant diseases in precision crop protection and plant phenotyping

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Hyperspectral imaging is one of the promising methods for a precise, reproducible and objective estimate of plant diseases for integrated pest management, precision crop protection or in plant breeding for the selection of disease resistant genotypes. This technique can further support the decision making process for integrated pest management practices in the field or in the greenhouse. Here the timing of pesticide application has to be precise with an appropriate active ingredient. For this purpose a detection and identification of plant diseases in an early stage is requisite. Since the response of visual disease rating by the human eye is not reproducible and depends on several factors, optical hyperspectral sensors are promising tools for detection and monitoring of plant diseases. One advantage of these measuring methods is the non-invasive and non-destructive nature. Innovative sensor systems can provide detailed and highly resolved information on crop systems and single plants. Plant diseases impact the optical properties of host plants depending on the host pathogen interaction in different ways. These modifications in plant biochemistry or physiology or pathogen specific structures can be assessed by hyperspectral imaging. The disease specific spectral pattern in time and space can be used for a monitoring of plant diseases.

The potential of hyperspectral imaging was evaluated using the model crops barley and sugar beet and their relevant foliar diseases. Spectral signatures were assessed with a hyperspectral VIS/NIR camera and with a hyperspectral SWIR camera from 400 to 2500 nm. The spectral data cubes were analysed with advanced automatic classification methods for a differentiation and quantification of diseased leaf tissue with high accuracy. First results further underline the potential of hyperspectral imaging for the detection of diseases resistant crop plants. In a next step the developed models will be carried forward to other crop plants and their relevant fungal diseases. It is assumed, that hyperspectral sensing in combination with powerful data analysis methods will be of essential support for Integrated Pest Management programs and the selection of resistant crop plants in a sustainable crop production.

O DIS 3

Metabolite Profiling and Hyperspectral Imaging of Sugar Beet Genotypes Responding to Fungal Pathogen *Cercospora beticola*
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The fungal pathogen *Cercospora beticola* is the cause of severe loss of sugar beet root yield worldwide. The infection of the leaf canopy with *C.beticola* is resulting necrotic lesions (leaf spot disease). Therefore the plants photosynthetic performance and the allocation of sucrose to the taproot is heavily reduced. The major toxin produced by *C.beticola* is a photosensitizer called cercosporin, which is essential for pathogenicity.

Our aim is to establish a fast non-invasive screening approach for *C. beticola* resistant cultivars using hyperspectral imaging. Further we characterize metabolite profiles of sugar beet genotypes with differing level of resistance and investigate the molecular mechanisms behind compatible and incompatible plant-pathogen interaction.

We conducted metabolite profiling with LC-PDA-Q-TOF-MS (liquid chromatography coupled with photodiode array detection and quadrupole time-of-flight mass spectrometry) and hyperspectral imaging of three genotypes with different degree of resistance towards *C.beticola*.

Comparison of the semi-polar metabolite (e.g. phenylpropanoids) showed distinct profiles for the genotypes. The total phenolics content could be correlated to the level of *C.beticola* resistance displayed by the genotypes. Additionally, hyperspectral imaging enabled a non-invasive method to distinguish between genotypes. We investigated the contribution of phenylpropanoids to the hyperspectral signature.

We also compared the abiotic stress response of the selected genotypes using different light intensity to mimic oxidative stress reaction and evaluate the genotypes' reaction to modified light conditions as cercosporin is light activated and pathogenicity is heavily depending on illumination. High resistance levels were associated with a pronounced phenylpropanoid metabolism and a strong reaction to increasing light intensity.

O DIS 4

The impact of infected spikelet position on the epidemiology of Fusarium head blight (FHB) evaluated by IR-thermography

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Precision plant protection involves non-invasive sensors for the detection of plant diseases. Implementing these sensors enables the site-specific application of pesticides and contributes significantly in rationalizing the use of pesticides. The threat of Fusarium head blight (FHB) is not only limited to quantitative yield but also includes the contamination with mycotoxins. The position of the primarily infected spikelet of wheat ears influences the development of FHB and consequently affects the grain yield and its contamination with mycotoxins. IR-thermography was used for detecting and assessing FHB of spring wheat (cv. Passat) in small scale measurement. Three spikelet within ears were chosen for inoculation with *Fusarium graminearum*: tip, middle and base. Randomly infection, resulting from spray inoculated ears, was also included in this study as additional control. Disease development was measured with a thermal imaging sensor and simultaneous visual ratings (FHB index). The temperature span within ears proved the best possibility in detecting FHB. Tip infected ears showed the highest temperature span 14 dpi. Exponential function showed high goodness of fit to diseased kernels gradients over single ears inoculated at the tip and base. There was no significant difference in thousand kernel weight in comparison to non-infected only in the case of tip infected ears. FHB index and ΔT (ear temperature -air temperature) were negatively correlated. The correlation varied from strong for randomly infected ears to weak for middle and base infected ears. Evaluating the yield loss and the potential contamination with mycotoxins caused by FHB is proved to be promising with the application of IR-thermography.

O DIS 5

New approaches for remote reading the information on the heterogeneity of the distribution of weeds in the areas of the field for a discrete application of plant protection products

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As part of the concept of intellectual crop, reduce pesticide load on agrocenosis actively working on the creation of high-precision technology differentiated application of plant protection products. These technologies are based on the concept of precision farming to geocode for the output of information processing field, taking into account the heterogeneity of the distribution of pests and weed infestation on the treated field.

Existing methods of information retrieval based on two approaches:

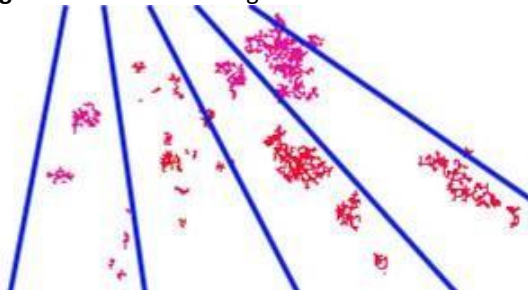
1. Geocoding mapping and data processing using geostatistical methods;
2. Removing the data on the distribution of harmful objects in the areas of the field and process control spraying is carried out in a single process mode. To implement this approach uses the sensitivity of optical or opto-electronic sensors and a database of reference samples of weeds for image processing. However, these methods of processing information received quite time-consuming. Therefore, for the processing of the data is discrete distribution of weeds in the field a new method for decrypting removable media.

Since the shape and color of weeds heterogeneous, automated its allocation is not a simple task. Simply solve the inverse problem. Typically, crops cultivated plants have uniformly periodic structure and strongly contrasted with the surrounding objects. In this regard, it is proposed to remove the original image is geocoded primary culture and then determine the level of infestation areas of the field. To solve these problems is an effective method of image analysis in the frequency domain. To convert the original image into the frequency domain using the device direct discrete Fourier transform. In the analysis of the original images and their representations in the spectral region can be seen that the objects with a periodic structure in the Fourier transform of the given pronounced light spots at some distance from the origin. whereas no such spots periodic missing objects. This property conversion further proposed for the analysis of agricultural fields with crops of the periodic structure.

Figure 1: Photo of cabbage field before treatment with filters.



Figure 2: Photo of cabbage field after treatment with filters.



O DIS 6

Monitoring of chestnut health condition using an Unmanned Aerial Vehicle

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Chestnut ink disease (*Phytophthora cinnamomi*) and chestnut blight (*Cryphonectria parasitica*) are diseases that cause important damages to European chestnut (*Castanea sativa*).

After two decades from the first occurrence of chestnut blight in Portugal, the hypovirulence began to be observed in some locations. The population of these strains is characterized by low diversity. Many of the sub-populations belong to the EU-11 group, which appears only in some orchards in Italy.

Successful treatment depends on the way the population of the fungus extends in the area to be treated. This study refers to the monitoring of inoculations in Padrela region (north Portugal).

The field evaluation was compared to remote sensing techniques that have the ability to collect information from various samples over a large area in a short time, especially with recent developments in sensors on spectral and spatial image resolution. The aerial images obtained by Unmanned Aerial Vehicles (UAVs) for vegetation monitoring has been motivated by the benefits as compared to full size airborne operation, namely the combination of high image quality and quick turnaround times together with lower operation costs and complexity.

For monitoring and evaluating the treated area and know the chestnut decline, were made in June 2014 aerophotogrammetric flights, covering 483 ha. It was used an UAV (*eBee*, *SenseFly*) and were obtained color and near infrared aerial photographs (Fig. 1). Those photographs were compared to aerial images obtained by piloted aircrafts in 2006.

In the period 2006-2014 occurred new chestnut plantations (67 ha), due to the eight multifunctional value of chestnut tree. However, in the study area the decline of chestnut was 56%. The biotic agents were the principal causes of the mortality and *C. sativa* decline, who was confirmed by field observations.

There are advantages on using UAV for the study purposes. Due to the low flying heights, resulting high resolution imagery, and lower image acquisition costs, compared to piloted aircraft or satellite images. UAV cover wide areas, and are virtually undetectable (flights 300 m, up ground), so animals won't be disturbed. The electric UAVs, do not have polluted emissions, resulting in benefits to the environment.

Figure 1: Chestnut tree affected by *C. parasitica* and aerial images obtained by UAV.



Chestnut blight (nº67)



Oral Presentations

Herbicides

O HERB 1

Glyphosate-use in North German arable farming differs regionally

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Glyphosate (N-(phosphonomethyl)glycine) is worldwide the most used herbicidal substance. Insouciant use of glyphosate in farming is currently criticized in science, society and politics. We conducted an on-farm study and analysed regional use of glyphosate in North German arable systems. Objective of our research is to reveal influencing factors on glyphosate use intensity. We hypothesise that the use depends on cropping systems, which are influenced by regional differences in farm management and socio-economic characteristics. We collected data on glyphosate use in four administrative districts of Northern Germany. All districts (Diepholz, Uelzen, Fläming and Oder-Spree) have similar sizes. Two regions (Diepholz, Uelzen) are located in West Germany and two regions (Fläming, Oder-Spree) are in East Germany. Especially farm structures differ between the West districts and the East districts. Data of all crops cultivated in the region were collected from 15 farms per district for the period 2005-2014, in total data of 20.000 fields are included in our study. We used the Standardised Treatment Index (STI) to quantify pesticide use intensity (Roßberg et al., 2002). Variance components of Standardised Treatment Index (STI) were analysed with a linear mixed-effects model. Glyphosate use intensity differs substantially between the study districts. Farmers in the Eastern districts (Fläming and Oder-Spree) used significantly larger amounts of glyphosate. We further prove that the variability of glyphosate use was mainly influenced by the factor "Farm". Glyphosate use was lower when conventional ploughing was applied. Hence, Glyphosate use and non-inversion tillage is strongly attributed to large farms. From the result, that non-inversion tillage and glyphosate use co-occurrences mainly on large farms in the East German Regions we conclude that these farms either regionally adapt their cropping systems due to climatic reasons or profit economically. The latter is very likely, because these cropping systems enable farms to save labor and hence, reduce costs. We analysed application, not motivation data - therefore, we cannot argue whether farmers in the Eastern region are more concerned to take care of their soil by implementing non-inversion tillage.

O HERB 2

Agronomic Consequences of Glyphosate Use - Field and Farm studies from Germany

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Herbicides containing the active ingredient glyphosate are important components of vegetation management in annual and permanent cropping systems worldwide. The German domestic market for glyphosate products, which are predominantly used in the aforementioned cropping systems, has shown a positive trend in recent years. Considering that the agricultural structure in Germany has not changed much in the last few years, the growing market must be due to changes in cultivation practices.

The current re-evaluation process by the European Union of glyphosate has been able to produce little evidence for health or environmentally-relevant problems related to glyphosate; nevertheless, there is an increasing insistence to turn around the trend in the domestic market. This would mean that cultivation practices would need to re-orient to strategies avoiding or minimising the use of glyphosate products. There is good reason to reverse the domestic trend when one considers preventing herbicide resistance in weeds towards glyphosate products, since long-term use in other countries has shown that it can lead to the development of resistance.

The conference paper attempts to provide an analysis of the current lively debate about glyphosate, outlines possible strategies to reduce the use of glyphosate products and identifies potential best management practices in the use of glyphosate products. The discussion takes place on the basis of weed and crop production aspects as well as economic and business aspects. Therefore, regional field studies and German-wide farm inventories were set up. The current inventory was carried out amongst 2000 professional farmers during winter 2014/15 and reflects the most recent status of glyphosate use and its interactions with farm management structure.

Oral Presentations

Herbicides

O HERB 3

Detoxification of chloroacetamide herbicide *metazachlor* and its relation with short- and long-term trade-off in yield and quality of *Brassica napus*

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Introduction: With the growing world population, the agricultural sector is increasingly relying on pesticide-use to ensure food production. However, the short- and long-term effects of herbicides on crop quality are not yet fully understood. Herbicides contain active compounds, which act specifically on plant physiology possibly inducing short-term chemical stress in crop plants.

Objectives: In this study, the occurrence of short-term phytotoxic effects of the pre-emergent chloroacetamide herbicide, metazachlor, are linked to the long-term life history parameters of *Brassica napus* (rapeseed).

Materials and methods: A semi-controlled mesocosm experiment was set up to study the short- and long-term effects on plant growth and development and cellular responses; such as pigment and nutrient profile, lipid peroxidation, antioxidative responses and detoxification processes in the leaves.

Results: In the short term, the aboveground biomass of *Brassica napus* was affected adversely by metazachlor, accompanied by morphological changes of the leaf disc. At cellular level, no signs of membrane lipid peroxidation nor changes in the pigment profile were detected. Total antioxidant capacity measurements in the leaves pointed towards an increase of hydrophilic antioxidants and a decrease of lipophilic antioxidants. Metabolite measurements of ascorbate (AsA) and glutathione (GSH) suggest the activation of the AsA-GSH cycle, which plays an important role in the antioxidant defense mechanism. At the end of the growing season, treated and non-treated crop plants are equal in stem length, aboveground dry weight and seed yield, suggesting *B. napus* crop does recover during its growing season from initial chemical stress induced by metazachlor.

Conclusion: *Brassica napus* displays clear signs of phytotoxicity under pre-emergent application of metazachlor, in the short-term, in the long-term, treated crop plants seem to recover during the growing season, resulting in comparable stem length, biomass and seed yield as non-treated crop plants. The results of this study can contribute to select a set of early response plant parameters, which reflect long-term effects on crop quality and which are consequently useful for herbicide development and selection of crop varieties.

O HERB 4

Development of an herbicide resistant tomato by mutagenesis techniques

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ALS inhibiting herbicides are characterized by a broad weed control spectrum, low mammalian toxicity, high selectivity and high activity with low application rates. There are now over 30 herbicides belonging to this group of herbicides that are registered for use all over the world. These herbicides act by inhibiting the enzyme acetolactate synthase (ALS), a key enzyme in the branched chain amino acid biosynthesis pathway leading to the formation of leucine, valine and isoleucine. Tomato plants are sensitive to these herbicides, except some herbicides of the sulfonylurea group which are detoxified by a *P450-type oxidase in tomato leaves and therefore are not effective in root parasites management*. Development of a tomato variety resistant to the imidazolinones herbicides may serve as a reasonable approach for broomrape control, the most troublesome tomato pest in the Middle East. EMS (ethyl methane sulfonate) mutagenesis was conducted on 20,000 seeds of the commercial tomato line M82. About 100,000 tomato second generation seedlings were screened for resistance to pulsar (imazamox). As a result, a novel tomato mutant HRT-1 was obtained. The mutant is resistant to high rates of imidazalinone herbicides pulsar, cadre (imazapic) and arsenal (imazapyr) in all stages of its vegetation, tissue culture, germinating seeds and tomato plants grown in the field. Several field experiments demonstrated that even a rate high as 144 g a.i. ha⁻¹ did not cause any visual damage or yield loss of HRT1 tomato plants. The resistance is due to a change in the herbicide's target site on the ALS molecule as a result of point mutation in the ALS gene located on chromosome three. The substitution of Alanine to Valine in position 194 which corresponds to Alanine₂₀₅ in *Arabidopsis* confers HRT1 resistance to the imidazolinones.

Oral Presentations

Herbicides

O HERB 5

Novel Herbicides from Some Plant Essential Oils: Activity, Chemical Constituents and Mode of Action

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Owing to the environmental and toxicological concerns coupled with fast appearing herbicidal resistance in weeds due to indiscriminate use of synthetic herbicides, a pressing need has arisen for searching new alternatives. The plant essential oils with little or no mammalian toxicity, less persistence in the environment and chemically and structurally diverse chemical constituents serve as the obvious choice. Though their use as pure components is uncommon, but their herbicidal formulations have become quite common especially in organic farming. The use of essential oils on the patterns of herbicides has another advantage as their active constituents may help in finding novel scaffoldings for the development of new herbicides. The essential oils extracted from *Eucalyptus citriodora*, *Callistemon viminalis* and *Melaleuca leucadendra* have been found to be promising for the management of weeds like *Echinochloa crus-galli*, *Avena fatua*, *Cyperus rotundus* and *Phalaris minor* that interfere with rice and wheat crops. The oils were active both at the post and pre-emergence level and their activity increased in a dose-response manner. In general, the effect was seen more on the grassy weeds compared to broad-leaved ones. Their effect at post-emergence level was comparable to that of synthetic herbicides. The mode of action of these essential oils has also been evaluated through various biochemical pathways. During the deliberations of the conference, it is proposed to discuss various aspects of essential oils as herbicides.

O HERB 6

C4 plant selective herbicides: A new approach to combat C4 weeds of arable crops

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Many of the world's worst weeds use C₄ photosynthesis whereas many economically important crops exhibit C₃ photosynthesis. One of the differences between these two photosynthesis modes is the enzyme for the first steps of carbon assimilation: C₄ plants use phosphoenolpyruvate carboxylase (PEPC) while C₃ plants use ribulose-1,5-bisphosphate-carboxylase/oxygenase. This difference provides the potential for developing novel and selective herbicides specifically controlling C₄ weeds infesting C₃ crops.

The aim of our project is to identify compounds that selectively inhibit PEPC of C₄ plants, whereas C₃ plants should remain unaffected; such compounds could become candidates for herbicide development. The project partnership consists of two groups at the HHU Düsseldorf as well as of two groups at Forschungszentrum Jülich and is placed within the strategy project BioSC funded by the State of North Rhine-Westphalia [<http://www.biosc.de/c4-psh>].

The project partners focus on identifying compounds through molecular modelling and screening of databases, as well as on identifying their binding mode, and mechanism of action and selectivity of the compounds in *in vitro* assays. Furthermore, the toxicology of selected compounds on soil bacteria is investigated. At IBG-2: Plant Sciences at Forschungszentrum Jülich, we screen pre-selected compounds on C₄ weeds and C₃ crops in leaf disc-assays using chlorophyll fluorescence measurements. Potential PEPC inhibitors are investigated in detail regarding their effect on plant growth. Gas exchange measurements and an automated phenotyping platform equipped with a hyperspectral line-scanner are used to assess the effect of the compounds on the physiological state of C₃ and C₄ plants.

Several compounds have been identified as potential PEPC-inhibitors in *in vitro* assays (Paulus et al., 2014) which are now screened and tested on a whole-plant level.

The development of selective PEPC inhibitors could contribute to solving the problems of lack of new herbicide modes of action and herbicide resistance development in weeds and broaden the options for effective weed control.

Paulus JK, Förster K, Groth G (2014). Direct and selective small-molecule inhibition of photosynthetic PEP carboxylase: New approach to combat C₄ weeds in arable crops. FEBS Letters 588 (12), 2101-2106. [<http://dx.doi.org/10.1016/j.febslet.2014.04.043>]

Oral Presentations

Integrated Pest Management III

O IPM III-1

Environmentally friendly apple fruits management system

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Growers participating in the “Agri-environment payments” program shall follow requirements of Regulation 1782/32003 as well as minimum requirements for fertilizer and plant protection product use and other relevant, mandatory requirements established by national legislation. According these requirements the same active ingredients of plant protection products must be used not more than two times per vegetation season and preharvest interval should be 1.5 times longer than indicated on the label. Plant protection products labelled as "Very toxic" and (or) "Toxic" are forbidden. At the same time, forecasting models should be incorporated in plant protection system.

The objective of trial is to achieve sustainable application of pesticides by reducing the risks and impacts on human health and the environment.

The research was carried out at the LRCAF Institute of Horticulture in 2011-2013 in intensive apple orchard. Seven cultivars 'Auksis', 'Alva', 'Connell Red', 'Ligol', 'Lodel', 'Rubin' and 'Shampion' were tested.

Diseases control program was based on internet supported forecasting system iMETOS[®]sm (Pessl Instruments, Austria). This system recorded meteorological conditions and calculated apple scab infections at three levels: light, medium and high. Susceptible to apple scab cultivars were sprayed when the risk of ascospores release or conidia light infection reached more than 70-80 %.

During the trial scab susceptible cvs. 'Alva' and 'Ligol' on average were sprayed twelve times but remaining cultivars on average were sprayed nine times. Reduced pesticide program does not guarantee total scab control, therefore damaged fruits should be thinned manually. Applying environmentally friendly fruit cultivation system high quality fruit yield reached on average 39 t/ha.

Acknowledgements: This work was carried out within the framework of the long-term research program “Horticulture: agro-biological basics and technologies” implemented by Lithuanian Research Centre for Agriculture and Forestry.

O IPM III-2

The Use of the Natural Volatile Compound to Manage the Pear Psylla *Cacopsylla bidens* (Šulc) in Commercial Pear Trees

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Introduction: Pear-feeding psyllids are the most damaging pests of commercial pear orchards in all pear-growing regions, in Asian, European, and USA. High-density populations of these insects can cause premature leaf and fruit drop, diminish plant growth, and reduce fruit size. In addition, their honeydew promotes sooty mold on leaves and russetting on fruits. Pear psyllas are also considered vectors of pear pathogens such as the bacterium *Erwinia amylovora* Burrill that causes fire blight and *Candidatus Phytoplasma pyri* causing pear decline. Pear decline has.

Since the pear psylla rapidly acquires inherited resistance to pesticides, the range of efficient chemicals for its control is narrowing down and the applied concentrations are constantly increasing, while consumers demand lower pesticide application in the orchards. Indeed, psylla control is one of the major obstacles to efficient integrated pest management (IPM) in pear orchards. Therefore naturally accruing volatile compounds from resistant pear trees as anti psylla insecticides are an attractive option.

Results: We have identified two pear accessions (Py.760-261 and Py.701-202) from the local Newe Ya'ar live collection as having resistance to pear psylla and found that these accessions, when used as inter-stock, provide the commercial Spadona scion with reduced susceptibility to pear psylla. GC-MS volatile metabolic profiling has been performed for the two pear accessions. We have identified several volatile compounds that accumulate in the leaves of Spadona grafted on these accessions but not in the leaves of Spadona grown on other rootstocks such as *P. Betulifolia* or *P. communis*. Laboratory experiments and applications of some these volatile compounds were very effective against psylla eggs, nymphs and adults. To the best of our knowledge, this is the first report on the accumulation and application of the volatile compound in Pear leaves against pear Psylla.

Conclusion: Our new discovery may provide a new concept to manage the Pear Psylla *Cacopsylla bidens* (Šulc) in commercial pear trees. This approach could be applicable for a variety of other fruits, crops specifically those, which show dramatically Psylla pest problems.

O IPM III-3

The Effectiveness of an Integrated Approach in Controlling Leaf and Ear Diseases in Winter Wheat

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An integrated approach to pathogen control in cereal crops involves combining fungicides with biocontrol agents. The objective of this study was to evaluate the effectiveness of integrated control strategies of infections caused by *Zymoseptoria tritici* on flag and penultimate wheat leaves. The composition of the microbiome on winter wheat grain was also analyzed. A field experiment was performed in north-eastern Poland in 2013-2014. Winter wheat (*Triticum aestivum* L. cv. Bogatka) was sown in 20 m² plots. The experiment had a randomized block design. During the growing season, wheat plants were protected twice or three times, at the stem elongation (first node) stage (BBCH 31), at the heading stage (BBCH 55) and at the watery ripe stage (BBCH 71) with triazole, morpholine, strobilurin and chloronitrile fungicides. The third protective treatment involved the use of yeast *Sporobolomyces* sp. or bacterial *Sphingomonas* sp. suspensions with the concentration of 10⁸ cells cm⁻³. Unprotected plants served as control. The severity of Septoria leaf blotch was higher in 2014, and all integrated strategies (two fungicide treatments and one with microbiological agent) proved effective in controlling flag leaf infections (Fig 1). Ear protection with tebuconazole (BBCH 71) and the bacterial cell suspension reduced the counts of *Fusarium* fungi on the surface of wheat kernels. Unlike bacterial and yeast suspensions, tebuconazole considerably inhibited the growth of *Penicillium* fungi on wheat grain. During the two-year experiment, wheat plants protected with fungicides (BBCH 31, BBCH 55) and yeast or bacterial suspensions (BBCH 71) produced the highest yield, by 9.4 and 10.5% higher, respectively, in comparison with the control (Fig 2).

Figure 1

Figure. 1. The severity of Septoria leaf blotch (leaf area with symptoms in %) Fun1 - chlorothalonil, epoxiconazole + piraclostrobin, tebuconazole, Fun2 – epoxiconazole + fenpropimorph, propiconazole, *Sporobolomyces* sp., Fun3 - fenpropimorph, propiconazole, *Sphingomonas* sp., Fun 4 – not treatment, epoxiconazole + piraclostrobin, tebuconazole.

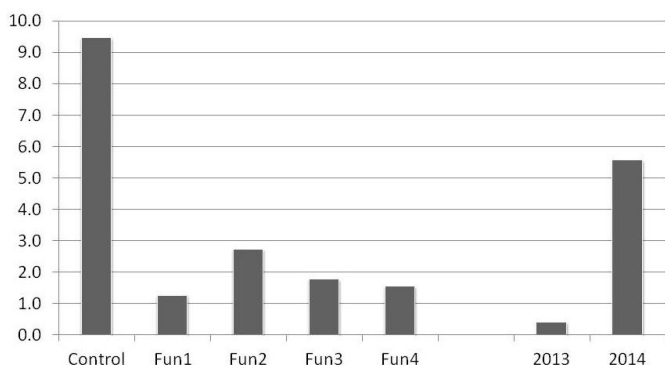
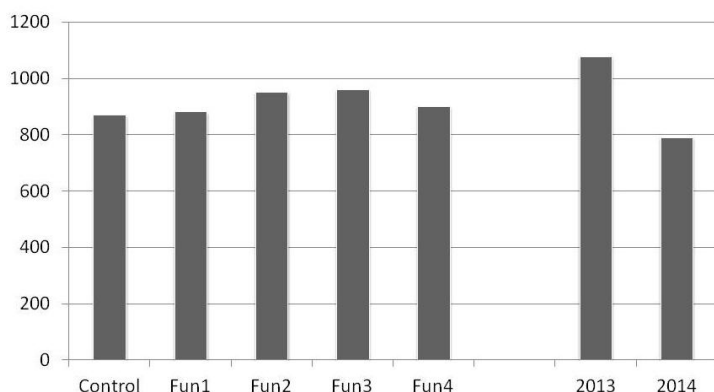


Figure 2

Figure 2. Grain yield of winter wheat (g / m²).

Explanation at Fig.1.



Oral Presentations

Integrated Pest Management III

O IPM III-4

Effectiveness of management practices on Fusarium wilt intensity in smallholder Gros Michel banana in Costa Rica

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Fusarium wilt (FW), caused by the soil-borne pathogen *Fusarium oxysporum* f. sp. *cubense* (Foc) is one of the destructive diseases of banana. Exclusion of the disease and resistant cultivars are considered the most effective management practices. The use of disease-free tissue culture planting material has also been recommended to reduce the spread of Foc into new fields through symptomless, but infected corms. Historically exclusion has not been achievable, especially locally and resistant cultivars are not always available or do not meet market requirements. Therefore, management strategies are needed to reduce current yield losses and increase plantation life in new plantings in infected soils. In this work the impact of different management practices on FW intensity was studied in 15 farms of 'Gros Michel' bananas in Turrialba, Costa Rica. Experiments were conducted in five farms from three production areas heavily infected by Foc race 1 (Grano de Oro, Guayabo and San Juan). Management practices included: a) Use of tissue-culture (TC) planting material; b) Inoculation of TC-plants with endophytes during acclimatization; c) Use of organic amendments; d) Use of chemical fertilization according crop phenology. Practices were divided according levels of intervention in Optimum [(OTP: (a+b+c+d)), Medium [MDP: (a+c+d)], Minimal [(MNP: (a))] and Farmer practices [FMP]. Plants of MNP were managed according to FMP. FW incidence was confirmed in all production areas with higher disease levels in Guayabo. Independent of production area, MNP plants showed higher FW intensity than all other practices, including FMP. OPT and MDP provoked a discrete FW reduction in some farms, manifested as longer incubation period and lower disease progression rate (DPR) when compared with MNP. Although there were not differences at the end of one crop cycle, OPT and MDP plants showed higher DPR than plants under FMP. This indicates that TC-plants were more susceptible than conventional planting material even when additional inputs were used. In conclusion, despite some effect of endophytes and organic amendments, these practices were not enough to reduce FW at field level. Work using different sets of multifunctional endophytes, soil microorganisms and additional soil-health oriented practices is on-going.

O IPM III-5

Ecologically-based Integrated Pest Management (IPM) Program for Food Security Crops in Central Asia

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Responding to the pest management and food security needs of recently emerged independent countries in Central Asia, a consortium led by Michigan State University (MSU) implemented a regional Integrated Pest Management (IPM) collaborative research and capacity building program from 2005 to 2014. Funded by the USAID and MSU, the project activities were implemented in three countries - Tajikistan, Kyrgyzstan, and Uzbekistan. The MSU-led consortium included University of California-Davis, Kansas State University, CGIAR/ICARDA regional program and a number of host-country organizations and local NGOs engaged in agricultural research and development in the region. The Central Asia regional IPM project was a part of the Global IPM CRSP Program managed by the Virginia Tech University (now referred as Feed the Future Food Security Innovation Lab: Collaborative Research on IPM). The project was implemented in two phases. The first five years (Phase I) focused on strengthening existing biolaboratories, introducing landscape ecological approaches for enhancing biological control of pests and IPM education and outreach. The second five years (Phase II) focused on the development of IPM packages for three food security crops - wheat, potato and tomato. IPM education and outreach through student training and farmer field schools was an integral component of the project. The training and capacity building strategy included in-country and regional workshops as well as farmers and students field schools with due consideration of gender equity. Furthermore, three young scholars from Tajikistan, Kyrgyzstan and Uzbekistan were trained at MSU through graduate degree programs in wheat, potato and tomato IPM. The project provided networking and short-term training opportunities for IPM professionals from the region to attend international IPM short courses, workshops and conferences. Scholarship, publications and dissemination of research results through electronic and print media were important outcomes. The project has broken isolation and helped established long-term relationships which will foster the continued development of ecologically-based IPM programs in Central Asia. More information on the project can be found at: http://www.ipm.msu.edu/international/central_asia_ipm

O IPM III-6

The model project “Demonstration Farms for Integrated Pest Management” - a suitable instrument for IPM implementation in Germany

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The model project “Demonstration Farms for Integrated Pest Management” is part of the German National Action Plan on Sustainable Use of Plant Protection Products. The project aims to implement innovative findings and suitable methods of integrated pest management (IPM) into practice, and demonstrate this to other farmers, advisors as well as to the public.

66 agricultural farms from different growing regions all over Germany participate in the project for 5 years. The farms represent the major production sectors apple growing, viticulture, arable cropping, vegetable growing and hop production. Specific requirements of IPM based on the general principles of IPM (Directive 2009/128/EC) were defined in project-related IPM guidelines formulated for each production sector. To ensure a high standard of IPM implementation, demonstration farms receive intense support and supervision by plant protection experts and hired advisors from the plant protection services of the federal states. They provide for comprehensive assistance when introducing new procedures. Furthermore, they are responsible for monitoring of crops, pests and diseases and data collection. Technical advice, monitoring systems and modeling of plant pathogen/pest systems is provided by the Central Institution for Decision Support Systems in Crop Protection (ZEPP).

The Julius Kühn-Institute (JKI) as a research institution coordinates the overall network and supports activities of the involved plant protection services. It is responsible for data processing and analysis (e. g. for treatment frequency, risk indicators, non-chemical measures or expenditures for monitoring) as well as interpretation and discussion of results.

Knowledge transfer and public relation work are key objectives of the project. The demonstration farms are encouraged to organise each year a farm day with field seminars and on-site demonstrations to motivate other farms within their region to adopt the demonstrated IPM procedures. The project website introduces the participating farms and informs about the project in general and its results (<http://demo-ips.jki.bund.de/>).

The work is financially supported by the German Federal Ministry of Food and Agriculture (BMEL) through the Federal Agency for Agriculture and Food (BLE), grant number 2810MD001.

Oral Presentations

Endophytes II

O END II-1

Kill or cure? Insights into the controversial interaction between *Paenibacillus* and *Serratia* strains with their host plants and with the plant pathogen *Verticillium* spp.

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Introduction: Verticillium wilt caused by *Verticillium* spp. is difficult to suppress and results in severe yield losses in a broad range of crops including oilseed rape and cauliflower. Five *Serratia* and five *Paenibacillus* isolates were previously shown to antagonistically affect fungal pathogens like *Verticillium* and have potential application in plant protection.

Objectives: The plant growth promoting (PGP) potential of *Serratia* and *Paenibacillus* isolates and their interaction with the *V. longisporum* were studied in order to develop a sustainable seed treatment that will protect oilseed rape and Brassica vegetables from Verticillium wilt.

Materials and methods: The selected *Serratia* and *Paenibacillus* isolates were applied to the surface-sterilized seeds of oilseed rape and cauliflower using bio-priming. The PGP effect and root colonization capacities of the isolates were compared under gnotobiotic conditions. One strain from each genus was selected and tested for its PGP qualities in sterile and non-sterile soil. The level of growth inhibition of *V. longisporum* by volatile organic compounds (VOCs) emitted by cultured *P. polymyxa* Sb3-1 was determined. Gas chromatography-mass spectrometry was used to identify VOCs produced by *P. polymyxa* Sb3-1 upon contact with VOCs released by *V. longisporum*, and vice versa.

Results: *Serratia* treatment resulted in different levels of PGP, while *Paenibacillus* strains damaged roots under gnotobiotic conditions. *P. polymyxa* Sb3-1 did not have a significant effect on plant growth in non-sterile soil, however it did promote plant growth in the sterile soil. It was demonstrated that VOCs produced by cultured *P. polymyxa* Sb3-1 significantly inhibited growth of the *V. longisporum*. Both microorganisms produced VOCs in reaction to one another.

Conclusion: The choice of growth environments in the investigation of plant-bacterium interaction is crucial. The interaction of *P. polymyxa* Sb3-1 with *V. longisporum* via emitted VOCs indicated an ongoing dialogue between these microorganisms resulting in growth inhibition of the plant pathogenic *V. longisporum* by the beneficial *P. polymyxa*.

O END II-2

Plant-Mediated Effects of Soil Amendment using Arbuscular Mycorrhizal Fungi on Colorado Potato Beetle

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Introduction: Plants grown on organically managed soils have been shown to be less favourable hosts for phytophagous insects, and it is explained by the mineral balance hypothesis. The hypothesis suggests that the organic matter and microbial activity associated with organically managed soils provides a buffering capability to maintain optimal nutrient and mineral balance in plants, which in turn affects the performance of phytophagous insects (Phelan, 1997). Soil microorganisms are an important factor in the health of plants. Symbiotic *Arbuscular Mycorrhizal Fungi* (AMF) form a key component of the microbial populations. By improving rooting and plant establishment, enhancing uptake of low mobile ions, improving nutrient cycling and enhancing plant tolerance to biotic and abiotic stress factors, AMF may be a useful tool to achieve an optimal nutrient balance that results in both good plant growth and resistance to herbivory in ecologically sound crop management systems.

Objectives: The overall objective of this research was to determine if AMF increases potato tolerance to Colorado potato beetle (CPB).

Materials and methods: Study was carried out at 24±2°C, 60±10 % RH, and a photoperiod of 14:10 (L:D) h in climate room. Development, survival and reproduction of CPB were investigated on potted potato plants (Granola cv) treated with manure+AMF (*Glomus intraradices*), synthetic fertilizer, and plants untreated. The life history raw data were analyzed using the age-stage, two-sex life table to obtain population parameters.

Results: Results indicated that AMF affected population growth of CPB. Values of population parameters (the net reproductive rate (R_0), intrinsic rate of increase (r) and finite rate of increase (λ)) were lower on plants treated with AMF. There were differences in concentrations of mineral in potato leaves, and mineral content of potato leaves explained 35% of the variation in developmental time and 38% of the variation in reproduction of the CPB among the treatments

Conclusion: We suggest the use of AMF in organic production systems for both good plant growth and resistance to herbivores

References:

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Oral Presentations

Endophytes II

O END II-3

A root endophyte induces tolerance to root herbivory in rice

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The root endophytic fungus *Piriformospora indica* recruits gibberellin (GA) signaling to colonize roots and systemically promotes plant growth under pathogen attack and salt stress. The rice water weevil (RWW) is a major insect pest of wetland rice. The adults feed on leaves without major impact on the plant, but the root-feeding larvae cause severe yield loss. We conducted two glasshouse experiments to investigate whether *P. indica* can protect rice plants against RWW. Root colonization by *P. indica* attenuated the negative impact of RWW on root and shoot biomass without affecting RWW performance, and suppressed larval induction of jasmonic acid (JA) in roots. Using the JA insensitive *COI1-18* and the GA-deficient *EUI1-OX* mutant, we observed that JA led to the depression of root growth by adult and larval feeding; an effect that was counteracted by GA. On the other hand, GA was required for the growth promoting effect of *P. indica*, while JA was uncovered to be a negative regulator of this function. We propose that crosstalk between GA and JA mediates the endophyte-induced tolerance towards root herbivory in rice.

O END II-4

The endofungal bacterium *Rhizobium radiobacter* RrF4 colonizes plant roots and induces growth and health independently of its fungal host *Piriformospora indica*

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The Alphaproteobacterium *Rhizobium radiobacter* F4 (*RrF4*) forms an intimate mutualistic tripartite symbiosis with the beneficial endophytic Sebacinalean fungus *Piriformospora indica* and a broad range of host plants. While attempts to cure *P. indica* from *RrF4* failed, the bacterium could be isolated nonetheless from the fungus and multiplied in pure culture. Here we report on *RrF4*'s genome and the beneficial impact the free-living bacterium has on plants. In contrast to other endofungal bacteria, the genome size of *RrF4* is not reduced. It shows a high degree of similarity to the plant pathogenic *Agrobacterium tumefaciens* C58, except vibrant differences in both the tumor-inducing (Ti) plasmid and the megaplasmid which can explain the loss of *RrF4*'s pathogenicity. Similar to its fungal host *P. indica*, *RrF4* colonizes roots of monocotyledonous and dicotyledonous plants without detectable host preference. Microscopic analysis of plant root colonization with GUS- and GFP-tagged *RrF4* identified distinct entry sites at maturation zones, and strong conglomeration at lateral root protrusions. *RrF4*-colonized plants have increased plant biomass and enhanced systemic resistance against bacterial leaf pathogens. *RrF4*-mediated resistance to *Pseudomonas syringae* pv. *tomato* DC3000 was compromised in Arabidopsis mutants indicative of the induced systemic resistance (ISR) pathway, a phenomenon that earlier also was observed with *P. indica*-mediated resistance. Consistent with this, *RrF4*- and *P. indica*-induced pattern of defence gene expression in barley roots were similar. In clear contrast to *P. indica*, but similar to other endophytic PGPR bacteria, *RrF4* colonized not only the root cortex but spread beyond endodermis into the stele. Together our data show that *RrF4* exhibits virtually the same mechanistic beneficial activity on plants as its host fungus *P. indica*, but shows differences in the root colonization pattern. Based on our findings we discuss the role of *RrF4* in the Sebacinalean symbiosis.

O END II-5

Effect of Chitooligosaccharides with Different Degrees of Acetylation on Wheat Seedlings under Salt Stress

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Soil salinity can cause abiotic stress and lead to significant inhibition of germination, growth and productivity of crops. Although chitosan appears to improve the tolerance of plants, the exact physiological mechanism for this mechanism is not currently understood. As DA is the most important parameter influencing the chitosans' various properties, how the DAs exactly work in altering activities of chitosan is unclear.

Accordingly, in this study, we exposed wheat seedlings to salt stress and investigated the effect of exogenous COSs with different DAs on wheat seedlings. Furthermore, we evaluated the expression of a series of salt-associated genes in wheat by quantitative RT-PCR to explore the physiological mechanisms of exogenous COS with different DAs on wheat tolerance to salt stress.

The results showed that treatments with exogenous COSs with different DAs increased the biomass of wheat seedlings; decreased the concentration of MDA; increased the contents of chlorophyll content, fluorescence and photosynthetic characters; and improved antioxidant activities of SOD, POD and CAT (Fig.1). RT-PCR analysis showed that expression of three antioxidant enzyme genes and two Na^+/H^+ antiporter genes in wheat were modulated to enhance the tolerance of plant to salt stress (Fig.2). The results illustrated that COS could protect plants from salt stress damage by modulating intracellular ions concentration and enhancing the capacity of antioxidant enzymes activities. Furthermore, COS with DA 50% had more effective activities of alleviating salt stress to wheat seedlings than those with other DAs, which indicated the degrees of substitution of acetyl group play important roles in the activities of COS.

Figure 1

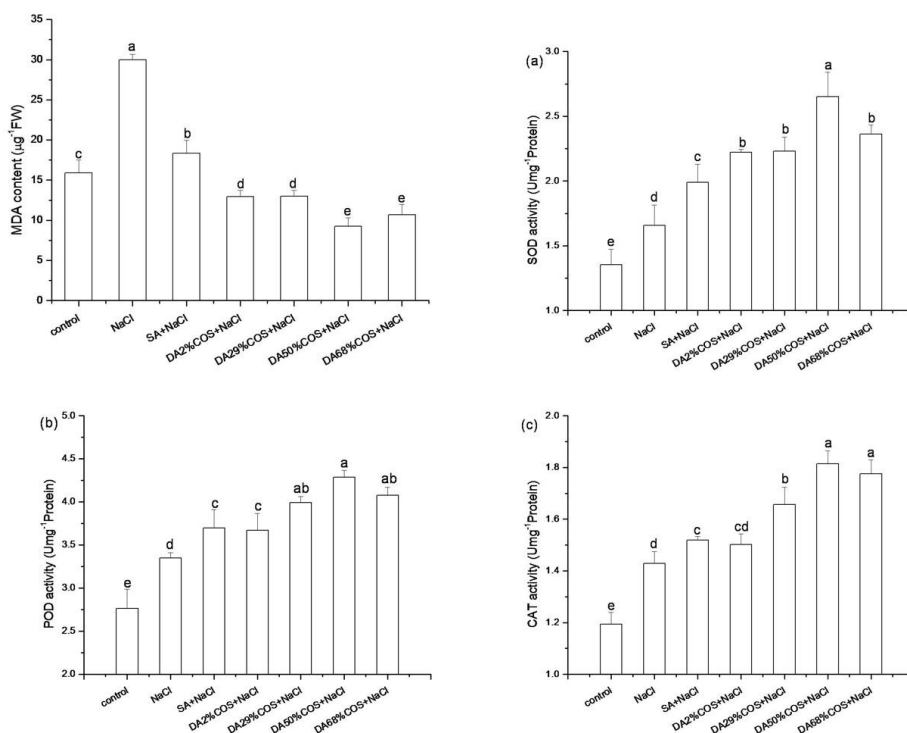
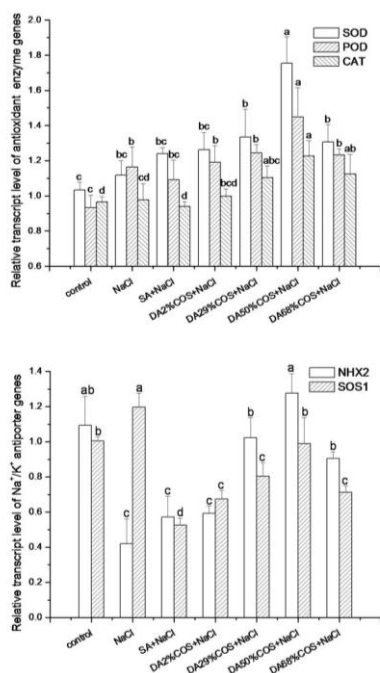


Figure 2



O END II-6

Evaluation of novel fungal formulations (*Metarhizium brunneum*) and botanicals (Neem) in an “Attract-and-Kill strategy” under field and laboratory conditions targeting wireworms

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Wireworms, the larvae of click beetles, are an important taxon of soil dwelling pests. They attack the subterranean parts of a wide range of crop plants and have recently evolved into a serious problem in many cultures, mainly due to the lack of specific control.

The joint projects ATTRACT and INBIOSOIL focus on the development of an innovative technology, taking advantage of the fact that wireworms orientate towards their host plants via detecting root CO₂-exudates. Providing an alternative CO₂-source (“Attract”-component) combined with a “Kill”-component may be the key for an efficient wireworm control, especially since insecticides in susceptible crops like potatoes and maize are on the brink of being phased out.

The entomopathogenic fungus (EPF) *Metarhizium brunneum* has found to be a potential biological control measurement for wireworms and may play an important role in the reduction of those. Furthermore, botanical insecticides such as Neem may also be a suitable means to control this pest.

Baker’s yeast, acting as CO₂-source was combined with nutrient additives and encapsulated in Ca-Alginate (“Attract”-formulation). For “Attract-and-Kill”-applications, NeemAzaI® technical (Trifolio-M GmbH) or *M. brunneum* spores were co-encapsulated or the attractive formulation was taken solely and combined with encapsulated *M. brunneum* spores within a co-application.

Experiments performed on a laboratory scale have shown that “Attract-and-Kill”-formulations affect wireworm vitality and have the potential to kill them. These preliminary results have been confirmed in field experiments conducted at four sites in Lower Saxony. The damage level of wireworm infestation in organically managed potato fields were significantly (although not always) reduced by the application of “Attract-and-Kill”-formulations.

Our current aim is to improve present formulations and enhance the attractiveness of the capsules by adding phagostimulants, aiming at leading the wireworm towards the capsule and provoking a bite reflex.

The development of a refined “Attract-and-Kill”-strategy to control wireworm infestation will contribute to reduced pesticide inputs in sustainable agricultural-horticultural systems and offers potential savings for growers.

Oral Presentations

Insecticides II

O INS II-1

Integrated Pest Management (IPM) in Grain Legumes: An update in Asia

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Introduction: Interactions with the farming communities revealed that majority of the farmers in Asia had adopted chemical control and initiate the plant protection based on the first appearance of the pest, irrespective of their population, crop stage, and their damage relationships. The cost of plant protection on various crops ranged up to 40% of the total crop production. Though IPM has been advocated for the past two decades, only 3.2% of the farmers adopted IPM practices in various crops. Recent farmer participatory approach adopted by ICRISAT showed positive outcome.

Objectives: To develop, evaluate and share IPM based research to minimize pesticide misuse in legume crops to provide residue free products to end users with better environment.

Materials and methods: Various IPM options such as resistant varieties, cultural, biological controls, evaluation of thresholds and need based application of chemicals developed at research stations were evaluated and shared with farmers in on-farm situations in a participatory approach.

Results: Insect pests are a major constraint in legume production and storage. Legumes pests are often sporadic, and at times cause complete destruction of crops. There has been a shift in pest spectrum in Asia over the past five decades. Farmer participatory approach adopted by ICRISAT in collaboration with national agricultural research systems, non- governmental organizations in a consortium mode proved effective in reducing pesticide use (20-100%) in different crops.

Conclusion: IPM research at ICRISAT in cooperation with NARS and NGOs in a participatory approach farmers adopted pest-resistant cultivars, potential agronomic practices, knowledge on natural enemies, and integrating various options and brought out several indigenous plant protection practices to the forefront to enhance the productivity of grain legumes along with environmental and operational safety.

O INS II-2

A landscape level monitoring study evaluating the risks posed by neonicotinoid dressed oilseed rape to three hymenopteran pollinator species

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Although higher tier studies did not show unacceptable adverse effects of oilseed rape (OSR) seeds-treated with the neonicotinoid insecticide clothianidin on honey bees, the EU commission suspended uses of clothianidin in bee attractive crops by applying the precautionary principle. For their re-authorization a landscape level monitoring study as proposed by the EU & SETAC Europe Workshop EPIF 2003 was conducted to further address evaluation uncertainties of the existing database for honey bees and risks posed to other hymenopteran pollinator species (bumble bees, Mason bees) under realistic worst case exposure.

A typical OSR cultivation area in Northern Germany was selected for this study. The study comprised 2 large-scale areas: a "control site" with all OSR varieties without an insecticidal seed treatment and a "treatment site" where all OSR varieties had been seed-treated with clothianidin. Both sites covered each an area of 65 km² (9 km in diameter). Residues of clothianidin were analysed in pollen and nectar collected from colonies of all test species as well as in pollen and nectar collected from honey bees in gauze tunnels on OSR fields.

Key conclusions from the study are as follows:

- The two landscapes serving as a control and a treatment area, were sufficiently similar in regard to topography, climatic conditions, soil properties and cropping history to scientifically support the study objective.
- The chosen landscape is representative for OSR cultivation areas across Western Europe.
- The residue level in rape pollen and rape nectar samples from the treatment area were at the upper range of residues found during previous studies in OSR pollen and nectar.
- No short- or long-term adverse effects were observed on the development of any of the three investigated test species (survival, population growth, reproductive performance) during or after the exposure to a Clothianidin seed treatment in the treatment landscape.

Oral Presentations

Insecticides II

O INS II-3

Insecticide resistance mechanisms and management in major pests of vegetable crops

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Introduction: The use of chemicals has resulted in selection of operational insecticide resistance in agricultural pests a phenomenon particularly striking in Mediterranean countries.

Objectives: We aim to investigate the spectrum of insecticide resistance, the underlying mechanisms and the dynamics of resistant alleles in field populations and to develop tools to facilitate resistance management of the spider mite *Tetranychus urticae*, the tomato borer *Tuta absoluta* and the whitefly *Bemisia tabaci*.

Materials and methods: Bioassays, biochemical assays and advanced molecular assays, such as next generation transcriptomics, were used to identify, characterise and analyse insecticide resistance mechanisms. Microsatellite markers, sequence polymorphism and simple molecular and ELISA based diagnostic tools were used to study the gene flow and the origin and spread of resistance in field populations.

Results: Striking abamectin, neonicotinoid and diamide resistant phenotypes have been identified in *T. urticae*, *B. tabaci* and *T. absoluta* respectively. In abamectin resistant *T. urticae* we identified mutations in the glutamate gated chloride channels and developed a Taqman assay for their early detection in field populations. Several overexpressed P450s have been associated with resistant phenotypes in *B. tabaci* and in *T. urticae* with variable cross spectrum catalytic efficiency. Sequence analysis of the putative diamide binding site region of the ryanodine receptor (RyR) in *T. absoluta* revealed a novel amino acid substitution that is associated with resistance. Analysis of the geographic distribution, gene flow and origin of known target site resistance mutations showed that although resistance mutations can arise several independent times their spread in large geographic areas seems highly dependent on human activities. Finally, we developed an ontology-based pesticide resistance database (Galanthus, <http://www.galanthos-prd.gr>) in order to facilitate decisions for resistance management strategies.

Conclusion: We investigated the spectrum and the mechanisms of insecticide resistance and the spread of resistance in field populations both in the context of local resistance management and population and evolution studies. Finally we developed tools to facilitate resistance management of major agricultural pests.

O INS II-4

Side-effects of pesticides on non-target organisms: 1- In Egyptian cotton fields

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The cotton sector remains one of the major importance to the Egyptian economy Destructive insect pests are the most limiting factor of the yield. Bollworms, leaf worm are the key pests and lately aphids and whitefly have become also a problem because of the wide use of pesticides. Although an IPM program is implemented in cotton fields in Egypt, pesticides are still the most effective tool for controlling the cotton pests. Pesticides' application has created several problems among such is pest resistance to pesticides which has become a critical problem in Egypt.

Side effects of some groups of recommended pesticides in cotton fields were evaluated on non-target pests, predators and pollinators through experimental field trials at two Egyptian Governorates for two growing seasons, 2013 and 2014. Direct counts of target and non-target organisms, pre-treatment and at three day intervals post treatments for each pesticide/ location were practiced. Reduction rate in insects' population was estimated. Side-effects of pesticides on numbers and activities of the honeybee, *Apis mellifera* (L.) workers under field conditions were studied. As well, a discriminating concentration technique was used for rapid monitoring of insecticidal resistance in field-samples of the pink bollworm (PBW), *Pectinophora gossypiella* (Saund.) larvae collected from sprayed cotton fields.

Rates of reduction in *P. gossypiella* population (target pest) ranged 63.2-72.5 and 61.9-74.8% in 2013 and 2014, respectively. Respective rates in non-target pest species; *Aphis gossypii* (Glov.), *Bemisia tabaci* (Genn.) and *Tetranychus urticae* Koch were 35.6-83.3 and 50.3-83.0%, while they attained 79.2-96.5 and 65.2-91.8% in the populations of the predatory species; coccinellids, *Chrysoperla carnea* (Steph.) and spiders. For honeybees, 59.2% mortality was recorded and foraging and nectar

Oral Presentations

Insecticides II

collecting workers were reduced by 14.5-36.6%. Resistance % in the PBW, collected from sprayed fields was estimated by 19.2-54.6%. Similar studies on other field and horticulture crops in Egypt have been carried out.

O INS II-5

Effect of emamectin benzoate and spinosad on some biological parameters of the *Chrysoperla carnea* (Stephens) (Neu.: Chrysopidae)

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The common green lacewing, *Chrysoperla carnea* (Stephens) is one of the best-known biological control agents. In this study, the lethal and sublethal effects of emamectin benzoate and spinosad were evaluated on the biological parameters of the *C. carnea*. Rearing of green lacewing and all experiments were done in growth chamber at 26 ± 1 °C, $70\pm 5\%$ RH and a photoperiod of 16:8 (L:D) h. Bioassay tests were carried out on 2nd instar larvae of green lacewing by residue contact method. The results showed that the tested insecticides at recommended field concentration had negligible toxicity on larvae of this natural enemy. According to IOBC standard method, emamectin benzoate and spinosad were classified as harmless. Sublethal effects of tested insecticides were studied at twice recommended field concentration of each insecticide. Emamectin benzoate and spinosad increased larval duration by 7.7 and 15.5 % and pupae duration by 7.6 and 11.4 % compared with control, respectively. The insecticides increased significantly larval mortality rate compared with control but there did not on pupal stage. Fecundity, fertility and adult longevity were not affected significantly by the insecticides compared with control. Results showed that these bioratioanal insecticides had the least adverse effects on the green lacewing. Therefore, they have a high potential to be combined with green lacewing as biological control agent in Integrated Pest Management (IPM) programs.

O INS II-6

Monitoring of acaricide resistance based on RCV and QS methods for adaptive management in *Tetranychus urticae* Koch

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Rapid resistance detection system is essential for a proper management of acaricide-resistant populations of *Tetranychus urticae*. Residual contact vial bioassay (RCV) and quantitative sequencing (QS) methods were employed to determine acaricide resistance levels in *T. urticae*. Among 19 acaricides tested, 12 were applicable to the RCV as they showed dose-dependent mortalities within 8 h post-treatment. The QS was able to determine the resistance allele frequencies of 12 point mutations associated with resistance to five acaricides (organophosphate, pyrethroid, abamectin, bifenthrin and etoxazole). The laboratory strains revealed high susceptibility to all the test acaricides with low resistance allele frequencies, but the field populations collected from rose greenhouses exhibited severe levels of multiple resistance and high resistance allele frequencies to almost all tested acaricides, indicating high levels of acaricide resistance in most rose cultivation areas in Korea. The RCV and QS methods would be useful for the selection of appropriate acaricides and for the establishment of an adaptive management system to control acaricide-resistant *T. urticae*.

Oral Presentations

Diagnosis

O DIA 1

B-Fast ELISA - a newly developed ELISA technique allowing large scale testing in two hours

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Intercontinental travel and trade have increased significantly in recent decades and resulted in the emergence of many plant diseases including those caused by viruses. Several new detection methods have been developed in the past to cope with these challenges in plant protection, but only very few got adopted for routine testing for various reasons. Enzyme-linked immunosorbent assay (ELISA) is probably still the most successfully established method, even if being limited in its sensitivity compared to molecular methods, the fact that many separate operating steps have to be carried out and that results are usually available only the second day. Regarding the latter two shortcomings, a superior ELISA method was developed, allowing obtaining reliable results within just two hours with a sensitivity being comparable to standard ELISA. Furthermore, the convenient protocol contains only very few individual steps and hands-on time. Following the simultaneous incubation of a mixture of capture and detection antibody (conjugate) together with the test sample for 1 hour, the ELISA wells are washed and substrate is added without any further operating steps. This new ELISA is fully compatible with standard ELISA buffers and equipment. The availability of such a newly developed rapid diagnostic tool which is suitable for large scale testing based on standard ELISA platforms will allow a faster response to future challenges in plant virus detection in a globalized environment.

O DIA 2

A case study of FD and BN phytoplasma variability in Croatia: multigene sequence analysis approach

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Introduction: Uncultivable bacteria from the genus '*Candidatus Phytoplasma*' are associated with grapevine yellows (GY) diseases worldwide. In Euro-Mediterranean viticultural areas, GY are the most frequently caused by Bois Noir (BN) phytoplasmas, recently assigned to '*Ca. P. solani*' species, and Flavescence Dorée (FD) phytoplasmas. Surveys of GY in Croatia have been continuously conducted since 1997, with BN being widespread, and recently discovered FD being present in restricted areas of the country.

Objectives: The aim of this research was to assess the variability of genotypes involved in GY pathosystems by using multilocus sequence typing (MLST) approach on a limited number of samples as well as to evaluate the MLST analysis in finer discrimination between closely related strains.

Materials and methods: Grapevine, weed and insect vector samples were collected from three locations in continental Croatia (central and north western part). All samples were tested by conventional PCR/RFLP assays amplifying phytoplasma 16S rDNA. In order to simultaneously detect FD and BN phytoplasmas in the infected grapevine, TaqMan triplex *real-time*-PCR assay amplifying house-keeping gene *map* was also performed. Species-specific *stamp* and *vmp1* genes together with house-keeping genes *tuf* and *secY* were amplified and sequenced from BN strains. In FD strains, *secY*, *map* and *uvrB-degV* genes were analyzed.

Results: MLST encompassing phylogenetic analyses and/or RFLP analyses revealed a diversity of BN genotypes, with one being prevalent and identified from both grapevine and the insect vector *Hyaletthes obsoletus*, corroborating their affiliation to the same pathosystem. Distinct BN strains found in bindweed and two grapevine samples indicated the presence of different BN pathosystems involving yet unidentified vector, possibly from the genus *Reptalus* or unidentified weed. Moreover, a co-occurrence of BN and FD phytoplasma in the same vineyard was identified. The genotyping of FD strains from both grapevine and *Scaphoideus titanus* has shown the presence of at least two distinct FD genotypes at two different locations, suggesting separate introductions of the disease in the country.

Conclusions: In this study, MLST proved to be a useful and informative tool allowing a step forward in better understanding of GY epidemiology in Croatia.

Oral Presentations

Diagnosis

O DIA 3

Development of multiplex PCR and qPCR assays for rapid, accurate and reliable detection and differentiation of select agent strains of *Ralstonia solanacearum*

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Ralstonia solanacearum causes bacterial wilt and attacks over 450 plant species including ornamentals such as geranium. It also limits the production of such economically important crops as tomato, tobacco, potato and banana. Traditionally, *R. solanacearum* is classified into five races and five biovars. Recent molecular classification, however, makes differentiating closely related *R. solanacearum* strains easier by grouping them into four phylotypes and subsequently into more than 50 sequevars. The phylogenetically coherent race 3 biovar 2 (r3b2) strains cause highly destructive brown rot of potato and bacterial wilt of geranium, and are capable of causing diseases under cooler temperature. Currently, *R. solanacearum* is listed as a select agent pathogen in the U. S. unless testing can verify that it is a non-r3b2 strain. A fast and reliable assay, therefore, is greatly needed to not only detect *R. solanacearum* at the species level, but at the same time also signal whether it is an r3b2 strain.

We improved current detection methods for *R. solanacearum* by developing both multiplex PCR and multiplex qPCR assays to simultaneously detect target *R. solanacearum* strains and differentiate r3b2 from non-r3b2 strains, as well as to exclude false-negatives associated with PCR/qPCR inhibition or unsuccessful DNA extractions in a single reaction. We took advantage of the publicly available complete and draft genome sequences of strains of the *R. solanacearum* species complex to target non-phage related DNA to ensure that the test is accurate. We also designed and included an internal plant DNA control primers and probes targeting the cytochrome oxidase gene into the multiplex PCR, to improve the confidence and reliability of the assays for r3b2 detection in plant extracts. Our multiplex PCR and qPCR assays were tested successfully against 34 r3b2 and 56 non-r3b2 strains of *R. solanacearum*, as well as five out-group bacterial species. Our assays also allowed detection of plant DNA and *R. solanacearum* from artificially infected tomato, potato, geranium, and tobacco plants. Our improved detection methods will help government officials to make timely and appropriate recommendations to exclude this select agent pathogen from the United States or from other countries where r3b2 is a quarantined pathogen.

O DIA 4

Use of droplet digital PCR and TaqMan assays for the detection and absolute quantification of *Penicillium verrucosum* in cereal grain

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Real-time PCR is commonly used for the detection and quantification of microbiological contaminants. In temperate climates, *Penicillium verrucosum* is the main causal agent of mycotoxin contaminations in cereal grain, which has been stored over a prolonged period of time. In grain silos, the growth of *P. verrucosum* often occurs in so called “hot spots”, small pockets providing conducive conditions for mould growth. The heterogeneous distribution of *P. verrucosum* and associated mycotoxins, such as ochratoxin A (OTA), present a challenge for testing large bulk loads of grain. In addition to representative sampling and homogenization, digital PCR (dPCR) in conjunction with TaqMan assays can be used to detect single copies of genetic markers in micrograms of template DNA. The new technology has an advantage over real-time quantitative PCR in terms of sensitivity and precision. For meaningful real-time PCR results, availability of reference materials is critical in order to generate standard curves, which are not required for dPCR. In this study, the RainDance RainDrop™ Digital PCR system was assessed for absolute quantification of wheat samples containing traces of *P. verrucosum*. Total amounts of template DNA used (1000 ng and 5000 ng DNA) for droplet digital PCR (ddPCR) were closely correlated with the occupancy of the single copy reference gene for wheat (PCR positive droplet count); but the variation observed in occupancy did not affect quantification of spiked samples. Combining the target and reference primers/probes in a single PCR reaction (duplex ddPCR) was successfully used for quantification of a range of low concentrations in spiked samples (ranging from 10 to 0.001%). In naturally contaminated samples, successful detection and quantification of *P. verrucosum* was also achieved using up to 5000 ng template DNA per ddPCR reaction. However, sheared DNA (size 3-5 kb) had to be used to allow for such high concentrations of template DNA, which also resulted in better separation of fungal and plant clusters from quenched droplets. Overall, positive droplet counts of *P. verrucosum* relative to reference counts of plant DNA correlated with levels of OTA contamination in representative wheat samples. In conclusion, the detection and low-level quantification of toxigenic fungi by droplet digital PCR can serve as a cost-effective proxy for the risk management of mycotoxin contaminations in shipments of bulk wheat.

Oral Presentations

Diagnosis

O DIA 5

Rhabdocline needle cast - most recent investigations on fungal distribution and genetic variation of *Rhabdocline pseudotsugae* Sydow

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Rhabdocline pseudotsugae Sydow is one of the major fungal pathogens in Douglas fir. To date, macroscopic and microscopic analyses of disease symptoms have resulted in the description of the pathogen as a highly specialized needle parasite. However, the knowledge of the ecology and biology of *R. pseudotsugae* is still in a very early stage.

The aim of the present study was to provide initial information on the pathway of infection and the investigation of population genetic structures of *R. pseudotsugae*, as a basis for an efficient and purposeful pathogen control and the long-term reduction in phytopathogenic risk of Douglas fir.

Recent results indicate that *R. pseudotsugae* causes latent infections in various Douglas fir tissue types (Morgenstern et al. 2013, 2014). Based on these results, seeds originating from German and North American areas were subjected to a systematic testing by *Rhabdocline*-specific polymerase chain reaction (PCR) in order to verify the occurrence of the fungus in seeds. *Rhabdocline pseudotsugae* was clearly detected in samples from six German and seven North American areas of origin. Twenty one percent of the tested seeds were infected. This indicates that infected seeds might represent another potential source of infection in addition to the ascospore-based distribution of *R. pseudotsugae*.

For the first description of the genetic variation between and within populations of *R. pseudotsugae*, Douglas fir needles with fruiting bodies were collected from sampling areas in Saxony and North Rhine-Westphalia, Germany. Different genotypes of the fungus could be distinguished by using Start Codon Targeted Polymorphism (SCoT)- and Branch Point Signal Sequences (BPS)-techniques. The results revealed high genetic diversity between and within the sampled areas. A correlation between fungus genotypes and infection intensity could not be detected.

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Morgenstern K., Döring, M., Krabel D. (2014). Rhabdocline needle cast - most recent findings of the occurrence of *Rhabdocline pseudotsugae* in Douglas-fir seeds. Botany92(6): 465-469.

O DIA 6

The Early Detection of Red Palm Weevil (RPW) in infested Date Palm Trees: A New Molecular and Proteomic Based approach for its detection and control

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Red Palm Weevil, *Rhynchophorus ferrugineus*, (Coleoptera: Curculionidae) is considered the most destructive pest of Date Palm trees. It feeds inside the palm and well hidden from human that makes detection process challenging. The early detection of Red Palm Weevil (RPW) is very important otherwise, it is impossible to save infested date palm. Intensive efforts have been made in the development of the RPW detection techniques but no single method is equally sensitive or cost effective and there is still no good solution for area wide detection. Biotechnology, Molecular biology and Proteomic based approaches are very useful for the early detection and control of RPW. Here, we designed a new approach based on host-plant resistance on molecular level. Upon infestation of RPW, the plant synthesizes some important enzymes/defense proteins/signaling molecules/involved in defense pathways that activate the defense response which can be studied by using molecular markers. Our results indicated that Red Palm Weevil infested and non infested Date Palms showed a particular response against RPW. By using Semi quantitative PCR, Real time PCR and SDS-PAGE, the study of early detection of RPW damage and Defense Genes Expression was very helpful to understand RPW and Date Palm specific Interaction. Our methodology can be a useful tool to study the insect-plant interaction and implementation in RPW management.

Oral Presentations IPM Components

O ICO 1

Ecological engineering approach for rice insect pest management in China

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Ecological engineering (EE) for pest management is a win-win approach for human society and nature to regulate crop herbivores through restoration of “natural” vegetation habitats in which the ecosystem service of bio-controls could be enhanced and therefore resulted in low population growth rate of pests. The major EE technical context includes: λ Landscape perspectives in farm design and planting, **M**onitoring target pests, **N**atural enemy conservation and enhancing, **O**ptimization of cropping, λ Pesticide use in need-based (abbrev. **LMNOP**). LMNOP will help natural enemies via the provision of **N**utrients (nectar and pollen), **A**lternative preys/hosts, **T**ouring roads, **U**mbrella, **R**eproduction supporting (**NATURE**). The EE is one of the smarter approaches to couple the different objective-driven farms, from conventional high yield, less-chemical input with ideal eco-, high-efficiency, and organic production farms. So the brief hypothesis is “**EE by LMNOP is a smarter NATURE**”. The field experiments in different production models from high biodiversity mountainous to lower biodiversity plain paddy confirmed this hypothesis. A smarter communication approach has been implemented for up-scaling this EE which has been applied by large scale farmers.

O ICO 2

“Lure & Kill”, “Stress & Kill”: Innovative Control Strategies for Herbivorous Pests

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Due to EU-regulations the use of synthetic insecticidal compounds has been restricted and will be even further restricted in the forthcoming years. Alternative, sustainable control strategies need to be developed and established to avoid control gaps for important crop pests. Various strategies have been proposed to improve pest control (i.e. lure and kill techniques using semiochemicals, visual cues or combinations of two of these compounds, and a kill component). Most of these control approaches have, however, been developed for targeting aboveground herbivorous insect pests. Given that over 70 % of insect species pass through at least one developmental stage in the soil there is an increasing demand for innovative control strategies for belowground herbivores. Targeting these life stages by controlling agents is, however, hampered by soil properties restricting the diffusion of control agents and the irregular distribution of the larvae or pupae, resulting in higher costs. Thus one strategy could be instead of bringing the control agents to the target pests, they could be attracted towards the killing agents. Since larvae need to orientate by chemical cues in the soil to find the roots of their host plants, artificial cues can be applied to the soil mimicking growing roots. When larvae are attracted to these application sites, the chances that they will come into contact with specific killing agents will be enhanced. The stress and kill strategy entails use of an agent (e.g. botanical, low dose insecticide) that stresses the target and increases its susceptibility to a biological control agent. The stressing agent may be an antifeedant which, through starvation, increases susceptibility to pathogens or an irritant that increases insect movement and exposure to potential control agents. The merits and drawbacks of the lure and kill and stress and kill strategies will be discussed.

O ICO 3

Pheromone blend analysis and cross-attraction among geographically-different *Maruca vitrata* populations

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The legume pod borer, *Maruca vitrata* (Lepidoptera: Crambidae), is a pantropical pest on leguminous crops. The larvae are controlled mainly by insecticides. Pest monitoring by pheromone lures can help to reduce the use of pesticides. (E,E)-10,12-

Oral Presentations

IPM Components

hexadecadienal was identified as the major sex pheromone compound of *M. vitrata*¹. (*E,E*)-10,12-hexadecadienol and (*E*)-10-hexadecenal were described as minor pheromone components². A blend of these components in a ratio of 100:5:5 attracted males in field trapping experiments in Benin², but not in Taiwan³, Thailand⁴, and Vietnam⁴. This finding indicates geographic variation in the sexual communication among *M. vitrata* populations. To create an effective lure in Southeast Asia, we investigated the pheromone composition of insect populations from Thailand, Taiwan, and Vietnam compared to a reference population from Benin. We developed a sensitive GC-MS method using selected ion monitoring to analyze single pheromone gland extractions. We confirmed the presence of (*E,E*)-10,12-hexadecadienal and (*E,E*)-10,12-hexadecadienol in all target populations, but we did not find (*E*)-10-hexadecenal. Moreover, the pheromone composition of both detected compounds did not vary significantly among the insect populations. In wind tunnel tests, we compared the responses of males from Taiwan and Benin to a) calling females and b) gland extracts of females from both origins in laboratory choice and non-choice bioassays. Taiwanese and Beninese males were similarly attracted to all pheromone sources. In conclusion, we found no distinct difference in the sexual communication between Asian and African insect populations. However, we did find a significantly higher attraction of males to live females than towards lures in the field in Taiwan. This indicates that additional, yet unidentified compounds may be present at least in the Taiwanese *M. vitrata* pheromone blend.

The research is part of the Project HORTINLEA, funded by the German Federal Ministry of Education and Research and the German Federal Ministry of Economic Cooperation and Development within the framework of the program GlobE - Global Food Security.

¹Adati and Tatsuki 1999. J. Chem. Ecol. 25

²Downham et al. 2003. J. Chem. Ecol. 29

³Schläger et al. 2012. Gesunde Pflanz. 64

⁴Srinivasan et al. 2015. In: New horizons in insect science

O ICO 4

Repellency effects of methyl eugenol on rice pests and their predators

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Question: Methyl eugenol (ME) is a phenolic compound produced by a number of plant species with a particular characteristic of attracting some insect species and repelling others. We investigated whether ME has repellency effects on rice pests and their natural enemies.

Method: We designed a laboratory study utilizing small cages containing food sources (e.g. live rice plants or brown planthopper eggs) with side openings to allow the individual insects a choice between feeding on- or leaving the food sources. Half of the experimental units received ME treatments while the other half served as controls. We conducted this study on brown planthopper and mirid bug as two representatives of rice pests and natural enemies commonly found in rice fields.

We also conducted paired-fields study with ME-impregnated papers deployed in half of the pairs while the other half served as controls. This setup is replicated four times and populations of rice pest species (brown planthopper, white-backed planthopper and green leafhopper) and their natural enemies (mirid bug and tetragnatha spiders) were estimated by sweep net sampling twice a season during vegetative and reproductive stages, subsequently. Here we report the results of our laboratory studies and one season of field study.

Results: In our laboratory study, the percentages of brown planthoppers staying in the cages to feed on the host plants were significantly lower in the ME-treated cages compared to controls at 1, 24 and 48 hours after the start of the experiment. Similarly we found that the percentages of eggs consumed by mirid bugs were significantly lower in the ME-treated cages compared to controls. These results indicated repellency effects of ME on both rice pests and their natural enemies.

In the field study, the population levels of brown planthopper, green leafhopper, white-backed planthopper, mirid bug and tetragnatha spiders were not significantly different on fields receiving ME treatments compared to controls at both vegetative and reproductive stages of rice growth.

Conclusion: Our study confirmed the repellency effects of ME on brown planthopper and mirid bug in a small and contained experimental system. Further investigation is needed to characterize the effect of ME on rice pests and their natural enemies in a larger scale.

Oral Presentations IPM Components

O ICO 5

Automated insect monitoring – what we learned so far

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Agriculture seems to be trapped in constant paradoxes: provide more food without significantly expanding farmable land and supply top quality products while reducing negative impacts on environment.

One of key issues is impact that pesticides have on environment. Therefore new pest control products are far more targeted and need to be applied in a timely manner in order to be effective. On the other side field scouting is usually still based on weekly visit of the traps as it has been for several years. That often results in a situation when development of pest population is not detected soon enough to effectively use modern (chemical or bio) pesticides.

During the presentation we will show how automated insect monitoring system like Trapview can address key issues in modern insect monitoring. These are: the need to have pest related data in nearly real-time, optimizing time spent on travel and providing tools to manage spreading of pests to non-infected areas due to import of food.

A few examples of key pests which can be efficiently monitored with automated traps will be shown and key challenges which had to be addressed and solved will be presented.

Key challenges include effective trapping of targeted insects, low maintenance requirements, simplifying detection/recognition of targeted pests and simplifying communication between all stakeholders needed to decide and to take pest control measures.

At the end we will address some of key future issues like how to efficiently deal with some of the most important invasive species (for example *Drosophila Suzukii*) and how to incorporate standard field scouting with early warning system provided by automated traps

O ICO 6

Digital pests monitoring in cole crops (*Brassica oleracea*)

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According to guidelines of integrated pest management (IPM), assessment of pest density is a meaningful tool for using in crop protection. The application of insecticides can be limited to the amount necessary due to estimated pest populations and corresponding thresholds. The currently available estimation methods (e. g. crop monitoring) for insect pests in vegetable crops under field condition are either very time consuming or not area-specific and for this reason rarely applied in practice. Therefore, the objective of this research is to develop automated area-specific and easy-to-handle decision support systems at the example of cruciferous vegetables. This research project is part of the “WeGa- Kompetenznetz Gartenbau” (<http://www.wega-online.org>) and is funded by the federal ministry of education and research.

In this study different types of digital decision support systems for detection of certain adult pests under controlled and field conditions were tested. TrapView, TriangelCameraSystem (TCS) and light grid were the most advanced systems. TrapView (Efos, Slovenia) is a technically modified delta pheromone-trap for the automatic detection of codling moth (*Cydia pomonella* L.) and is commercially available. For the first time, TrapView was used in combination with pheromones for pest of cole crop (e.g. *Plutella xylostella* L.). The TCS as well as the light grid were developed in collaboration with the Osnabrück - University of applied science. The TCS is a video surveillance system for cabbage root fly (*Delia radicum* L.). Light grid is a photoelectric light system, which operates on the basis of infrared beams and was used to monitor flying insects. The preliminary results indicate that the three tested systems are generally well suited for the detection of adult pest. However, further optimizations and testing must to be done to evaluate the quality of the systems.

Oral Presentations Technology Transfer

O TTR 1

Twenty years of experience of the IPM Wheat Model in Northern Germany (Schleswig-Holstein)

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Increasing intensity of wheat cultivation has been achieved through shorter rotations, high plant populations, and multiple nitrogen applications. These agronomic practices result in substantial grain yields but also increased pressure from diseases. Pathogens that have become economically important are *Mycosphaerella graminicola*, *Phaeosphaeria nodorum*, *Pyrenophora tritici-repentis*, *Puccinia recondita*, *Puccinia striiformis*, *Tapesia yallundae*/*T. acuformis*, *Fusarium* spp. and *Blumeria graminis* f. sp. *tritici*. Control of the diseases caused by these pathogens has a high priority in minimizing yield losses (average 2 tons). The occurrence of individual pathogens and the economic significance of the diseases they cause can vary substantially in different climatic zones and production systems. Epidemiological development of pathogens strongly depends on the weather. Together with differences among cultural practices, this leads to differences in the onset, course, and severity of the disease complex from one year to the next. Decisions on the need to apply fungicides, application timing, and choice of active ingredient have hitherto been guided largely by experience. Such decisions, however, often cannot be regarded as optimal when economic and ecological aspects are also taken into account. Spray decisions are often made according to a “precautionary” principle based on the crop’s growth stage. It cannot be assumed, however, that this system will always control the pathogen population at the most sensitive stage of its population dynamics at the transition from the epidemiological accrescence to the progression phase. From the epidemiological point of view, growth stage-oriented treatments are randomly timed and therefore may only be partially successful, and the possibility of economically and ecologically unjustifiable applications cannot be ruled out. In a multi-year cooperation of the plant protection services of the German states Bavaria and Schleswig-Holstein and the Universities of Munich and Kiel, an Integrated Plant Protection System (IPM Wheat Model) has been developed for the most important fungal wheat diseases. From 1995 to 2014, epidemiological and yield studies were carried out within the framework of annual regional monitoring programs in three different varieties at 10 sites, equipped with online weather forecasting stations. For optimizing plant protection treatments, an important basic principle was the development of functional pathogen-specific control thresholds and prognosis systems. Averaged over the years and locations, the fungicide-untreated control achieved a yield of 8.7 t/ha, the stage-oriented, ‘professional’ variant (3.7 applications, full application rate) achieved 10.4 t/ha, and the IPM version (2.0 applications, reduced application rate) 10.2 t/ha. The differences in yield between the stage-oriented and IPM variants were not significant. In summary, the amount of fungicide active ingredients was reduced by 48% in the IPM variant compared to the ‘professional’ variant, with a comparable yield and disease control.

O TTR 2

Plant Medicine (Phytiatry), a University science and Plant Doctor, a necessary profession for the benefit of global agriculture: Arguments and actions for its establishment

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Agronomy is continuously evolving according to the needs of Agriculture. Since several related disciplines are gradually emerging from classical Agronomy to create distinct professions, urgent actions have to be taken for establishing Plant Medicine (Phytiatry) as a new University Science.

We enlighten the major problems related to the current situation in Plant Medicine concerning education and application and analyse arguments, emphasizing the fundamental particularity of sciences related to Plant Medicine, along with actions for upgrading education and specialization and establishing a new profession of Plant Medicine doctors.

Plant Medicine could be a distinct University multidisciplinary science that can deal with basic and applied research in studying plant diseases and pests, abiotic diseases, plant nutrition and generally management of plant pests and diseases. As a five-years University course could include over forty different scientific disciplines having as core plant pathology, agricultural entomology and nematology, weed science, phytopharmacy, disease and pest diagnosis, plant protection strategies, identification of new diseases, pests and weeds, disease and pest monitoring.

Strong arguments in favor of establishing Plant Medicine as a separate University science are presenting to highlight problems related to scientifically accurate plant disease and pest diagnosis, related to the need of consulting agronomists or farmers on quarantine pathogens and pests, deal with complicated problems of postharvest diseases, with mycotoxin aspects on food safety and suggest measures for managing relative pathogens, able to provide the appropriate plant nutrition instructions and to guide the farmers to produce quality products. Plant Medicine Doctors able to contribute in ameliorating the negative impact of farming on the Agricultural Environment and instructing farmers to be in vigilance in protecting both their farms, the agricultural environment and their health and reduce high costs in crop production. Plant Medicine Doctors able to eliminate the

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problem of amateurism in disease and pest diagnosis and management. As for international actions these must be focused on the education at Universities, the alarming cases for lack of specialists, the extension plant clinics and the joint International congresses under the auspices of relative International Societies

O TTR 3

The changing role of science in soil and plant health extension programs

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Introduction: A review of the long history of soil health extension and research programs across the NE region of Victoria, Australia, demonstrates a number of clear shifts and trends in extension program design. These shifts and trends bring a number of new challenges for extension programs.

Objectives: This paper explores these shifts and trends, the challenges for extension and proposes some changes to our understanding of the role of science in supporting extension programs for soil and plant health.

Materials and methods: This paper draws from a review of soil health extension and research programs across the NE region of Victoria.

Results: The review demonstrates a clear shift from 'fixing up' single issues with soil health such as acidity, salinity, soil fertility and plant health issues to taking a more integrated holistic farm and catchment system approach. This in part has been driven by increasing landholder interest in soil health and sustainable plant production systems and in particular the role of soil biology and organic matter in agricultural systems. The linking of soil health with plant health and human health clearly demonstrates a shift towards the adoption of systems thinking into problem solving processes used on farms.

There has also been a clear shift from government driven research and extension programs to community led initiatives. Philanthropic, government and industry/RDC funding bodies have also begun to recognise the role of community groups as key extension program delivery vehicles.

These shifts and trends create challenges for crafting and delivering clear soil and plant health extension messages, particularly when management of soil and plant health often requires understanding of complex soil, farm, catchment systems and markets.

Conclusion: These shifts and challenges have led to a change in our understanding of the role of science in supporting extension programs for soil and plant health. We have learnt the importance of extension programs fostering discussion, learning and the development of connections between participants, and the science experts or specialists. Most importantly we have learnt the importance to work with land managers and producer groups to develop their own solutions.

O TTR 4

Empowering Farmers to Reduce Pesticide Risks in Asia

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FAO has a long-standing record of technical assistance and support to its member countries for empowerment of rural communities in the Asia region to tackle pesticide-related problems from a risk reduction perspective. Use of the most hazardous pesticides must be eliminated and overuse reduced in favour of effective and environment-friendly alternatives, such as Integrated Pest Management. In tandem with pesticide management regulatory reform, grass-root level education efforts, including Farmers Field Schools, are of vital importance to facilitate risk reduction in rural communities. Using innovative and science-based research methodologies, this paper will outline results of impact assessment work done on community education for pesticide risk reduction efforts implemented in Cambodia and Vietnam during the 2007-2012 period.

O TTR 5

Increasing grower awareness and monitoring spread of *Heterodera glycines* in North Dakota, USA

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Introduction: Planted hectares of soybean (*Glycine max*) in the U.S. state of North Dakota (ND) has increased from approximately 0.5M ha to nearly 3M ha since the 1990's. As a result of this rapid expansion many soybean pathogens common in other U.S. states have not been identified in ND, and many ND growers are unfamiliar with many soybean diseases. In 2003, the invasive soil-borne nematode *Heterodera glycines* (Soybean Cyst Nematode [SCN]) was identified in ND. Soybean cyst nematode is one of the most important yield-limiting pathogens in the United States but yield losses can be mitigated if the pathogen is detected early and actively managed before the level of infestation becomes high. However, a lack of awareness and a negative social stigma associated with SCN has limited grower interest to test for SCN infestation, and made it difficult to ascertain the geographic spread of the pathogen.

Objective: The objectives of this study were to increase SCN awareness and sampling by growers, and determine the geographic distribution of SCN in ND.

Materials and methods: In 2013 and 2014, a free and anonymous SCN soil-testing service was provided to growers who submitted soil-samples in pre-paid sample bags distributed through the Cooperative Extension Service. Distribution of bags was accompanied by an Extension awareness campaign that included press interviews, advertising, educational seminars, and field day events. Egg count data (eggs/100cm³) were mailed directly to the submitting grower and a low-resolution map of egg counts was created and publically distributed.

Results: In 2013 and 2014, 193 and 579 SCN-samples, respectively, were submitted by growers using the program. Nematode eggs were identified in approximately 30% of the samples received and were located in 21 counties, including 11 where SCN had not been confirmed previously.

Conclusion: Distribution of the SCN maps coincided with an increase in SCN sampling and requests for educational seminars, interviews and management information; particularly in areas not known to have infested with SCN prior to map distribution. It is likely that this program will result in proactive SCN management and a reduction of economic loss from this invasive pathogen.

O TTR 6

Tomato growers' perception of biocontrol as a driver for adoption and improved extension services

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Introduction: In spite of the decennia-long use of biocontrol in greenhouse tomato production in Flanders (Belgium) and the legal obligation to practice integrated pest management (IPM) since 2014 (according to the Sustainable Use Directive 2009/128/EC), their implementation in commercial greenhouses still is not optimal and the adoption of innovative techniques remains slow.

Objectives: Therefore, we study tomato growers' perceptions and notions on biological pest control and IPM and of the consecutive steps in their implementation. The acquired insights should provide suggestions to improve extension and advisory services on crop protection. A higher success in knowledge transfer, should in turn increase the sustainability of tomato production.

Material and methods: To gain insight in growers' perceptions of biocontrol, we conduct a series of in-depth interviews, covering a list of open-ended topics linked to the production practices in general and pest control in particular, such as the glasshouse holdings' structure, the people making decisions, their notions about IPM, pest prevention, pest and predator monitoring, biological or chemical intervention and their opinions and visions about knowledge transfer. The sample group of tomato growers started with greenhouse holdings' crop protection managers proposed by extension research centres, and continued by using snowball sampling. While conducting and analysing the interviews we use a grounded theory approach. This approach focuses on the social psychology of the actors studied. Data are collected and analysed to identify concepts, ideas and views and to assess the actors' interpretation of the problem and the way they solve it. Grounded theory is generated through the abstraction of concepts and the relations between them from qualitative data, *in casu* interview transcripts.

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Results: Preliminary results from this study will be presented. The revealed concepts and relations provide insight in the tomato growers' perceptions of biocontrol, their attitudes and their motivations for adopting specific practices.

Conclusion: Suggestions can be made to improve crop protection extension services' interaction with tomato growers and make their communication about innovations in crop protection practices more effective.

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Legal Issues I

O LEG I-1

Update on the implementation of the EU legal framework for plant protection products

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Introduction: Update on the implementation of the EU legal framework for plant protection products with some highlights on recent issues.

Objectives: Present to the participants an update on some issues that have recently emerged in the implementation of the EU legislation on plant protection products.

Issues can cover a broad range of topics such as data requirements, renewal of authorisations, data protection and confidentiality.

Short Résumé: Since June 2011, I am working in Directorate General Health and Food Safety of the European Commission in Brussels. I am the legal advisor to the unit E3 in charge of the implementation of EU law on plant protection products (Regulation 1107/2009) and maximum residue levels (Regulation 396/2005). I provide advice on different aspects of the implementation of the legislation to Member State Authorities and companies, draft implementing legislation and guidances.

From 2009 to 2011, I worked in the unit in charge of implementing the REACH Regulation in Directorate General Enterprise. I was in particular involved with restrictions to the placing on the market and use of dangerous chemicals. Before that, I have been a legal advisor in a unit in charge of different pieces of EU legislation pertaining to chemical substances: fertilisers, drug precursors, detergents, pyrotechnic articles, and explosives.

I have a law degree from Université de Paris Pantheon Sorbonne (France) and a Master in Comparative Law from George Washington University (US).

O LEG I-2

Contesting the Use of the Precautionary Principle: Hope over Experience?

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The European Commission has broad discretionary powers when it adopts measures on the basis of the precautionary principle. In theory, such powers do have boundaries, as the measures adopted must be proportional to the chosen level of protection, non-discriminatory in their application, consistent with similar measures already taken, and based on an examination of the potential benefits and costs of action or lack of action. In practice, however, arguing that the European Commission has acted in breach of the precautionary principle may prove to be a challenging undertaking because *inter alia* the threshold of judicial review by the Court of Justice of the EU is set at a “manifestly inappropriate” (and therefore very high) level. Our presentation will review the most recent case-law of the Court of Justice of the EU on challenges to measures adopted on the basis of the precautionary principle (including, but not limited to plant protection products) and offer insights on when such challenges are most likely to succeed.

O LEG I-3

Registration of plant protection products in the EU: evaluation within the zonal procedure and national authorisations

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EU Regulation (EC) No 1107/2009 concerning the placing of plant protection products on the market was published 21 October 2009 and is applied in all member states since 14 June 2011. As laid down in Article 1 (3) the purpose of the Regulation is harmonization between the Member States: “The purpose of this Regulation is to ensure a high level of protection of both human and animal health and the environment and to improve the functioning of the internal market through the harmonisation of the rules on the placing on the market of plant protection products, while improving agricultural production.” Harmonization is key to establish the system of zonal evaluation which is requested by the Regulation as a main instrument to grant national authorisations of plant protection products. Industry experiences so far a lack of harmonization. Examples will be provided showing that EU-decisions are not yet completely accepted and that consistency throughout the Member States is a still critical issue. It will be outlined that the lack of harmonization results in severe consequences for the growers and has economical implications. By describing the current situation in the Member States potential solutions will be provided.

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Legal Issues I

O LEG I-4

Refusal of zonal registrations or mutual recognition by a national regulatory authority - legal aspects

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The EU Regulation (EC) no 1107/2009 provides for the harmonization of registrations within geographic zones of the EU. Such harmonization shall be achieved on the basis of the zonal registration or mutual recognition procedure. The relevant Guidance document provides that other Member States “must not re-evaluate the application.” Instead, the conclusions reached by the Member State assessing the application for registration shall prevail. The Member State that is requested to issue a national registration may, however, impose specific risk mitigation measures (Art. 36 para 3 subparagraph 1) or refuse authorization, if due to specific local conditions, the product in question poses an unacceptable risk to human or animal health or the environment (Art. 36 para 3 subparagraphs 2-4). This paragraph is interpreted by the German authorities in the sense that an authorization may be refused due to its own risk assessment. The scope of Art. 36 para 3 subparagraphs 2-4 shall be explained, and the interpretation of the German regulatory authorities in case of risk assessments that deviate from the assessment report prepared by another Member State shall be reviewed and legally evaluated in the light of EU law.

O LEG I-5

Suitability criteria for authorisation of products for non-professional users in Germany - industry view

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In Germany, suitability criteria for the authorisation of plant protection products for use in home gardening have been in place since the 1990s. They consisted mainly of use restrictions (e. g. highly toxic products) and specific risk mitigation measures like package size limitations or ready-to-use formulations.

Following the implementation of Regulation (EC) No. 1107/2009 and Directive 2009/128/EC, the national plant protection legislation for amateur products was adapted in 2012. Plant protection products for non-professional users should now preferably fulfil the criteria of Article 47 of Regulation 1107 for low-risk products. In the absence of such products, however, authorisation is still possible if the updated suitability criteria are met.

The authorisation of products for "non-professional users" in Europe is still handled on a national level and therefore even less harmonised than that of products for agricultural use. This presentation highlights the regulatory situation in Germany from an industry perspective.

O LEG I-6

Pesticides for non-professional use - Industry perspective on the regulatory framework

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Introduction: The home and garden market is a small but important sector of the total pesticide market. Gardeners expect a variety of products be available for the management of the land that is considered an extension of the living area and sometimes a means to supplement food supply. There is wide political misconception that all pesticide products are toxic and hazardous to the environment. Whereas, in many countries regulatory frameworks require that products destined for the home and garden be safe to use. In the EU, there is a stringent and effective regulatory framework in place based upon the sound principles of risk evaluation and risk mitigation; however, inaccurate NGO led campaigns and communications place undue political pressure on the regulatory process and add to wider public and stakeholder misunderstanding as to the benefits and safety of pesticides.

Results: Available data on the safe use of garden products reveals that incidents of human intoxication are very rare; likewise, home and garden use of pesticides is a minor contributor to the occurrence of pesticide residues in water. Evidence also reveals that impacts on bees have been reduced in recent years. These favourable results can be in-part attributed to consumer awareness of the potential risks of pesticide use, and adherence to produce use instructions. In addition, industry investment in research and development and best practice stewardship initiatives sees the marketing of reduced risk products and special guidance to improve the safe and sustainable use of products.

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Industry position: Industry calls for a harmonized approach. Risk assessments should be based on reality, based on science without discriminatory measures. Industry has every interest in continuing to market products that are safe to use. Continued improvements are born through innovation, but innovation needs time and especially predictability.

Conclusion: Under the current regulatory framework, home and garden pesticides are available as low hazard-limited risk solutions. Users should be given the freedom of choice of safe-to-use products to maintain their homes and gardens. Pesticide producers innovate through research and development programmes to further reduce risks, but this is best achieved under a stable and predictable legislative framework.

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Biocontrol of Insects I

O BI I-1

Progress and uses of Microbial Control in Chilean Berries

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The Chilean Berries Industry, including blueberry, raspberry, blackberry, strawberry and currants, has been constantly growing in the last 30 years. Thus, the country is the main exporter of berries from the South hemisphere and the fourth in the world, either in volume and value. To keep this industry productive and open to the world, insect and disease management are a major concern. Mealybugs (*Pseudococcus*) is the main aboveground pest, for its direct damage and the quarantine restriction at the importer countries. Belowground the most challenging pests are the Vine weevils (Coleoptera: Curculionidae), which produce severe damages at the root system. They are also quarantine pests, making mandatory their control to avoid fruit rejections in foreign markets. Farmers base their management in chemicals pesticides, but the growing restriction for chemicals and the public concern about pesticides, encourage the farmers to use Microbial Control, like entomopathogenic fungi (EPF) and nematodes (EPN). Research has showed that the use of EPF (*Beauveria* and *Metarhizium*) are an effective alternative to manage mealybugs in the orchards. Control range 60, 70 and 92% with 1, 2 or 3 applications before the pest move to the aerial part. Vine weevils are difficult to control, because larvae are deep into the soil or dwelling the main roots, avoiding physical contact with pesticides. Therefore, EPN is the most effective measure to control these kinds of insects, because of their ability to search larvae into the soil or inside the dwellings. EPN has been used against the most important vine weevils: *Aegorhinus nodipenis* (Plum weevil), *Aegorhinus superciliosus* (Raspberry weevil), *Asynonychus cervinus* (Fuller's rose weevil), *Naupactus xanthographus* (Grapevine weevil) and *Otiorynchus sulcatus* (Black vine weevil). EPN like *Steinernema australe*, *S. feltiae* and *S. unicornum* are able to reduce about 70% the adult emergencies from the soil. Research continues looking for better isolates of entomopathogens to control these and other pests related to berries. Also, mass rearing *in vivo* and *in vitro* has been accomplished with yields above 2×10^9 spores/g of dry rice and 35,000 dauers/ml of EPF and EPN, respectively. Formulations such as granules, gels and clays are available for berry farmers facilitating the use of Microbial Control.

O BI I-2

Efficacy of using *Harmonia axyridis* (Coleoptera: Coccinellidae) on *Myzus persicae* (Hemiptera: Aphididae) on vegetables under greenhouse conditions

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Myzus persicae is one of the most biologically diverse and widely distributed pests principally due to its ability to transmit plant viruses. In order to evaluate the control ability and benefit of *Harmonia axyridis* on *M. persicae*, releasing *H. axyridis* to control *M. persicae* were investigated as comparing with application of biological insecticide in pimiento and eggplant greenhouse. The results showed that *H. axyridis* could continue to depress population density of *M. persicae* in pimiento greenhouse. The colonization rate of *H. axyridis* was 64% which was the peak of *M. persicae* outbreaks. In addition, the yield of pimiento and benefit that releasing *H. axyridis* control *M. persicae* were higher than biological control. In eggplant greenhouse, releasing *H. axyridis* delayed the peak of aphids for one week. The population density of *M. persicae* was declined by 79% and the colonization rate of *H. axyridis* was reach to 86% after increased the number of released *H. axyridis*. These results indicated that monitoring the population dynamics of natural enemies and pests in entire stage should be definitely facilitated the ratio of natural enemy and pest per week after planting 15 days. Releasing *H. axyridis* in the early two to three months could be cost-effective and sustainable in controlling *M. persicae* in vegetable greenhouses.

O BI I-3

Evaluations of entomopathogenic nano- *Beauveria bassiana* and Nano beauvericin against rice insect pest under laboratory and field conditions

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Chilo agamemnon and *Hydrellia prosternalis* are the main rice pests they cause a lot of damage to the rice crop. The entomopathogenic fungi nano-*Beauveria bassiana* were tested against these two insect pests. LC50 recorded 187 X 104 and 198 X104 spores/ml for *Chilo agamemnon* and *Hydrellia prosternalis*, respectively under laboratory conditions. When the toxin

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nano- beauvericin were tested against the two pests, the Lc50 reduced to 44X 10⁴ and 49X 10⁴ spores/ml for both the tested insect pests, respectively. Results showed that the percentage of infestation were significantly decreased to 9 and 8% under field conditions in the plots treated with nano- *B. bassiana* and 1 and 2% in the plots treated with nano- beauvericin. The yield significantly increase to 6631± 36.80 and 6331± 33.70 and 7456± 30.40 ton/ feddan in the plots treated with nano- *B. bassiana* and nano- beauvericin, respectively .

O BI I-4

Improved biopesticide application strategies for insect pest management in Africa

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The development of entomopathogenic fungi as biopesticide for the management of arthropod pests is gaining prominence across Africa and a number of products have emerged as a result of strong partnership between research institutions and the private sector. Despite the interest, a number of challenges such as susceptibility to climatic factors, poor shelf life and need for effective and economical mass production and formulation technologies exists and require technical backstopping. The failure to address these challenges might preclude adoption of biopesticide by small-scale farmers. Climatic factors such solar radiation, temperature, relative humidity, rainfall *etc* affect biopesticide efficacy and persistence. Additionally, the high cost of biopesticide production is a critical factor in terms of affordability by small-scale farmers limiting their adoption. A crucial balance between biopesticide efficacy and cost needs to be achieved to ensure affordability and wide adoption by farmers. Over the past 20 years, *icipe* has carried out various biopesticide R&D activities to address the aforementioned challenges and improve their use by growers across Africa. Several approaches that include the use of autoinoculator, spot sprays and other application strategies have been explored to either prolong the persistence or reduce the required amount of inoculums for field application. Most of these strategies are developed based on information on pest ecology and using opportunities offered by new developments in pest management such as attractants (visual and odor-based). Taking into account the versatility of microbial agents, the use of endophytes has also become a prominent new area of interest. Identified endophytes have been observed to stimulate plant growth and confer resistance to pest attacks. In the process of improving biopesticide application, issues related to incompatibility between IPM tools have arisen and strategies to avoid or overcome such obstacle to their utilization and application will be discussed in the presentation.

O BI I-5

Encapsulation of *Metarhizium brunneum* as basis for an attract and kill strategy

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Several soil-borne herbivorous insect pests such as wireworms and western corn rootworm larvae cause tremendous losses in different crops like potato and maize. The INBIOSOIL project aims at developing innovative beads containing entomopathogenic fungi (EPFs) as well as novel synergistic co-formulations of EPFs with efficacy enhancing agents to control soil-borne insect pests. These innovative formulations should display properties such as high mechanical stability, high entrapment efficiency, high shelf life and optimal nutrient additives for growth and sporulation of EPFs (C/N ratio, pH). For application in the field these formulations were dried with a focus on high cell survival, low a_w values and good re-swelling properties. For scale-up, high throughput encapsulation technologies like jet cutting and several drying processes e.g. fluidized bed dryer, drum dryer or the Innojet dryer were investigated, respectively.

Here we report on the encapsulation and drying of novel formulations on lab and technical scale. Furthermore data will be presented on investigations on the influence of nutrients on sporulation, re-swelling of dried capsules as well as the influence of fillers on a_w value and survival of dried encapsulated aerspores of the *M. brunneum* isolate BIPESCO 5 and virulence against *Tenebrio molitor* larvae.

Dry beads with additives such as starch or other nutrients showed survival up to 80 % for *Metarhizium brunneum* spores and a_w values down to 0.1 depending on the formulation and drying method. Depending on the composition, dried beads rehydrated in water to 70-90 % of their initial bead diameter. Furthermore we encapsulated *M. brunneum* aerspores together with CO₂-releasing *Saccharomyces cerevisiae* in a co-formulation to implement an "attract and kill" - strategy to control wireworms and other soil-borne insect pests in agricultural fields. Selected field results, using *M. brunneum* (isolate ART 2825) formulated beads

to control wireworm larvae will be presented by the collaborating group of S. Vidal, demonstrating that these novel formulations are capable to significantly reduce the wireworm populations in the field.

O BI I-6

Endophytic entomopathogenic fungi for biological crop protection: novel integrated fermentation and formulation strategies

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Introduction: Endophytic entomopathogenic fungi like *Beauveria* spp. or *Metarhizium* spp. are characterized by a cosmopolitan distribution, are pathogenic to many different insect orders and are able to endophytically colonize different plant species and plant parts. Biocontrol of insect pests with these fungi is challenging because of the low efficacy, difficult application and limited shelf life of the usually insufficiently formulated “active ingredients”. A solution for these problems could be the combination of a fermentation and formulation strategy to selectively mass-produce fungal biomass for an optimized formulation.

Objectives: The current research project aims at developing novel integrated submerge fermentation and formulation approaches, namely sprays, beads or seed coatings that will enhance germination, penetration and plant tissue colonization of these fungi thus systemically protecting plants against herbivorous insects.

Materials and methods: To investigate the impact of formulation on *B. bassiana* ATP-02 and *M. brunneum* spp., fungal biomass was selectively produced as mycelia, microsclerotia, aero-, blasto- and/or submerged conidiospores fundamentally differing in their cultivation requirements. To develop a spray formulation, different adjuvants were combined with fungal biomass. Germination and penetration assays were conducted with a GFP-labeled *B. bassiana* isolate. Furthermore, novel formulations based on biopolymers were developed.

Results: By using molasses and titanium dioxide as UV-B protecting agents, the viability of *B. bassiana* spores could be increased to 57±14 % and 77±11 %, respectively. An improved spray formulation increased germination by over 35 % and germ tube growth was stimulated leading to a 45 fold increase in length on tomato leaves. Furthermore, penetration was enhanced up to 50 % using a spray formulation containing 0.1 % Triton X-114, 1 % molasses, 1 % titanium dioxide and 10⁶/ml spores. Based on these promising findings, an integrated fermentation and formulation strategy will also be developed for specific *Metarhizium* spp. isolates.

Conclusion: By developing suitable integrated fermentation and formulation strategies, penetration and colonization of plant tissues by endophytic entomopathogenic fungi can be substantially improved.

Oral Presentations

Digital Technologies

O DIG 1

Digital technologies for diagnosing insect pest, disease and weed problems

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Correct detection and diagnosis of pest, disease and weed problems is a critical component of best management practice. This applies at all levels of decision making, including decisions made by farmers, advisors and by national and international biosecurity agencies. In recent years, however, a world-wide decline in practical taxonomic and diagnostic expertise has made correct identification and diagnosis of crop disorders more challenging and this, together with expected changes in the distribution and abundance of pests, diseases and weeds driven by climate change, has the potential to make plant and crop diagnosis even more difficult in the future.

To support this diagnostic process, various information technology (IT) approaches have been developed, including image databases, remote microscopic diagnostics, and various dichotomous and matrix key programs and products. This presentation describes one of these approaches, based on the Lucid matrix system for identification and diagnostics. Initially launched over 15 years ago, the Lucid software system has been used world-wide to create and deploy a wide variety of multimedia based identification and diagnostic tools or keys. Since its initial release, Lucid has been continuously updated and improved and a complementary product, Fact Sheet Fusion - a fact sheet builder - has been developed and released. Using these two software systems and with support from a help desk and dedicated website at www.lucidcentral.org, domain experts are able to easily create and provide plant protection decision makers with multimedia identification and diagnostic tools and fact sheets in one package.

A range of these Lucid based tools will be demonstrated, including USB and DVD based products, online Java and web-server applications, and Android and iOS smartphone apps based on the latest Lucid Mobile deployment technology. One project involving the International Rice Research Institute and a number of national rice programs has resulted in the development of rice diagnostic apps tailored to local languages and situations. As part of this project we are extending Lucid Mobile to include data collection and reporting functions based on user observations, a facility that we believe could be of value to a range of plant protection and biosecurity activities in the future.

O DIG 2

Temperature-dependent age- specific demography of grapevine moth (*Lobesia botrana*) (Lepidoptera: Tortricidae): jackknife vs. bootstrap techniques

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Grapevine moth, *Lobesia botrana* (Lep. Tortricidae) is a key pest of Iran vineyards that lives in a wide range of area with broad range of temperature fluctuations. Temperature has a key role in population dynamics of insects. This effect may realize *via* change in speed of biochemical reactions related to development, survival, and reproduction that consists life history components of an animal. Hence the best manner of studying this effect is surveying reproductive-life table parameters under different temperature regimes. In this study eight constant temperatures (5, 10, 15, 20, 25, 30, 32, and 35 ± 1 °C), 60 ± 10% RH, and 16:8 (L: D) h photoperiod was chosen for demographic studies of the grapevine moth. No development of immature stages was occurred at 5 and 35°C and emerged moths were unable to mate successfully at 10, 15, and 32°C. Overall immature development decreased from 320.7±3.4 d in 30 °C to 34.2±0.2 d in 10°C and further increased to 42.5±0.6 d in 32 °C. Based on the stable population growth parameters it seems that 25°C is being more close to optimum temperature. Maximum value of intrinsic rate of increase, gross and net reproductive rates were 0.0719 d⁻¹, 55.5 and 23 females per generation in this temperature. Both jackknife and bootstrap estimates of mean and standard error were largely similar, hence both may use for uncertainty estimates. The data suggests cold storage of grape to prevent damage. The certain damage may be expected in the first generation in spring. Hot summer of our area itself may restrict the pest development of subsequent generations.

Oral Presentations

Digital Technologies

O DIG 3

Using Empirical Knowledge to Create Decision-Making Tools for Peanut Pest Management

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Introduction: Providing tools to assist farmers in making cost effective and environmentally-sound pest management decisions is a goal of IPM specialists. Valuable information can be communicated by risk indices derived from empirical data, where numerical index values are assigned to indicate an enhanced or diminished likelihood of pest damage. This paper presents our effort to assemble empirical data and apply it in an interactive decision-making tool that provides farmers with information for more effective pest management decisions.

Objectives:

- Aggregate available research and observational data that provide insight into pest management decision making
- Adapt data to a knowledge base that provides insight into lower or higher risk scenarios

Materials and methods: Program developers relied on results of published research studies and many years of research and extension experience to create or modify existing risk indices for peanut. Peer-reviewed literature was consulted and any factor that was documented to increase, decrease or otherwise affect the risk of certain pest problems was organized into a spreadsheet for each pest. Overall risk values were calculated, and values were adjusted as necessary so that outcomes would conform to our experience and expectations. As a user changes inputs for cultural practices, pesticide applications, and cultivars, the program automatically adjusts the risk category for all pests.

Results: The compilation of information was used to develop an “expert” on-line system as a decision-making tool. Growers can use this tool <http://www.peanut.ncsu.edu/riskmgmt/> to weigh relative risks and cost associated with various agronomic and pest management practices in the southeastern United States.

Conclusions: The acceptance and use of the on-line risk management tool has been valuable for both extension workers and farmers. Both groups are also educated through use of the program relative to the interactions among various crop management practices.

O DIG 4

Enhancing the pest identification experience through custom-designed digital products

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USDA's Identification Technology Program (ITP) develops web-based interfaces and applications that directly support pest screening and identification by the plant protection community. ITP delivers a wide diversity of products to its broad clientele base (off-shore, ports, domestic, and trade) that are custom-designed for specific responsibilities and knowledge of end-users. Product lines and various interfaces and applications within these lines will be highlighted that address specific stakeholder requests for products and the profiling of end-users for the future product. A three-year plan will be discussed that is directed at: 1) increasing efficiency and turn-around time for product development and 2) enhancing services, products, and end-user experiences.

O DIG 5

Diagnosis, monitoring and advice: smartphones respond to these challenges of plant health.

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Plant diseases remain a threat to crops and always cause significant losses if they are not well identified, monitored, and controlled. With Information and Communication Technology (ICT), several complementary applications in plant health have been developed at INRA and grouped under the e-phytia website. These applications allow the user to identify diseases of several crops through an image diagnosis module, and to consult comprehensive sheets on symptoms, biology of diseases and pests, but also on control methods.

Mobile applications are distributed under the name Di@gnoplant: they provide diagnosis and advice in the field with a smartphone or tablet. Another mobile application, Vigi@nt, is now available: it allows epidemics and biomonitoring, image diagnosis. The observations are dated and geotagged with the smartphone, and then transferred to e-phytia database. Furthermore, it is now possible to combine the performance of these two types of applications to provide in the field diagnostic-

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monitoring-advice mobile tools in plant protection. Using these performances, the application of citizen science AGIIR allows you to map in France invasive insects such as the Asian hornet, and the pine processionary caterpillar.

The continuum of knowledge and observations organized from research to the field, and vice versa, through e-phytia website and associated mobile applications will be presented (cf. Figure), this in the context of a sustainable crop protection.

Figure 1: INRA website e-phytia and associated mobile applications



O DIG 6

Improving information flow within the Plantwise programme using Information and Communication Technology

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Introduction: Plantwise is a global programme, led by CABI, to help smallholder farmers get the knowledge they need to reduce crop loss caused by pests and diseases. This is achieved by working closely with national partners to establish sustainable networks of local plant clinics, run by trained plant doctors, where farmers can find practical plant health advice. Plant clinics are reinforced by the Plantwise knowledge bank, a gateway to online and offline actionable plant health information, including diagnostic resources, pest management advice and front-line pest data for effective global vigilance.

Objectives: Exchanging information with plant doctors is a crucial element of Plantwise but can be highly problematic logistically. This work was to understand how mobile technologies could improve the current clinic model.

Materials and methods: Countries were assessed for their readiness for use of tablets and Kenya was selected for initial trials. Appropriate apps were licenced or created for exchanging information. A training course for plant doctors was written and delivered to selected plant doctors during a pilot phase. Following a lessons learned assessment, the use of tablets was rolled out to a wider set of plant doctors for regular use.

Results: Plant doctors were generally very quick to understand the technology and determine how it could improve their workflows. Many benefits were seen, such as quality and speed of delivery of information from clinics, accuracy of diagnosis and treatment of crop problems, and the exchange of images. Plant doctors also found new ways of using the tablets, such as setting up self-help groups. Some further challenges were met, including ensuring that the treatment advice could be easily shared with farmers through SMS.

Conclusion: Tablets were found to improve the work of the plant doctors and feedback from them and farmers was very positive. Tablets will now be tested under a full Monitoring and Evaluation process and trialled in further Plantwise countries in 2015.

Oral Presentations

Fungicides I

O FUN I-1

The formula for fungicide sustainability

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Fungicides are a critical component of food security worldwide contributing about \$8 extra productivity for every \$1 spent. However this fungicidal crop protection is threatened by ever-increasing regulatory stringency and the development of resistance. The implementation of validated strategies for the maximisation of the effective life of existing and new fungicides is of the upmost importance.

Our research brings together two strands; one, an analysis of the status of fungicide resistance in pathogens of grain and grape crops in Australia and two, reviews of experimental and modelling data on resistance management strategies. The results show that Australia is suffering from resistance issues in a way that is entirely in agreement with the modelling predictions. Pathogens with short generation times and large population sizes are showing high levels of fungicide resistance that is having major impacts (>\$100m p.a.) on both the grain and grape industries. Pathogens with moderate generation times are showing worrying if sporadic signs of resistance. If current fungicide regimes are continued, we can confidently predict that further resistance problems will develop and intensify.

The modelling can be used both to protect so-far effective fungicides and to suggest solutions when resistance has developed. The results show that

- When resistance can be detected at > 1%, the only remedy is to significantly reduce use of the affected fungicide or even the entire mode of action group. This implies that phenotypic screening methods are generally unsuitable.
- When resistance is present at levels < 1%, lower doses, mixtures and alternations of fungicide modes of action will prolong the effective life of the at-risk fungicide. Intensive monitoring will then be needed to gather the information need to adjust the fungicide regimes.
- Negative cross resistance within a mode of action has potential to add to the armoury of tools available for prolonging effective life of products

O FUN I-2

Mixtures or alternation? Assessing fungicide anti-resistance strategies

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Introduction: Fungicide resistance threatens agricultural disease control. Two anti-resistance strategies are applying a high- and low-risk fungicide in series (alternation) or parallel (mixtures). These strategies have been compared extensively in the modelling literature² but results have been inconclusive. The structure of the underlying epidemiological models appears to strongly affect the conclusions.

Aims: To identify under which conditions mixtures or alternation are more likely to lead to slower spread of resistance, considering the aspects of mathematical models which might bias results in either direction.

Methods: We use a series of computational and mathematical models (Fig 1). We compare predictions from analytically-tractable models with progressively more complex simulation models and particularly focus on how the shape of fungicide dose-response curves affects strategy performance. We use a range of metrics to compare the strategies, from the simple based purely on the spread of resistance to the more complex including economic considerations.

Results: We develop an analytic criterion which allows the better strategy to be identified in our simplest models. This allows us to predict for which combinations of doses of fungicide either strategy out-performs the other. The broad predictions hold in the more complex models, but exact results depend strongly on the particular parameters used (Fig 2).

Conclusions: Our results contrast with the recent modelling literature which favours mixtures¹ and show that robust predictions require models to be selected and parameterised carefully. We plan to fit our models to field data, allowing predictions for specific host-pathosystems.

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Fungicides I

Figure 1: The models used in our analysis. Dotted lines on the dose-response curve show that a half-dose gives more than half the effect.

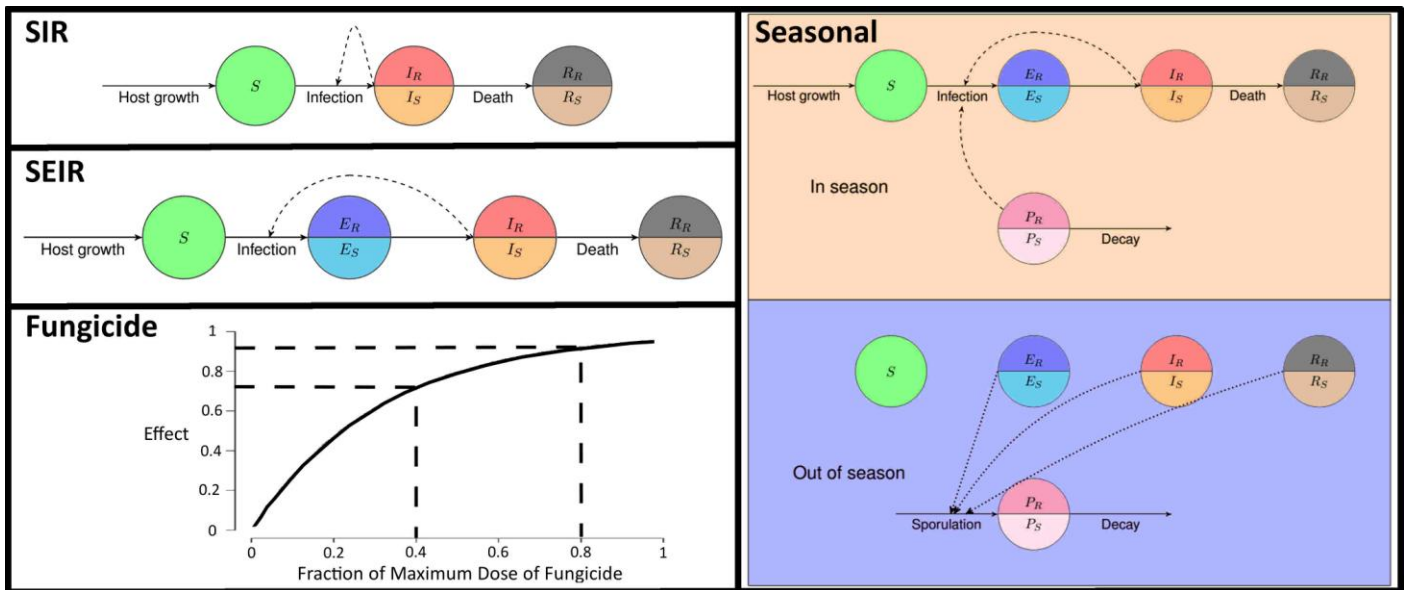
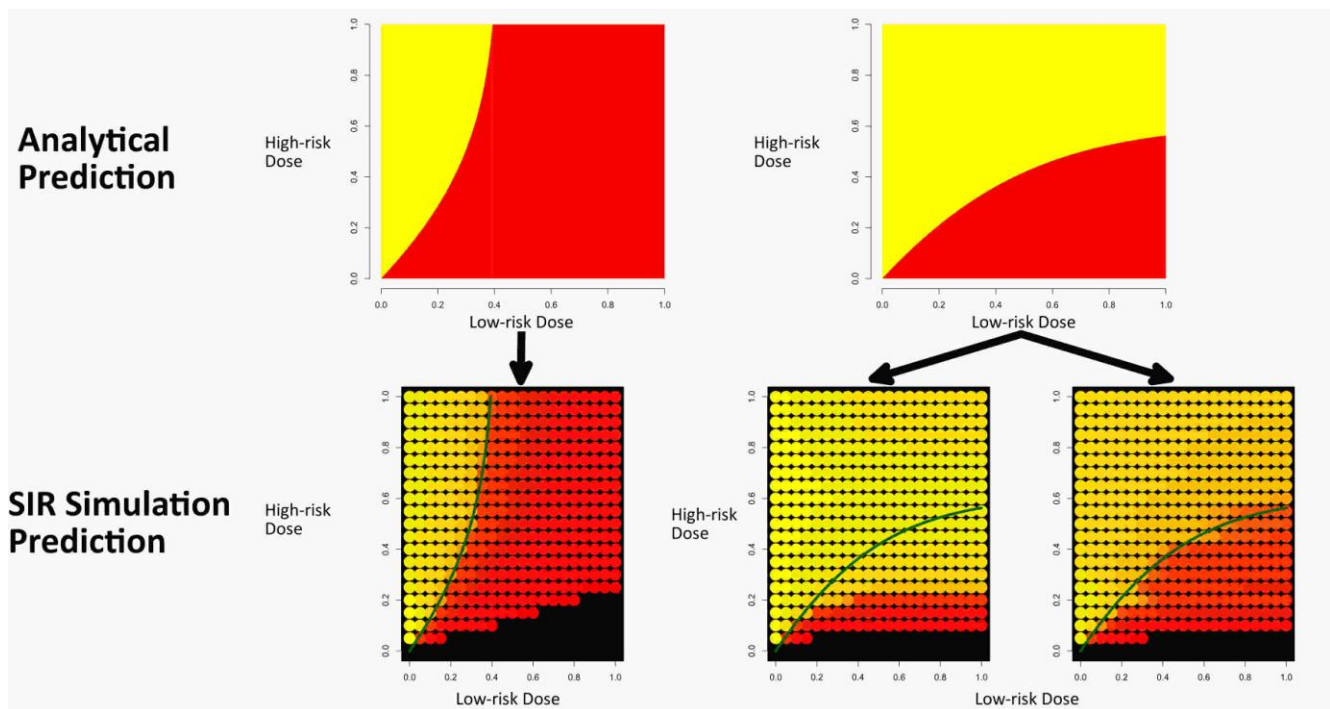


Figure 2 Predictions from a simple analytical model and the SIR model for different parameter combinations. On the y-axis is the dose of high-risk fungicide relative to the maximum legal dose, and on the x-axis is the dose of the low-risk. In the red area mixtures perform better and in the yellow, alternation. The green line shows the predicted boundary between the areas.



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Oral Presentations

Fungicides I

O FUN I-3

Resistance mechanisms to anilinopyrimidines fungicides in *Botrytis cinerea*

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Anilinopyrimidines (AP) fungicides (cyprodinil, mepanipyridin, pyrimethanil) were first introduced in various European countries more than 20 years ago. These fungicides still constitute an integral part of the plant protection program in various crops worldwide such as grapes, apple and cereals. Anilinopyrimidines fungicides display good efficacy for the control of a range of Ascomycetes and Adelomycetes pathogens such as *Botrytis cinerea*, *Sclerotinia sclerotiorum*, *Venturia inaequalis*, *Pyrenophora teres* and *Rhynchosporium secalis*. In most of these pathogens resistant field isolates have been reported at low to moderate frequency but so far the underlying resistance mechanisms have not been deciphered. In order to characterize AP resistance mechanisms in *Botrytis cinerea*, two complementary approaches were undertaken. First approach was the induction of resistance by random mutagenesis in the laboratory followed by their molecular characterization using Illumina[®] sequencing and reverse genetics. Second approach was the generation of mapping populations from crosses between resistant field isolates and reference strains, the fine mapping of the genetic factors and their validation were performed by reverse genetics.

The characterization of our collection of laboratory mutants led to the identification of nine different resistance mechanisms affecting nine different genes. Amongst these, one was also found in the field, and another, not present in our collection of laboratory mutants was mapped *de novo* from a cross between a sensitive and a resistant field isolate. Based on our monitoring of *Botrytis cinerea* field populations we could determine that these two resistance mechanisms account for over 80% of the resistance in the field.

Such diversity of possible Fungicides resistance mechanisms is so far unique. These results, in addition to AP treatment-related metabolomics and transcriptomics information enable us to progress our understanding of the mode of action for this class of fungicides.

O FUN I-4

Dynamics of fungicide resistant *Botrytis* populations in strawberry fields

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Introduction: *Botrytis cinerea* is the major pathogen on strawberries and causes high yield losses. Fungicides are used to control grey mould, but resistance frequencies in *Botrytis* field populations have dramatically increased over the last years.

Objectives: We have investigated the dynamics of fungicide resistance, the corresponding mutations, and the genetic diversity of *Botrytis* field populations.

Methods: *Botrytis* strains were isolated from infected tissue, purified, and tested for fungicide resistance. DNA was prepared and used for analysis of resistance mutations and genetic markers. A PCR-based protocol was developed to quickly differentiate between *Botrytis* groups and species for a high number of isolates.

Results: *Botrytis* isolates collected from German strawberry fields in 2012 to 2014 showed high resistance frequencies and increasing numbers of multi- and superresistant strains, with resistances against several or all registered site-specific fungicides. Only fludioxonil remained partly active against these strains in greenhouse experiments, because no highly fludioxonil-resistant strains appear in the fields. Striking seasonal changes in resistance frequencies were observed. Resistance frequencies increased after spraying and decreased in the following spring. They remained at high levels in intensively sprayed fields, whereas low levels of resistance were usually found in unsprayed fields. *Botrytis* populations on strawberries were genetically heterogeneous, they consisted of several subgroups of *B. cinerea* and, in low abundance, the related species *B. pseudocinerea*. *B. pseudocinerea* was detected more often in unsprayed fields, and before treatments in sprayed fields. Surprisingly, all *B. pseudocinerea* strains analysed were fungicide sensitive and, in contrast to *B. cinerea*, contained an intron in *cytB*, which prevents the development of QoI resistance.

In the course of our studies, a new species, called *Botrytis fragariae*, was discovered, which was observed in high frequencies in one of the strawberry fields.

Conclusions: The increasing frequencies of fungicide resistance in strawberry fields and other crops present a serious challenge for grey mould control, which demands new disease management strategies.

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O FUN I-5

Fungicide resistance in cereal pathogens

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Various fungal pathogens in cereals have significant impact on yield and quality. In wheat, *Zymoseptoria tritici*, *Blumeria graminis* f.sp. *tritici*, *Puccinia triticina*, *Oculimacula* spp., *Pyrenophora tritici-repentis* and *Fusarium* spp. are important pathogens and in barley *B. graminis* f.sp. *hordei*, *Rhynchosporium secalis*, *Pyrenophora teres* and *Ramularia collo-cygni*. Fungicides for cereal disease control include Qo inhibitors (QoIs), sterol biosynthesis inhibitors (SBIs) and succinate-dehydrogenase inhibitors (SDHIs). QoIs were first introduced in 1996 and in countries with intensive QoI use, resistance has developed in *Z. tritici*, *B. graminis*, *P. tritici-repentis* and *R. collo-cygni*, which is conferred by mutation G143A in the cytochrome *b* gene. This mutation has so far not been detected in *P. triticina*, *P. teres* and only rarely in *R. secalis*. These pathogens are still controlled by QoIs. SBIs have been used for more than 30 years for control of cereal diseases and changes in SBI-sensitivity have been described for most target pathogens. Resistance development follows a stepwise mode with low to moderate resistance factors. Mutations in and overexpression of the *cyp51* gene are the main mechanisms for reduced sensitivities; interestingly, in some cases of mutations, SBIs are differentially affected. Nevertheless, SBIs are still the backbone in cereal disease control programs. More recently, the new generation of SDHI fungicides has been introduced worldwide against many cereal diseases. From field isolates of *P. teres* and laboratory mutants of *Z. tritici* it is known that mutations in different SDH-subunits which make up the binding site of ubiquinone and SDHs influence binding affinity thus reducing sensitivity. Extensive monitoring programs are running to identify sensitivity changes for all fungicide classes and their target pathogens. Newest monitoring data and mechanisms responsible for sensitivity changes are presented.

O FUN I-6

Multi-drug-resistance (MDR) in septoria leaf blotch

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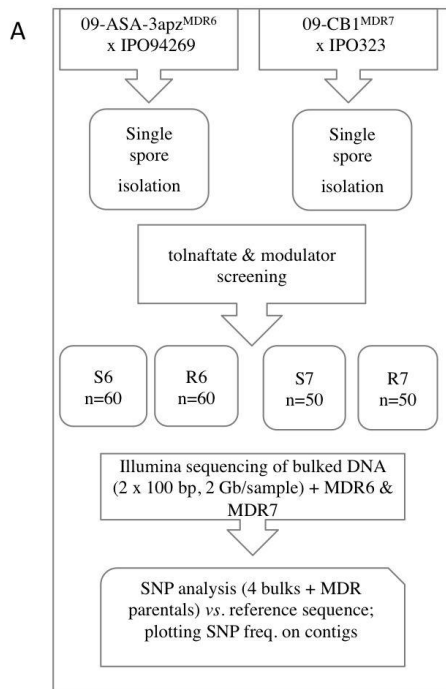
Context: Multidrug resistance (MDR) is a common trait developed by many organisms to counteract chemicals and/or drugs. The basic MDR mechanism relies on an overexpressed efflux transport system that actively expulses the toxic agent. In fungi, MDR has been extensively studied in *Saccharomyces cerevisiae*, but also plant pathogenic fungi, e.g., *Botrytis cinerea*, are concerned by this phenomenon. In agriculture, it is currently under investigation if MDR strains may threaten the efficacy of fungicide treatments. MDR strains were detected in septoria leaf blotch (*Zymoseptoria tritici*) field populations since 2008 (Leroux & Walker, 2011). These strains are cross resistant to fungicides with different modes of action.

Results: We have shown that this resistance is due to active fungicide efflux, potentially through the overexpression of one membrane transporter gene, *MgMFS1* (Omrane et al., 2015). The inactivation of *MgMFS1* abolished the MDR phenotype in at least one field strain. A bulk-segregant analysis (BSA) of two MDR isolates coupled to next generation sequencing showed a clear co-segregation between the MDR phenotype and the left arm of chromosome 7 covering *MgMFS1* (Fig.1). We identified a 519 bp insert (LTR-like) in both MDR strains as well as in other MDR field strains. Genotyping of the progenies for the promoter insert showed a clear, but not exclusive correlation between the *MgMFS1* promoter insert and the MDR phenotype. Current investigations are underway to analyze if the LTR-like insert - alone or in combination with a second mutation - confers the MDR phenotype through *MgMFS1* overexpression.

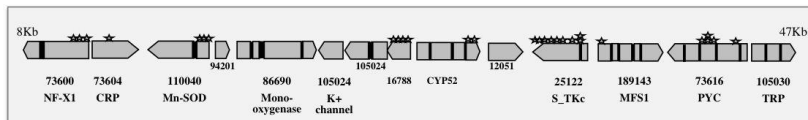
Conclusions: MDR is new resistance phenomenon in *Z. tritici*. It operates through fungicide efflux and involves overexpression of at least one major membrane transporter. Its evolution may be due to retrotransposon reminiscence, but additional mutations may be involved. The combination of *mdr* mutation(s) to target site mutations may threaten most fungicide treatments against septoria leaf blotch.

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Fungicides I

Figure 1: A/ Flow chart of the applied BSA procedure; B/ Region of chromosome 7 with the highest distortion. SNPs and INDELS inducing non-synonymous substitutions are indicated by the stars.



B



Reference:

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Oral Presentations CABI/Plantwise

O CABI 1

Plantwise - a global alliance for plant health support

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Plantwise is a global programme, led by CABI, to increase food security and improve rural livelihoods by reducing crop losses. Working in close partnership with relevant actors, Plantwise strengthens national plant health systems from within, enabling countries to provide farmers with the knowledge they need to lose less and feed more. As Plantwise addresses all problems on all crops, it applies equally well to a specific crop like maize. This is achieved by establishing networks of plant clinics, based on a similar approach to human health clinics, where farmers can find advice to manage and prevent crop problems. Agricultural advisory staff trained as plant doctors learn methods to identify any problem on any crop brought to the clinics, and provide appropriate recommendations guided by national and international best practice standards. Plant clinics are reinforced by the Plantwise knowledge bank, a gateway to actionable plant health information with online and off-line resources for pest diagnostic and advisory services. Plant clinic records are collated and analysed to support the quality of advice given to farmers and inform decision-making. By putting knowledge into the hands of smallholder farmers, Plantwise is able not only to help them lose less and feed more, but also to gather data which can assist all stakeholders in the plant health system- from research, agro-input supply, extension and policy-making. Most importantly, Plantwise is a development programme which cooperates with a number of international and national organisations working to remove constraints to agricultural productivity. By enabling partners to embed and sustain networks of plant clinics to help farmers, national plant health systems are growing stronger and building functioning link between actors. Countries are now utilizing knowledge bank resources to improve national vigilance. As a result of the Plantwise programme, increased numbers of women and men working in agriculture will have access to appropriate, timely and locally-relevant knowledge on plant health management for generations to come.

O CABI 2

Plantwise knowledge bank - a key crop pest information resource for developing countries

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The Plantwise knowledge bank plays a central role in the provision of crop protection information to all stakeholders in the Plant Health System. It holds validated information on the diagnosis, treatment and distribution of crop pests from global and local sources. Available as an open access online resource at www.plantwise.org/knowledgebank the information contained can also be made available in many different formats, such as print, offline, apps and SMS messages. Content can be readily translated and filtered to allow each Plantwise country to access appropriate content that focuses only on those pests which are present in the country or a threat to the region. This information is also available to be integrated with other local projects aimed at improving extension work, e.g. it has been included in the e-extension development in Kenya.

By agreement with each country, the knowledge bank also acts as a central store for information being collected from the plant clinics, under appropriate access control. These data include farmer details, crop seen, problem diagnosed and solution recommended. Plantwise assists with developing in-country capacity in the processes of data collection, management, validation and analysis. Local experts then assess the: distribution of pests; crops grown; and research needs. Importantly this also helps in the assessment of the quality of advice provided by the plant doctor and can identify any on-going development and training needs. The data held can also form a basic source for Monitoring and Evaluation. It is crucial to incentivise the countries to recognise the value of the data in order to secure long term sustainability.

O CABI 3

Improving the national plant health systems of countries through plant clinics: the cases of Rwanda and Malawi

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Crop losses to pests are a great concern in agriculture all over the world. Farmers can experience crop losses as high as 80% in cotton, 40% in maize, and in worst incidences, maize lethal necrosis disease (MLND) can result in 100% yield loss. Since 2011 the CABI led Plantwise initiative has been working to provide the support needed to make plant health systems of countries more responsive to crop losses including those due to pest damage in small holder farms. Plant clinics operated by agricultural extension services are the main pillars employed by Plantwise to contact farmers and provide them with advice needed to manage plant health problems. This service is supported by Plantwise Knowledge Bank, a technical resource for supporting diagnosis at the farmer, extension worker, agricultural research and regulatory service (phytosanitary and pesticide) levels. Plant clinics were started in Rwanda and Malawi in 2011 and 2013, respectively. The suitability of employing the use of plant clinics as part of the strategy for building capacity of countries to develop integrated plant health systems needed for improved management of pests in countries by targeting interventions at small scale farmer fields in the two countries is discussed. The 62 and 42 plant clinics that are currently operational in Rwanda and Malawi, respectively have addressed a total of 3,721 plant health queries arising from visits to plant clinics by 3,510 farmers (1,734 in Rwanda, and 1,776 in Malawi) as at December 2014. The role of plant clinics in early detection of new pests, exemplified for maize lethal necrosis (MLND) is discussed. Some other pests whose occurrence on diverse crops has been detected at clinics in the two countries are also presented and discussed. Steps for making clinics functional and future opportunities are presented. Types of data which are generated from plant clinics and their potential uses by diverse stakeholders in national plant health systems are presented and discussed.

O CABI 4

Lessons learnt on information needs for plant health advisory services through monitoring and evaluation and use of gender disaggregation of plant clinic data

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The development of plant clinic networks as part of national plant health systems is one of the aims of Plantwise programme. Plant clinics are designed for easy access to advice on plant health problems by smallholder farmers. Performance of plant clinics is monitored through monitoring and evaluation (M&E) processes conducted at local, national and programme levels focussing respectively on plant clinic operations; relevant policy questions and assessment of the programme. Under Plantwise, all data collected during M&E are gender disaggregated to facilitate detailed analysis and to enable good understanding of the different information needs of male, female, young and old farmers that use plant clinics. Analysis of the data also makes it possible to evaluate the effectiveness of the Plantwise approach in reaching different farmers. Studies in Kenya and Uganda indicate that agro-dealers are a key source of advice to most farmers. However, farmers who use plant clinics prefer getting such advice from plant doctors. A separate study in Uganda found the effectiveness of plant clinics is due to the direct contact of extension officers with farmers and the ways information from plant clinics can be shared through other channels. The same studies indicate that attendance at plant clinics by women farmers is variable between and within countries. This confirms that plant health systems go beyond the formal government systems and include private sector and civil society actors. Since these actors operate in a changing environment, the plant health system should be adaptive and owned by all actors. Such a system can allow greater inclusion of women. However, further research is needed to understand how to address system challenges to increase inclusion of women. Since some partners seem to perceive M&E as a policing tool, there is need for shared learning on the purpose and objectives of M&E to enable improved engagement with Plantwise implementing partners and a shift of local and national processes towards self-assessment and peer review.

Oral Presentations CABI/Plantwise

O CABI 5

Institutionalization of plant clinics within national plant protection services: Experiences of Uganda

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Pests present the greatest challenge in endeavours to increase crop based agricultural production in Uganda. They contribute 30-40% of all crops losses at pre- and post-harvest stages of production chains. Supporting farmers to improve the management of pests is largely dependent on agricultural extension services. In Uganda these services are decentralised and are provided by district local governments (DLGs) rather than the national government. Plant clinics were introduced in Uganda in 2005 to support extension services in providing information to farmers on pest diagnosis and management. At the time, the department of crop protection, (DCP) in the Ministry of Agriculture, Animal Industry and Fisheries (MAAIF) along with non-governmental organizations (NGOs) viz. Caritas Uganda and SASAKAWA Global 2000 and the districts of Mukono and Soroti implemented started operating a few clinics. Shortly afterwards, SOCADIDO, Self Help Africa and Rwenzori Information Centred Network also became active players plant clinic operations in the districts of Kayunga, Iganga, Katakwi, Serere and Bukedea, Ngora and Kumi. Currently 160 plant clinics have been established in 88 districts and are operated majorly by DLGs with the DCP as the custodian of plant clinic data. Plantwise initiative is moving towards sustainability because all trainings and backstopping of clinic operations is now done by Ugandan institutions including Makerere University, National Agricultural Research Organisation and DLGs. Having included Plantwise approach in the development strategy and investment plan of MAAIF (2010-2015), it is envisaged that sustainability of the initiative as a government function and the resultant operational integrated plant health systems for Uganda will be one of the key achievements of this strategy. The experiences in P approach in Uganda and potential benefits of an integrated plant health system are presented in this paper.

O CABI 6

Transfer of technology towards food security

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Food security has become a matter of concern due to unabated rise in population, reduction in arable land and losses due to pests, causing 40% yield reduction worldwide. Many diseases have caused famine and changed the history of mankind. Lack of timely diagnosis is responsible for losses. Therefore, knowledge transfer to growers is vital to boost food security. Transfer of technology/implies extending knowledge to the growers by traditional tools and modern information technology repeatedly to boost their knowledge bank in saving the crops from pests favouring sustained productivity to meet food requirement of ever-growing millions. Traditional tools employed were training - general or theme-based, demonstration, field days, campaigns, advisory, communication- print & electronic media (multimedia, radio, SMS and satellite channel). Vibrant communication has greater impact on technology transfer While electronic devices are effective, print had its own relevance, as these can be referred in need. The print material included leaflets, handbills, posters, diversified bulletins on crop diseases, question-answer series, booklets and innovative periodicals- *Plant Protectionist in Hindi*, *Plant Disease Warning and Plant Pathology Courier*. *Plant Disease Warning* was issued as and when outbreak of disease was anticipated and messages contained were frequently flashed by All-India Radio (AIR) as a result of tie-up between Haryana Agricultural University & AIR, which helped in averting the epiphytotics. *Plant Pathology Courier* was issued twice a year and was loaded with latest information of practical relevance and was mailed to all concern. The three publications were much in demand beyond Haryana. Several problems were solved during plant health camp. Farmers were advised to prefer IPM and shed total reliance on pesticides. To sensitize users and dealers week-long Pesticide Safety was observed. As a result of continuous motivation, seed treatment in rice, pearl-millet, wheat/barley and gram, peas and vegetable crops has become a common cultural practice amongst farmers besides use of resistant varieties. Empowering growers with latest know-how and knowledge helped in reducing losses and minimizing expenditure on pesticides and maintaining bio-diversity and realizing sustained productivity and improving food security.

Oral Presentations

Legal Issues II

O LEG II-1

Hazard v. Risk in EU Chemicals Regulation

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Hazard is the potential of something to cause harm, whereas risk is the likelihood of harm occurring. Chemicals regulation is generally focused on minimizing the risks associated with chemicals. In the EU, however, the hazard classification of individual chemicals can impact significantly the regulation of products containing those chemicals, regardless of the actual risks that the products may pose to human health or the environment. A discussion is needed on how the EU can move towards a better and more coordinated legal framework for the regulation of chemicals through sound risk assessment.

Risk assessment in the EU rests on three components. *Hazard identification* assesses the nature and severity of the possible adverse effects that a chemical can cause humans or the environment. *Exposure assessment* is the evaluation of the nature and probability of human or environmental exposure to the chemical. Finally, *risk assessment* determines the likelihood that adverse effects may occur in the light of the intrinsic hazard of chemicals and the anticipated exposure.

EU regulations are based both on hazard identification (e.g. REACH, plant protection products, cosmetics) and on risk assessment (e.g. medicinal products, general product safety). However, regulatory action triggered by *hazard identification* (without a risk assessment) can lead to inappropriate regulatory consequences downstream; intrinsic hazard does not determine by itself whether a product is 'safe' (the extent to which it poses risks), and such regulatory action may lead to restrictions on beneficial products, entail a loss of consumer benefits, discourage innovation and increase the use of products with equal or greater risks. Next to these unwanted consequences downstream, the hazard-based approach tends to be at odds with WTO rules and has raised concerns with EU trading partners.

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O LEG II-2

Challenges, recent developments and need of more harmonization in human health risk assessment

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Introduction: The evaluation and approval of active substances and the authorisation of plant protection products in the European Union (EU) in accordance with Regulation (EC) Nr. 1107/2009 places high demands on all parties involved in this complex process. This holds true for the applicants, who must accommodate for new data requirements and provide the necessary documentation; competent authorities in the Member States and EU (European Food Safety Authority, EFSA), who verify and evaluate the provided documents; and the EU Commission, who make the final decisions on the approval of the active substances. Therefore a high level of harmonisation is necessary in order to observe the deadlines set forth by Regulation (EC) Nr. 1107/2009.

Objectives: Both the latest developments in health assessment made in the process of harmonization of the approval of active substances and authorization of plant protection products, as well as the existing need for action will be presented here. Two examples of currently challenging issues will be discussed: cut-off criteria and negligible exposure.

Results: The harmonisation of authorisation procedures for plant protection products in the EU is on the right track. However, in terms of a further optimisation of all process steps, additional efforts are needed.

Conclusion: In the European Union (EU) the authorisation of plant protection products and approval of the active substances contained therein in accordance with Regulation (EC) Nr. 1107/2009 places high demands on all parties involved in this complex process. At the same time, a high level of protection for consumers, operators, workers, residents and bystanders is ensured and the decision-making process is transparently represented.

O LEG II-3

New approaches and better harmonisation of operator, worker, bystander and residential risk assessment

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A prerequisite for the approval of plant protection products in Europe is the estimation of operator, worker, bystander and resident exposure as the basis for a quantitative risk assessment. Because no harmonised European approach existed so far, the Member States developed their own strategies and models resulting in different estimates for the same exposure scenarios.

Oral Presentations

Legal Issues II

With the implementation of zonal registrations this practice has become questionable as different exposure estimates are not compatible with a joint authorisation of plant protection products in Europe.

The European Food Safety Authority (EFSA) recently addressed this problem in a guidance document for operator, worker, bystander and resident exposure ^[1]. In this guidance a systematic review of all available exposure data and models was presented and, where possible, recommendations for a standard approach were given. New developments such as the Agricultural Operator exposure Model (AOEM) or the Bystander and Resident Exposure Assessment Model (BREAM) were taken into account. Both models will replace exposure data that were collected in the 1980ies and will contribute to more appropriate and accurate exposure estimations. The concept for bystander and resident exposure was amended in the guidance: four exposure pathways - spray drift, vapour, surface deposits and entry into treated crop - will be considered separately and in case of resident exposure also added up.

Although some questions remain open the guidance document is a major step forward to a harmonisation of evaluation principles. Further work such as from the BROWSE project or the greenhouse AOEM project will close gaps and improve current exposure calculations.

[1] EFSA (European Food Safety Authority), 2014. Guidance on the assessment of exposure of operators, workers, residents and bystanders in risk assessment for plant protection products. EFSA Journal 2014; 12(10):3874, 55 pp., doi:10.2903/j.efsa.2014.3874

O LEG II-4

Challenges and concepts for cumulative risk assessment of pesticides

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Introduction: Cumulative risk assessment (CRA) is of major importance and one of the biggest challenges for the near future as a legal requirement within the EU for active substances used in plant protection products (PPP). It is important to develop a methodology to take into account cumulative effects for combination of active substances or combination of active substances with co-formulants. The implementation of cumulative aspects in regulatory decisions is in high demande and promoted by EU parliament, EU Commission, European Food Safety Authority (EFSA) and national authorities.

Objectives: Based on EFSA's work on CRA, the Federal Institute for Risk Assessment (BfR) drafted a concept on how to account for cumulative aspects in the regulatory context in risk assessment. The BfR draft concept uses dose-addition of individual active substances/co-formulants as the toxicological standard concept for CRA and proposes a tiered approach. It recommends starting with the calculation of a hazard index (HI) for all relevant substances contained in the PPP. Proceeding to higher tiers is currently foreseen if the HI is larger than 1, i.e. an unacceptable risk cannot be excluded. In higher tiers, the HI should be calculated with respect to common targets and might consider effect-specific NOAEL's (No Observed Adverse Effect Level) or relative potency factors, if available. Refinements should consider both the toxicity and the exposure of the CRA and will depend on availability of relevant data. Chronic exposure assessment needs to take into account all relevant substances contained in the PPP under consideration, but also the residue background of other pesticides in food, which have to be derived from representative food monitoring programmes.

Results and conclusion: Application of this concept as part of the routine risk assessment for PPP is envisaged as soon as suitable experience has been gained in a testing phase and after discussion with risk assessors, regulators and stakeholders interested or directly involved in risk assessment. It is planned that BfR will review the chronic CRA for each active substance and each CAG regularly as soon as all essential monitoring data are available. It is planned to carry out case studies on the impact on regulatory decisions.

The BfR concept for cumulative dietary risk assessment is part of the publication "Human health risk assessment from combined exposure in the framework of plant protection products and biocidal products", Bernd Stein, Britta Michalski, Sabine Martin, Rudolf Pfeil, Vera Ritz and Roland Solecki in J. Verbr. Lebensm (2014) 9:367-376.

Oral Presentations

Legal Issues II

O LEG II-5

Bayer SweepAir: A concept study of a technology for mitigation of dust emission during planting of treated seeds

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Significant efforts are currently oriented at minimising the possible negative effects of dust from seed drilling operations. Although the main focus lies on the questions related to the environmental exposure, the experience shows that any reduction in the amount of dust available for emission in the environment will also have a positive effect in the area of operators and bystanders.

In the overall strategy aimed at dust reduction through the overall process chain (from untreated seeds to the sowing operation), the mitigation of dust emissions during sowing of treated seeds plays a very important role. The Bayer SweepAir technology, developed as a concept prototype, shows that it is possible to reduce the amount of dust emitted by a corn planter by more than 99 %.

The presentation will provide an overview of all aspects related to dust mitigation, with particular focus on sowing machinery and SweepAir technology. Comparison with other technological modifications will be provided.

The final part of the talk will be dedicated to questions associated with the implementation of dust reducing solutions in agricultural practice.

O LEG II-6

Mitigating Environmental Risk for Plant Protection Products - Results of the SETAC MAgPIE workshop

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Introduction: Risk mitigation measures are of increasing importance to allow the use of plant protection products (PPP) in compliance with the requirements for environmental protection as defined by regulatory authorities in Europe (EC Regulation 1107/2009). In order to promote implementation and acceptance of risk mitigation measures in decision making as well as in the risk assessment procedures, a workshop was organised under the auspices of SETAC and European Commission..

Objectives: The objectives were to (i) address environmental risks to wildlife including vertebrates and invertebrates, flora and microorganisms, biodiversity as well as surface- and groundwater quality; (ii) discuss current practices and future developments with all stakeholders, i.e. experts from national authorities, research sector, industry and farmers to eventually (iii) provide Stakeholders with a toolbox of appropriate mitigation measures and a network to share information.

Materials and methods: The workshop was organised in 2 sessions during 2013: the 1st to identify the most promising risk mitigation tools and explore ways to improve harmonisation and a 2nd to determine how to account for these risk mitigation options in the risk assessment and check their efficacy. Approximately 75 experts from 23 European countries, the US EU Commission and EFSA participated.

Results: For each of the areas considered i.e. groundwater, surface water, in-field terrestrial and soil organisms, and off-field terrestrial organisms, existing risk mitigation measures were collected and analysed for their efficiency, their potential use in risk assessment, and their practicality for farmers and regulators. An inventory of voluntary initiatives and stewardship programmes, experiences in risk management, proposals for the new or adapted S phrases was also performed and the needs for further developments identified. The outcome of these analysis constitutes a risk mitigation toolbox from which the tools being the most appropriate for specific legal, agronomical and environmental conditions can be chosen by individual Member States whilst ensuring a European harmonised level of protection.

Conclusion: The proceedings are in preparation and are intended to be finalized in 2015. Additionally a website will be installed to share information between stakeholders, and provide communication to farmers.

Oral Presentations Biocontrol of Insects II

O BI II-1

Introduction and acclimatization of entomophagous: pro et contra

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Traits, determining effectiveness of entomophagous in biological control, are often related with its high invasion potential. Because of it, as promising biocontrol agents we select from the natural environment species that are potential invaders. In recent decades, frequency of cases of entomophage invasion, introduced earlier for biological plant protection, has increased.

In some cases invasion activity of former agents of biocontrol caused negative consequences, including replacement or disappearance of several indigenous species, which previously occupied the dominant or subdominant position in the species communities. Among polyphagous predators the Multicolored Asian lady beetle *Harmonia axyridis* is a demonstrative example of introduced entomophagous, this became an invasive species. In this regard, one of the important aspects of the work on development of entomophagous bioresources is their invasive potential evaluation. In the present work attempted to define features that distinguish potential invaders of promising biological control agents. Screening was based on our collection entomophages (including *Cheilomenes sexmaculata* and *Propylea dissecta*).

Currently, in the south of Russia we found several populations of entomophagous (*Cryptolaemus montrouzieri*, *Lysiphlebus testaceipes*, *Perillus bioculatus*, *Serangium parcesetosum*, *H. axyridis*), that were introduced about 30-40 years ago. Some biological traits of these entomophagous and approaches to its applying are discussed.

O BI II-2

Steps in introducing *Lathrolestes ensator*, a parasite of the apple sawfly, *Hoplocampa testudinea*, in North America

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The Apple Sawfly, *Holocampa testudinea* Klug (Tenthredinidae), origins from Northern Europe. Its only known host is the apple tree. It was found for the first time in North America in 1939 in Long Island, NY, and, from there invaded New England States and entered Quebec, Canada, in 1979. As of 2014, it was found in Ontario, New Brunswick and Nova Scotia. Because it disrupted protection programs of apple orchards, and because it had no known natural enemies in North America, a classical biological program was initiated by importing the larval endoparasite *Lathrolestes ensator* Brauns (Ichneumonidae) from Western Europe through a contract to CABI (Delémont, Switzerland). From 1994 to 2001, a total of 604 living adults were released in an insecticide-free apple orchard of Frelighsburg, Qc, Canada, after a passage through a quarantine facility in Ottawa, Ont.. This procedure, that aimed to exclude any undesirable species, had two major drawbacks. Firstly, some shipments experienced high (ca. 50%) mortality of adults before they were released. Secondly, the procedure failed to provide optimal synchronism of adult parasitoids with their hosts. In 2001, it was demonstrated that *L. ensator* successfully established in Frelighsburg, Qc. It has also invaded an adjacent orchard such that two sources orchards (hereafter orchards A and B) provided *L. ensator* for dissemination in orchards of other localities from 2002 to 2014. *L. ensator* was disseminated by collecting applets showing secondary damage and releasing them under the canopy of apple trees in selected apple orchards of Quebec, Ontario and New Hampshire. Dissection of sub-samples of applets collected in orchards A and B revealed that *H. testudinea* larval parasitism ranged from 24 to 87%. *L. ensator* is now established in several orchards of Quebec.

Oral Presentations

Biocontrol of Insects II

O BI II-3

Enhancement of Natural Enemies through the Use of Flowering Medicinal Plants in Syria

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Enhancement of natural enemies through habitat management is an important component of conservation biological control. Many predators and parasitoids require non-prey food such as pollen and nectar to complete development. The availability of suitable nectar may also enhance one or more natural enemy life history traits, such as longevity and fecundity, which may in turn increase their impact on herbivore populations. This study investigated the attractiveness of eight species of medicinal flowering plants (*Nigella sativa*, *Cuminum cyminum*, *Coriandrum sativum*, *Foeniculum vulgare*, *Pimpinella anisum*, *Linum usitatissimum*, *Carthamus tinctorius*, *Fagopyrum esculentum*) to beneficial insects in Syria in 2010 and 2011. The results showed that flowers of *C. sativum*, *F. vulgare*, *P. anisum* and *C. tinctorius* were very attractive to natural enemies, mainly *Chrysoperla* sp., *Trissolcus* sp., *Spharophoria* sp., *Bracon* sp., and Coccinellidae. *C. sativum* was also found to increase % parasitism by *Trissolcus grandis*, an egg parasitoid of Sunn pest (*Eurygaster integriceps*). These flowering medicinal plants could be incorporated into cropping systems to conserve and enhance natural enemies and also provide a direct source of income for farmers. Our study suggests a potential interest in growing specific plant species which attract natural enemies of the pests that are most common in the surrounding crops, but do not provide resources to crop pests themselves. Screening individual plant species for their attractiveness to natural enemies allows selection of the best plants. With this information, a community of native plant species can be developed for this purpose.

O BI II-4

The stability of classical biological control in New Zealand simplified pastoral ecosystems

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Introduction: New Zealand's pastoral landscapes can superficially appear similar to grassland areas elsewhere but in reality they are significantly different. The antipodean ecosystems comprise very low levels of biodiversity and although they may indeed contain some New Zealand-endemic invertebrate species, these usually do not include key exotic pest-suppressing species such as parasitoids, generalised predators and predatory spiders. The lack of biotic resistance to invasive species coincides with unfilled niches and explains why 90% of the country's pasture pests comprise exotic invaders.

It is against this background that over the last 30 years in New Zealand, there has been a major biological control triumph in perennial ryegrass (*Lolium perenne*) pasture ecosystems in that the endoparasitoid wasp *Microctonus hyperodae* Loan has controlled the severe pest of Gramineae the Argentine stem weevil *Listronotus bonariensis* (Kuschel).

Objectives: This contribution discusses now-emerging evidence of a breakdown in the biological control of the Argentine stem weevil (ASW), possibly through the acquisition of some kind of host resistance to the parasitoid.

Materials and methods: For the purposes of this study, an abundance of historical parasitism data collected in the 1990s provided a strong basis against which to compare 2014 parasitism levels.

Results: Data collected have now shown that annual ASW parasitism levels by *M. hyperodae* are less than half of those measured in the 1990s when there was good evidence for very significant pest suppression. This result is highly consistent with earlier findings that showed that overwintering levels of parasitism had dropped by the same amount over the last 10-15 years.

Conclusions: Based on these results, the question then arises as to what the mechanism might be. Given both the ecological setting and biological characteristics of both the weevil and the parasitoid, it is fair to speculate that selection by the wasp has led to resistance by the weevil. Thus, it may well be that the very thing led to the success of *M. hyperodae* in suppressing *L. bonariensis* could now be the cause of its undoing. The empty habitat, devoid of biotic resistance and refugia has after 20 years permitted both the weevil and the parasitoid to thrive to the extent that resistance to the parasitoid has started appear.

Oral Presentations
Biocontrol of Insects II

O BI II-5

The role of green spaces in biological control of Sunn Pest (*Eurygaster* spp. (Scutellaridae: Heteroptera)) Konya Province Turkey Sample*

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Natural or man-made, green spaces has now taken on a new value and function, the importance of which is widely acclaimed within the parameters of sustainable development. The protection of natural balance and biodiversity are one of the most important functions of green spaces. The increasing importance of biological control against pests had also recently increased the importance of green spaces in terms of plant protection. Then, the improving of environmental conditions of local natural enemies located in the food chain is vital precaution in the biological control. Afforestation and arrangement of green space is the first and most important step to be taken in this regard.

In Konya Province of Turkey, 10-12.5 million hectares of cereal are grown in every year, and therefore it called as "Cereal store of the country". Sunn pest (*Eurygaster* spp.) is the most important cereal pest in Turkey. Chemical control measures started in 1950's have been carried out increasingly. *Trissolcus* spp. (Hymenoptera: Scelionidae) is the most effective biological control agent to decrease the population level of the Sunn pest. However these parasitoids are generally present in Turkey, except a few parts where polyculture farming is widespread; they are insufficient to solve the problem. Thus, to increase their population and be more effective they should be supported.

It is a known fact that there are few studies on "protection and creation of green spaces in nature regarding to natural enemy-host relations" which is thought to provide great contributions to biological control of the problem. However, in near future, in terms of frame of IPM, the importance of this subject will definitely further increase

The Turkish ministry of agriculture has started national sunn pests project in 2004 to develop and dissemination of biological control.

In this study, the afforestation efforts aimed supporting IPM studies against sunn pest in Konya province were summarized. Some recommendations were also developed about green space planning and plant species which can be useful in that programme.

This study was supported by Selcuk University, coordinatorship of scientific research projects

O BI II-6

Biological control: challenges and opportunities for controlling cowpea insect pests in West Africa

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We are presenting challenges and opportunities for the development and deployment of a 'biological control pipeline' addressing insect pest problems in cowpea (*Vigna unguiculata*) in West Africa. Biodiversity and population genetic studies have been carried out to guide the identification of novel biological control candidates, which are subsequently assessed for their potential in sustainably reducing pest populations. Pre-release assessment studies are targeting critical questions such as potential impact on biodiversity and biosecurity in general, together with factors leading to successful establishment such as host finding capacity and intra-guild competition. Also, experience from the field has indicated the importance of the right deployment system for establishing a population of the released natural enemy through inoculative releases. Using the case study of the legume pod borer *Maruca vitrata*, the presentation leads through the various steps of this development-to-deployment process, including partnerships with e.g. social enterprises. The same approach is also presented for the development of bio-pesticides against the same target pest, as one of the valuable components of integrated pest management (IPM) for cowpea.

Oral Presentations

Modelling/Forecasting I

O MF I-1

Implementation the decision support system for early warning of cucumber diseases in solar greenhouses

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Question: Solar greenhouses are one of the most popular facilities for vegetable and fruit production in north of China, which have significant contribution to solve the problem of fresh vegetable supply in winter for China's citizens. However, with proper temperature and high relative humidity, the diseases are the important limited factor for vegetable production and quality in solar greenhouses. Then the improper treatments, such as over-use of pesticides after disease occurrence and regular spraying irrespective of the diseases happen or not, may result in product pesticide residue and quality safety risk. Thus we developed an early warning system for managing the disease and reducing pesticide usage, which is also a decision support system for precision plant protection in greenhouses.

Methods: The system has several characteristics for implementation: real-time data collection, primary infection model, integrated management. The outside greenhouse weather parameters, temperature, relative humidity and solar radiation were recorded by the weather stations every day at 30 min intervals. The data were transferred from the greenhouses to the database server using GPRS. Based on the input parameters that were both readily available and appropriately limited in number, EWMPICD (early warning model for primary infection of cucumber diseases, e.g. downy mildew, powdery mildew and grey mildew) was developed based on monitoring data, early warning theory and plant disease epidemiology. Because the leaf wetness duration (LWD) played an important role in disease warning systems for crops in solar greenhouses and was difficult to monitor, the estimation model was investigated to form a practical estimation solution for LWD based on other parameters. The infection condition early warning submodel was developed by using a threshold method based on the combination of LWD and mean temperature in LWD. The temperature was chosen as the warning indicator for incubation, and the incubation early warning submodel was defined using nonlinear regression methods. The traceability algorithm of warning sources was developed in relation to expert knowledge and in terms of a mode of disaster mitigation that involved cutting the disaster chain from the headstream. The methods for controlling the diseases were based on good agricultural practices (GAP).

Results: The system has been implemented in Beijing and Tianjin involving potential users during the system development and testing to obtain a better effect.

Conclusions: Feedback collected during development, testing and practical use of the system suggested that potential users were likely to use the decision support system.

O MF I-2

Using expert knowledge to estimate risk priorities: an organism ranking tool

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Introduction: Assessing the biosecurity importance of an organism or disease is essential when decision-makers and risk managers have to prioritise resources across the biosecurity system. The Ministry for Primary Industries has developed an Organism Ranking System to provide an organisation-wide view on priority organisms after considering the impact of that organism to New Zealand. Plant pests currently assessed include those whose invasion biology is well known as well as emerging risks.

Objectives: Two methods were assessed for their ability to incorporate expert knowledge and uncertainty in calculating the risk ranking score for plant pests. The outputs of the risk ranking model are also summarised and potential uses illustrated.

Materials and methods: A modification of the Group Delphi technique was used to populate the parameters of a risk ranking model with expert knowledge. This method produces a range of possible values to be used for each parameter in the model. The model ranks the organisms using a combination of economic impact, probability of entry into New Zealand and probability of spread once in New Zealand. To generate values for each parameter in the model, two methods were used: calculating a median value for each parameter and fitting a Beta-PERT distribution to the data. The outputs from each method were compared and assessed in terms of their usability for decision making and work prioritisation.

Results: Populating the organism ranking model with median values from the expert answers provided a quick and simple method to estimate the risk ranking score. However, the median value was only representative when there was agreement among experts and did not capture important differences. Values generated by the beta-PERT distribution represented the range of answers more effectively, and therefore uncertainty could be incorporated into the final ranking score.

Conclusion: The organism ranking system enables expert knowledge to be used in assessing the potential risk of pest species of concern. Fitting a distribution to the range of expert answers enables uncertainty in individual parameters to be accounted for in

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the ranking score. Overall outputs as well as individual parameters can be examined to further understand aspects of the risk ranking score for specific organisms.

O MF I-3

A comparison of two ecological niche modeling software in predicting potential distribution of *Ectomyelois ceratonia* Lepidoptera: Pyralidae

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Ectomyelois ceratonia is one of the most important pests on a wide range of products around the world and a major pest in pomegranate garden Iran. In this study, we used two ecological niche models, Genetic Algorithm for Rule-set Production (GARP) and Maximum Entropy (Maxent), along with the geographical distribution of its host pomegranate (*Punica granatum*) to predict the potential geographical distribution of *Ectomyelois ceratonia*. The results suggested that the suitable distribution areas based on GARP were generally consistent with those based on Maxent, but GARP predicted distribution areas that extended more in size than did Maxent. The results also indicated that the suitable areas for carob moth infestations were mainly west, south west, north and north western Iran. In addition some provinces in central and south east of Iran were ranked as low suitability to unsuitable areas. A jackknife test in Maxent showed that the precipitation of wettest month and Annual mean temperature played the most important roles in distribution modeling of carob moth, while mean diurnal range and maximum temperature of warmest month were less important.

O MF I-4

Artificial neural networks for forecasting development of wheat disease

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Modeling and subsequently based on this forecasting the development of wheat disease is difficult from nonlinear dependencies and large-scale factors influence the process. One of the possible ways to overcome these and other difficulties in the construction of forecasting systems is the use of artificial neural networks. Prediction problem can be divided into two main classes: classification and regression. Regression problems can be solved using the following types of networks: multilayer perceptron, radial basis function, generalized regression network and linear network. As parameters in our study were used:

- The observed degree of development of wheat disease (septoria tritici blotch of wheat (*Mycosphaerella graminicola* (*Septoria tritici*)), powdery mildew of wheat (*Blumeria graminis*), leaf rust of wheat (*Puccinia triticina*)) at the time of the forecast;
- The average temperature;
- The number of days with precipitation at;
- Phenological stage of development wheat at the time of the forecast;
- Average moisture content of the atmosphere;
- Average annual rainfall and other factors.

Projected output parameter - the degree of development of wheat disease to phase 75. Used software packages Neuroph, KNIME et al., with built in neural networks forecast of the following types: linear networks, radial basis function and multi-layer perceptron. Obtained results showed that for such a set of input parameters, there is a tendency receive neural network satisfactory prediction of the disease. So with the use of neural network - multilayer perceptrons managed to accurately predict septoria leaf blotch and leaf rust epidemic of wheat on the territory of Russia.

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O MF I-5

Improving the Degree-day model for forecasting *Locusta migratoria manilensis* (Meyen) (Orthoptera: Acridoidea)

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The degree-day (DD) model is an important tool for forecasting pest phenology and voltinism. Unfortunately, the DD model is inaccurate, as is the case for the Oriental migratory locust. To improve the existing DD model for this pest, we first studied locust development in seven growth chambers, each of which simulated the complete growing-season climate of a specific region in China (Baiquan, Chengde, Tumotezuqi, Wenan, Rongan, Qiongzong, or Qiongsan). In these seven treatments, locusts completed 0.95, 1, 1.1, 2.2, 2.95, 3.95, and 4.95 generations, respectively. Hence, in the Baiquan (700), Rongan (2400), Qiongzong (3200), and Qiongsan (2400) treatments, the final generation were unable to lay eggs. In a second experiment, we reared locusts for a full generation in growth chambers, at different constant temperatures. This experiment provided two important findings. First, temperatures between 32 and 42°C did not influence locust development rate. Hence, the additional heat provided by temperatures above 32°C did not add to the total heat units acquired by the insects, according to the traditional DD model. Instead, temperatures above 32°C represent overflow heat, and can not be included when calculating total heat acquired during development. We also noted that females raised at constant 21°C failed to oviposit. Hence, temperatures lower than 21°C should be deducted when calculating total heat acquired during adult development. Using our experimental findings, we next mimicked 24-h temperature curve and constructed a new DD model based on a 24-h temperature integral calculation. We then compared our new model with the traditional DD model, results showed the DD deviation was 166 heat units in Langfang during 2011. At last we recalculated the heat by our new DD model, which better predicted the results from our first growth chamber experiment.

O MF I-6

Modeling academic knowledge using semantic networks in integrated pest management of cereal stem borers

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Question: In conservative biological control, the local landscape is managed in a way that ensures the survival of natural enemies. Our hypothesis is that some components of the landscape, especially secondary host plants of pests, can be identified using trophic chains and food webs published in the literature. This hypothesis was evaluated considering cereal stem borers in a lowland region of Benin characterized by a complex agro-ecological context. A mosaic of crops (maize, sorghum, rice, cotton, gardening...) occupies 49% of the area in the rainy season, the rest being comprised of fallow and natural areas.

Methods: The first step was to construct a semantic network using data from 70 scientific papers published between 1957 and 2014, among them 11 review articles, concerning lepidopteran cereal borers in Africa. The data introduced in the semantic network are trophic chains, their geographical location and the bibliographic references. The second step was to extract, from this semantic network, the part related to food webs including the cereal borers studied (*Busseola fusca*, *Sesamia calamistis* [Noctuidae] and *Coniesta ignefusalis* [Crambidae]) and their wild host plants (9 species of Cyperaceae and 23 species of Poaceae) observed in the landscape. This subnet included location and bibliographic reference. The last step was to check the consistency of the combination of geographic locations juxtaposed in the subnet, as well as the bibliographical references.

Results: The resulting semantic network describes 3004 trophic chains distributed in 40 territories and 13 regions. The extraction of the food web related to the three species of borers helped identify 15 species of plants able to host borers. Among them, *Rottboellia cochinchinensis* is able to host, indirectly via the host borer, two Hymenoptera that are larval parasitoids of the borers, i.e. *Goniozus indicus* (Bethylidae) and *Xanthopimpla stemmator* (Ichneumonidae).

Conclusion: The pertinence of our analysis is determined by the accuracy of the data provided by the authors. Some studies lacked information on the precise location of the observation or gave an incomplete description of the local ecology. In addition, some reviews did not cite previously published work, raising questions about the value of these reviews or of the uncited studies.

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O FUN II-1

Fungicide sensitivity status and Resistance Management of *Phakopsora pachyrhizi* after first detection of QoI target site mutants in Brazil

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Introduction: Asian soybean rust (*P. pachyrhizi*) has turned out since 2003 to be a very devastating soybean disease in Brazil and fungicides used for control belong mostly to QoI and DMI compounds. During 2008, a weaker efficacy of straight applied DMIs was reported for the first time in regions of Mato Grosso and Mato Grosso do Sul whereas DMI-QoI mixtures performed very well. Since then, such mixtures were preferably applied to ensure proper disease control and resistance management, especially for DMIs. The resistance risk for QoIs was regarded to be low due to the presence of an intron, which does not allow the occurrence of the target site mutation G143A. However, in season 2014, reduced field performance of QoI containing products has been reported in some Brazilian soybean growing regions. DNA analysis of respective samples carried out at Bayer CropScience identified for the first time presence of the mutation F129L. Adherence to FRAC QoI and SBI guidelines is discussed under the light of these new findings.

Objectives: This study show results of latest DMI and QoI resistance research and discuss consequences of the new F129L mutants in regard to their relevance in practice as well as options for future sound resistance management strategies for the use of DMI and QoI fungicides in soybean.

Materials and methods: Sensitivity analysis of Brazilian soybean rust populations were performed *in vivo* by EC₅₀ evaluation, molecular biological analyses by pyrosequencing.

Results: Molecular methods were developed to detect target site modifications in the *cyt b* gene of *P. pachyrhizi* and the mutation F129L has been detected for the first time in Brazilian soybean rust samples. First greenhouse studies with selected populations bearing high frequency of F129L did not show reduced sensitivity towards trifloxystrobin *in vivo*.

Conclusion: Current investigations showed presence of the target site mutation F129L in soybean rust samples, but give up to now no hint for an impact on the efficacy of trifloxystrobin *in vivo*. Further studies are initiated to evaluate possible sensitivity changes towards other QoIs as well as to characterize the F129L mutants in more detail, e.g. in regard to their virulence and competitiveness towards wild-type populations and *in vivo* sensitivity towards other fungicide classes. As sensitivity shifts of soybean rust towards DMIs have been described earlier, strict adherence to FRAC QoI and SBI guidelines and sound anti-resistance management strategies remain of high importance for future disease control.

O FUN II-2

Decreased sensitivity of *R. solani* to Quinone outside inhibitor (QoI) fungicides did not adversely impact control by QoI and other classes of fungicides

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Introduction: Rhizoctonia damping-off and crown and root rot caused by *Rhizoctonia solani* are the major diseases affecting sugar beet in the United States. Growers rely on fungicides to control these diseases. Azoxystrobin, a quinone outside inhibitor (QoI) fungicide was used since 1999 for controlling *R. solani*. Resistance became a concern after resistance to *R. solani* AG-1-1A was reported.

Objectives: Determine baseline sensitivity of *R. solani* AG-2-2 IIB to different classes of fungicides; determine any shift in sensitivity over time; and efficacy of fungicides at controlling the pathogen in the greenhouse.

Materials and methods: Sensitivity of *R. solani* isolates to different classes of fungicides were evaluated *in vitro* using mycelium radial growth assay and by evaluating disease severity 3 weeks after inoculating plants treated with different fungicide classes in the greenhouse. One hundred and five *R. solani* isolates collected from sugar beet were used in this study; 27 were collected before the use of QoI fungicides and were used for the baseline study and 78 isolates collected between 2005 and 2012 were evaluated for shift in fungicide sensitivity.

Results: The mean EC₅₀ values for baseline isolates were 49.7, 97.1, 0.3, 0.2, and 0.9 µg ml⁻¹ and for non-baseline isolates were 296.1, 341.7, 0.9, 0.2, and 0.6 µg ml⁻¹ for azoxystrobin, trifloxystrobin, pyraclostrobin (QoIs), penthiopyrad (SDHI), and prothioconazole (DMI), respectively. The mean EC₅₀ values of azoxystrobin, trifloxystrobin, and pyraclostrobin increased with a change factor of 6.0, 3.5, and 2.7, respectively. Frequency of isolates with EC₅₀ values >10 µg ml⁻¹ for azoxystrobin and trifloxystrobin increased in non-baseline isolates by 30%. There was no change in sensitivity between baseline and non-baseline isolates for penthiopyrad and a slight increase in sensitivity for prothioconazole. All fungicides used at labeled rates effectively controlled *R. solani* *in vivo*.

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Conclusion: Over time, sensitivity of *R. solani* to Qols decreased, but the Qols, SDHI and DMI fungicides at labeled rates effectively controlled the pathogen in vivo. Monitoring and testing for fungicide sensitivity, along with the baseline data, can be used to determine when different fungicide classes should be used to control *R. solani* and manage fungicide resistance.

O FUN II-3

Solatenol™, an SDHI fungicide setting new standards in disease control

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Solatenol™ is a new broad spectrum foliar fungicidally active ingredient discovered and developed by Syngenta. It is the third Syngenta succinate dehydrogenase inhibitor (SDHI), and the second in the benzonorbornene amide subclass.

The strong affinity to the target enzyme succinate dehydrogenase results in very high intrinsic activity on various pathogens (rusts, powdery mildews and leaf spots). Combined with a strong association with the plant's wax layer, it provides reliable, long lasting disease control even under unfavourable conditions (e.g. wet weather). Solatenol™ has been tested on many important crops and has shown excellent activity on a wide range of destructive plant pathogens.

Solatenol™ was selected and has been proven to have an outstanding and reliable activity against soybean rust (*Phakopsora pachyrhizi*) in extensive greenhouse and field trials. The high intrinsic activity as well as the slow uptake and translaminal movement into the plant result in a long lasting effect with protection outside and inside of the plant tissue. It has consistently delivered good performance at rates as low as 30 g/ha with superior soybean rust control to all current, commercially available fungicides. In mixture with azoxystrobin, it is registered and sold as Elatus™ in the major South American soybean markets (Brazil, Paraguay, Uruguay, Argentina and Bolivia).

In addition to its excellent performance against the soybean rust, Solatenol™ at 75 g/ha is also active on a broad spectrum of wheat (*Zymoseptoria tritici*, *Puccinia striiformis*, *Puccinia recondita*) and barley diseases (*Puccinia hordei*, *Ramularia collo-cygni*, *Rhynchosporium secalis*, *Pyrenophora teres*). In 2014, during the rust epidemic in Europe, it has shown outstanding and longer protection than the current standards. Solatenol™ is also very active against key diseases in other crops including pome fruits (*Venturia inaequalis*), potatoes (early blight), vegetables (powdery mildew, early blight, anthracnose), corn (*Puccinia sorghi*, *Cercospora zea-maydis*), peanuts (*Puccinia arachidis*, *Sclerotium rolfsii*).

Solatenol™ is safe to the crops when applied alone and in mixtures, e.g. with DMI, Qol compounds, or a range of other partners. Solatenol™ has been submitted for registration in key markets and on multiple crops.

O FUN II-4

The threat of Succinate-Dehydrogenase Inhibitors (SDHIs) resistance evolution in cereal pathogens

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The evolutionary forces shaping fungicide resistance varies with respect to the different fungicide classes and between different fungal genera or species. The chemical group of the pyrazole-carboximides represents the new generation molecules of Succinate DeHydrogenase Inhibitor (SDHI) fungicides, known as complex II respiration inhibitors. SDHI were discovered more than 40 years ago. However the first generation of compounds had narrow spectrum, therefore these substances remained limited to few diseases. Next generation SDHIs display broader spectrum controlling a variety of diseases in different crops. To date, resistance has been reported in 14 fungal pathogens. The target of SDHI fungicides is the succinate dehydrogenase (SDH) complex in the mitochondrial respiratory chain. SDH consists of four subunits (sdh-A, to sdh-D). Resistance was described as monogenic and several target site mutations have been detected targeting three sdh subunits (sdh-B to sdh-D). In *Pyrenophora teres*, the major barley pathogen, ten mutations associated to sensitivity reduction were reported (sdh-B H277Y, sdh-C K49E, R64K, C75S, G79R, H134R, S135R and sdh-D D124E, H134R, D145G). The most frequent mutations in natural population were sdh-C G79R and sdh-B H277Y. Resistance factors were low for sdh-B H277Y and moderate for sdhC-G79R. To the present recombination did not combine multiple mutations in one genotype. *Zymoseptoria tritici*, the principal wheat pathogen, isolates harboring mutations sdh-B N225T and sdh-C T79N, W80S, N86S were reported in natural population at low frequency. Resistance factors associated were low. Today field performance reduction of SDHIs were not reported, however this depends on the strength of the resistance reduced sensitivity and its frequency in a particular population. Here we describe the

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evolutionary forces shaping SDHI resistance in field and we will discuss the evolution of single target genes and how their combination into a functional enzyme might shape the response to fungicide.

O FUN II-5

Relevance of point mutations in the target gene of SDHI fungicides for growing cereals

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Apart from demethylation inhibitors (DMIs) and quinone outside inhibitors (QoIs), succinate dehydrogenase inhibitors (SDHI) nowadays belong to the most important fungicide classes being used in crop protection. The quaternary structure of the target enzyme SDH consists of four distinct subunits and the ligand binding site is located at the interface of the subunits B, C and D.

Since 2003, extensive monitoring programs have been implemented and mutation analysis initiated. Several target site mutations have been detected at different positions in the subunits B, C and D of different pathogens in both, lab mutants and in field isolates. However, as the positions of mutations can differ from pathogen to pathogen and even different substitutions of one and the same amino acid may occur within one species, the cross-resistance pattern between different SDHI fungicides is quite complex.

Since 2012, occurrence of only single field isolates of *Mycosphaerella graminicola* with target site mutations were reported, while in *Pyrenophora teres* a higher number of isolates with diverse mutations were detected and, furthermore, found more frequently in Europe. However, the field performance of products containing SDHIs was not affected, neither for the control of *M. graminicola* nor for net blotch.

Objectives were to (i) determine the sensitivity status of current pathogen populations and (ii) to evaluate the relevance of different SDH genotypes for agricultural practice.

Sensitivity analysis of fungal strains with different genotypes was performed *in vitro* by EC₅₀ evaluation or *in vivo* by evaluation of the level of efficiency of formulated products. For molecular biological analyses, pyrosequencing was used.

Reduced sensitivities of mutated isolates *in vitro* were also confirmed *in vivo*. At the same time, differences in virulence could partly be observed, which may be linked to fitness penalties.

Current investigations showed differences in the virulence of lab mutants and suggest fitness penalties, especially of mutants with highest EC₅₀ *in vitro*. However, the occurrence and spread of possible future SDHI mutants is not foreseeable. Therefore, there is further need for intensive sensitivity monitoring and resistance research, including detailed investigations on resistant lab or field strains, to analyze the relevance of putative mutations. Moreover, strict adherence to FRAC SDHI guidelines and sound anti-resistance management strategies remain of high importance for future disease control.

O FUN II-6

Impact of carboxamide intensity on disease control in wheat and SDHI & DMI sensitivity of *S. tritici*

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Introduction: With the introduction of the Pyrazole-Carboxamides into the German market in 2011, the question was raised, if the SDHI mode of action is prone to a similar risk of resistance development as QoIs and if the resistance management guidelines of the FRAC were strict enough. Especially the maximum number of applications with 2 x SDHI per season was discussed intensively in regard to *S. tritici* in wheat.

Objectives: A trial program was started in 2011 by Bayer CropScience Germany in cooperation with official advisors from several federal states to assess the benefit of SDHI on the disease control and yield in wheat and monitor the impact of SDHI applications on the sensitivity of *S. tritici* towards Bixafen and Prothioconazole under different spray strategies and environmental conditions.

Materials and methods: 31 trials were conducted since 2011 all across Germany, containing five treatment lines, representing 0%, 50% and 100% of the maximum number of SDHI applications per season:

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	BBCH 30-37	BBCH 39-59
untreated	-	-
0 x SDHI	SBI	SBI + QoI
1 x SDHI late	SBI	SBI+QoI+SDHI
1 x SDHI early	SBI+SDHI	SBI+QoI
2 x SDHI	SBI+SDHI	SBI+QoI+SDHI

Leaf samples were collected and tested at Epilogic (Freising) resp. Bayer CropScience for their EC50 values for Bixafen and Prothioconazole *in vitro*.

Results: The results show clearly a benefit of SDHI application in the disease control in wheat. The efficacy against *S. tritici* rises from 66 % without SDHI, to 70-75% with 1x SDHI and finally to 80% with 2x SDHI, correlated with a significant lower variation and higher yields. In regions with an early disease pressure, a timely application of SDHI at BBCH 30-34 followed by a SDHI free solution at BBCH 49-55 reduced the severity of *S. tritici* over the whole season more than the vice versa strategy.

The resistance monitoring of *S. tritici* in all trials showed no differences for Bixafen and Prothioconazole with regard to the number of SDHI applications, locations or years.

Conclusion: Especially with early disease pressure, the timely application of a SDHI at early growth stages is crucial for the control of *S. tritici* over the whole season. It's superior efficacy delays the disease development and reduces the curative burden of the following application. Overall under continuous disease pressure, the application of two times SDHI per season is the best way to control *S. tritici* and safeguard yields. According to the monitoring results, there is also no hint for this strategy for an increased danger of a resistance development.

O EDU 1

Educational needs for integrated management of sustainable production systems

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A myriad of national and international issues challenge the economic and environmental sustainability of plant production systems, e.g. globalization, resiliency in the face of a changing climate, feeding an ever increasing population, etc. Improved student capacity (including increased student numbers and educational direction) is repeatedly cited as a necessary component in addressing these challenges. In order for these production systems to be sustainable at all levels, they must be more knowledge intensive, creating a greater need for individuals with comprehensive skills necessary for management, diagnostics, and problem solving. There is also a need to integrate educational and extension experiences into these programs to produce graduates with the translational skills. Internationally, the Food and Agriculture Organization of the United Nations (FAO) has presented a new paradigm called sustainable crop production intensification to produce more but continuing to conserve natural resources. This approach is also considered 'knowledge intensive' and will require highly trained practitioners capable of dealing with management, diagnosis and problem solving across the entire production system.

The historical single-discipline model for graduate student training, while still relevant, is not sufficient to meet these challenges. There is a shortage of broadly trained professionals who understand the complex interactions affecting plant production systems and are capable of diagnosing problems and managing production systems. These professionals also need to effectively adapt technology advances to real world production situations and translate these advances to the producers. These issues have created an opportunity to embrace an additional graduate education model developed to insure the economic competitiveness and environmental sustainability of plant production systems.

The Doctor of Plant Health (DPH) program at the University of Nebraska - Lincoln and Doctor of Plant Medicine (DPM) program at the University of Florida are professional doctoral-level programs that focus on providing interdisciplinary training across all aspects of plant health. Comprehensive education across disciplines is coupled with the requirement for extensive internship experience. These plant practitioners (i.e. plant doctors) will have the knowledge, skills, and abilities to provide industry, government, and academia comprehensive diagnostic and integrated management expertise for all plant production systems. The DPM and DPH programs provide a dramatically different model for graduate education to supply professionals capable of meeting a variety of applied needs. Graduates of these professional programs, i.e. plant doctors, will help provide the knowledge intensive leadership required for sustainable plant production systems in the 21st Century.

O EDU 2

From the establishment of ENDURE to C-IPM: the importance of networking in IPM implementation in Europe

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Among numerous challenges that integrated pest management (IPM) faces in Europe, lack of budget, knowledge transfer into practice and communication both at country level and between the Member States are major obstacles hindering IPM research and implementation. There is an increasing awareness that networking is the most powerful means to overcome such problems in European agriculture. IPM is a complex and multi-faceted approach which need continuum of integration of new and innovative crop protection measures. Consequently, a large number of actors are involved in the sector of crop protection who need to be coordinated through effective communications and dynamic collaboration to make any IMP action successful. To this aim, the European Network of Excellence ENDURE, which was built almost a decade ago, successfully links leading European institutes committed to IPM research and implementation. Many European IPM research projects, such as PURE, take stock of the outcomes and lessons learned from ENDURE. In addition, ENDURE paved the way for C-IPM, a new networking project of 21 European countries focusing to create added value and synergies by coordinating national research and extension efforts and by pooling national resources. The aims are the identification of future research and development priorities; provide recommendations on national and jointly executed European research to ensure food security and simultaneously adopting sustainable crop protection strategies.

O EDU 3

Agronomic evaluation of IPM strategies in European winter-wheat and maize production

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Within the framework of the EU project PURE, long-term experiments were conducted to evaluate two IPM strategies (IPM1 and IPM2) against current practice (CP) in winter wheat based (6 locations) and maize-based (3 locations) cropping systems. Furthermore 15 on-farm experiments were set up in grain maize to study the effect of specific IPM tools on weeds and the European corn borer (ECB) (*Ostrinia nubilalis* Hübner).

In winter wheat weeds, diseases and pests were effectively controlled in CP and IPM1 while unsatisfactory control was sometimes observed in IPM2. IPM1 yields were either comparable to or lower than CP while IPM2 yields were generally lower. Pesticide use was significantly lower in IPM1 and IPM2 compared to CP. In some cases yield losses could be attributed to changes in cropping practice, e.g. delayed sowing, while in other cases it was caused by an insufficient pest control. Lessons learnt were that variety mixtures can be a very effective tool to reduce disease pressure and thus fungicide use and that inter-row cultivation can be an alternative to herbicides in winter oilseed rape. In contrast the performance of weed harrowing was variable. Omitting the use of pesticides can result in pronounced yield losses in winter wheat but the study revealed that there is considerable scope for reducing pesticide use without significant yield penalties by adopting IPM.

Overall CP provided better weed control and higher maize yields than IPM2 whereas no differences in weed control and yields were observed between CP and IPM1 at 2 of the 3 locations. Total broken plants by ECB were higher under the IPM2 strategy compared to IPM1 and CP. Overall, the IPM tools tested in the on-farm experiments provided sufficient weed control without significant differences in yields and greatly reduced the reliance on herbicides. Only exception was mechanical weed control with no supplemental use of herbicides. Against the ECB all tested IPM tools provided the same level of control as the conventional use of insecticides. It can be concluded that IPM implementation and success in maize-based systems depend on specific local conditions as well as the level of weed and pest pressures. Knowing the history of the field in terms of weeds and monitoring pests and weeds during the growing season will determine the choice of IPM tools and the level of IPM implementation.

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Education and Science Networks

O EDU 4

Economic and environmental evaluation of IPM strategies in winter wheat and maize cropping systems (PURE 2011-15)

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Introduction: The European Directive 2009/128/EC requires the reduction of risks for human health and the environment as well as the mandatory implementation of the general principles on IPM. To meet the challenge European farmers may need to adapt their cropping systems.

The EU project PURE (Pesticide Use-and-risk Reduction in European farming systems with Integrated Pest Management, 2011-15) tested practical IPM solutions to reduce the dependence on pesticides in major European cropping systems, including arable cropping systems based on either winter wheat (typical for northern Europe) or grain maize (typical for central-southern Europe). The evaluation of the economic feasibility and the environmental impact of IPM systems are important to ensure the uptake of IPM by farmers.

Methods: Three-year on-station experiments were conducted to evaluate wheat- and maize-based rotations with different IPM level (IPM1 and IPM2) against the conventional crop protection system (CS). The winter wheat and maize systems were tested in typical regions in northern Europe (6 regions) and central-southern Europe (3 regions), respectively. The IPM strategies included in modification of crop rotation, selection of resistant varieties, use of advanced decision support systems, band application of herbicides, mechanical weeding, reduced dose rates, use bio-pesticides and non-chemical alternatives.

The ex-post assessment of the sustainability of IPM systems was conducted using an adapted version of the DEXiPM model, including SYNOPSIS for the environmental risk assessment and a cost-benefit-assessment.

Results: Overall, IPM strategies were found to have lower environmental impact mainly due to significantly reduced pesticide use in both IPM systems. Whereas their economic sustainability depended on possible yield reductions, changes in costs of IPM tools and methods and type of crops in the rotation compared to conventional systems. Winter wheat and maize across the tested regions yields in IPM1 were comparable to or in some cases lower than CS while yields in IPM2 were generally lower. The results indicate that IPM systems can achieve a reduction of the environmental risks. The economic results deviate from conventional systems depending on the region and the possible yield penalties of the new IPM elements.

O EDU 5

Multimedia instructional AIDS for teaching Nematology

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Teaching in general, and in the plant protection sciences in particular, is a challenging endeavor in the 21st century. A large blackboard and a package of multi-colored chalk have been replaced by a large monitor and a range of multi-media resources. Hardbound textbooks are increasingly being replaced by or supplemented with software bound instructional modules. In order to successfully “engage” students, instructors must come to class armed with seasoned experience in the subject, a keen awareness of the students foundation in science and a genuine enjoyment for the teaching activity. Introduction to Nematodes, a multimedia, multi-platform, multi-layered slide production by E.C. McGawley, C. Overstreet, M.J. Pontif and A.M. Skantar brings this new technology to the teaching of nematology. The presentation, two years in production, is available for free (for educational purposes) download from the websites <nematologists.org>, <ontaweb.org> and <nematode.net>. Additionally, a full color poster depicting Common Genera of Plant-Parasitic Nematodes has been published to these websites very recently and is also available for free download. To date, there have been over 5,000 downloads of the “Intro” presentation and almost 300 of the “Common Genera” one. The nematology community is encouraged to contribute to these ongoing education aids

Oral Presentations

Education and Science Networks

designed to foster quality instruction in nematology and produce an increased awareness of the importance of nematodes in agriculture.

O EDU 6

International Association for the Plant Protection Sciences

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The first International Plant Protection Congress was held in Louvain, Belgium in 1946 and subsequent congresses have been scheduled at 4-year intervals. At the XIII IPPC, held in the Hague in 1995, a recommendation was made to establish the International Association for the Plant Protection Sciences (IAPPS). The purpose of IAPPS was not only to provide an umbrella organization for the IPPCs but also to provide a forum and structure for the coordination and integration of the plant protection sciences on a global basis. This recommendation was acted upon and IAPPS was formally established at the XIV IPPC held at the International Conference Centre, Jerusalem 25-30 July, 1999.

IAPPS Mission: Promote the Integrated Pest Management (IPM) approach in research and in the practical application of the plant protection sciences worldwide.

IAPPS Goal: To insure production of sufficient food, feed and fiber for a growing world population. With the need for increased food production, effective and sustainable plant protection practices will have an increasingly important role to play.

IAPPS Activities: To achieve its goals IAPPS organizes the following activities:

- Operates through a Governing Board whose members represent 15 world regions.
- Provides oversight of the International Plant Protection Congresses (IPPCs), a major international forum for integrating and disseminating crop protection information and technology.
- Sponsors the Elsevier published *Crop Protection* journal that includes articles on integrated pest management research and technology transfer.
- Collaborates with regional and national plant protection organizations to organize symposia and workshops on specific plant protection topics.
- Manages a collaborative website (www.plantprotection.org) dedicated to providing online access to information and training resources for plant protection.
- Produces Global Plant Protection News (<http://iapps2010.wordpress.com>) with the latest news on all aspects of plant protection.
- Presents International Plant Protection Awards of Distinction (IPPADs) to eminent scientists that have made an internationally recognized contribution to the plant protection sciences.
- Promotes individual memberships for 1) *Students*, 2) *Developing country Scientists*, and 3) *Industrialized Country Scientists*; *Corporate Memberships* for national, regional and international (CGIAR, AIRCA, , governmental, NGOs) organizations and agencies with a negotiated fee; and *Affiliate Memberships* with national, regional, and international societies without fees.

Oral Presentations

Legal Issues III

O LEG III-1

Minor Uses

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Speciality crops are a prerequisite for healthy nutrition. They represent a market worth €70 billion/year and 22% of the total value of annual EU agriculture output. While the magnitude of pest problems faced in these crops is similar to major crops, many efficient plant protection solutions are often unavailable for economic reasons. Therefore, helping new minor uses authorisations play an essential role in supporting the food chain. My presentation will provide an overview of EU regulatory framework for minor uses and will highlight the industry's view on barriers to the authorisation of products for minor uses; some of those barriers include the data requirements and the onerous product authorisation process, which includes a zonal process and mutual recognition. Furthermore, the presentation will present the recent developments in the European Commission plans to establish EU platform for Minor uses as well as EU Minor Uses Database (EUMUDA); EU Technical Working Group. Finally, I will include an outline of stakeholders activities.

O LEG III-2

Overview jurisdiction on PPP-imports in Germany

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Introduction: Due to the reform as a result of EU-VO 1107/2009 and the new German PflSchG 2012, the legal conditions for EU-Parallel Trade and the import of plant protection products to Germany have changed. The legal provisions for EU-Parallel Trade have been modified in the way that in order to attain an import licence the importer has to fulfill stricter requirements as in the past. Furthermore illegal imports are now subject to criminal liability.

Objectives: This lecture is giving the audience an overview of the new legal requirements for EU-Parallel Trade and the import of plant protection products in detail as well as the actual impact of legislative changes on the market for plant protection products in general.

Materials and methods: The lecture will be held orally combined with a power point presentation.

Results: According to transitional provisions the old import licences remain valid until the end of authorisation of the reference product and therefore for many years. The legality of imports is therefore depending on the issuing date of the authorisation. The main difference between the old and the new law is since June 14th 2011 the mandatory "manufacturer identity" between the import product and reference product as well as the criminal liability of illegal imports.

Conclusion: As a conclusion, we support the legal change and the stricter requirements for EU-Parallel Trade in respect of the high rate of illegal imports to Germany in the past. The new additional legal requirements protect not only the original producer of plant protection products and their financial investment but also the ultimate buyer against the health and environmental risks resulting from illegal imports.

O LEG III-3

Access to regulatory data (Aarhus transparency rules for environmental information)

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Plant protection products cannot be placed on the market without approval by public authorities. Companies have to submit a large amount of regulatory data in the approval procedures to demonstrate the safety of their products. Such data represent a substantial investment and contain confidential business information (CBI). However, the data may be the target of requests for access under the so-called transparency rules. In particular, the Aarhus Convention, an international treaty signed by more than 45 states, allows any person to request, without giving any reason, access to environmental information in a broad manner. Further, public authorities have to ensure that such information increasingly becomes available via the internet.

The Aarhus provisions allow public authorities to refuse disclosure of information based on the protection of CBI, after having balanced the interests involved. However, this exception is interpreted narrowly and cannot be claimed for information on emissions into the environment. The scope of the access to information rights to regulatory data is strongly disputed and challenged in court. Industry is concerned that any failure to effectively protect CBI contained in regulatory data from disclosure bears the risk of misuse when access is sought by competitors.

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Consequently, a diligent weighing of the interests involved is required in access to regulatory data cases, respecting proportionality and also any overriding legal framework. Many legislations provide for sector-specific rules regarding the protection of CBI contained in regulatory data which may not be undermined. A confirmation of the precedence of such rules will help to stimulate the innovativeness of the industry and promote better plant protection products for a sustainable future.

O LEG III-4

Content of and experiences with the plant protection products advertisement provision of Article 66 of the EU Regulation 1107/2009

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The Regulation (EC) No 1107/2009, which replaced the former EU Plant Protection Directive 91/414 by the 14th of June 2011 and which has direct legal effect in all EU member states, contains regulations for advertisement of plant protection products in Article 3 no. 31 and in Art. 66.

The key term "advertising" is defined in Article 3, No. 31 of Regulation (EC) No 1107/2009: 'advertisement' means a means of promoting the sale or use of plant protection products (to anyone other than the authorization holder, the person placing the plant protection product on the market and their agents) by printed or electronic media ". This definition of the key term "advertising" is to be analyzed and the resulting questions shall be answered.

Art. 66 of Regulation (EC) No 1107/2009 contains a set of specific regulations for advertisement of plant protection products. The individual statutes and requirements are presented and analyzed. The resulting practical problems are raised and answered.

O LEG III-5

Demarcation pesticides of biocidal products

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Demarcation pesticides of biocidal products is of practical importance because it gives decision whether phytosanitary legislation or biocidal (chemical) legislation shall apply. This has direct impact on all regulated areas from approval of active substances over authorisation of products to sales and use as well as official controls. The EU Commission published a guideline which is not legally binding but nevertheless has far-reaching consequences for borderline cases and especially for rodents.

O LEG III-6

State of scientific and technical knowledge

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Art. 36 para 1 of EU Regulation 1107/2009 (Plant Protection Products Regulation) "*Examination for authorisation*" states that the Member State examining the application shall make an independent objective and transparent assessment in the light of current scientific and technical knowledge using guidance documents available at the time of application. In the presentation it will be discussed who has the task to define the current scientific and technical knowledge and how the current scientific and technical knowledge has to be implemented into authorisation procedures.

Oral Presentations

Biocontrol of Insects III

O BI III-1

Investigating of diversity and species richness of the wheat aphids and introduce of their coccinellid predators in Urmia, West-Azerbaijan

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West Azerbaijan Province is a mountainous area in the northwest of Iran that has a high potential for the production of grains/cereals. The diversity of active aphids in the wheat farms of West Azerbaijan was studied for two years during 2010 and 2011 and the diversity of aphids species and their coccinellids predators was examined. The present study identified 6 harmful species of aphids and 5 species of lady-bugs that are their main enemies at different vegetative stages of wheat growth. In 2010, *Schizaphis graminum* (Rondanii) species was the most frequently found species and was considered the dominant species in the area. The highest species richness of the collected aphids of the grains was 5 species in the mid-May in 2010. The highest degree of Shannon diversity index (1.81) was also related to 2nd of May, in 2010 and the least amount of the same index (0.65) was found in 1st of July in the same year. In 2011, *Metopolophium dirhodum* (Walker) species had the most frequency rate and was regarded as the dominant species. The highest species richness of the collected aphids of the grains was observed during the mid-June in 2011. The highest degree of Shannon diversity index (1.25) was related to 15th of April in 2011 and the least amount of the same index (0.81) was found in 11th of March in that year.

O BI III-2

Can endophytes be used more extensively for biocontrol?

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Introduction: Research groups around the world are investigating the occurrence and potential usefulness of entomopathogenic fungi, known to have direct infective ability on a range of insects, which also have endophytic lifestyles. Originally thought an oddity of a strain of *Beauveria bassiana*, over 20 years of steadily increasing research has determined that a range of insect-killing fungi are able to colonise plants at least some of the time.

Objectives: Our laboratory has been investigating the endophytic occurrence of *Beauveria* spp. which are common pathogens of insects in New Zealand. It is surprising how many plant species have *Beauveria* endophytes. In New Zealand, however, all occurrences appear to be one species, *B. bassiana*. We have recovered *B. bassiana* from plants as diverse as *Pinus radiata*, brassica, maize and dandelions.

Materials and methods: Our investigations centre on occurrence, genotypic association, effect on plant and pests, and stability of *Beauveria* and other endophytes. Through genome sequencing, transcriptomics, PCR, fluorescent assisted microscopy and bioassay, we are examining where fungi occur in plants and how they operate.

Results: In some cases *B. bassiana* presence can affect the growth of the plant, either positively or negatively. It is much harder to determine effect on insects. We have found some small effects in specific instances, rather than large impacts, but also effects on gene expression in the plant. This work is ongoing, but the multiple actions of some fungi as endophytes against both pests and disease may have utility for biocontrol.

Conclusion: The aim of investigating these entomopathogens as endophytes is to attempt to replicate the success of the grass endophytes *Ephialöe*, which provide pest resistance to plants and are present in almost all ryegrass sold in New Zealand.

O BI III-3

Trichopria drosophilae: a potentially successful control agent of *Drosophila suzukii*

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Drosophila suzukii (Matsumura, 1931) (Diptera: Drosophilidae) native to South East Asia is one of the most important invasive pests of unripe fruits which invaded European countries from 2008. Insect damage is due to egg-laying inside unripe cane fruits and stone fruits, high reproductive capability and numerous overlapping generations per year. During 2013 and 2014, different field and laboratory surveys had been developed in order to detect the natural enemies of *D. suzukii* in Lombardy and Emilia Romagna (Northern Italy). Among the parasitoids detected, *Trichopria drosophilae* Perkins, 1910 (Hymenoptera: Diapriidae)

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appears to be one of the most promising enemies of this hazardous pest in these areas. Laboratory tests were developed to improve the poor knowledge on *T. drosophilae* biology and the relationship between this parasitoid and drosophilids (*D. suzukii* included). A trial was aimed at evaluating the duration of the pupal development of *D. suzukii* and of the natural host *Drosophila melanogaster* Meigen, 1830. Following this experiment, one-day old pupae of both species were exposed to *T. drosophilae* under no-choice conditions. Furthermore, trials at $25\pm 0.5^\circ\text{C}$ allowed to evaluate the reproductive capability of *T. drosophilae* on *D. suzukii* and on native drosophilids, while trials at different temperatures (between 0°C and 40°C) allowed to evaluate adult survival in different environmental conditions. The results showed a good adaptability of *T. drosophilae* on *D. suzukii*. One-day old *D. suzukii* and *D. melanogaster* pupae were successfully parasitized by *T. drosophilae* with no significant difference between these two species. Adults were able to survive from 5 to 33°C . Therefore, 34°C were established as the upper thermal limit for adult survival. At lower temperatures, adults were able to survive more than four months but did not reproduce. The information acquired so far is in line with the biology of *D. suzukii* which prefers milder temperature, and confirm the hypothesis that *T. drosophilae* can be a successful control agent of this hazardous pest.

O BI III-4

High temperature, plant water stress and performance of an aphid parasitoid

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Introduction: Regional increases in heat waves and drought due to climate change may alter the distribution and abundance of affected insects. Water-deficit stress can enhance the nutritional value of plants to phloem-feeding insects such as aphids, leading in turn to higher rates of parasitism. Heat stress may influence growth parameters of insects negatively, but insects may recover during cooler periods. Little is known about their combined effect on parasitoids of insect herbivores.

Objectives: We evaluated the effect of high day-night temperatures and moderate water-deficit stress on the development, survival and reproduction of *Aphidius ervi* (Hymenoptera: Braconidae) parasitizing the potato aphid *Macrosiphum euphorbiae* (Hemiptera: Aphididae), on potato, *Solanum tuberosum* (Solanaceae).

Materials and methods: Life history parameters of *A. ervi* were determined at ambient and high day-night temperatures ($25/15^\circ\text{C}$ and $30/20^\circ\text{C}$) using aphids on preconditioned potato plants subjected to either water deficit stress (25-30% pot capacity) or well-watered plants (80-100% pot capacity). Stomatal conductance and amino acid concentrations were measured as indicators of plant water stress and nutritional status.

Results: Percentage of mummies formed reached 61% on water-stressed plants at ambient, 35% and 33% on well-watered plants at high and ambient, and 27% on water-stressed plants at high day-night temperatures. Egg to adult development time and adult longevity were not affected by high day-night temperatures. More parasitoids emerged from parasitized aphids reared on water-stressed plants at ambient temperature than any other treatment. The lowest number of offspring was recorded from parasitized aphids on water-stressed plants at high temperatures. Stomatal conductance was lower and total essential amino acid concentration was higher in water-stressed than well-watered plants at both regimes.

Conclusion: Heat waves and drought frequently occur concurrently. The combined effect of high day-night temperatures together with water deficit-stressed plants may reduce parasitoid population growth and any beneficial effects that higher amino acid concentrations in water deficit-stressed potato plants may have on *A. ervi* parasitizing *M. euphorbiae*.

O BI III-5

Effect of host unavailability durations on parasitism behavior of *Ooencyrtus fecundus* Ferriere & Voegelé (Hym.: Encyrtidae) egg parasitoid of sunn pest

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Sunn pest *Eurygaster integriceps* Puton is the most important pest of wheat and barley in Iran. Egg parasitoids (Hym., Scelionidae and Encyrtidae) are the most promising natural enemies of this pest. Unfortunately these parasitoids are in a high risk of encountering with durations of host absence early season; hence in this study we evaluated the effect of different intervals of host unavailability on the egg parasitoids in terms of parasitism rate. *Ooencyrtus fecundus* Ferriere & Voegelé (Hym.: Encyrtidae), which is a widely and continuously distributed species in wheat fields were chosen for experiments. The wasp specimens were collected from Garamalek and Kujuvar villages, located in west of Tabriz, East Azarbayjan province, Iran. The experiments were conducted under laboratory conditions ($25\pm 1^\circ\text{C}$, $50\pm 10\%$ RH and 16L: 8D h photoperiod) with five treatments including a control (complete access of wasps to the hosts), access every other days, every third days, once a week and complete

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access following a six day prevention period in 15 replications. The results revealed that parasitism rate decreases by increasing unavailability intervals of host. The least value of total fecundity as well as instantaneous population growth rate was occurred in fourth treatment (access once a week). These values were 42.93 ± 4.11 egg/female and $0.129 \pm 0.004/d$ respectively. The least value of net reproductive rate recorded for the same treatment is being 23.57 ± 2.25 . In the case of compensation of the lost fecundity during the access days, results showed that a partial compensation was occurred in all treatments.

O BI III-6

Signals of Significant Evolution Revealed by Sequence Analysis of Intragenomic rDNA-ITS2 sequences of *Diadegma semiclausum* (Hymenoptera: Ichneumonidae)

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The parasitoid, *Diadegma semiclausum* (Hymenoptera: Ichneumonidae) is one of the most effective parasitoids of diamondback moth (DBM), *Plutella xylostella* in the lowland areas of Homs, Syria. Molecular evolution studies are useful tools to shed light on the molecular bases of insect geographical spread and adaptation to new hosts and environment. In this study, molecular evolution analysis was performed based on the 42 ribosomal internal transcribed spacer-2 (rDNA-ITS2) sequences representing the *D. semiclausum* and eight other *Diadegma* spp. from Syria and worldwide. Possible recombination events were identified by RDP4 program. Three potential recombinants of the *D. chrysostictos*-UK1, *D. chrysostictos*-UK2 and *D. rapi*-Australia were detected. After detecting and removing recombinant sequences, the ratio of non-synonymous (dN) to synonymous (dS) substitutions per site ($dN/dS = \omega$) has been used to identify codon positions involved in adaptive processes. Bayesian techniques were applied to detect selective pressures at a codon level by using five different approaches including: fixed effects likelihood (FEL), internal fixed effects likelihood (IFEL), random effects method (REL), mixed effects model of evolution (MEME) and Program analysis of maximum likelihood (PAML). Among the 43 positively selected sites that differed significantly between clades of *Diadegma* species, fifteen sites under positive selection were only found in *D. semiclausum*. Additionally, all *D. semiclausum* branches tree were highly found under episodic diversifying selection (EDS) at $p \leq 0.05$. Our study provides evidence that both recombination and positive selection have significantly contributed to the molecular diversity of *Diadegma* spp., and influence in their fitness.

Oral Presentations

Modelling/Forecasting II

O MF II-1

Development and validation of environmental disease predictive model for chickpea blight (*Ascochyta rabiei*) under semi-arid conditions

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Due to lack of resistance in indigenous chickpea (*Cicer arietinum* L.) germplasm of Pakistan, chickpea blight is controlled through fungicides. Excessive use of fungicides is causing resistance in the pathogen and creating fatalistic effect on the semi-arid environment of the country. Disease predictive model under such situation is the dire need, so that, excessive use of fungicides could be avoided by forecasting early onset of disease. Objective of this study was to develop a chickpea blight disease predictive model based upon environmental variables i.e. maximum and minimum temperatures, relative humidity (RH), rainfall and wind speed for timely application of fungicides. For this purpose, environmental factors and chickpea blight disease severity data of five years (2006-10) were used. Correlation analysis was performed to determine the relationship of environmental variables with disease severity. Significant correlation was found between all environmental variables and disease severity. Stepwise regression analysis was used to develop model. In the model, maximum and minimum temperatures, RH, rainfall and wind speed caused significant variability in disease development ($R^2 = 72\%$). Model was then validated with two years (2011-12) environmental and disease severity data. Two year model validated the model on the basis of closeness of coefficient of determination i.e. $R^2 = 82\%$, slope and intercept. Statistical indices i.e. root mean square error (RMSE) and error (%) were used to check the performance of the model. Overall, RMSE and error between observed and predicted values were $\leq \pm 20$ representing the model as a good model. Multiple regression model developed through this study is first time study under semi-arid conditions of Pakistan, and would help in predicting accurate blight disease on chickpea crop.

O MF II-2

Evaluation of strawberry grey mould management using iMETOS[®]sm forecasting model

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Research on the efficiency of forecasting models for pest and diseases in horticultural plants under Lithuanian climate conditions using internet forecasting system iMETOS[®]sm was started at the Institute of Horticulture in 2007. Fruit rot pathogens reduce the yield and quality of plant production and cause material economic loss. Forecasting model presents the favourable *B. cinerea* risk periods on the basis of the interaction between air temperature and leaf wetness duration. Development a decision support system to help producers decide when to apply fungicides.

The aim of study was to evaluate strawberry grey mould management using iMETOS[®]sm.

The research was carried out at the LRCAF Institute of Horticulture 2008-2009 and 2013-2014. There were compare two plant protection systems: conventional and the forecasting model. Strawberry field experiments carried out in cv. 'Elkat', planted at a spacing of 0.8 x 0.3 m. on two rows white film-mulch. The treatments were replicated four times at randomized complete block. iMETOS[®]sm *B. cinerea* model recommends using fungicides when the infection risk is more than 60% for 3 days. The trial design involved the following active ingredients at rates boscalid + piraclostrobin 1.8 kg ha⁻¹ and ciprodinil + fludioxonil 1.0 kg ha⁻¹. The incidence and frequency occurrence of contamination fruits were assessed after 4 and 8 days after storage.

Using forecasting model there was least rotten strawberry in 2008-2009 and 2013. The model in 2008 was more efficient 8.68 points compared with conventional system, 7.05 in 2009 and 4.67 in 2013. Forecasting model reduced the spread of grey mould in strawberries after 4 and 8 days of storage compared with control.

Acknowledgements: This work was carried out within the framework of the long-term research program "Horticulture: agro-biological basics and technologies" implemented by Lithuanian Research Centre for Agriculture and Forestry.

Oral Presentations

Modelling/Forecasting II

O MF II-3

iMETOS[®]sm *Botrytis cinerea* forecasting model harmonisation for onion IPM

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Botrytis cinerea Pers.: Fr cause two important onion diseases leaf fleck and neck rot. The development of pathogens infection highly depends on inoculums of the pathogen, spore release and the influence of parameters as air temperature, duration of leaf wetness, relative humidity. Disease forecasting models indicate favourable conditions for infection risk, therefore fungicides application are made more accurate. iMETOS[®]sm *B. cinerea* forecasting model indicates the possibility to develop leaf fleck on the basis of the interaction between air temperature and leaf wetness duration. The aim of study was to evaluate the favourable conditions for onion leaf fleck spread according forecasting model.

The research was carried out at the LRCAF Institute of Horticulture in 2012-2014 on onion cv. 'Stuttgarter Riesen'. In order to evaluate the fungal infections incidence and occurrence assessed after 2, 4 months of onion bulbs storage. Model recommends using fungicides when the infection risk is more than 60% for 3 days. According this point were calculated risk development periods when infection risk was at least 3 and more days.

B. cinerea forecasting model data showed, that risk periods for onion leaf fleck depends from meteorological conditions through plants growth season and differ every year. In 2012 and 2014 were similar conditions. It was calculated four risk periods of *B. cinerea* development in 2012: in June -1, in July -3 risk periods; and six periods in 2014: in June -4, July -2. Due to different conditions in 2013 were two risk periods in July. Mycological analysis of onions bulbs showed high of density *Botrytis* spp., the occurrence of damage was detected 41% of bacteria, 7% - insects, 9% - physiological and 44% - fungi damage all three experimental year.

Acknowledgments: This work was carried out within the framework of the long-term research program "Harmful organisms in agro and forest ecosystems" implemented by Lithuania Research Centre for Agriculture and Forestry.

O MF II-4

Take-off time of the small brown planthopper, *Laodelphax striatellus* (Hemiptera: Delphacidae) in East China

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Introduction: The small brown planthopper, *Laodelphax striatellus* (Fallén) transmitting *Rice stripe virus* (RSV) is an important economic insect pest of rice in East Asia. Recently, occurrence of *L. striatellus* and RSV disease have re-emerged in East Asia. In early June 2008, an outbreak of the first generation of overwintering *L. striatellus* occurred in the eastern part of China. Then, they migrated oversea into the western part of Japan and caused serious disease damage to rice. Prediction of such overseas immigration provides useful information for controlling immigrant insects and their disease efficiently.

Objectives: Take-off time of the first generation of the overwintering population is necessary for the immigration prediction simulation and the objective of this study is to investigate the insect's take-off timing in Jiangsu Province, the source area, in late May to early June.

Materials and methods: A tow net trap mounted at the top of a pole 10 m above the ground was placed by a wheat field in Tongzhou, Jiangsu Province during May 25 to 13 June in 2012.

Two canopy traps with a digital video camera were placed in wheat fields in Dongtai, Jiangsu Province, and monitoring started on June 1 and ended on June 6, 2013. Insects in the video movie were counted hourly.

Results: Insects were caught in the net trap during June 8 to June 12, 2012. The number of trapped insects peaked at 1800 h CST. There were also some catches during the daytime, from 0900 to 1500 h CST. The catches during the night (from 2100 to 0440 h CST) and dawn (from 0440 to 0700 h CST) were relatively small.

During the day and evening, the number of the insects that entered into the canopy traps changed hourly, but increased gradually towards the evening and peaked at 1700 h. Few insects were trapped in canopy traps during the night, at dawn or in the early morning (1935-0700 h CST);

Conclusion: *Laodelphax striatellus* emigrated from wheat fields mainly in the early evening, before dusk. The insects also emigrated during the daytime but rarely emigrated at dawn, showing a pattern that is unlike the bimodal emigration at dusk and

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dawn of two other rice planthoppers, the brown planthopper, *Nilaparvata lugens* (Stål), and the white-backed planthopper, *Sogatella furcifera* (Horváth). This take-off timing is used as basic information for the immigration prediction simulation.

O MF II-5

Annual Yield Losses Associated with Southern Rust of Maize and their Use for Disease Management Decisions

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Global distribution of southern rust of maize is primarily tropical or subtropical, mainly due to the pathogen's temperature and moisture requirements. Historically, worldwide epidemics have caused severe losses (up to 85%) leading to localized starvation primarily in West Africa. Epidemics are common for the tropics and subtropics while in temperate areas disease development is observed annually but epidemics are intermittent, making disease management decisions difficult for growers. Annual yield loss information (yield and quality impact) was lacking as an input for growers to make informed rust management decisions. The objective of this study was to determine yield losses due to southern rust as a component of a rust management system. Therefore, yield losses were determined annually in multi-location plots across the Gulf States of the United States each measured by multiple techniques (plot experiments and on-farm surveys) to determine presence and impact of southern rust on local maize plantings. Yield losses were determined to be 15.2, 1.0, 3.2, 3.9 and 18.9% from 2010 through 2014, respectively. In years where southern rust losses were highest, adequate, but not excessive moisture occurred during the grain development period. Whereas, in low yield loss years, high rainfall occurred during the reproductive period leading to the speculation of urediniospores being washed from their traditional aerial distribution or from the leaf lamina after deposition but prior to infection. Correlations were high ($R^2 = 80.9\%$) comparing high yield loss to high southern rust development. Work continues to determine yield losses associated with rust development at various maize growth stages.

O MF II-6

Migration prediction systems for three rice planthoppers and a wind-borne immigration analysis system for the oriental fruit fly

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A variety of insect pests fly overseas into Japan that consists of many islands. Rice planthoppers, important pests of rice plant, are such examples. The brown planthopper and the white-backed planthopper migrate mainly from southern China into Japan in summer rainy season from late June to early July, whereas the small brown planthopper sometimes immigrates from eastern China in early June and transmits *Rice stripe virus*. It is essential for pest management to know the timing and area of their immigration into rice fields.

The oriental fruit fly is an insect pest that attacks tropical fruits and vegetables. This species was eradicated from Okinawa Prefecture, the most southwestern part of Japan, in 1986. Although the state of eradication in Okinawa is maintained, insects are sometimes caught in monitoring traps every year. One of the possible re-invasion routes is wind-borne immigration from neighboring source areas, Taiwan and the Philippines. It is a natural phenomenon. The other route is accidental importation of infested fruits carried by human. It is possible for the former case to analyze wind data in order to suggest whether trap catch is due to wind-borne immigration. Such suggestion may be useful for plant quarantine officers who are responsible for keeping the state of eradication.

Therefore, migration prediction systems of rice planthoppers for plant protection officers, and a wind-borne immigration analysis system of the oriental fruit fly for plant quarantine officers have been developed.

For the migration prediction systems, there are two types of prediction models: one for the brown planthopper and the white-backed planthopper, the other for the small brown planthopper. The both models predict the arrival timing and area of planthoppers using weather forecast data, but have several differences in modeling of take-off time, source areas, flight duration and so on. The predicted information is presented to plant protection officers by email and web-based service called "JPP-NET" operated by the Japan Plant Protection Association.

The wind-borne immigration analysis system for the oriental fruit fly is windows software that presents re-invasion risk index and trap catch. The index is calculated based on the daily-basis frequency of arrival of airstreams from the possible sources to islands of Okinawa for previous 14 days. The period corresponds to an interval of sample collection in the monitoring traps. The index, therefore, becomes 0 to 14. Larger values indicate that winds from the source arrived in Okinawa Prefecture more frequently, and zero shows there was no winds from the source. If an insect was caught in a monitoring trap during the risk

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index was high, the insect is suggested to be a wind-borne immigrant. Plant quarantine officers use the system to suggest the fly's wind-borne immigration and to understand the situation.

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O FUN III-1

Study on fungicide-induced/primed molecular and physiological effects on barley (*Hordeum vulgare* L.)

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Plants can be induced/primed by various biotic and abiotic stresses giving unique physiological states like enhanced disease resistance or tolerance to certain abiotic stresses. This effect has been observed by infection with necrotizing pathogens or colonization of plant roots by beneficial microorganisms as well as by stimulation of various natural and synthetic compounds. Understanding of the molecular mechanisms underlying might provide novel approaches to exploit the genetic potential of plants for increasing plant resistance to pathogen attack as well as tolerance to environmental stresses in the practice, and is therefore of great scientific and practical importance.

It has been demonstrated that several fungicides are able to enhance plant tolerance to various biotic and abiotic stresses and give positive physiological effects on plant in addition to their fungicidal activity. To understand the mechanism underlying, we analysed the transcriptome change of barley in response to the fungicide application. Transcriptomes of the samples treated by the fungicide were investigated by RNAseq in comparison with the reference. Differentially expression genes (DEG) in response to the treatment were identified and selected. Sequence analysis and functional annotation *in silico* as well as lab.-validation identified a subset of candidate genes of interest. Here, we report our recent results of analysis of transcriptome data and the identification of candidate master-switch genes, and pathways, which are associated with the fungicide effects observed in barley and *Arabidopsis thaliana* as well.

O FUN III-2

Enhance endogenous plant defences by isotianil, a new resistance inducer

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Introduction: Isotianil is a new plant defence inducer that controls leaf blast and bacterial leaf blight in rice and black sigatoka in banana. Discovered by Bayer in 1997, isotianil has been jointly developed by Bayer CropScience (BCS) and Sumitomo Chemical Co., Ltd., and is being distributed under the brand name ROUTINE by BCS. Isotianil does not exhibit by itself any fungicidal activity against pathogens but protects plants against infection when applied at an early developmental stage.

Objectives: Elucidation of the mode of action of isotianil

Material and methods: To elucidate the molecular mechanisms of isotianil-induced resistance, gene expression profiling experiments were performed using an Affymetrix array of the rice whole genome. This data was verified using qPCR. Further experiments supporting the current mode of action hypothesis focusing on the NPR proteins have been carried out and will be described in more detail during the presentation.

Results: Isotianil, applied at a dose range used in agronomical conditions and without pathogen pressure, induces only few changes in gene expression. These genes directly responsive to isotianil are involved in salicylic acid catabolism and pathway. Upon pathogen challenge, isotianil primes rice plants for an increased defense genes activation compared to simple infection. The onset of resistance is accompanied by a potentiated SAR response including enhanced activation of PR genes and of genes involved in secondary metabolisms such as phenylpropanoid and flavonoid pathways.

Conclusion: Isotianil mode of action very probably involves the salicylic signaling pathway in plants but acts differently from other host defense inducers also targeting this defense pathway, e.g. acibenzolar-S-methyl.

O FUN III-3

Elicitor screening to protect wheat against *Zymoseptoria tritici*

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Plants face an array of biotic and abiotic stresses in their environment, making it necessary to use various chemical inputs to maintain satisfactory yield. Today, conventional agriculture is evolving towards more sustainable practices, out of respect for human health and the environment. Elicitors are considered as promising biological control tools and draw major interest in IPM

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strategies. These plant-immunity triggering compounds, also called “stimulators of plant natural defenses”, induce a general and systemic resistance in the plant to various diseases.

Although numerous elicitors have already been identified and some of them reached the market since the late 1970s, further investigations are still required to better understand the mode of action of these molecules in the plant and ensure a consistent efficiency under various field conditions. Few elicitors have yet been successfully tested and formulated to protect monocotyledonous crop plants such as wheat, which is cultivated over large areas in Europe.

This study focuses on the screening of ten potential elicitor products of various origins and structures to protect winter wheat against the fungal pathogen *Zymoseptoria tritici*. Greenhouse trials were carried out to measure the ability of the different products to reduce disease foliar symptoms (necrosis, chlorosis and pycnidia). Topical spraying treatments with 3 different concentrations of each product were carried out 5 days before pathogen inoculation. Disease severity (% of symptoms on the total surface of the third leaf) was then scored every 2 days up to 28 days post-inoculation. In addition, phytotoxicity and biocide activity of these products was evaluated under greenhouse and laboratory conditions, respectively.

The corresponding results will be presented and discussed with the perspective to choose the best elicitor candidates and to undertake investigations on the signaling pathway and the influence of environmental parameters on the elicitation capacity.

O FUN III-4

Chemical control of the late root and crown rot in sugar beet caused by *Rhizoctonia solani*

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Introduction: The late root and crown rot (RCR), caused by *Rhizoctonia solani*, is a severe disease in sugar beet world-wide and is responsible for considerable economic losses in Germany every year. In Germany control measures are restricted to cultivation management and growth of resistant cultivars, which show a high yield penalty under non-diseased conditions. In other countries where RCR is prevalent, fungicides are used successfully for disease control. However, no fungicides are registered in Germany.

Objectives: The efficacy of two fungicides and the interaction of fungicide treatment and plant resistance level was analyzed in six independent field trials with artificial as well as natural infestation.

Materials and methods: Two fungicides containing active substances belonging to the triazole and strobilurin group in different formulations were applied at BBCH16 and BBCH31 to sugar beet cultivars carrying different levels of resistance. Disease development was monitored during the vegetative growth of the plants. At harvest the influence of fungicide treatment on final disease severity as well as on beet yield and quality was evaluated.

Results: The application of both fungicides resulted in a comparable level of disease control and disease severity was decreased from up to average 82% rotten beet surface to 16% in artificially inoculated plots. In the trial, showing the highest disease severity, fungicide application increased white sugar yield from 7.6 to 13t/ha in the resistant cultivar, from 5.7 to 13.7t/ha in the intermediate resistant cultivar and from 3.3 to 14.1t/ha in the susceptible cultivar.

Conclusion: The results of this trial series demonstrate the great efficacy of both tested fungicides to control RCR in sugar beets. Currently available control measures (agronomic measures and plant resistance) are not able to achieve a sufficient level of protection. Therefore an integrated approach combining plant resistance with chemical control can significantly improve RCR management in sugar beets.

O FUN III-5

Evaluation of a novel fungicide for management of *Plasmopara halstedii* on sunflower (*Helianthus annuus*)

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Introduction: Sunflower, *Helianthus annuus*, is an economically important crop cultivated on more than 25 million hectares worldwide. Sunflower is grown for vegetable oil, human food, bird-food markets, and ornamental purposes. Downy mildew, caused by *Plasmopara halstedii*, is a yield-limiting disease occurring on every continent where sunflowers are cultivated except Australia. Downy mildew is particularly a problem for sunflower producers in the north central United States (U.S.) and in most

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European Union (E.U.) countries. In the past, two FRAC 4 fungicides, metalaxyl and mefenoxam were used as seed treatments to manage downy mildew, until insensitive *P. halstedii* isolates became widespread in both the U.S. and the E.U. Currently, only one FRAC 11 fungicide, azoxystrobin, is labeled in the U.S. for the suppression of downy mildew. Thus, there is a need for other fungicides, with a different mode of action, which might offer better management of downy mildew.

Objective: The objective of this study was to evaluate the field efficacy of a novel fungicide compound, oxathiapiprolin, applied as a seed treatment for the management of downy mildew on sunflower.

Materials and methods: Thirteen field trials were conducted from 2011 to 2014 in three locations of north central U.S. The trials were planted in a randomized complete block design, artificially inoculated with *P. halstedii* zoospores, and irrigated to facilitate the infection process. Incidence levels were determined by recording systemically infected plants three times throughout the growing season.

Results: In trials with measurable disease pressure, all treatments that contained oxathiapiprolin had significantly lower incidence levels than the non-treated, inoculated checks and had the same or significantly lower incidence levels than azoxystrobin at the current U.S. labeled rate.

Conclusion: The results indicate that oxathiapiprolin could provide another fungicidal seed treatment option for the management of downy mildew on sunflowers. If oxathiapiprolin was used in combination with a compound of a different mode of action, it could help forestall or delay fungicide resistance development which could have significant long-term management impacts.

O FUN III-6

Controlling *sclerotinia sclerotiorum* in oilseed rape by use of a dropleg device

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Introduction: In the past, different fungicides and insecticides have been found in the stored pollen in beehives, resulting from pesticide applications during oilseed rape flowering. This causes an area of conflicts between farmers and beekeepers. Field trials from University of Hohenheim showed that it is possible to significantly reduce the amount of pesticides in beehives by spraying below the canopy with dropleg nozzles. But there is a lack of information, whether *sclerotinia sclerotiorum* can effectively be controlled with droplegs.

Objectives: Trials were carried out in 2014 on three oilseed rape fields in North-, Middle and East of Hesse, Germany to study whether *sclerotinia sclerotiorum* can effectively be controlled with dropleg nozzles.

Figure 1: Sprayer with dropleg nozzles



Material and methods: Randomized block designs with the variants “no pesticide (control)”, “dropleg nozzles” and “standard nozzles” were established with three and four repetitions. Each “pesticide variant” was treated with a mixture of a fungicide (Boscalid) and an insecticide (Tau-fluvalinate) to control *sclerotinia sclerotiorum* and cabbage seed weevil in BBCH 63-65. The beetles were rated short time before spraying and damage on seed pouches thirty days after spraying. *Sclerotinia* was rated in BBCH 75-80. Yield was measured at harvest.

Results: The fields were “cabbage seed weevil free” before spraying. No damage on seed pouches was observed. In the unsprayed controls up to 40% of the oil seed rape plants were infested with *sclerotinia sclerotiorum*. With dropleg- and standard nozzles high reductions of *sclerotinia sclerotiorum* near zero could be realized. The differences of the sprayed compared with the

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unsprayed control were significant. Both nozzle types reached equal efficiency factors. Yield was significantly higher in the sprayed compared to the unsprayed variants. No significant differences in yield were observed between the nozzle types.

Conclusion: *Sclerotinia sclerotiorum* could be effectively controlled with dropleg nozzles. This technology may be a promising way to couple the interests of beekeepers and farmers. However, further experiments have to be conducted to study the control effect of dropleg nozzles on cabbage seed weevils.

Oral Presentations

Workshop • Management of the South American Leafminer, *Tuta absoluta*

O W S T A 1

First level IPM practices for *Tuta absoluta* Meyrick (Lepidoptera: Gelechiidae) according to the cropping system in two far regions in Algeria

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In Algeria, *Tuta absoluta* (Meyrick) has been first reported in 2008 in the province of Mostaganem where farmers were among the first suffering strong attacks on tomato crops. It became the first major pest because it has found all suitable conditions to spread over the country. Since 2009, the Algerian Ministry of Agriculture has planned a strategy according to a regional IPM program executed by FAO with a system of Farmer Field School providing practical training to tomato growers. Six years after its invasion, the incidence of *T. absoluta* became less severe. Because our greenhouses differ strongly from those in European countries it will be very hard to apply adequate IPM strategy used in Europe. Despite an increasing interest on conservative biological control to manage *T. absoluta* we faced a very strong insecticide market limiting the development of the Integrated Pest Management. The aim of our paper was to evaluate alternative methods of control including trapping, use of natural enemies and bio-pesticides and to analyze results of fields and laboratory experiments conducted in different agrological conditions with traditional practices. We listed constraints and advantages. In unheated plastic greenhouses equipped with sex pheromone traps and insect proof nets we tested the role of both systems. Male captures were not always correlated with damage on leaves or fruits because the farmers should open the greenhouses for reducing high temperatures.

Therefore we need more knowledge on biology of the pest and its natural enemies in order to avoid mistakes. We also should explain how the abundance of weeds around tomato crops could play an important ecological role in biodiversity by harboring a complex of beneficial arthropods. The most direct way of preventing the infestations of *T. absoluta* is to cultivate healthy plants in early season (under plastic houses) or in open fields. In the Oued Righ Valley (Southeastern Algeria) farmers cultivating tomato under palm date trees with a traditional system cropping, combining several vegetables with aromatic plants were able to produce tomatoes for the local market without any chemical control. We should persuade farmers to believe in IPM strategy encouraging them to increase biodiversity for reducing vulnerability for environment.

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Workshop • Behavioral and Biological Control of Stink Bugs

O WS BUG 1

Population dynamics and IPM of mirid bugs in multiple crops in northern China

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The mirid bugs, predominantly, *Apolygus lucorum* and *Adelphocoris suturalis* (Hemiptera: Miridae), became major pests on cotton after several years of widely adoption of transgenic *Bacillus thuringiensis* (Bt) cotton expressing Cry1Ac toxin in northern China. The *A. lucorum* damage not only cotton, but also various vegetables and fruit trees, and has become an area-wide pest in multiple crops in northern China. The frequent spray of pesticides for controlling mirid bugs increased input of farmers that counteracted the benefits of planting Bt cotton, and caused negative effects to the environment. The biology and ecology of these mirid bugs were intensively investigated in recent ten years to lead effective IPM strategies. The investigation of population dynamics of these mirid bugs indicated that they overwinter with diapause eggs in weeds and fruit trees adjacent to cotton fields and produce one or two generations on them in the following spring, thus, we suggested to eradicate weeds by burning, spraying herbicide and/or mechanical methods, remove branch stubs of fruit trees, and spray pesticides on the newly hatched nymphs in early April. The adults were found to move into cotton fields during squaring stage in mid-June and reproduce two subsequent generations causing severe damage on cotton, thus we suggested to spray pesticides in squaring stage to control the immigrants. Mung bean and red bean were found attractive to adult *A. lucorum* and *A. suturalis*, respectively, and blooming carrot was attractive to adults of both species, thus we suggested to plant these crops as trapping crops. Artificial diets for both species were invented and used for screening effective pesticides, monitoring pesticide resistance, and mass rearing mirid bugs to produce parasite wasps leading biological control.

O WS BUG 2

Stink bug management in macadamia orchards: Updates and latest recommendations

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Stink bug monitoring was carried out during the past season in macadamia orchards with the branch tapping as well as the thermal fogging methods. Three nymphal peaks could clearly be defined. The availability of food is possibly the main driver of immigration of *Bathycyrtus distinctus* into and out of the orchards. When young developing nuts were present increasingly higher numbers of this insect was recorded in the orchards. After maturity, population numbers of the two spotted bug decreased but interestingly, the winter complex consisting of a range of lesser important stink bugs quickly filled this rapidly emptying niche. In order to understand migration patterns of stink bugs, other subtropical crops (avocados, mangos and litchis) were also monitored with a thermal fogging machine. Although *Pseudotheraptus wayi* was prevalent throughout summer in macadamias, considerably larger numbers were recorded during April. It would also appear as if this insect utilizes litchis as a temporary winter refuge because relatively large numbers of adults were recovered in this crop during June/July. A discrete spray consisting of an environmentally friendly product during this time might break this seemingly continuous cycle in subtropical crops and could possible help to reduce overall spray applications in the subtropical fruit industry in general. A case of apparent resistance against synthetic pyrethroids was investigated in a macadamia orchard (cv. 791) just outside Nelspruit. Due to the continuous bearing tendency of this cultivar, bugs were regularly exposed to pyrethroids. The effect of Cypermethrin EC 200g/L was quantified on these insects as well as insects that were obtained from a traditionally unsprayed area. Approximately 30% and 15% of the suspected resistant individuals died after receiving double and single rates of Cypermethrin respectively. During the same period 100% mortality was observed after applying the standard registered rate to the natural population. This reinforced the belief that some form of resistance might currently be present in South African populations of *B. distinctus*. Reasons for resistance as well as implications for the industry are discussed. Future plans in managing apparent resistance is also highlighted.

O WS BUG 3

Spectral preference and use of light traps for the population monitoring of the southern green stink bug, *Nezara viridula*.

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Introduction: The southern green stink bug, *Nezara viridula* (L.) (Heteroptera: Pentatomidae) is a serious agricultural pest throughout the world. In recent years, *N. viridula* has been expanding its range northward in Japan, probably due to the global warming. In Japan, automatic daily monitoring using light traps has been conducted at agricultural experiment stations or plant protection centers in every prefecture for forecasting pest outbreaks. However, there is little information on *N. viridula* caught in light traps, and therefore light trap data have not been well utilized for this species.

Objectives: Our objectives are to develop a monitoring system and monitoring traps for *N. viridula* using light sources.

Materials and methods: We analyzed light trap (60W incandescent lamp) data set around Kyushu. We also investigated the behavioral preference for wavelengths using light-emitting diodes (LEDs) of five different peak wavelengths (373, 444, 464, 534 and 583 nm).

Results: There are strong correlations between winter temperatures and total numbers of bugs caught before August. Total numbers of bugs caught after August strongly correlate with those of before August. In free-flying preference experiments, both male and female adults strongly preferred the ultraviolet light (373 nm) among five LEDs.

Conclusion: Winter temperatures and numbers of bugs caught before August are good indicators of monitoring *N. viridula* population. Ultraviolet LED could be useful light source to develop the monitoring traps.

Figure 1: The relationships between winter temperatures and total numbers of *N. viridula* caught before August.

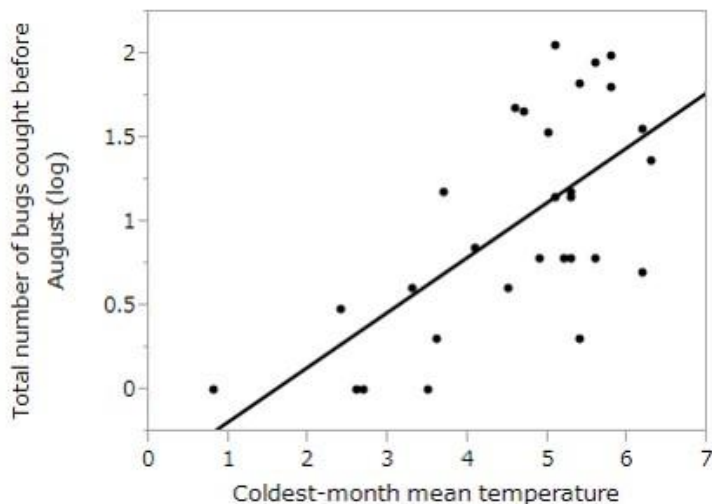
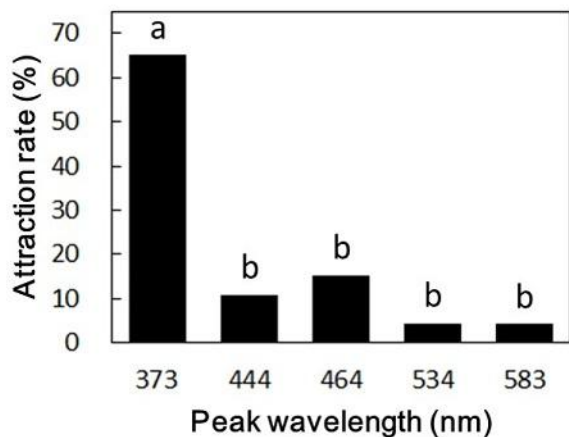


Figure 2: Spectral preference of *N. viridula* males for five different wavelengths of light. Different letters above columns indicate significant differences ($p < 0.05$) using multiple comparison test for proportions (Zar, 1996).



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O WS BUG 4

Role of male seminal products in regulating female reproduction in *Lygus hesperus*

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Lygus hesperus Knight (Hemiptera: Miridae) is a highly adaptable polyphagous plant bug that threatens numerous crops in the southwestern United States. Despite their importance, the development of new control measures is hampered by limited knowledge of their basic biology. One potentially exploitable aspect of their life history is that of reproduction. Newly mated females enter a multi-day refractory period during which their sexual receptivity and attractiveness to courting males is greatly reduced, while their rate of egg-deposition increases. These behavioral changes appear to be induced by male-derived factors delivered in the spermatophore during copulation. Investigation of the spermatophore composition revealed several unique proteins in the water soluble fraction, one of which is similar in size to the *D. melanogaster* sex peptide, a male derived compound known to inhibit receptivity in female flies. *L. hesperus* spermatophores also contain substantial quantities of juvenile hormone and the fatty molecule myristyl acetate. The former is a key endocrine regulator of reproductive behavior and physiology in most insects, and topical application of the latter can reduce the attractiveness of virgin females. Some of the behavioral effects of mating on females appear to be mediated by induced expression changes in brain biogenic amines, including induced changes to dopamine levels by juvenile hormone. The results support the hypothesized role of males in manipulating the post-mating behavior of females and male competitors, and suggest this is achieved through multiple components that act in concert to induce both short- and long-term effects. It may be possible to exploit this system to develop highly targeted control measures.

O WS BUG 5

Seasonal abundances of a bean bug and its natural enemy in seminatural and arable habitats in agricultural landscapes

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Introduction: Recent studies have demonstrated that the abundances of insect pests and invertebrate natural enemies in crop fields are largely influenced by land use patterns, such as the amount and spatial arrangement of source (i.e., seminatural) habitats. Insect pests and their natural enemies move to crop fields from surrounding source within a species-specific spatial scale. Thus, the availability of natural pest control can be affected by their own dispersal patterns and associated distribution patterns. Such natural pest control service is important for designing environmentally friendly crop protection systems.

Objectives: To determine differences in distribution patterns between the soybean pest *Riptortus pedestris* (Hemiptera: Alydidae) and its egg parasitoid *Ooencyrtus nezarae* (Hymenoptera: Encyrtidae) in source and cultivated habitats.

Materials and methods: We compared their abundances in soybean fields and forest edges, which were assumed to be the overwintering sites of *R. pedestris*. We set synthetic attractant-baited traps for both species over 2 years. During one of the two years, we also examined the rate of parasitism using an egg trap.

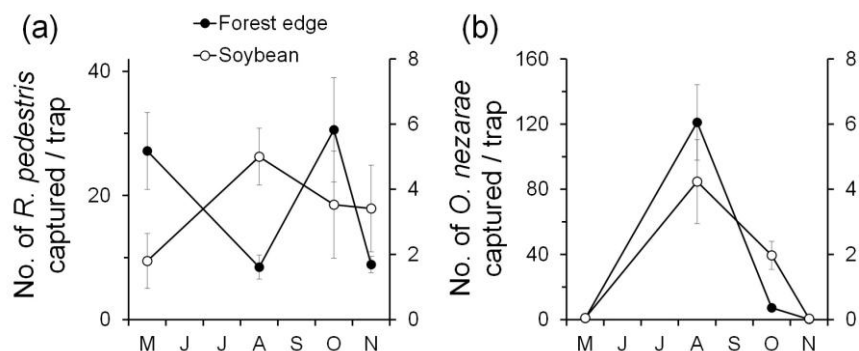
Results: The numbers of both *R. pedestris* and *O. nezarae* trapped at forest edges were higher than the numbers caught in soybean fields, suggesting that forest edges are important source habitats. Compared with *R. pedestris*, the abundance of *O. nezarae* in soybean fields was considerably lower than in forest edges, presumably due to differences in their dispersal abilities and their responses to landscape structure and resource distribution. Better pest control service by *O. nezarae* was provided at forest edges than in soybean fields.

Conclusion: Spatial arrangement and distance from the forest edge should be considered when using pest control by *O. nezarae* in soybean fields.

Oral Presentations

Workshop • Behavioral and Biological Control of Stink Bugs

Figure 1: Mean (\pm SE) numbers of (a) *R. pedestris* and (b) *O. nezarae* caught per trap in soybean fields and forest edges in 2009. The data, obtained from one or two traps per site in 2009 were averaged to represent each site. Seventeen study sites were located in soybean fields in 2009, and 11 sites were located in forest edges. Each star besides plots indicates GLMM results ($p < 0.01$).



O WS BUG 6

Evaluation of aggregation pheromone trap for the monitoring and controlling bean bug

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Introduction: Bean bug, *Riptortus pedestris* (F.) (Hemiptera: Alydidae), is an important pest of soybeans in Korea and Japan. A synthetic aggregation pheromone trap has been commercialized and used in soybean fields in Korea for both monitoring and mass-capture of this bug.

Objectives: As the trap's efficacy in reducing the pest population or crop damage is unknown, in a series of studies we evaluated the aggregation pheromone trap in both experimental and commercial soybean fields located in Andong, Korea.

Materials and methods: Two treatments, one with traps deployed for the entire cultivation period and one with no traps, were tested in six small experimental fields. Separately, in a second experiment, three treatments (one with traps deployed for the entire cultivation period, one with traps deployed until September, and one with no traps) were tested in 12 commercial fields.

Results: While the numbers of *R. pedestris* were not different between the treatment and control until August, the pest's abundance increased significantly in the treatment in fall period. Relative to the density of *R. pedestris* in the field, trap catch rate was low during the fall when the field population was abundant and high in early summer when the field population was very low. Installation of aggregation pheromone traps in plots caused crop damage from *R. pedestris* to increase compared to the control in experimental fields, while similar levels of damage among the treatments were observed in the commercial fields.

Conclusion: *R. pedestris* populations increased significantly during the fall in the presence of the aggregation pheromone trap, which should therefore be used with great caution. The reasons for the higher population level of *R. pedestris* observed during the fall period were discussed.

O WS BUG 7

Occurrence and Genetic Diversity of the Brown Marmorated Stink Bug (*Halyomorpha halys*) in Europe

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The Brown Marmorated Stink Bug (BMSB), *Halyomorpha halys*, has recently established in Europe and continues to spread to new regions. This bug is a serious pest of a wide variety of economically important field, fruit and vegetable crops, as well as ornamental plants. With a broad host range of over 200 host plants, the BMSB threatens the a number of agricultural crops. In addition, this pest overwinters in man-made structures and has become a nuisance pest in urban areas. Here we discuss the genetic diversity of this pest in the native range of Asia, as well as in recently invaded areas in Europe and North America. Information on the genetic diversity may help pinpoint the source of the invasive population(s) and helps track the movement and spread of this pest in newly invaded areas.

Oral Presentations

Workshop • Knowledge Transfer through School Projects, Neighbourhood Gardening and Plant Health Clinics

O WS KT 1

The Planting Potato with Science Project: Potato Virus Science for Children Plant Protection Perception.

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Introduction: The Brazilian agro-business stands on top of a positive trade balance; linking economical-social-agro-ecological prosperity to plant protection, demanding plant health science knowledge. Such conscience has driven us to volunteer on a project named: "Planting Potato with Science - PPwS". Since 2008 at Prof. Jamile's Elementary Municipal School, in Limeira, SP, 40-60 students (9-10 yrs/4th-5th grades) have been on a hands-on learning of basic plant health science (ref. 1).

Methodology: In a donated greenhouse with concrete benches, each student (2-3 x/week) monitor 2-4 health x 1 virus infected potato plant (*Solanum tuberosum*), comparing: stem-leaves-tuber size, weight, quantity. The role insects (aphids, whiteflies, trips) play on transmitting viruses from sick to health plants (from home garden to farm crops) is demonstrated at the "Transmission Day", which takes place at the 40-50th days.

Results and discussion: The potato has shown to be an ideal plant for the goals of this educational & technology transfer project: (1) cycle of 80-90 days = school period; (2) susceptible to many insect-transmitted virus diseases (ex.: PVY, PLRV), allowing comparative (health x sick) notes on symptoms/growth; mathematics and metric system application; (3) vegetative (tuber) propagation, demanding annual import of virus-free tuber/seed-potato stocks renewal, adding a real risk of introducing-spreading new or quarantine tuber/soil-borne pathogens and pests. The last is a fact for potato production in Brazil. Thus, instead of planting whole tuber/seed-potato (conventional), students learn that planting sprouts only, detached from virus-free tuber/seed-potatoes, can be a more sanitary safe "seed-potato". This is what the Sprout/Seed-Potato Technology has been revealing to the seed-potato import-export market, a world-wide movement (ref. 2). The digging potato tubers for final comparisons are followed by taking them to the kitchen: cooking and tasting what the students grew.

Conclusion: The PPwS project has been an innovative approach toward exposing elementary school students into basics of scientific work through an early hands-on plant health x diseases (viruses) manipulation. The PPSW project have tutoring discussions among plant pathologists, teacher, parents, education authorities, agronomists and common people on the importance and impact of plant protection to the Brazilian agro-economical-social-environmental continued prosperity. The PPwS project can be instrumental on effective-integrated plant-protection actions, raising young plant protection guardians whose uniform is conscience and perception of what plant protection is all about.

References:

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2- Souza-Dias, et al., 2008. http://www.potato.ro/en/eapr2008-brasov.com/files/EAPR08-AB-pdf/EAPR2008-ABSTRACTS_BOOK.pdf

Figure 1

The "Planting Potato with Science Project"

At Profa. Jamile Elementary Municipal School, in Limeira, SP (Brazil), children (9-10yrs – 4-5th grd) take early and first contact on plant (potato) virology (health x disease) science . A basic step to build a strong **Plant Protection** conscience and perception on agro-economical-social-environmental impact



Oral Presentations

Workshop • Knowledge Transfer through School Projects, Neighbourhood Gardening and Plant Health Clinics

Figure 2

The Planting Potato with Science Project – has been presented to other municipal schools: The Semear - Beneficent Association - Vila Olimpia – Campinas, SP(Brazil)
(Coordinated by Mr Jairo Oliveira; assisted by Mrs. Rosi Giampaoli)



O WS KT 2

Non-Government Organization and Plant Health Clinic: Beacon for Plant Health of Urban Horticulture

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Ever-growing population and urbanization has been responsible for reducing greenery and ecological balance. Urban horticulture is aimed at orchestrating a brighter urban world by recreating greenery and ecological balance by promoting cultivation of fruits, vegetables, plant on road side, park and kitchen garden etc. Non-government Organization (NGO) and Plant Health Clinic (PHC) play a vital role in dissemination of knowledge on growth and protection of plants, which not only provide fruits/vegetables/ornamentals, glorify environment and decrease pollution. Here I will cite the contribution of Peepal and online PHC towards knowledge transfer for boosting plant health and environment. Association for Promotion of Peepal and *Neem* (APPAN) - a NGO headed by me was formed to reduce pollution and improving ambience by promoting *Peepal* (*Ficus religiosa*) and *Neem* (*Azadirachta indica*). With long experience of diagnostic/ advisory experience and passion to serve the people, after retirement, prompted me to start www.xsgrowth.com in 2008 to provide online diagnostic and advisory service free of cost. While APPAN, promoted *peepal* and *neem*, by providing technology to residents/builders and free saplings for plantation on roadside, park and temple premises, XSGrowth PHC offered technology for protection. These plants did not suffer from any ailments except man invasion on *Neem*, detaching tender shoots as disposable tooth brush with inherent germicidal property. In kitchen garden, damping off was commonly observed. Fruit- and shoot borer appeared prevalent in eggplant and okra, where detaching affected shoot/ fruits and burial in soil was advised. Eradication of *Cuscuta*, parasitizing a wide range of plants, involved mechanical removal followed by sun-baking/burning. Lime, guava and papaya are the main fruit plants for which owners are apprised about prevalent / expected problem and provide the prescription. Pesticide use was discouraged until necessary; rather bio- and neem-based pesticides and reliance on IPM was encouraged. Solution to most problems is provided online. Urbanites sought solution online or through telephony. Online solution may be effective if the stakeholder is able to project right symptoms and history of the problem. Tango between NGO & XSGrowth was helpful in promoting urban horticulture and their protection. The people conversant with internet sought online advice, while others approached traditional plant clinic. NGO and Plant Clinics truly are the beacon for disseminating knowledge.

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Poster Presentations

Mycotoxins

P MYC 1

The specific composition of micromycetes-contaminants of children foods in Syria and their toxigenic activity

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For the investigation of micromycetes-contaminants in children foods and their toxigenic activity, 173 samples of children foods traded in local markets in al-Hasakah governorate -northeastern Syria- were collected and tested during the period (2011-2012). The tested samples were included in 4 food groups: oily seeds (35 samples), potato & maize chips (53 samples), biscuits (45 samples) and powdered milk & sugars (40 samples). The Sample investigation was undertaken in the plant diseases laboratory in Al-Qamishli Agricultural Research Center. The results of the analysis showed that most of the samples tested were contaminated with fungi in varying degrees ranged between 1×10^2 and 5×10^7 spore / gram of food substance. Only 2.3% of the samples tested exceeded 10^4 spore / gram of food substance. The results of the investigation also led to isolating and identifying 566 isolates to 21 different species of fungi, belonging to 8 fungal genera, 4 families, 3 orders & 2 classes of fungi (*Zygomycetes* & *Hyphomycetes*). The largest number of fungal species belonged to the genera: *Aspergillus* (7 species) & *Penicillium* (6 species), to a lesser extent the genus: *Fusarium* (3 species) and one species for each of the genera: *Alternaria*, *Rhizopus*, *Mucor*, *Stemphiliium* & *Cladosporium*. The species (*A. niger*, *A. flavus*, *P. cyclospium*, *R. stolonifer* and *A. alternata*) were detected on all the tested samples. The toxicity test conducted by biological methods for 82 pure fungal isolates: 28 of *Aspergillus flavus*, 10 of *A. niger*, 5 of *A. ochraceus*, 15 of *Penicillium spp*, 7 of *A. alternata* and 17 of *Fusarium spp*. in laboratory conditions indicated that 26 isolates (31.7%) showed different rates of toxicity on germination of chickpea and corn seeds and on activity of *Bacillus sp.* in lab conditions, which indicates that the tested isolates had produced toxic substances. There is a need to undertake suitable actions to reduce fungi activity to prevent the potential health hazards of the poisonous mycotoxins on the consumer.

P MYC 2

Assessment of plant diseases and mycotoxins in organic and conventional farming

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The Norwegian Scientific Committee for Food Safety assessed the effect of organic and conventional production systems on plant health based on a review of scientific literature relevant for temperate climate. Some studies found higher root rot incidence in organic than in conventional cereals, while others report on no difference. For leaf and head diseases of cereals no consistent differences are found. There are reports on lower incidence of head blight caused by *Fusarium spp.* in organic than in conventional cereals, but there are also studies that find no difference in *Fusarium* incidence between the cultivation systems. Results from comparison of mycotoxin content in organic and conventional cereals vary. Most studies found no difference in DON content between the cultivation systems, and the majority of the remaining studies reported on lower levels of DON in organic than in conventional cereals. Organic cereals contain lower levels of T-2 and HT-2 toxin than conventionally grown cereals in most studies. Late blight is severe in susceptible potato varieties, and only resistant cultivars can be cultivated organically in climate with annual epidemics. The limited number of comparative studies on other potato diseases does not provide sufficient data to conclude on their incidence in the two production systems. Apple scab and grey mould of strawberries are severe in organic production because less efficient control methods are available than in conventional orchards. Four studies showed higher mycotoxin contamination in organic than in conventional apples, while seven studies reported on no difference in contamination. The few comparative studies on vegetable diseases do not provide sufficient information to conclude on differences between the production systems. Only few comparative studies of quality in organic and conventional seed and seed potatoes have been published, and it is not possible to conclude on quality differences. However, in some studies a higher proportion of seed borne diseases were found in organic than in conventional seeds.

P MYC 3

Effects of water supply and atmospheric CO₂ enrichment on the *Fusarium* toxin contamination of maize

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As a consequence of rising atmospheric CO₂ concentrations [CO₂] a significant global increase of air temperature is predicted and more frequent summer droughts are expected for Central Europe. These changes will have a pronounced impact on cropping systems because climate and weather conditions influence growth and health of cultivated plants decisively. Major fungal pathogens causing infectious diseases which endanger food and feed safety by mycotoxin production in maize belong to the genus *Fusarium*. In cooler regions of Europe *F. graminearum* and *F. culmorum* producing toxins like deoxynivalenol (DON) are the most important toxinogenic species which infect maize in the field. Multiannual experimental field trials were performed in Braunschweig cultivating maize either under different water supply (either site-specific precipitation or reduced to 50 %) using rain-out shelter or in combination with different [CO₂] (either ambient: 378 µl CO₂ l⁻¹ or set to 550 - 600 µl CO₂ l⁻¹ during daylight hours) using free air CO₂ enrichment (FACE) equipments to evaluate the potential effects of water availability and CO₂ enrichment on *Fusarium* toxin contamination of maize. The level of DON representing the main *Fusarium* toxin found in whole plant, ear or grain samples ranged considerably between cultivars and years. High DON concentrations of >2 up to 9 mg kg⁻¹ were observed in the years 2007, 2008 and 2013 with clearly lower levels, however, ranging between 0.1 and 1.8 mg kg⁻¹ in 2009, 2010, 2011 and 2012. Concentrations of 3-acetyl-DON detected in 2012 and 2013 reached about half the level of DON, whereas traces of zearalenone or nivalenol were occasionally found in some ear samples. With respect to DON concentrations detected in maize regarding all seasons, no statistically significant main or interactive effects were observed for the [CO₂] and water treatments. These results indicate that other factors (e.g. cultivar, year) might have a stronger influence on *Fusarium* toxin contamination than drought and the increase of atmospheric CO₂ concentration.

P MYC 4

RNA interference-mediated control of *Aspergillus flavus* in maize

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Introduction: Aflatoxigenic *Aspergillus flavus* is a frequent contaminant of agricultural commodities such as corn, peanut, tree nuts and cottonseed. Ingestion of foods, especially corn, contaminated with aflatoxins has been implicated in acute toxicoses while chronic, low-level exposure can lead to immune suppression and liver cancer in humans. RNA interference (RNAi) is a form of host-induced gene silencing (HIGS) that has evolved in most eukaryotes as a means of defense against viruses. RNAi silences targeted genes by degrading mRNA before it is translated into protein. RNAi has been used to increase disease resistance in plants susceptible to infection by pathogenic fungi. In these studies, fungal mRNAs destined for translation into proteins critical for growth and virulence were degraded by small interfering RNAs (siRNAs) produced by the plant RNAi machinery and subsequently translocated into the invading fungal pathogen.

Objective: Develop and evaluate maize transformed with an RNAi vector targeting *A. flavus* alpha-amylase (*amy*) mRNAs that are critical for fungal growth and aflatoxin production.

Materials and methods: A binary RNAi vector expressing hairpin RNA (hpRNA) targeting the *A. flavus amy* gene was constructed and transformed into Hi-II maize via *Agrobacterium*. R₀ seed from transgenic maize events was infected with a GFP-expressing *A. flavus* strain in an *in vitro* kernel screening assay (KSA). After 7 days infected seeds were analyzed for fungal growth, aflatoxin and *amy* gene expression. Fluorescence emanating from the fungus was used as a measure of fungal growth.

Results: Preliminary results indicated significant reduction in both fungal growth and aflatoxin levels in several *amy*-RNAi transgenic lines compared to control. qRT-PCR of *A. flavus amy* gene expression correlated well with those *amy*-RNAi corn lines that demonstrated the greatest reductions in *A. flavus* growth and aflatoxin production.

Conclusions: *In vitro* studies indicate that host induced gene silencing of *A. flavus amy* gene expression resulted in a significant inhibition of *A. flavus* growth and aflatoxin production in some of the transgenic maize lines.

Poster Presentations

Mycotoxins

P MYC 5

Generation of recombinant antibodies against fumonisins and fumonisin-producing pathogens

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Fumonisin and fumonisin-producing fungi are distributed worldwide and particularly interested to agriculture and food safety. Fumonisin B analogs, particularly FB₁, FB₂, and FB₃, are major mycotoxins found in cereals. A simple analytical method to detect poisonous mycotoxins and mycotoxin-producing pathogens is essential for forecasting pathogens and controlling mycotoxins. We describe methods for convenient and sensitive detection of fumonisins and associated fungal pathogens with recombinant antibodies. Highly reactive scFv antibodies specific to surface targets of *Fusarium verticillioides* and fumonisin FB₁, respectively, were selected from immunocompetent libraries by phage display. The antibodies against *Fusarium* pathogens were verified to bind on the surface of conidiospores and on their hyphal surface while scFvs against FB₁ were isolated and cross-reactivated to FB₂, and FB₃. Surface plasmon resonance measurements confirmed that the generated recombinant antibodies display a high affinity to their respective antigens. The coding sequences of pathogen-specific scFv antibodies were genetically fused to an alkaline phosphatase gene and the scFv-AP fusions were expressed in bacteria. Both the antibody properties and enzymatic activity was retained and used for immunoassays of naturally contaminated samples. Fumonisin-specific scFv antibodies had no cross-reactivity to deoxynivalenol, nivalenol and aflatoxin that are frequently present in food/feed samples together with fumonisins. Validation assays with naturally contaminated samples revealed a good agreement between the antibody-based assay results and chemical analysis results of fumonisins. These results indicated that the isolated recombinant antibodies can be used for the rapid, accurate, and specific detection of contamination of fumonisins and fumonisin-producing fungi in agricultural samples.

P MYC 6

Isolation and preliminary study of aflatoxin B1-destroying metabolites secreted by *Phoma glomerata* and *Gliocladium roseum*

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Pre- and post-harvest infection of crops by *Aspergillus flavus* can result in contamination of feeds, other agricultural output and foodstuffs with aflatoxin B1, a highly durable hepatotoxic and carcinogenic mycotoxin. Different chemical and physical agents that destroy B1 are currently available, but all of them are imperfect in terms of safety, economy or the treated product quality. Aflatoxin-destroying fungi or bacteria look more promising as alternative decontamination agents, but they often produce undesirable metabolites, which worsen taste, aroma, and nutritional value of the products. At the same time, use of microbial B1-catabolizing enzymes would avoid disadvantages of decontamination by microorganisms.

The aim of this research was to isolate and investigate some properties of B1-degrading metabolites from culture liquid filtrates (CLF) of *Phoma glomerata* (*Pg*) and *Gliocladium roseum* (*Gr*), which we previously found to be B1 destructors and secret the target activity extracellularly.

To isolate high-molecular weight metabolites (HWM), the CLF were fractionated by ultrafiltration. HWM, which precipitated with ammonium sulfate at 70% saturation, were dissolved in and dialyzed against a multicomponent buffer (1) followed by change the buffer to distilled water and freeze drying. To assay toxin degradation, B1 was incubated (27°C, 72 h) in the obtained samples, which were previously sterilized through 0.22-µm Millipore filters. Residual B1 was extracted and quantified by HPLC (1).

B1-destroying activity secreted by the fungi was strongly associated with HWM (above 5 kDa).

Pg- and *Gr*-HWM were pH-sensitive, demonstrated different degradation dynamics, and destroyed 78% (*Pg*) or 69% (*Gr*) of added B1 at pH 8.3. Anion-exchange chromatography of

Pg-HWM allowed revealing a fraction that destructed 98% of B1. Proteinase K significantly reduced catabolic activity of both *Pg*- and *Gr*-HWM. Addition of PMSF to the reaction mixture congaing B1, HWM and the proteinase resulted in reactivation of the toxin degradation process.

These results showed that B1 was destroyed by fungal proteins, which most likely were extracellular enzymes.

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1. Shcherbakova L. et al. Jundishapur J Microbiol., 2015, 8(1) e24324.

Poster Presentations

Mycotoxins

P MYC 7

Regulation of the Deoxynivalenol-Induced Toxicity Response in *A.thaliana*

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Mycotoxin, such as the trichothecene deoxynivalenol (DON) produced by some *Fusarium* species, are not always essential for initiating disease but are often linked with increased aggressiveness. The effect of DON on cell function has been most extensively studied in animal cells in attempts to understand its toxicity. We aimed to assess the effects of DON on plant such as *Arabidopsis thaliana* and how plant defence responses happened. The growth and morphology of *A.thaliana* growing on media containing DON was investigated. The inhibition of germination of *A.thaliana* seeds decreased. DON exposure inhibited plant growth obviously, especially preferentially inhibited root elongation. Trypan blue staining for measuring of cell death, 3,3'-diaminobenzidine (DAB) staining for reactive oxygen species (ROS), method of Nakano for measuring of SOD, CAT and POD activities were used. It showed that DON exposure could cause necrotic lesions in detached leaves and ROS production was stimulated, suggesting DON is toxic to *A.thaliana* and ROS pathways involved in the DON-induced toxicity. Ultrastructural examination of *A.thaliana* leaves exposed to DON showed that the separation of the plasma membrane from the cell wall, fold formation, chromatin condensation, destruction in the structures of the mitochondria and chloroplasts, uneven thickness of the membrane, and distorted nuclear membrane and nuclei. When *A.thaliana* seedlings exposed to DON, the number of starch granules and peroxisome increased. qRT-PCR was used for the relative expression level of the salicylic acid-inducible marker gene *PR1*, respiratory burst oxidase homologue *AtrbohC* and *AtrbohD*, and ascorbate peroxidase *APX*. A continuous increase in the ROS content with the increase of toxin concentration was observed. The application of toxin to excised *A.thaliana* leaves significantly accelerated the increase in MDA.

P MYC 8

Fusarium and Gibberella ear rot of maize:

Susceptibility of varieties cultivated in Switzerland and impact of coinfections on mycotoxin contamination

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Question: Maize ears are often infected by fungi belonging to the genus *Fusarium*. These pathogens produce mycotoxins which can contaminate the harvest so severely that it can not be fed to animals. Many factors contribute to disease development, such as cultural practices (e.g. crop rotation), meteorological conditions and host resistance.

- Can hybrid resistance help manage Gibberella ear rot and mycotoxin contamination?
- How can we evaluate hybrid resistance?
- What is the impact of infections by multiple *Fusarium* species on mycotoxin biosynthesis?

Methods: Macroconidial suspensions of *F. graminearum* and *F. verticillioides* were used to inoculate maize hybrids in the field at flowering either by injecting the suspension in the silk channel or by perforating the grains with needles previously dipped in the suspension.

Results: Artificial inoculation allowed a stable evaluation of Gibberella ear rot resistance. A strong correlation between symptoms expression and deoxynivalenol (DON) levels was observed. For some hybrids, there were discrepancies in disease levels between inoculation methods (silk channel or grain inoculation). Results from two varieties with very different resistance profiles inoculated over seven years with *F. graminearum* showed that the meteorological conditions during and after flowering explained slightly more variation than the factor "variety". Among the 38 maize hybrids tested between 2008 and 2014, significant differences in resistance were observed. Symptoms development was lower for the 17 hybrids co-inoculated with the two *Fusarium* species compared to single-species inoculation. *F. graminearum* and *F. verticillioides* coinfection reduced DON levels in all hybrids, but increased fumonisin levels in 12 hybrids.

Conclusions: Artificial inoculations of maize ears allowed a stable ranking of the varieties according to their resistance to *F. graminearum* infection. Silk channel and grain inoculations evaluate most likely different types of resistance. After crop rotation and weather conditions, disease resistance may be a valuable tool to manage mycotoxin contamination. The impact of coinfection on mycotoxins biosynthesis may explain why symptoms expression and mycotoxins levels sometimes don't correlate well in naturally infected ears.

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Trichothecene mycotoxin levels in winter wheat in Ontario, Canada

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Fusarium head blight caused by *Fusarium graminearum* is a serious disease of wheat (*Triticum aestivum* L.). Deoxynivalenol (DON) is the mycotoxin most commonly detected in contaminated wheat grain in Ontario, Canada. The objective of this study was to evaluate the level of trichothecene mycotoxins in winter wheat grain in Ontario from 2009, 2010 and 2013. The harvested grain was sampled to determine DON, 15-acetyl DON, 3-acetyl DON, nivalenol (NIV), T-2 and HT-2 toxins using a GC-MS system with a detection limit of 0.06, 0.05, 0.05, 0.12, 0.06 and 0.04 µg/g, respectively. The average DON level was 0.7 µg/g, 0.3 µg/g and 3.3 µg/g in 2009, 2010 and 2013, respectively. NIV was not detected in any sample in 2009 and 2010, while was detected just in one sample in 2013 at level 0.14 µg/g. T-2 and HT-2 toxins were detected in one sample in 2009 at level 0.07 µg/g and 0.06 µg/g, respectively, while they were not detected in 2010. In 2013, T-2 and HT-2 ranged from 0.08 µg/g to 0.14 µg/g and from 0.04 µg/g to 0.80 µg/g, respectively. In 2013, DON level was high in general, but lower mean levels of DON were detected in hard red wheat than in soft white wheat (1.5 vs. 4.1 µg/g, 3.3 vs. 11.8 µg/g and 5.2 vs. 19.8 µg/g at Ridgetown, Inwood and Centralia, respectively). 15-acetyl DON and 3-acetyl DON were not detected in 2009 and 2010 in Ontario. In 2013, 15-acetyl DON were detected at all three locations, while 3-acetyl DON were detected in soft white winter at one or two locations. Several times higher average levels of DON were detected in 2013 compared to previous years, with some winter wheat lines showing a level of tolerance to mycotoxins accumulation. We recommend future monitoring of trichothecene mycotoxins in winter wheat in Ontario, Canada.

P MYC 10

Connection among host of origin, Patulin production and aggressiveness in a *Penicillium Expansum* population

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Penicillium expansum is the causal agent of blue mould, one of the most economically damaging postharvest diseases of pome fruits, which can affect even several other hosts as sweet cherries and table grapes. Although several reports on the role of mycotoxins in plant pathogenesis were published, their influence on host preference amongst producing fungi is still largely unknown. In the present study the complex interaction host/aggressiveness/patulin production in *P. expansum* was investigated. Three *P. expansum* strain groups, originating from apples, sweet cherries, and table grapes (7 strains per host) were grown on their hosts of isolation and derived artificial media. Strains within each group proved to be more aggressive and produced more patulin when grown on their host of isolation. Table grape strains were the most aggressive (81% disease incidence) and strongest patulin producers (up to 554 µg/g). The difference in aggressiveness was appreciable only in the presence of a living host, suggesting a significant influence of the host on *P. expansum* ability to cause the disease. Incidence/severity of the disease and patulin production proved to be positively correlated, supporting the role of patulin as virulence/pathogenicity factor. The existence of genetic variation amongst isolates was confirmed by the High Resolution Melting (HRM) method that was set up herein, which permitted to discriminate *P. expansum* from other species (*P. chrysogenum* and *P. crustosum*) and, within the same species, amongst the host of origin. Host effect on toxin production appeared to be exerted at a transcriptional level.

P MYC 11

Ecology, epidemiology and control of the mycotoxigenic fungi *Aspergillus* spp. in pistachio orchards in Greece

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Mycotoxin contamination of agricultural commodities is considered a serious food quality and safety issue worldwide. One of the most highly carcinogenic mycotoxins is aflatoxin (AF) produced by *Aspergillus flavus* and *A. parasiticus*. AF has often been detected in high concentration levels in pistachio nuts. The goal of this study is to evaluate the AF contamination of pistachio nuts in a main pistachio producing area of Greece, the Island of Aegina and to propose sustainable integrated management solutions. The specific objectives are to: a) assess the geographical and physiological population divergence and distribution among *Aspergillus* spp. in pistachio nuts and orchards, b) assess the dynamics of the population composition of AF producers

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during the pistachios growing season, c) determine the AF content in nuts and study the epidemiology of AF contamination in correlation with meteorological data, d) evaluate novel biocontrol strategies with the study of antagonistic activity of a collection of yeasts and atoxigenic *Aspergillus* isolates against *A. flavus* in laboratory and field experiments and e) evaluate the efficacy of several fungicides in laboratory and field experiments against *Aspergillus*. Surveys and sample collection from 20 pistachio orchards located at different regions on the Island of Aegina showed that both *Aspergillus* section *Flavi* and section *Nigri* could be isolated from all parts of healthy and damaged pistachio fruits (hull, shell, nut). However, the population dynamics of the mycotoxigenic fungi were greatly differentiated among the Island orchards. A collection of 500 *Aspergillus* section *Flavi* strains has been created and analysed for morphological differentiation, sclerotium size and AF production in order to estimate the diversity of fungal population. One of the most efficient strategies to reduce aflatoxin levels in several crops at pre- and post-harvest stage is the application of native biological control agents in the field. One of the mechanisms of contamination reduction is through competitive exclusion of the aflatoxin-producing fungi by the application of non-aflatoxigenic strains. The ultimate aim of the current research is to reduce aflatoxin contamination in pistachio orchards by selecting and applying the most suitable non-toxic strains of *Aspergillus* in the field.

P MYC 12

The role of the global regulator of secondary metabolism *AclaeA* in *Aspergillus carbonarius* physiology, virulence and ochratoxin A production

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Aspergillus carbonarius is considered one of the main fungi responsible for the sour rot in grapes and for the production of the carcinogenic mycotoxin ochratoxin A. The regulatory mechanisms of OTA production by *A. carbonarius* remain still largely unknown. In *A. nidulans* the global regulator of secondary metabolism *laeA* encodes a nuclear methyltransferase protein that is required for the expression of secondary metabolite genes while its presence is considered indispensable for mycotoxin, antibiotic and mycelial pigment biosynthesis. BLAST analysis of the genome of *A. carbonarius* with the *laeA* gene of *A. nidulans* resulted in the presence of an orthologous gene named *AclaeA*. The goal of this study was to investigate the role of the regulatory gene *AclaeA* in physiology, virulence and ochratoxin A production by deleting this gene from the genome of the wild type *A. carbonarius* strain 5010. *AclaeA* was deleted by targeted gene replacement using *Agrobacterium tumefaciens* mediated transformation. The evaluation data on morphological characteristics, virulence experiments in red and white grape varieties and ochratoxin analysis of $\Delta AclaeA$ mutants showed that $\Delta AclaeA$ strains were defective in growth and in OTA production and were less virulent producing 40-50% less conidia in three different cultivars of grape berries. Current studies are focused on the global regulatory role of *AclaeA* in secondary metabolism of *A. carbonarius* at transcriptional and metabolomics level. The study of the regulatory gene *AclaeA* can contribute to a broader understanding of the role of secondary metabolites during the *A. carbonarius* - grapes interactions.

P MYC 13

Elucidation of the interactions between the mycotoxigenic fungi *Fusarium proliferatum* - *Fusarium verticillioides* and maize germplasm

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Fusarium verticillioides and *Fusarium proliferatum* cause ear and grain rots and produce the carcinogenic mycotoxins fumonisins in maize that are very harmful to human and animal health. *Fusarium* infection and fumonisin contamination occur as maize kernels reach to physiological maturity, and increase during the season up to the average harvest date. A major strategy to control these pathogens and to reduce the detrimental effects of fumonisins is breeding of less susceptible plant genotypes. The objectives of the current study were to characterize at species level different *Fusarium* species isolated from maize plants in Greece and to evaluate a number of hybrid lines after infection with a mixture of four different *F. verticillioides* and *F. proliferatum* strains. The goal was to collect data on disease incidence and severity and fumonisin production and if there is a positive association between visible symptoms caused by *Fusarium* infection and mycotoxin concentration. In *in vitro* experiments of corn kernels infections, a number of hybrids showed a significant reduction in symptom development and in conidia and fumonisins production. The hybrids that showed significant resistance in *in vitro* tests were further evaluated in field trials and several lines showed a significant reduction in fumonisin contamination and *Fusarium* disease severity. A better

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understanding of the resistance mechanisms would facilitate the implementation of strategic agriculture to breeding of resistant germplasm and to contribute to reduction of ear rot and production of fumonisins in maize.

P MYC 14

Isolation of mycotoxigenic fungi of *Aspergillus* spp. and *Fusarium* spp. and detection of aflatoxins and fumonisins from maize fields in Greece

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Various species of *Aspergillus* and *Fusarium* cause ear and grain molds in maize, and produce carcinogenic mycotoxins (i.e. aflatoxins and fumonisins) that are particularly harmful to humans and animals. *Aspergillus* spp. and *Fusarium* spp. infection starts usually at physiological maturity of the seed at preharvest level and increase up to storage at postharvest level. The goals of this study are to estimate the disease incidence and severity of ear rots and evaluate the aflatoxin and fumonisin contamination in maize fields in major production areas of Greece, with varying climatic conditions, and to propose sustainable management strategies. Maize ear samples were collected from 70 different fields from the counties of Thessaly (central Greece) and Macedonia (Northern Greece). The principle objectives of these surveys are to assess the geographical and physiological divergence and distribution among *Aspergillus* spp. and *Fusarium* spp. in maize, to assess the dynamics of the population composition of aflatoxins and fumonisins producers during the maize growing season, to determine aflatoxins and fumonisins content in maize ears and to study the epidemiology of toxins contamination in correlation with meteorological data. Experimental data showed that both *Aspergillus* and *Fusarium* could be isolated from all parts of corn ears. A collection of about 1000 fungal isolates has been created and analyzed for morphological, microscopic and molecular differences. The mycotoxin contamination levels were assessed by homogenization of corn kernels and determination of the toxins by the ELISA method. Analysis showed that a very critical step for mycotoxin production and the disease severity is maturity of the seed, humidity levels and environmental conditions depending on the mycotoxin. A better understanding of the epidemiology of *Aspergillus* spp. and *Fusarium* spp. and their associated mycotoxins in Greece would facilitate the implementation of an integrated management approach to control and reduce ear rots and mycotoxins contamination in maize

P MYC 15

Biological and chemical control of the aflatoxigenic fungus *Aspergillus flavus* in maize

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Aspergillus species cause significant diseases (molds) in field crops (including maize), grapes, nuts and dried fruits at both pre- and post-harvest level. These molds are usually associated with the production of various mycotoxins such as the carcinogenic aflatoxins. In this study, several formulations and non-pathogenic biotic factors were evaluated for the control of *Aspergillus flavus* and aflatoxins in maize crops. *In vitro* experiments were performed on maize kernels with the following factors: a) Zeolite, a mineral with special physicochemical properties, b) Agri-Fos 600[®], a commercial product based on potassium phosphonate anions that induce the immune system of plants, c) Trianium[®], a commercial product based on the fungus *Trichoderma harzianum* that works by stimulating the growth of the root system and inhibiting the infection and colonization of pathogenic fungi, d) Botector[®], a commercial product containing the yeast *Aureobasidium pullulans* whose action is based on inhibition of the pathogen colonization due to space and nutrient, e) *Paenibacillus alvei* K-165, an antagonistic rhizobacterium that induces systemic resistance of plants, f) Serenade Max[®], a bio-fungicide/bio-bactericide that also stimulates natural plant defense mechanisms and has demonstrated increased plant growth effects, g) Vacciplant[®], a commercial product that contains laminarine, a promoter of the immune system of plants, and h) a non-toxigenic strain of *Aspergillus flavus*. Finally, the fungicides Switch[®], Geoxe[®], Granuflo[®], Cantus[®], Chorus[®] and Quadris[®] were tested. All biocontrol and chemical products were applied in maize kernels by immersing the seeds for 30 min at highest label doses. The infection with conidia of *A. flavus* was carried out 24h later by applying on each corn kernel a droplet of 10 µl (10⁶ conidia/ml). In preliminary field experiments, Zeolite, Agri-Fos 600[®], Trianium[®], Botector[®], a non-toxigenic strain of *A. flavus* and the chemical Switch[®] were applied on maize plants in the field by spraying 3 days before infection with *A. flavus*. The above formulations were also applied by injection on the cob with a second booster dose on the day of infection with the toxigenic strain of *A. flavus*. The role of these treatments on disease incidence and severity as well as aflatoxin contamination data will be presented.

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Effect of fungicides on fitness and ochratoxin A production in *Aspergillus tubingensis* wild population

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Ochratoxin A (OTA) is a mycotoxin commonly present in cereals, grapes, coffee, cocoa and their processed products. The toxin is produced by several species of the *Aspergillus* and *Penicillium* genera. In the last decade, OTA has received increased attention worldwide because of its hazard to human and animal health. Due to this toxicity, the International Agency for Research on Cancer (IARC) has classified OTA as a group 2B carcinogen. The use of fungicides is a strategy to prevent mycotoxin production but it has been reported that some influence positively or negatively OTA production.

In this study we investigated how selective (cyprodinil and fludioxonil) and non-selective (sulphur and copper) fungicides can affect OTA production by *A. tubingensis* isolates. In addition the rate of mycelia growth of these isolates was also studied and whether they can develop resistance to cyprodinil and fludioxonil. For both resistant and sensitive isolates, EC50 values as well as baseline sensitivities were measured and six resistant isolates for each fungicide were tested for fitness parameters (mycelia growth, spore production and germination).

Our results show that OTA production for all tested isolates was generally low, showing higher values at 30°C incubation. Sulphur induced higher mycelial growth while copper did not show any difference from control. Copper also induced in some isolates higher OTA production values. Lastly, resistant isolates to both selective fungicides, produce higher levels of OTA than wild type. In all fitness parameters we observed the negative effect of both selective fungicides. The results will be further discussed in view of literature review and deeper statistical analysis.

P MYC 17

Fusarium species complex and fumonisin on maize grains in Ethiopia

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Fusarium species are the most frequently isolated fungi in maize in several growing areas. Infection of maize by *Fusarium* species reduces grain yield, and quality due to mycotoxin contamination that poses a health risk to humans and animals. Two-hundred maize grain samples were obtained from 20 different locations of Ethiopia, analyzed for the composition and prevalence of *Fusarium* species in addition to fumonisin contamination. About eleven *Fusarium* spp. were identified based on morphological criteria and by sequencing the partial translation elongation factor 1-alpha (*EF-1α*) gene. *Fusarium verticillioides* and *F. graminearum* species complex were the dominant species contributing for 42% and 22.5% of all isolates, respectively. The spectrum and prevalence of *Fusarium* species differed among the locations investigated. Fumonisin analysis showed 77% of the samples were positive for the toxin with concentrations ranging from 25 to 4500 mg kg⁻¹ (mean: 348 mg kg⁻¹ and median: 258 mg kg⁻¹). Slight variation in fumonisin concentration was also observed among locations. Overall results indicate wide spread infection of maize by several *Fusarium* species and contamination by fumonisin toxins. These findings demand for measures to reduce the impact of these fungal species and the associated toxic compounds by implementation of good agricultural practices.

P MYC 18

Cultural, Morphological Variability and Biological Control of Aflatoxins Produced by *Aspergillus flavus* Isolates in Maize

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Different isolates of *Aspergillus flavus* cultured from soil and seed samples of maize collected from different districts of Andhrapradesh and Kolar district of Karnataka state were evaluated with potential fungal antagonist, *Trichoderma viride* isolate TIF -16 showed highest inhibition percentage (95.55) with high mycoparasitic activity. The seeds being colonized with *A. flavus* isolates were subjected to quantification of Aflatoxins using High Performance Liquid Chromatography (HPLC). The *A. flavus* isolate AFT-16 produced highest B1 toxin level (4625.32 ppb) and AFT-19 produced highest B2 toxin level (5427.58 ppb) which are isolated from seed. From these studies, prevalence of afB1 toxin and also the presence of afB2 toxin in one sample of infected maize kernels was documented. Treatment of maize plants with talc based formulation of *T. viride* significantly reduced

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A. flavus infection and production of aflatoxin in maize kernels. When the cobs were challenge - inoculated with *A. flavus* 3 days after application of *T. viride*, the aflatoxin content in maize kernels was decreased from 11.5 to 0.12 ppb.

P MYC 19

Study of the contaminant Mycoflora of the Biofilms of the docks of storage of cereals and research of Aflatoxins and the Ochratoxine A

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Food diseases constitute one of their current public health problems most prevalent in the international scale. These diseases are caused by various agents, especially pathogens. In addition to viruses and pathogenic bacteria, toxinogen fungi pose a danger to the health of man and animals by the secretion of highly toxic substances during their proliferation in foods of plant or animal origin.

The bad conditions of grain storage are a favorable climate for the development of a big number of microorganisms, and this increases the susceptibility of attack by molds and biofilm formation.

In this context, achieving a mycological and mycotoxicological comparative study on the grain biofilm in storage silos in the regions of Bechar is necessary.

The results of various mycological and mycotoxicological analyzes showed the severe contamination of our samples with a higher rate (13.5×10^5 UFC/g).

The dominance of *Penicillium* and *Aspergillus* were very common on all samples, and species identification revealed different species that we cite: *A. niger*, *A. flavus-parasiticus*, *A. fumigatus*, *A. candidus*, *A. clavatus*, *P. rubrum*, *P. verrucosum*, *P. expansum*, *P. lilacinum*. The diversity of these species is an indicative of bad storage. The genres *Fusarium*, *Ulocladium*, *Geotrichum* and *Rhizopus* were also revealed.

Research of mycotoxins on different samples by the CCM method reveals the presence of Aflatoxin B1, Aflatoxin G1 and Ochratoxin A.

P MYC 20

The relation under the productions of blastospores and destruxins in *Metarhizium anisopliae* with virulence against *Plutella xylostella*

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Destruxins are kinds of cyclo-peptidic mycotoxins produced by entomopathogenic fungus, *Metarhizium anisopliae*. This fungus also produces blastospores in submerged culture. Both blastospores and destruxins are candidates for insecticide, but, the relation under the both productions and the impact factors are not clear yet. The current experiment surveyed the effects of inoculum, rotation, dissolved oxygen (DO) on the productions of blastospores and destruxins A and B (DA and DB) in submerged culture of *M. anisopliae*. The results indicated that DO levels were influenced by inoculum amounts and rotation speeds, meanwhile, the productions of DA, DB and blastospore were also closely influenced by those factors. Totally, when DO values >40%, destruxins and blastospores achieved higher productions, while at DO values <40%, obtained lower productions. The regression analysis suggested that the productions of blastospores, DA, and DB were positively correlated with DO levels. Meanwhile, the positive correlations between the productions of DA or DB and blastospores were also found. In addition, the virulences of blastospores and conidia were bioassayed. The LC₅₀ value of blastospores was 8.07×10^6 spores/mL at 4 d after treatment, while the conidia had a LC₅₀ of 12.28×10^6 spores/mL. The experiment will give new insights into production of destruxins and blastospores of *M. anisopliae*.

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Identification of novel sources of mycotoxin contamination of Shea butter along the processing chain

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Introduction: Shea butter is useful in drug and cosmetic industries, among others. It is also a source of income for the local people in the communities where it is produced in Nigeria. It has been observed that microbes which contaminate shea butter reduce its quality and render the butter inappropriate for certain uses. Identification of microbes and sources of contamination are important.

Objectives: To determine the novel sources of mycotoxin contamination of shea butter along the processing lines with the aim of improving the quality of the butter.

Materials and methods: The identification of postharvest and processing practices of local shea butter processors in Niger and Kwara States of Nigeria using the semi-improved and traditional methods of shea butter processing was carried out by the personal interviews and observation methods. The enumeration of moulds and yeast associated with the sources of cross-contamination was determined using the serial dilution the method.

Results: The results revealed that the method of shea butter processing was a key to determining the level of hygienic practices of processors, butter storage, vulnerability of the processing lines to microbial contamination and livelihood improvement and interventions from shea butter development agencies. The identified sources of contamination were dirty processing environments and utensils, poor hygienic conditions of the processors and the use of dirty water. The micro-organisms isolated included *Aspergillus* sp, *Penicillium* sp and yeast cells. This processing method provided a platform for cross-contamination of the processing equipment/utensils, raw materials and finished products by fungi and possibly, disease transmitting vectors. The combined effect of the continuous cross-contamination process caused quality deterioration of Nigerian shea butter.

Conclusion: For the quality of shea butter to be improved on, there is the need to develop measures for controlling the processing stages against direct and indirect cross-contamination by microbes and disease vectors.

P MYC 22

Biocontrol strategies to reduce Fusarium Head Blight and deoxynivalenol accumulation in wheat

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Fusarium head blight (FHB) caused by *Fusarium graminearum* species complex is a devastating disease that causes extensive yield and quality losses to wheat in humid and semi-humid regions of the world. The disease can cause not only yield losses but also grain contamination with mycotoxins such as deoxynivalenol (DON). Several epidemics have occurred in Argentina in the last 50 years. Control of the disease is partially achieved by crop rotation, fungicide application, breeding for resistance and tillage practices, but reduction of FHB is not always successful. Biocontrol strategy can be used as part of an integrated pest management. The objectives of this study were: 1- to evaluate the biological control effect of two bacterial formulations on FHB disease incidence, severity and DON accumulation under a field trial and 2- to evaluate the formulated strains on wheat stubble to reduce *F. graminearum* inoculum after harvest. *Bacillus methylotrophicus* RC 218 and *Streptomyces* sp. RC 87B were applied both at anthesis period for FHB parameter's control and over wheat stubble left after harvest for *F. graminearum* control. FHB incidence and severity were visually estimated, DON content was assessed by HPLC after harvest. On stubble, *F. graminearum* prevalence was monitored by TaqMan PCR during three months. A significant disease severity and DON reduction were observed at the field trial with the formulated strains applied alone or in combination (25.8-30% and 85-100% for severity and DON reduction, respectively); but no reduction on disease incidence was achieved. On wheat stubble, *Streptomyces* sp. 87B reduced *F. graminearum* inoculum after 40 days (82%). After three months, *F. graminearum* inoculum was reduced to undetectable levels under the *Streptomyces* sp. 87B treated plot, meanwhile in the control plot the pathogen was detected (9.85 pg DNA/mg). No biocontrol activity with *Bacillus methylotrophicus* RC 218 was observed on stubble. The two formulated biocontrol strains showed high potential for contributing to the reduction of FHB on wheat at different levels in the *F. graminearum* life cycle.

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Potential insecticide chitinases and proteases isolated from *Trichoderma asperellum* on *Cerataphis brasiliensis*

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Nowadays many replacement control alternatives to chemical control has been researched. One of fungi commonly used in disease control plants is *Trichoderma*. However, little research has been reported on insects. Aiming to evaluate the potential of the insecticide *T. asperellum* on *Cerataphis brasiliensis* through chitinase enzymes and proteases, six Brazilian isolates were selected. The isolates were grown in liquid medium with and without production of enzymes, individually in vials containing selective medium (0.3 KH₂PO₄, 0.03 g MgSO₄, 0.2g yeast extract and 0.3 g NaCl) containing chitin to Induction of chitinase and protease protein, remained for 5 days and 10 days of shaking at 26 ° C (\pm 2°C), respectively. Then filtering was performed in Millipore filter (0.45 micron), and 1 ml of each isolate filtrate was applied on 10 insects with 4 replications, put in petri dishes and kept in a room with 26 ° C temperature (\pm 2°C). The treatments were: sterile water, *Beauveria bassiana* fungus as standard and isolates of *T. asperellum* UFRA T 129, 166, 127, 192, 175 and 57. We evaluated mortality for 15 days. The results showed that the liquid containing the enzymes chitinase and protease caused mortality from 80% to 100%, not differing from standard treatment with *B. bassiana*, while the treatment with water ranged from 10 to 20%, however, the liquid no enzyme induction growth caused mortality lower than 60%. It is suggested that the chitinase enzymes and proteases are involved in insecticide produced by *T. asperellum*, similar to that produced by *B. bassiana* entomopathogenic fungi.

P MYC 24

Deoxynivalenol glucosylation in commercial durum wheat cultivars under field conditions

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Fusarium head blight (FHB) is a major fungal disease affecting wheat worldwide and it is caused mainly by species within the *Fusarium graminearum* species complex. The disease can severely reduce grain yield and quality and the infected grains could be contaminated with mycotoxins such as deoxynivalenol (DON). DON is phytotoxic and it is considered a virulence factor of the fungus, affecting the protein synthesis in plant cells. As a consequence, the formation of the "masked" mycotoxin deoxynivalenol-3-glucoside (D3G) results from a defense mechanism the plant uses for detoxification. This ability has been associated to resistance to FHB. The objective of this study was to evaluate the capacity of commercial durum wheat varieties to glucosylate DON to DON-3G under natural conditions in order to select promising varieties for wheat breeding programs screening for FHB-resistant germplasm. Wheat grain samples were obtained from commercial cultivars from 2013 and 2014 harvest seasons in different localities (Balcarce, La Dulce, and Miramar) from the main durum wheat-producing area in Argentina. DON, its acetylated forms (3/15 ADON) and DON-3G were analyzed by HPLC MS/MS. A three way ANOVA was used to compare total DON content and % glucosylation versus variety, year and location. There were statistically significant interactions between year x location, year x variety and location x variety ($p \leq 0.001$) in DON accumulation. There was a significant difference in the total DON concentration between years, with a media of 1554.8 and 1954.8 $\mu\text{g}/\text{kg}$ in 2013 and 2014, respectively. Also significant differences were found between locations with the highest levels in Miramar and Balcarce. There was a positive correlation ($r=0.89$) between the DON and DON-3G content in the grains, and a negative correlation between DON content and % glucosylation. The level of glucosylation was not significantly different, and was not affected by the factors year and location. Furthermore, Buck Granate showed a good glucosylation activity towards DON and it is a promising candidate for further studies in wheat breeding programs.

Poster Presentations

Nematodes

P NEM 1

In vitro and Screenhouse Toxicity of Derivatized Citrulline from Water Melon (*Citrullus lanatus*) on *Meloidogyne incognita*

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Root-knot nematodes are vegetable pests. The most destructive responsible for yield loss in *Corchorus olitorius* is *Meloidogyne incognita*, with characteristic pronounced galls on roots of plant. A nematicide used for control is carbofuran. Because of its high water solubility, low adsorption coefficient, it is mobile in soil, has potential to contaminate lakes, streams, and groundwater. Hazards caused by synthetic nematicides justify the search for bionematicides. Derivatized citrulline from water melon was investigated on *C. olitorius* infected with *M. incognita* *in vitro* and screen house, to establish its potential as substitute for carbofuran. White outer part of *C. lanatus* (2kg) was macerated and extracted with ethanol. Extract was decanted, allowed to stand for 2hrs. 2g of methylamine hydrochloride was added, and refluxed for an hour; allowed to cool to room temperature. 1g of sodium nitrite was added. Mixture was cooled (ice/NaCl). This cold mixture was added slowly into an ice-cold solution of 10mL conc. H₂SO₄ and 10g ice. Whitish crystals were obtained, filtered by suction and dried. *In vitro* experiment consisted of three treatments (citrulline, carbofuran, crude extract of *C. lanatus*) at four levels with three replicates. Randomised complete design was used with 36 counting dishes. Treatment was dissolved in water at 3, 5 and 8mg. Screenhouse had 36 buckets; treatments were dissolved in 100mL water and applied in banded form at the base of plant. In the laboratory, highest (8mg) concentration of treatments after 30mins was more effective than the lowest concentration (3mg). 96.12% mortality was observed for citrulline, as opposed to 45 and 11% observed in carbofuran and crude extract of *C. lanatus*. Result was consistent with screenhouse, where plants treated with the highest concentration of materials had reduced nematode population and improved vegetative growth. Plants treated with all the levels of citrulline had the best vegetative growth and there was no root galling. Infrared result of citrulline showed bands at 3431cm⁻¹ which is characteristic of a primary amide. Bands at 1506 and 1456cm⁻¹ supports presence of amide group. From the C-NMR (200MHz, CDCl₃), resonance at 0.8-1.37ppm is attributed to methyl, and methylene protons. Down field signals at 4.6ppm can be ascribed to olefinic protons. Citrulline can serve as an alternative to the toxic synthetic nematicide.

P NEM 2

Vegetable Production Sustainability: Controlling nematode infection in *Corchorus olitorius* using *Eucalyptus officinalis*

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Synthetic nematicides often very expensive cause environmental damage when used indiscriminately. Consequently, the nematicidal properties of chromatographic fractions from *Eucalyptus officinalis* were investigated. The fractions were partially characterized using Fourier transform infra-red (FT-IR) and gas chromatography mass spectroscopy (GCMS) analysis. The constituent of *E. officinalis* as revealed by the GCMS result include eucalyptol (14.30%), α -pinene (3.80%), citronellol (3.20%), α -terpeneol (22.30%) and 1, 8-cineole (28.00%). The IR spectral data obtained also supports that 1, 8 cineole and α terpeneol are the main constituents of the *Eucalyptus officinalis* fractions. In the laboratory, the fractions obtained from the methanol extract chromatographed on silica gel 100-120 mesh grade produced significantly ($p < 0.05$) higher nematicidal activity with 40.70% mortality as against 23.60% recorded for the n-hexane partition of the aqueous extract. This was followed by dichloromethane partition of the residual aqueous extract and n-hexane crude extract filtered on aluminium oxide column which had 35.11% and 30.12% respectively. On the field, the chromatographic fraction of the methanol extract (MeOH/si-gel/cc₁ and MeOH/si-gel/cc₂) produced significantly ($p < 0.05$) higher plant height, number of leaves and branches. *Corchorus olitorius* plants that were treated with synthetic nematicides and the chromatographic fractions were significantly taller with more leaves per plant and they had neither nematodes nor galls compared to the untreated control. Few nematodes were recovered from the soil treated with the fractions. Growth stimulation was highest in *Corchorus olitorius* plants which were treated with chromatographic fractions from methanol extract and carbofuran at the highest concentration. *Eucalyptus* extracts thus have bio-nematicide potential.

Poster Presentations
Nematodes

P NEM 3

Evaluation of two species of water ferns, *Azolla*, *Caroliniana* and *A. Pinnata* as soil amendments against *Meloidogyne javanica* infecting tomato in Egypt

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The impact of dry materials of *Azolla caroliniana* and *A. pinnata* on controlling *Meloidogyne javanica* on tomato cv. Super Strain-B was carried out under greenhouse conditions 25±5°C. The treatments were applied at the rates 25 and 50 gm of dry materials of each species / pot. Application of *A. caroliniana* and *A. pinnata* succeeded in reducing the development and reproduction of *M. javanica* and improved tomato growth when compared with those of the check. *A. pinnata* was more efficient in reducing number of nematode stages based on galls, egg-masses, females, developmental stages in roots, as well as, number of juveniles in soil per plant at both rates as compared with *A. caroliniana* did. Also, the growth of tomato plants was affected due to the application of azolla. Addition of azolla to the plant soil caused remarkable increase in all plant growth parameters. The higher dose was more effective than the lower one. However, *A. pinnata* resulted in increasing the plant growth much more than *A. caroliniana*.

P NEM 4

Sustainable Phytonematode Management through Meliaceae plants

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Phytonematodes are ruinous soil antagonists of vegetables causing enormous productivity loss to world agriculture thus leading to food insecurity. Synthetic nematicides although form a primary strategy to weed out these pests but due to their high cost and inherent environmental hazards many developed countries have withdrawn them from the market. More emphasis is given nowadays on plant based organics for nematode management for their easy availability and ecological feasibility. In the present study the soil amendment with fresh floral parts and decomposed fruits, leaves and bark of *Azadirachta indica* A. Juss. /margosa/ neem and a related species *Melia azedarach* L. / persian lilac/ bakain were found to be highly effective in reducing the natural infestation of plant parasitic nematodes around *Solanum lycopersicum* and *Solanum melongena*. In separate experiments the root-knot development caused by *Meloidogyne incognita* and population of *Rotylenchulus reniformis* on *S. lycopersicum* and *S. melongena* were also managed significantly by the above treatments. Highest inhibition was observed when plants were treated with the decomposed fruits of margosa/ neem and persian lilac. As a consequence of reduced root galling and populations of plant parasitic nematodes, the growth of the test plants improved significantly. Hence, it may be concluded that plant parts as biomanagement are effective tools in controlling phytonematodes and are ecologically safe and feasible for sustainable agricultural productivity. These findings may go a long way for the plant protection programme.

P NEM 5

Inducing the systemic resistance of tomato plants by root-knot nematode females extract against *Meloidogyne javanica* infection

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Females of *Meloidogyne javanica* were homogenized with distilled water in the proportion of 20 individuals / 3 ml of water. The dilute homogenate was applied as foliar spray at the rate of 3 ml / plant to tomato plants inoculated with *Meloidogyne javanica*. While some nematode females extract (NFE) treatment were pre-inoculation, others were post-inoculation. NFE treatment was also given to uninoculated groups of pots. Evidently, nematode females extract (NFE) significantly ($P \leq 0.05\%$ and or 0.01% levels) increased growth of plants and reduced nematode infection. The pre-inoculation treatment was more effective than post-inoculation one. Clearly, the nematode females extract is thought to induce systemic resistance in tomato plants.

Poster Presentations

Nematodes

P NEM 6

Association and impact of nematode pests on indigenous leafy vegetable *Amaranthus* species

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Indigenous leafy vegetables (ILV), i.e. *Amaranthus* species, significantly contributes towards traditional diets and food security of people in urban and rural areas due to their medicinal properties and rich micronutrient levels. However, nematode pests reduce their yields. Objectives of this study were to i) conduct a nematode audit in the Gauteng province in areas where *Amaranthus* spp. are used as a food source, ii) assess the effect of initial population levels (P_i) of two root-knot nematode species (RKN; *M. incognita* and *M. javanica*) on a good- (*A. arusha*) and poor-host (*A. thohoyandou*) domesticated *Amaranthus* sp. and iii) determine the effect of the two RKN species on the nutritious value of the two accessions. Nine parasitic nematode genera were identified from soil and root samples, the predominant genera being RKN. P_i -level trials showed that root masses of poor-host plants were significantly ($P \leq 0.05$) higher than that of good host plants. The number of egg masses/per root system were significantly ($P \leq 0.05$) higher for the good host versus that of the poor host. Nutrient trials showed that root and leaf masses for the two inoculated accessions were not significant ($P \leq 0.05$). However, egg masses as well as eggs and J2 numbers/root system for the good host were significantly higher than those for the poor host and the un-inoculated controls. Significant differences were only recorded for nitrogen and protein between the un-inoculated control treatments of the two accessions, values for the poor host being higher than that for the good host. Data of this study already assist people to plant an ILV accession which reduce RKN soil populations, contributing towards sustainable production of ILV and other food crops. Higher protein levels in leaves of the poor-host accession will benefit dietary requirements of consumers, while the same trend for nitrogen will favour sustainable crop production since no or lower nitrogen fertiliser could be used.

P NEM 7

Nematicidal potential of extracts from some selected plants against the root-knot nematode, *Meloidogyne incognita*

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The effects of water extracts of leaves of *Tagetes erecta*, *Tithonia diversifolia*, *Chromolaena odorata* and *Occimum gratissimum*, each at 3.3, 5.0, 6.6, 8.3 and 10.0% w/v, on eggs and second stage juveniles of *Meloidogyne incognita* were investigated *in vitro*. The efficacy of dry milled leaves of these plants at 1 t/ha and 2 t/ha and carbofuran at 1.5kg a.i./ha and 2.5kg a.i./ha were also evaluated against *M. incognita* in a screen house.

Fifty *M. incognita* eggs in water suspension were dispensed into glass blocks and 1ml of each extract at different concentrations were added. Fifty freshly hatched juveniles were also dispensed into glass blocks to which 1 ml of each plant extracts at the different concentrations were also added. Distilled water served as control. Hatched eggs were counted every 24 hours for 10 days while juveniles were observed for mortality every 24 hours for five days. In the screen house, 48 pots were filled with sterilized soil. Treatments were carbofuran (1.5 and 2.5 kg a.i./ha), milled dried leaves of these plants at 1t/ha and 2t/ha each and untreated control. Two days later four seeds of cucumber were sown in each of the 48 pots. One week after germination, the seedlings were each inoculated with 10,000 *M. incognita* eggs. The treatments were arranged in a completely randomised designed with four replicates. Data were collected on Vegetative Growth (VG), Gall Index (GI), nematode reproduction and yield (g). LC_{50} was also determined. All data were analysed using ANOVA ($p=0.05$) and means were separated using Duncan Multiple Range test at 5% probability.

Water extracts of *T. erecta* inhibited egg hatch by 90.5% at the highest concentration and was significantly higher than egg hatch observed in *O. gratissimum* which produced the lowest egg hatch inhibition of 70.7%. *T. erecta* also caused 100% juvenile mortality within 24 hours of exposure. *T. erecta* extract was the most potent among the plant extracts used with LC_{50} of 0.31mg/ml^{-1} . In the pot experiment, *T. erecta*, *C. odorata*, carbofuran and *O. gratissimum* reduced GI by 62.5%, 65%, 75% and 75.5%, respectively. Similarly, root-knot nematode (RKN) population was reduced by 85.4% in *T. erecta*-treated pots; *C. odorata* caused 87.6% reduction and Carbofuran 93.1%. The results of this study suggest that application of these plants as botanical pesticides in the management of RKN is highly promising, especially as they are readily available in Nigeria.

Poster Presentations
Nematodes

P NEM 8

The Development and Life Cycle of Root-*Meloidogyne incognita* in Cucumber (*Cucumis sativus* L.) roots

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The knowledge of biology of any pest is very essential in the formulation of appropriate management strategies. With this information, it is possible to identify the most vulnerable stage of the pest. From this, decision can be taken on the timing and the type of control measure(s) to adopt. Therefore, decision can be taken on the timing and type of control measure(s) to adopt. Therefore, life cycle and development of the root-knot nematode, *Meloidogyne incognita*, in the roots of cucumber (cv *Marketmore*) were studied in a screen house of the National Horticultural Research Institute, Ibadan, Nigeria.

Two seeds of cucumber were planted in nursery bags containing 5 litre of steam-sterilized sandy-loam soil. Plants were inoculated with 5,000 freshly hatched second stage juveniles of *M. incognita* three days after germination. Twenty four hours later, and subsequently on a daily basis, two seedlings were randomly uprooted and the roots were cleaned and stained using 0.1% acid fuchsin in lactoglycerol method. After de-staining, roots were examined for nematode penetration and stages of nematode development. Environmental temperature during the study ranged from 30.3±2.3°C to 31.1±3.0°C, relative humidity ranged from 65.6±9.6% and 88.1±5.2%, and the soil temperature was 27.5°C and 34.1°C.

The development of *M. incognita* spanned 22 days after inoculation. Penetration was first observed 24 hours after inoculation. The second stage juveniles (J2) (405.0µ) moulted to third stage juvenile (J3) (428.57µ) 10 days after inoculation. Two days later, fourth stage juvenile (J4) emerged and measured a mean length of 457.14µ in length. The young females were noticed 16 days after inoculation and adult mature females were observed 18 days after inoculation with gelatinous matrix or egg sac. Eggs were observed for the first time in the egg sac at 20 days after inoculation and two days later that is 22 days after inoculation, the eggs hatched to J2 with a mean length of 500.0 µ and the width of 28.57 µ.

This suggests that two or more generations of root-knot nematode are possible in a cucumber farm within a growing season. The short generation cycle observed in cucumber production plants may lead to a quick population build up of root-knot nematode which would severely attack cucumber causing yield and quality reduction. This will also leave a large population of root-knot nematode in the soil for subsequent crops.

P NEM 9

Occurrence of dagger nematodes (*Xiphinema* spp.) in the Republic of Armenia and region of Nagorno Karabakh.

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Grapevine is a traditional and the most important crop for Armenian agriculture. Nematodes of the genus *Xiphinema* are migratory root ectoparasites that feed mainly on the root-tips of a wide range of wild and cultivated plant species. Several species also transmit nepoviruses.

There is few information regarding the occurrence and distribution of *Xiphinema* species in the Republic of Armenia, especially virus vector species. The data concerning fauna of plant parasitic nematodes of Nagorno Karabakh region is absent.

The aim of this research was to analyze the occurrence of *Xiphinema* species in vineyards of the Republic of Armenia and region of Nagorno Karabakh. For that, plants and soil samples were collected from four mountain regions (Tavush, Vayots-Dzor, Aragatsotn, Kotayk) and two regions of Ararat valley (Armavir, Ararat) of the Republic of Armenia and two regions of Nagorno Karabakh (Martuny and Askeran).

Soil samples including fine roots in the rhizosphere of *Vitis vinifera* "Areni", "Ararati", "Mskhali", "Rkatsiteli", "Voskehat" varieties with symptoms Grapevine fanleaf virus (GFLV) infection were collected.

Morphological and molecular identification of the isolated species was carried out. Morphological measurements were performed on temporary and permanent glycerin slides. Molecular identification was carried out by using the D2-D3 expansion segments of the 28S rRNA gene and the ITS region of individual nematodes from Vayots-Dzor, Aragatsotn regions and Ararat valley.

Poster Presentations

Nematodes

The occurrence of *Xiphinema* species was 85% and 100%, respectively, in vineyards of Armenia and in the region of Nagorno Karabakh. Four species of *Xiphinema* (*X. brevicolle*, *X. diversicaudatum*, *X. index*, *X. vuittenezi*) were identified in the Republic of Armenia. *Xiphinema index* and *X. pachtaicum* were detected in Nagorno Karabakh region for the first time. The occurrence of *X. index* and *X. vuittenezi* in Ararat valley, Vayots-Dzor and Aragatsotn regions was, also, confirmed by molecular methods. Many plants of grapevine showed typical symptoms of GFLV. Identification and distribution of GFLV in the Republic of Armenia and role of *Xiphinema* species in transmission of viruses are the objectives of our future scientific researches.

P NEM 10

What role do common weeds play in sugar beet rotations in the presence of *Heterodera schachtii*?

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Weeds can serve as alternate host of nematodes and constitute an additional problem in nematode management. In Germany, intensive cultivation of sugar beet in 3-year crop rotations with small grains provides long fallow periods. In previous studies, only few weed species and then at high population densities developing during this fallow period increased population densities of *Heterodera schachtii* whereas most of the frequently observed weeds did not. This lack of reproduction could be related to the time of year and also differ among geographically distinct weed populations. In a one-year microplot experiment during the typical vegetation period of sugar beet, *Capsella bursa-pastoris*, *Chenopodium album* and *Thlaspi arvense* were assessed for their suitability as host plants of *H. schachtii*. Only limited reproduction was found under the weed species compared to sugar beet. In a greenhouse study, the reproduction of *H. schachtii* on ten *Chenopodium album* populations collected from different geographical origins of Germany was determined. Reproduction of *H. schachtii* on the populations of *Chenopodium album* was compared with that of susceptible and resistant cultivars of sugar beet and oilseed radish. Reproduction of *H. schachtii* varied among the *C. album* populations, but at a much lower reproduction level than on the susceptible sugar beet and oilseed radish. Population parameters were similar to those under resistant sugar beet. The results of low reproductive potential on *Chenopodium album* supported the hypothesis that the risk for *H. schachtii* population buildup under these weeds may be limited, and support previous recommendations that under typical field conditions during stubble periods, weed management for nematological reasons appears not necessary.

P NEM 11

Use of organophosphate nematicides to increase efficiency of *Xiphinema index* extraction by the Baermann funnel method

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The dagger nematode *Xiphinema index* causes serious damage to grapevines in many Mediterranean countries, and monitoring its populations in soils is important its management. Nematodes are most easily extracted by the Baermann funnel method, but extraction efficiency of *X. index* is generally low.

Question: Will combining the Baermann funnel method with nematicide treatment increase the efficiency of *X. index* extraction?

Methods: *X. index*-infested soil was treated with nematicides, and incubated for 3 or 7 days before extraction by the Baermann funnels. Alternatively, Baermann funnels were filled with nematicide solutions instead of water. Extraction efficiency of the Baermann funnel method combined with nematicide treatments was compared with that of an elutriation and sieving method.

Results: Treatment of *X. index*-infested soils with the organophosphate nematicides, fenamiphos and cadusafos, at concentrations of 2.0 and 4.0 mg/L soil, followed by incubation for 3 or 7 days, increased the number of *X. index* extracted in Baermann funnels to as much as four times the number from untreated soil. Fluensulfone, a heterocyclic fluoroalkenyl sulfone, did not increase the number of extracted *X. index* at these concentrations, but it did at 8.0 mg/L soil. When fenamiphos solutions of 1.0 and 2.0 mg/L were used in the Baermann funnels instead of water for the nematode extraction, the number of extracted *X. index* was up to three times higher than that from funnels filled with water. The extraction efficiency of these two methods using fenamiphos was not lower than that of the elutriation and sieving method.

Conclusions: These methods, using organophosphate nematicides, are simple, practical and provide efficient extraction of *X. index* from soil using Baermann funnels.

Poster Presentations
Nematodes

P NEM 12

Resistance in sunflower to South African root-knot nematode species

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Sunflower is an important oil-seed crop in South Africa and is parasitized by root-knot nematodes, which is an economical-important pest in local crop-production areas. At present no synthetically-derived nematicide is registered for nematode control *per se* on sunflower and the crop is used in maize cropping systems, with potato and soybean also being included as rotation crops. All these crops are highly susceptible to root-knot nematode pests. Genetic host-plant resistance is an effective management method in keeping root-knot nematodes below economic threshold levels. Currently there are no known root-knot nematode-resistant sunflower cultivars available on the South African market. The objective of this study was to test 21 commercially-available sunflower cultivars from various seed companies for resistance to *Meloidogyne incognita* and *M. javanica* in the greenhouse, respectively. A susceptible tomato cv. Floradade was included in both trials as the susceptible standard. Parameters used to select for resistance included reproduction factors (Rf), egg-laying female (ELF) indices and number of eggs and second-stage juveniles (J2) per root system. Nematode data was collected 56 days after inoculation and subjected to statistical analysis (ANOVA). The degree of resistance was assigned to each genotype according to Canto-Saenz's quantitative scheme. Tomato plants in both experiments had high root-knot nematode parameters, indicating the successful execution of the experiments. Substantial variation existed among the sunflower cultivars in terms of their host status to the two respective *Meloidogyne* spp. Cultivar Procol 101CL showed resistance to *M. incognita* since it had a mean Rf of 0.5, ELF-index value of 0.6 and 2 534 eggs and J2 per root system. None of the sunflower cultivars tested, however, exhibited resistance to *M. javanica*. This is the first report of root-knot nematode-resistance in sunflower in South Africa. This sunflower cultivar can be included in the sunflower breeding programme to breed for root-knot nematode resistance. Ultimately, it can already be used by producers to reduce population levels of *M. incognita* where it poses problems and this way contribute towards sustainable crop production.

P NEM 13

Efficacy of three species of entomopathogenic nematodes against the corn stem borer, *Sesamia cretica* Led. (Lep.: Noctuidae)

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The corn stem borer, *Sesamia cretica* Led. (Lepidoptera: Noctuidae), is a main pest of maize in Iran. Attacks by *S. cretica* are usually high on early plantations of May, when the first generation of adults emerges after a period of larval hibernation. The present study aimed to evaluate the efficacy of three species of entomopathogenic nematodes including *Heterorhabditis bacteriophora*, *Steinernema carpocapsae* and *S. feltiae* as biological agents. During the study, the responses of larvae at 25±2°C for three periods of 24, 48 and 72 h with different concentrations of 0, 50, 100, 200, 400, 800, 1600, and 3200 third instar larvae of nematode (infective stage=IJs) per milliliter into 10 cm Petri dishes containing filter paper soaked with 1 ml of nematodes suspension was performed. Also the stem contains a number of pest larvae soaked in a 10 ml suspension and placed in plastic containers. The results showed that highest mortality caused by these nematodes was 100% both in stem and Petri dishes. With increasing nematode population level and exposure time mortality of *P. xylostella* larvae was increased. Based on the obtained results *S. feltiae* species caused the greatest mortality both in stem and Petri dishes condition and then *S. carpocapsae* and *H. bacteriophora* had the highest effect. Among the tested three times of exposure, 72 hours has caused the highest mortality in *S. cretica* larvae. In general it is recommended to apply these nematodes in suitable condition for controlling the corn stem borer.

Poster Presentations

Nematodes

P NEM 14

Efficacy of entomopathogenic nematode, *Heterorhabditis bacteriophora* against the diamondback moth, *Plutella xylostella* (L.) in laboratory condition

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The diamondback moth, *Plutella xylostella* (L.), is a global pest which has a large spread in tropical and subtropical and around the world does damage to cabbage and other plants of Brassicaceae family. This pest is able to attack their hosts in the greenhouse and field. In vitro studies was carried out on the diamondback moth, *Plutella xylostella* larvae using an entomopathogenic nematode isolate, *Heterorhabditis bacteriophora* obtained from the Koppert company, Netherlands. Larvae of *P. xylostella* were collected from cabbage farms around Mashhad city of Iran. During the study, the responses of larvae at 25 °C for three periods of 24, 48 and 72 h with different concentrations of 0, 50, 100, 200, 400, 800, 1600, and 3200 third instar larvae of nematode (infective stage=IJs) per insect into 10 cm Petri dishes containing filter paper soaked with 1 ml of nematodes suspension was performed. Maximum mortality caused by *H. bacteriophora* nematode was 60% at 24 h, 80% at 48 h and it was 100% at 72 hours. With increasing nematode population level and exposure time mortality of *P. xylostella* larvae was increased. Based on probit analysis, LC₅₀ values of *H. bacteriophora* nematode in three test periods were 1484.57, 544.97 and 242.67 IJs per insect, respectively. Initial ANOVA was performed for *H. bacteriophora* nematode. The effect of both nematode population levels (IJ) and exposure time (ET in hour) on third instar larvae of the diamondback moth, *P. xylostella* (df = 6; F <0.001) and (df = 2; F <0.001) was significant respectively. In general it is recommended to apply this nematode in suitable condition for controlling diamondback moth.

P NEM 15

Potency of entomopathogenic nematodes on the tomato leaf miner *Tuta absoluta* (meyrick) (Lepidoptera - Gelechiidae)

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The tomato leaf miner *Tuta absoluta* (meyrick) is considered as causing the greatest loss of tomato plantations in Egypt since its introduction in 2009. The potential of entomopathogenic nematodes in suppressing the insect developmental stages has been evaluated under laboratory conditions. The tested native isolates were *Heterorhabditis bacteriophora* (Hb), *Heterorhabditis indica* (Hi), *Steinernema carpocapsae* (Sc) and the imported isolate *Heterorhabditis bacteriophora* (Hb88). It appears that the first larval instar is more susceptible to infection with all tested nematodes strains judged from the LC50 values. These values were 57.17, 57.61, 62.57 and 72.49 IJs after treatment with *H. bacteriophora* (Hb), *H. bacteriophora* (Hb88), *H. indica* (Hi) and *S. carpocapsae* (Sc), compared to 58.9, 61.95, 114.7 and 76.95 IJs, after treatment of fourth larval instar with the same isolates, respectively. The pupal stage was least susceptible. Value of LC95 run in the same way. So these nematodes can be recommended for use in IPM strategies and which may alternate the application of hazardous chemical insecticides.

P NEM 16

Effects of amino acid treatments on nematodes

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Amino acids (aa) are naturally occurring substances that are important for all living organisms. The application of certain aa at different concentrations affects various life stages of a broad spectrum of nematode species. However, the mechanistic details for the observed effects remain elusive till now. To investigate this question, we analyzed the effects of methionine (Met), lysine (Lys), threonine (Thr), isoleucine (Iso), 2-ketobutyric acid (Ket), homoserine (Hom) and tryptophan (Try) on the free-living nematode *Caenorhabditis elegans* and the plant parasite *Heterodera schachtii*. The activity and development of *C. elegans* was decreased by Try applications. No aa had an effect on the activity of *H. schachtii*. Interestingly, soaking J2 stage nematodes in aa solutions for twenty-four hours, led to more female nematodes per plant for Lys, and less for Thr. The strongest effects were observed when aa were supplemented to the nutrient-medium in a monoxenic culture of the host plant, *Arabidopsis thaliana*. This approach reduced the number of female nematodes per plant for Iso, Met, Thr, and Ket. Additionally, slight negative effects could be detected on the adult female sizes. Interestingly, these effective aa all belong to a group of metabolites that are

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derivatives of Hom. In this study we were able to separate the direct effects of aa applications to pre-infective juveniles, from effects that may also involve the host plant.

P NEM 17

Potential of *Crotalaria juncea* as cover crop for nematode suppression under Central Europe conditions

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In Central Europe, cruciferous cover crops are grown for suppression of plant-parasitic nematodes prior to planting a main crop for example sugar beet. However, repeated cropping of crucifer in rotations can increase incidence of some soil-borne diseases, e.g., clubroot, and the availability of alternative cover crops is highly desirable. The tropical legume *Crotalaria juncea* acts as non-host or resistant host to several plant-parasitic genera, produces nematotoxic secondary metabolites, and can enrich soils with nitrogen. The potential for cultivating *C. juncea* under Central Europe climate conditions for nematode-suppressing purposes was examined. Two cultivars of this plant were available for this study. In a first step under controlled conditions, the host status of *C. juncea* to *Globodera pallida*, *G. rostochiensis*, *Heterodera avenae*, *H. filipjevi*, and *H. schachtii* was examined. In a second step, two planting date studies were conducted plots: (I) in steamed soil in small plots, (II) in steamed soil inoculated with *H. schachtii* after the soil heating in microplots. In each experiment, Rhizobium-inoculated seeds were planted at the beginning of April, May, June, July and August. Plant measurements including height and nodulation were taken during the season and at harvest in the second half of September. In the nematode-infested plots, nematode population densities were determined at plant harvest and one month after incorporation of the plant material. Overall, *C. juncea* grew well after most planting dates and was a non-host or poor host for the cyst nematodes tested. Infectivity of *H. schachtii* was low in the microplot experiment. The combined benefits of nitrogen fixation with nematode suppression of this tropical legume appear a valuable enrichment of intensive productions systems.

P NEM 18

Influence of aqueous ozone treatments on tomato-*Meloidogyne incognita* interaction

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Annually significant tomato yield losses are caused by climate changes and pests and diseases attacks. Root-knot nematodes (*Meloidogyne* spp.) are the most damaging pests on the base of their wide distribution in tropic and sub-tropic climates and their wide host range. They modify host root tissue using effector proteins to create feeding sites as their source of nutrition. To develop alternative control strategies to nematode infections, we have considered aqueous ozone treatments (AOT) as a possible tool. Although gaseous ozone is phytotoxic, previous studies demonstrated that ozone in aqueous phase is effective to contain pests. The purposes of this study were to confirm the impact of AOT on tomato-*M. incognita* interaction and to analyse its role in plant defence response.

Ozonated water was produced *in situ* by an ozone generator at 10 ppm and directly applied to tomato roots. Treatments were performed daily (10 ml/pot) for 4 days as soil drench on 14 days old tomato plants maintained in a growth cabinet (25 ± 2 °C). Nematode infection control by ozone treatments was set up by infecting untreated and treated plants with J2s of *M. incognita*. A number of plants were used 2, 4 and 7 days after nematode infection for biochemical and molecular analysis. Other plants were transferred in a glasshouse at 25 ± 2 °C and after 60 days they were uprooted and dry shoot and root fresh weights recorded. Root gall index, eggs and J2s/g root, total nematode population density and reproduction rate were evaluated. AOT significantly decreased severity of root gall index and soil nematode population in comparison to untreated plants.

Tomato sensitivity to AOT was verified as reactive oxygen species production (ROS) in both treated and untreated root galls and compared with uninfected treated and untreated roots. A higher significant ROS production was observed in ozone treated uninfected roots in comparison to treated and untreated infected roots. As antioxidant mechanisms play an important role in the response of plant to the combination of abiotic and biotic stresses, the effect of AOT on the expression of different genes involved in ROS scavenging, such as CAT, SOD and APX, was also evaluated. AOT by altering gene expression, ROS production and inducing a non specific defense response can be considered a useful tool to contain nematode infection.

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The fungicide fluopyram exhibits nematocidal activity toward *Rotylenchulus reniformis*

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Multi state trials observed cotton (*Gossypium hirsutum* L.) cultivar and nematicides as they influenced reniform nematode (*Rotylenchulus reniformis*) population development and cotton yield. Stoneville 4946 B2RF (ST4946) is tolerant to reniform nematode and Fiber Max 1944 GLB2 (FM1944) is highly susceptible and these cultivars were selected based on these yield performances. Two standard nematicide treatments, Temik 15 G (aldicarb), applied as an in furrow granule at 4.4 kg/ha was included as the historical standard, and Aeris (thiodicarb) applied as a seed treatment at 0.75 mg ai per seed was included at the standard seed treatment nematicide. Velum Total (imidacloprid + fluopyram) was applied at planting as an in furrow spray (216 ml/ha) over the seed treatment Aeris (0.75 mg ai/seed). Gaucho + Fluopyram (0.375 mg ai/seed + 0.175 mg ai per seed) comprised a new premium seed treatment nematicide option. Aeris (0.75mg ai per seed) followed by a foliar sprays of Vydate CLV (205 ml/ha at the 2 - 8 leaf stage). Gaucho 600 (0.375 mg ai/seed) represented the non nematicide control and was included to assess the nematode disease pressure in each field. Analysis of the data found no significant cultivar by nematicide interactions indicating the nematicide treatment responses were similar on both cotton cultivars. Cotton plant stand was similar across cultivars. The nematode tolerant variety ST 4946 vigor ratings were similar to the susceptible variety FM 1944. Nematicides did affected vigor with the Velum Total in furrow spray over the Aeris seed treatment, and Aeris seed treatment plus the foliar Vydate CLV spray, and the industry standard Temik 15 G supporting the most vigorous plants compared to the Gaucho seed treatment. ST 4946 supported lower nematode population densities than FM 1944 at the 30 day after planting sampling period. The Velum Total plus Aeris nematicide treatment reduced nematode densities ($P < 0.05$) 54% compared to Gaucho seed treatment. Seed cotton yields were affected by cultivar and nematicide. ST 4946 produced 5% more seed cotton than the susceptible variety FM1944. Velum Total plus Aeris produced similar yields to Temik 15G which was significantly more cotton than the Gaucho seed treatment. The Velum Total plus Aeris increase was 10-40 % higher or an average of 275 kg/ha of seed cotton averaged over all tests.

P NEM 21

A best way of chemical epigenetics to enrich secondary metabolites of *Aspergillus niger* y-61 with nematocidal activity

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A fungal metabolites from strain of *Aspergillus niger* y-61 can be used to control the root knot nematode but no more value compounds were obtained from metabolites indicated by bioassay. Many of bioinformatically discovered secondary metabolism gene clusters are kept silent under standard laboratory conditions including fungi. The chemical epigenetics is a useful way to activate the dormant of secondary metabolite biosynthetic pathways. In our earlier studies on the secondary metabolites of *A. niger* y-61, oxalic and citric acids with the function of anti-nematode have been identified. To obtain new compounds from this strain, the DNA methyltransferase inhibitor 5-azacytidine were used in medium with concentration of 50 μ M and the result of test showed the secondary metabolite products have changed a lot compared with control samples, the products from treated by 5-azacytidine one including Tensyucic acid C, Pyrophen, Cyclo-L-Ala-L-Leu, Aspernigrin A and a new compound were found; The secondary metabolite products from untreated control samples showed no one of them have above compounds appears. All of the metabolites products have nematocidal activity against second Junile (J2) of *Meloidogyne incongnita* *in vitro* test. The result confirmed that the use of chemical epigenetic modifies is an effective technique for screening and discovering new bio-agents by promoting the expression of silent biosynthetic pathways to produce unique secondary metabolites from fungi.

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Nematicidal effect of composted organic wastes and fertilizer applications on potato-cyst nematodes

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The addition of organic material to soil can be an effective alternative to environmentally unsafe chemical treatments for the control of plant parasitic nematodes. The effects of vermicompost and aqueous solutions of vermicompost (vermicompost tea) alone or mixed with urea on the development and survival of two potato-cyst nematodes, *Globodera rostochiensis* (pathotype Ro1) and *G. pallida* (pathotype Pa2) and on the growth parameters of the host potato plants were evaluated. Amendments to the soil with these materials significantly decreased the number of cysts/100 g of soil, the number of eggs and juveniles/cyst and the number of eggs and juveniles/g of soil of both species in comparison with the untreated controls. The suppressive effect was significantly higher at the highest than the lowest treatment dose for all tested materials. *G. rostochiensis* was more sensitive than *G. pallida* to all tested materials. The aqueous solutions of vermicompost alone or in combination with urea were more effective than the solid vermicompost for controlling both species. Vermicompost and the vermicompost teas had positive effects on plant fresh stem weight and stem height. The application of vermicompost tea substantially decreased the amount of material needed compared to the solid vermicompost. These amendments are thus promising for the control of potato-cyst nematodes in sustainable agricultural systems.

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P NEM 23

Host-status and host-sensitivity of African ginger and African geranium to *Meloidogyne incognita*

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Sustainable development in the context of international restrictions of synthetic nematicides calls for urgent introspections on uses of indigenous plants as alternative crops for management of highly destructive root-knot (*Meloidogyne* species) nematodes. African ginger (*Siphonochilus aethiopicus*) and African geranium (*Pelargonium sidoides*) - indigenous to Southern Africa, have attractive attributes to serve as alternative crops. However, the degree of nematode resistance in the two plant species is not documented. Two separate tests were conducted to assess the degree of nematode resistance in the two plant species. In each trial three-week-old seedlings were inoculated with 0, 35, 70, 175, 350, 875, 1750 and 4375 eggs and second-stage juveniles of *M. incognita*, arranged in a randomised complete block design, with 6 replications. Fifty-six days after inoculation, the reproductive factors (RF) for *M. incognita* on *S. aethiopicus* were less than one, whereas, *P. sidoides* recorded RF values above one at higher inoculum levels. Generally, when RF is less than one and nematode infection does not lead to plant injury, the target plant is viewed as being resistant to the test nematode. Also, when the RF values above one occur and the plant does not suffer any damage, the target plant is tolerant. In conclusion, *S. aethiopicus* and *P. sidoides* were resistant and tolerant, respectively, to *M. incognita*. Therefore, *S. aethiopicus* is an ideal crop for use as an alternative crop in areas with high *M. incognita* levels, whereas *P. sidoides* is not suitable for use in crop rotation systems since it will inherently enhance the build-up of nematode population densities for subsequent crops.

P NEM 24

Degree of nematode resistance in *Moringa oleifera* and *Artemisia annua* to *Meloidogyne incognita*

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Moringa oleifera and *Artemisia annua* are widely used as alternative crops in various tropical and subtropical regions, particularly in developmental projects for ameliorating malnutrition and malaria, respectively. However, the root-knot (*Meloidogyne* species) nematodes are detrimental in the successful implementation of crop-related developmental projects. Increasing restrictions on synthetic nematicides have since dictated that in any crop-related developmental projects the

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potential uses of host resistance in nematode management be assessed prior to large scale production. Two trials were established separately to determine the host-status and host-sensitivity of *M. oleifera* and *A. annua* to *M. incognita*. Eight nematode levels were arranged in a randomised complete block design, with 6 replications. Fifty-six days after inoculation, the reproductive factor (RF) values on both plants were below unity at all levels of inoculation and there was no nematode effect on plant growth and development. In plant nematology, when RF is less than unity and the host plant does not suffer any yield loss in response to nematode infection, the test plant is viewed as being resistant to the test nematode. In conclusion, *M. oleifera* and *A. annua* were resistant to *M. incognita* and could therefore serve as alternative crops for management of population densities of *Meloidogyne* species in smallholder farming systems of South Africa.

P NEM 25

Characterization of nematode pests of Enset (*Ensete ventricosum* Welw. Cheesman) and their management

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Enset (*Ensete ventricosum* Welw. Cheesman) is an important starch staple crop, cultivated primarily in southern and south west Ethiopia. Related to the banana family, enset is similarly infected by plant parasitic nematode. From previous survey studies *Pratylenchus goodeyi* appears to be the dominant nematode pest, which is believed to contribute to reduced productivity of enset. However, while surveys have demonstrated high *P. goodeyi* infection levels, there is relatively scant information on how damaging the nematode is to enset production. There is also little information on the variability of the nematode pest in terms of levels of pathogenicity on enset and if so, how this may relate to variability in climate and temperature zones under which enset is grown. Our study is being undertaken to assess the possible damage of nematode pests, with emphasis on *P. goodeyi*, and in relation to the presence of other diseases and how climate and agro ecology may affect this.

P NEM 26

Occurrence and geographical distribution of root lesion nematodes, *Pratylenchus thornei* and *Pratylenchus neglectus*, associated with wheat in Turkey

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Root lesion nematodes (RLN), *Pratylenchus thornei* and *P. neglectus* are widespread among wheat fields with high population density, particularly where cultivation is intensive and wheat is grown after wheat without crop rotation in Turkey. The occurrence of root lesion nematodes (RLN) among major cereal growing areas in Eastern Mediterranean, Central Anatolian, and Eastern Anatolian, Marmara and Ege region were mostly observed. *P. thornei* and *P. neglectus* were identified as the common root lesion nematodes in these regions. The cereal cultivating areas in Eastern Mediterranean, Central Anatolian, and Eastern Anatolian, Marmara-Ege region were infected by root lesion nematodes 83.5, 37.0, 33.0 and 73,23 %, respectively. Population densities of *P. thornei* and *P. neglectus* were low during the cold snow period and it increases gradually until June/July, whereas it rapidly decreases over the summer period in Central Anatolian. Root lesion nematodes are known to cause yield losses by 19-32% in Turkey. Because of wide host range and lacking information on resistance, there are few chances to control these nematodes by crop rotation and use of resistance cultivars. Nowadays, most of studies have been focused on screening of wheat germplasm for resistance for *Pratylenchus thornei* and *P. neglectus*.

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Root-knot nematodes, *Meloidogyne* spp. from AL-Qassim Fields, Saudi Arabia

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Root-knot nematodes, *Meloidogyne* spp. are the most important group of plant parasitic nematodes that occur in Saudi Arabia. A total of 11 populations of *Meloidogyne* spp. were collected from representative horticultural fields of Al-Qassim region of Saudi Arabia and maintained at the Experimental Center of College of Agriculture and Veterinary Medicine, Qassim University. Populations were collected from the root system and a single egg mass of each population were picked and reproduced by inoculating pot-growing tomato. *Meloidogyne* species were characterized using perineal morphology. The result indicated the presence of *M. incognita* (8), *M. javanica* (3). *Meloidogyne incognita* populations were tested for identifying races of the nematodes using modified North Carolina Differential Host Test. Races 2 and 4 of *M. incognita* were identified. These findings are essential for developing cropping system designed for Root-knot management in Al-Qassim Fields.

P NEM 28

Revealing the resistance response of common bean genotypes to the root knot nematode

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Common bean (*Phaseolus vulgaris* L) is important food crop with a rich source of nutrient contents; however they are highly susceptible to root knot nematodes. Characterization the resistance reaction of common bean sources against root knot nematodes is extremely important in terms of improving resistant cultivars. In this study, over 80 common bean genotypes were screened against the root knot nematode to reveal the resistance response under the growth chamber conditions. The evaluation was determined by using the root galling severity, the nematode egg mass production, roots and shoot weight. Results indicated that 14% of common bean genotypes were found to be resistant (immune + resistant and moderately resistant) and 60% of bean genotypes were shown to be susceptible (moderately susceptible + susceptible + highly susceptible) reactions. Moreover, positive correlation was observed between the resistance and plant weight. This work provides important information for plant breeders to enable the nematode resistant common bean materials for breeding studies.

P NEM 29

Long-introduced woody plants in insular botanical garden: the effect on the complex of plant-parasitic nematodes

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The effects of prolonged cultivation of introduced plants on soil nematode communities in general and on plant parasites in particular have not been studied enough. Investigation of parasitic nematodes has the high practical significance for agriculture and plant growing: they may represent quarantine objects, pests dangerous to crops, virus transmitters, etc. The aim of this study was to explore the complex of plant-parasitic nematodes in the rhizosphere of introduced woody plants in botanical gardens as compared with that in natural forest habitats. Surveys were carried out in the botanical garden on Valaam Island (Lake Ladoga, Republic of Karelia, Russian Federation, 61°23'25" N, 30°57'17" E). Soil samples were collected from the rhizosphere of 10 introduced tree species: *Abies balsamea*, *Abies sibirica*, *Pinus sibirica*, *Larix sibirica*, *Acer platanoides*, *Fraxinus excelsior*, *Quercus robur*, *Tilia platyphyllos*, *Ulmus glabra*, *Malus domestica*. Natural biocenoses (*Picea abies* and *Pinus sylvestris* forests) were taken for reference. Nematode extraction from the soil, fixation and identification were performed according to classical methods. The soil nematode fauna associated with introduced woody plants on Valaam Island was represented by 65 taxa; ten of them were obligate and five were facultative plant parasites. Nematode species rare for Karelia were found: *Cephalenchus leptus*, *Longidorus elongatus*, *Paratrichodorus pachydermus*. Plant parasitic nematodes accounted for 8.0 to 48.5% of the total fauna. The highest diversity and numeric dominance of this group in the nematode community were detected under young *Abies balsamea* (7 taxa, 48.5%) and in the orchard with *Malus domestica* (7 taxa, 41.6%). Natural forests, on the contrary, featured a prevalence of nematodes associated with plants, and a low contribution of plant parasites to the fauna (0.1-5.9 %). It

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was established that prolonged cultivation of plants introduced into botanical gardens affected soil nematode communities: plant-parasitic nematodes became more diverse and abundant; their contribution to the community structure was obviously increased as compared with natural forests. The study was co-funded by the Russian Foundation for Basic Research, grant № 15-04-07675.

P NEM 30

Dimethyl Disulfide (DMDS) in the Control of the Cyst Nematode *Heterodera carotae* on carrot in Italy

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Dimethyl disulfide (DMDS), found in nature in several plants belonging to *Alliaceae* and *Brassicaceae* families, is able to control soilborne pathogens, weeds and phytoparasitic nematodes. To verify its efficacy three field trials were carried out on carrot in Abruzzo, Apulia and Lazio regions (Italy) (trials A, B and C, respectively) against the carrot cyst nematode *Heterodera carotae*. The soil was subdivided in plots 50 x 4.8 m for shank application with machines. Each plot was subdivided in 5 sub-plots to provide replications for each treatment. In trials A and B treatments were: i, ii and iii, DMDS as shank formulation (Accolade 99.1%) at the rates of 180, 280 and 380 L/ha. In the trial C treatments were DMDS at rates of 280 and 470 L/ha. In all trials 1,3 D (Telone 97) at 140 L/ha was used as standard with untreated as controls. DMDS was applied three weeks before sowing in a wet soil (70% of the water holding capacity, soil T > 20 °C). Fumigated plots were covered with plastic films virtually impermeable (VIF) for 2 weeks. One week later soil aeration, carrot seeds were sown in each plot. Soil samples were collected before and after treatments and at the carrot harvest. Cysts were extracted by the Fenwick can and their number/100 g dried soil and number of eggs and juveniles inside cysts determined. In the trial B, after fumigations, the extracted cysts from soil of all plots were subjected to an hatching test to verify the vitality of the eggs inside the treated or untreated cysts. At the harvest the weights of marketable and unmarketable carrots were recorded. After treatments and at the harvest no significant differences were evident among treatments including the untreated controls in the number of cysts/100 g dried soil. However, from the hatching tests resulted that the number of viable eggs in the untreated cysts was significantly higher (35%) than those in the different treatments (0.01 - 0.36%). Marketable carrot yield in all treatments and trials was significantly higher than those in the untreated controls. In the trials A and C no significant difference was evident among the DMDS tested rates also compared with 1.3 D, whereas in the trial B the significant highest marketable carrot yield was observed in the highest DMDS rate (380 L/ha). Finally, from our data, DMDS can be considered a very effective fumigant to control carrot cyst nematode.

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Dimethyl Disulfide (DMDS) in the Control of the Cyst Nematode *Globodera pallida* on potato in Italy and in The Netherlands

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The potato cyst nematode (PCN) *Globodera pallida* causes considerable yield losses to the main Italian and Dutch potato growing areas. Dimethyl disulfide (DMDS), on the base of recent studies can control phytoparasitic nematodes, soilborne pathogens and weeds. Two field trials were carried out to verify its efficacy on potato in Italy (Conversano, Bari province) and in The Netherlands (Nieuwe Pekela, Groningen province) (trial IT and NL, respectively) against *G. pallida*. The soil was subdivided in plots of 200 m² for shank application with machines. Each plot was subdivided in 5 (IT) or 4 (NL) sub-plots to provide replications for each treatment. In both trials DMDS treatments were: i, ii and iii, Accolade in shank formulation (DMDS, 99.1%) at the rates of 180, 280 and 380 L/ha. Mocap (ethoprosfos 10%) was used at 100 Kg/ha and Monam (metam sodium 510 g/L) at 300 L/ha, as standard controls in Italy and The Netherlands, respectively. The lowest dose of DMDS in the trial NL was also applied mixing the

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soil by a spading machine and then pressing it with a roller. In both trials untreated soils were used as controls. DMDS was applied three (IT) and five (NL) weeks before sowing. Fumigated treated plots were covered for two (IT) and four (NL) weeks with plastic films virtually impermeable (VIF) with the exception of the metam-Na. One week later soil aeration, potato were sown in each plot. Soil samples were collected before and after treatments and at the potato harvest. Cysts were extracted by the Fenwick can and their number/100 g dried soil and number of eggs and juveniles inside cysts determined. At the harvest the weights of potato tubers were recorded. No significant differences were evident among treatments including the untreated controls in the number of cysts/100 g dried soil in the I trial. In NL trial all treatments significantly reduced the number of cysts in comparison to the untreated control. Marketable potato yields (MPY) in all DMDS and Ethoprosfos (IT) and metam-Na (NL) treatments were significantly higher than in the untreated control. Among DMDS treatments at different rates in both trials no significant differences were recorded. MPY in Ethoprosfos treatment was significantly lower than those recorded in all DMDS treatments. Concluding, DMDS treatments could be positively and favourably considered in PCN sustainable control.

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Status of the root-knot nematodes, *Meloidogyne* spp. (Goeldi) in Turkey

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Root-knot nematode (*Meloidogyne* spp.) are one of the most important harmful agent in many economically important crops in world. These nematodes pose a serious agricultural threat due to their large host range and because many plants lack natural nematode resistance mechanism. In temperate climates of the world *Meloidogyne incognita*, *M. javanica* and *M. arenaria*, whereas *M. hapla*, *M. chitwoodi* and *M. fallax* are seen as common in the cool climate. So far 8 root-knot nematode species, *Meloidogyne incognita*, *M. arenaria*, *M. chitwoodi*, *M. exigua*, *M. hapla*, *M. javanica*, *M. thamesi*, *M. ethiopica* and *M. artiellia*, were found in various agro-ecological regions in Turkey. Results of a survey connected with 79 *Meloidogyne* samples showed that four root-knot nematode species, *M. incognita*, *M. arenaria*, *M. javanica* and *M. chitwoodi* were the most common in Turkey in range of 28, 27, 35 and 10%, respectively. Several races of some above mentioned species had been identified in various agro-ecological regions in different particularly vegetable and horticultural crops in Turkey. The studies indicated that *M. incognita* race 1, race 2, race 4, race 5, race 6; *M. arenaria* race 2, race 3; *M. javanica* race 1, race 5 and *M. chitwoodi* race 1, race 2, were found in Turkey. There are many tomato varieties having resistance gene (*Mi*), also grafted seedlings on the market. Recently alternative control methods like soil solarization alone, solarization+low dose fumigant, solarization+biofumigation applications are being recommended for greenhouses in Turkey.

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Influence of nemarioc-al phytonematicide soil residues on rhizobium nodulation, growth of *Vigna unguiculata* and population of root-knot nematode

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Nemarioc-AL phytonematicide consistently stimulated growth of tomato (*Solanum lycopersicum*) and suppressed population densities of *Meloidogyne* species under diverse environments. However, information on its soil allelochemical residual (SAR) effects on growth of successor crops and related population nematode densities is scant. A field trial was therefore conducted to investigate the SAR effects of nemarioc-AL on growth of cowpea (*Vigna unguiculata*) as a successor crop and the related population nematode densities. The SAR conditions were created in a 5 × 5 factorial tomato experiment, with the first and second factors being concentrations and application intervals, respectively. The concentration × application interval contributed 21% to total treatment variation (TTV) in number of nodules, with permutations consistently reducing number of nodules. The concentration factor contributed 12% and 8% to TTV in dry seed mass and number of nematodes, respectively, increasing (39-83%) dry seed mass and decreasing (88-94%) nematode densities. The application interval contributed 7% to TTV in dry shoot mass and number of nematodes each, increasing (39-69%) dry shoot mass and reducing (45-92%) nematode densities. In

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conclusion, appropriate permutations of concentration and application interval are necessary to allow for cowpea production as a successor crop to a tomato crop after using nemarioc-AL phytonematicide.

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Biological efficiency of Fluopyram on potato tuber against Colombia root knot nematode (*Meloidogyne chitwoodi*) in Turkey

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The Columbia root-knot nematode (*Meloidogyne chitwoodi* Golden et al.) is a quarantine organism in Turkey as well as Europe. This nematode is of economic importance to several crops, especially in potatoes, that tuber quality is seriously decreased. *M. chitwoodi* is identified in potato fields in Niğde, Nevşehir and İzmir, where almost half of total potato production in Turkey is located. Unfortunately, there is no registered product to control this nematode in Turkey. In this study biological efficiency of Fluopyram product (Velum Prime 400SC) against *M. chitwoodi* had been assessed on potato tuber in naturally infested fields in Niğde and İzmir. Fluopyram was sprayed onto soil just before planting of potatoes, using 60 ml+60 ml/da (divided dose, applied at planting time and one month after) and 80, 100, 120 ml-cc/da doses (at planting). This experiment was performed in a completely randomized design with four replications. Tuber evaluation was done at harvest time and two months after harvest, using a 0-6 scale to describe tuber quality. The result clearly showed that the efficacy of Fluopyram against root knot nematode damage on tubers was 78.35, 75.25, 82.02 and 93.06 % in Niğde, 83.65, 69.46, 76.21, 89.19 % in İzmir respectively ($P < 0.05$). The findings of these experiments show, that using Fluopyram is a practical and effective way to control the *M. chitwoodi*.

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Population dynamics and damage threshold levels of *Meloidogyne hapla* to rose rootstocks

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Introduction: Ethiopia developed to one of the top producers of roses worldwide. However, this booming business is facing severe threats such as caused by the root-knot nematode *Meloidogyne hapla*. Ensuring high quality rose production in the future requires management strategies for *M. hapla*. One such strategy is seen in resistant and/or tolerant rose rootstocks.

Objectives: To study the population dynamics and damage threshold levels of *M. hapla* on common rose rootstock species.

Materials and methods: In greenhouse experiments, the relationship between initial population densities (P_i) of *M. hapla* on growth of three rose rootstocks (*Rosa corymbifera* 'Laxa', *R. multiflora* and *R. canina* 'Inermis') and nematode population development was studied. Each plant species was inoculated with ranges of nematode densities of 0, 0.062, 0.125, 0.25, 0.50, 1, 2, 4, 8, 16, 32, 64 and 128 second-stage juveniles (J2) g^{-1} soil and were allowed to grow for 80 days. At evaluation, plant growth and nematode parameters were recorded. Population dynamics of *M. hapla* were calculated based on the Seinhorst population model ($P_f = (M * P_i) / (P_i + M/a)$) and damage threshold levels were calculated using the Seinhorst yield model ($y = Y_{max} * (m + (1 - m) * 0.95^{(P_i - T/P_i)})$).

Results: Tolerance limits (T) for *M. hapla* were 0.04, 0.09 and 0.01 J2 g^{-1} soil and minimum yields (m) were 0.65, 0.471 and 0.427 for *R. corymbifera*, *R. multiflora* and *R. canina*, respectively. The reproductive factor (P_f/P_i) was higher at low initial nematode population densities for all rootstocks and then decreased to below maintenance level with increasing initial population density. Root gall severity consistently increased with initial nematode population density. Further, number of root-galling against final nematode population per gram root fresh weight showed a strong positive relationship. *Rosa multiflora* supported the population of *M. hapla* to a maximum population density of (M) 27.53 J2 g^{-1} soil with an estimated average multiplication rate (a) of 24.39. For *R. corymbifera* and *R. canina* the maximum population densities were 6.08 and 4.78 J2 g^{-1} soil and the multiplication rates 4.34 and 3.62, respectively.

Conclusions: It was demonstrated that all three rootstocks are sensitive to even low initial nematode densities and are excellent host for *M. hapla*.

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Identification and Incidence of Plant Parasitic Nematodes at Vegetable Production Areas in Lakes Region of Turkey

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Lakes region having a number of lakes is located in the high plateau of the western Taurus Mountains of West Mediterranean region. This region is very important for vegetable production areas as significant portion of total vegetable needs of Turkey on the summer and early fall season. Plant parasitic nematodes and soilborne pathogens have a limiting effect on the vegetable production. Also, there was limited knowledge about plant parasitic nematodes in this region.

The aim of this study was to determine identification and incidence of plant parasitic nematodes in the Lakes region. A hundred sixty soil and root samples were collected from intensive vegetable growing areas in September and October, 2014. Free living and migratory nematodes were extracted from soil by using modified Baermann Funnel technique and sedentary endoparasitic root-knot nematodes were obtained from roots under stereo binocular microscope by using a small needle and forceps. Free living and migratory nematodes were made preparation according to Seinhorst (1959) procedure and identified by morphological characters. For root-knot nematodes, perineal pattern model was prepared into a drop glycerine and processed as described by Hartman and Sasser (1985).

Meloidogyne spp., *Pratylenchus* spp., *Geocenamus* spp., *Paratylenchus* spp., *Helicotylenchus* spp. and *Tylenchus* spp. were common plant parasitic nematodes in vegetable growing areas of Lakes region and their frequencies were detected 51.8 %, 31.2 %, 38.7 %, 8.1 %, 16.8 % and 21.3 %, respectively. Root-knot nematodes were the most important genus and caused significant crop losses in the region annually. Dissemination ratios of root-knot nematodes were 39.1 %, 26.5 %, 23.4 and 3.2 % for *Meloidogyne hapla*, *M. incognita*, *M. javanica* and *M. arenaria*, respectively. The other 7.8 percent of root-knot nematodes were not identified according to perineal pattern model. Additionally, *Pratylenchus penetrans* formed high population densities in several locations having sandy loam soil and caused serious crop damage with soil borne fungal and bacterial pathogens.

P NEM 37

Morphological and molecular characterisation of *Meloidogyne hapla* populations from roses in Ethiopia

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Introduction: Roses are grown in more than 80% of the existing cut-flower producing farms in Ethiopia, and are increasingly facing serious nematode problems. During a survey in 2011 and 2012, *Meloidogyne hapla*, considered mainly a temperate species, was detected in several greenhouses in Ethiopia parasitizing rose plants.

Objectives: To characterize the *M. hapla* populations from Ethiopia using morphological and molecular parameters and compare them with populations from outside Ethiopia.

Materials and methods: Soil samples were collected from 12 randomly selected farms distributed in six districts around Addis Ababa, Ethiopia. Pure isolates of 125 *Meloidogyne* spp. from six farms were established from single egg masses on tomato cv. Moneymaker. Morphological characters based on light and scanning electron microscope images together with morphometric measurements of females, males and second-stage juveniles (J2) were compared with populations of *M. hapla* from different countries. In addition, molecular characterization was performed based on the 28S D2-D3 expansion segments within the ribosomal DNA and the region located between cytochrome oxidase unit II and the 16S rRNA gene of the mitochondria (mtDNA).

Results: Based on molecular and morphological data, eighty-two of the isolates were identified as *M. hapla*. Morphological characters of females, males and J2 were in line with descriptions of other *M. hapla* populations with few exceptions. The female perineal pattern of the Ethiopian populations fitted the original description but was smaller than that of the population described by Jepson. J2 body size was larger compared to previous descriptions from Hawaii and East Africa and the *a* ratio value was much greater than for the East African population but similar to the Hawaiian population. Phylogenetic relationships of the Ethiopian *M. hapla* population with other related *Meloidogyne* species on the bases of both mtDNA and D2-D3 expansion segment sequence analysis revealed highly supported clades containing the Ethiopian isolates as well as other published isolates from different countries.

Conclusions: The Ethiopian populations of *M. hapla* shared common morphological and molecular characteristics with populations from outside Ethiopia.

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Novel galling patterns of *Meloidogyne Incognita* on selected Maize Genotypes

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Root-knot nematodes are associated with galls which are indices of root damage and host suitability. *Meloidogyne incognita* is a threat and serious challenge in crop production. This research work investigated galling patterns in maize. In this study, maize seedlings were inoculated with *M. incognita* one week after germination at a density of 5,000 eggs and second stage juveniles per plant in a sterilized top soil volume of 5,000ml per pot. Six elite maize genotypes were used: Oba Super 1, 8535-23, 9134-14, Sint Marzoca Larga, Oba Super 2 and Western Yellow. The treatments were replicated four times. Plants were observed daily and watering of plants was carried out as needed. The experimental set-up in the greenhouse was terminated twelve weeks after planting. Roots were washed with tap water and damp dried with paper towel. Galling index was rated on a scale of 1 to 5, reproductive factor (Rf) was calculated by dividing final nematode density with initial nematode density and galling patterns were determined under the compound optical microscope. The mean galling index ranged from 1.2 to 2.5. Rf ranged from 0.04 to 0.62. The following galling patterns, some of which were novel, were observed: sub-terminal globose gall, tentacle-like terminal galls, coiled gall, club-like gall, bead-like galls, massive galls, tumour-like galls, sub-terminal trio-globose galls, broad terminal curved galls, sparingly galled roots, elbow-like gall, ruminant (Goat) heel-like galls, hyper galls (galling of the galls), single sided degenerative massive galls, terminal spear-like galls, terminal degenerative galls, clustered galls and curved club-like spotted gall (compound eye) gall. The galling patterns were as a result of the maize genotypes reactions to *M. incognita* infection.

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Comparative virulence of *Dactylella oviparasitica* strains for the control of *Heterodera schachtii*

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The fungus *Dactylella oviparasitica* (syn. *Brachyphoris oviparasitica*) is a hyperparasite of the sugar beet cyst nematode *Heterodera schachtii*. It was discovered as the primary biological entity responsible for a long-term (>35 years) population suppression of *H. schachtii* in field 9E at the University of California Riverside's Agricultural Operations. The objective of this project was to compare the virulence of three genetically different strains of *D. oviparasitica* (DO50, ARF, DOST) for their potential as biocontrol agents against *H. schachtii*. Fungal cultures grown on water agar were tested for their ability to inhibit egg development and maturation in white females of *H. schachtii* reared on cabbage in greenhouse cultures. White females were washed from roots 3 or 5 weeks after inoculation with J2 and surface sterilized in 0.6% hypochlorite for 5 minutes. After 5 days incubation on the fungal plates, females were removed onto water agar plates and crushed to release and enumerate eggs. DO50 was significantly more virulent than either ARF or DOST. No viable eggs were present after incubation of 3-week-old females on a water agar culture of DO50, while DOST and ARF reduced the number of eggs by 60% compared to females incubated on water agar plates without fungi. The susceptibility of eggs within females to fungal parasitism declined as the females matured. Neither ARF nor DOST significantly reduced the number of eggs in 5 week old *H. schachtii* females while DO50 reduced the number of eggs by 60%. These results confirm our earlier findings that biological control of *H. schachtii* by *D. oviparasitica* is primarily targeted to developing females and eggs. Moreover, the findings indicate that efficacy of parasitism differs considerably among strains of the fungus.

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Nematode assemblages associated with soybean-based cropping systems in South Africa

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Soybean (*Glycine max* (L.) Merr.) is an oilseed crop that is increasingly expanding in terms of its production in South Africa. Various plant-parasitic nematode species parasitise local soybean crops, with *Meloidogyne incognita* and *M. javanica* being listed as the predominant nematode pests that parasitise crops that are included in soybean-based cropping systems. Information on nematode-soybean associations exists locally for conventional soybean crops but not for their Roundup Ready

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peers, which constitute more than 65% of modern plantings. Nematode surveys were thus conducted during 2012 and 2013 during which soil and root samples were obtained at 11 localities where conventional and Roundup Ready soybean crops were grown in close proximity. Grass in natural areas adjacent to soybean fields was sampled concurrently to assess the status of both plant-parasitic and non-parasitic nematode assemblages in such ecosystems. Results indicated that *Meloidogyne incognita* and *M. javanica* was generally the predominant nematode pests associated with both conventional and Roundup Ready soybean roots. Population levels of these root-knot nematode pests ranged from ca. 23 000/50g roots of Roundup Ready to ca.175 000/50g roots of conventional soybean cultivars. Interestingly, natural grass hosted up to ca. 1 800 *Meloidogyne* spp. eggs J2/50g roots. Other plant-parasitic nematodes that were recorded from root and soil samples from soybean fields and natural vegetation were *Pratylenchus* spp *Helicotylenchus* spp., *Rotylenchus* spp., *Scutellonema* spp., *Criconemoides* spp., *Criconema* spp., *Tylenchorynchus* spp and Neotylenchidae. In terms of non-parasitic nematodes a variety of fungivores, bacterivores, omnivores and predators were recorded in the soil samples from soybean as well as natural veld sites. Bacterivores generally dominated in terms of their population levels and diversity. Results of this study suggested that there is in essence no difference in nematode assemblages present in soils where conventional and Roundup Ready soybean crops are cultivated in South African production areas.

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Evaluation of management methods for root knot nematode on carrot and tomato

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Introduction: The northern root knot nematode, *Meloidogyne hapla* Chitwood, causes severe damage to carrots, tomatoes and many other crops. In Canada, some fumigants have been withdrawn from the market, and new regulations will restrict the use of fumigants. Vegetable growers will benefit from effective, non-fumigant methods for nematode management.

Objectives: To identify products that provide effective control of root knot nematode and reduce nematode damage on carrots and tomatoes.

Materials and methods: Growth room trials were conducted with carrots and tomatoes grown in muck soil (pH~6.8, organic matter 70-80%) inoculated with *M. hapla* eggs. The treatments were: metam sodium 210 L a.i./ha, capsaicin plus oleoresin of capsicum (C+OC) 59 L a.i./ha, oriental mustard seed meal 840 kg a.i./ha, abamectin 2 L a.i./ha, and flufenbutyl 4 kg a.i./ha. Dazomet 392 kg/ha, was applied in some trials. Inoculated and non-inoculated checks were included. Products were applied to soil in bins, sealed for 3 weeks, then opened prior to seeding. Damage to roots was assessed 3 months after seeding. Marketable yield for carrots and top growth of tomatoes was also recorded. Field trials were conducted with products applied using label rates, methods and time prior to seeding. These were compared to chloropicrin shank-injected below the carrot seed at seeding. All of the products, plus some combinations were assessed in one trial. In a second trial, chloropicrin was compared to C+OC and an untreated check.

Results: In the field, all products reduced nematode damage. Standard fumigants chloropicrin and metham sodium, and the non-fumigant nematicide flufenbutyl increased plant growth and marketable yield of carrots. Results in growth room trials were similar; metham sodium, abamectin, dazomet and flufenbutyl reduced nematode damage compared to the untreated, inoculated check.

Conclusions: Standard fumigants effectively protect vegetable crops from nematode damage. New product, flufenbutyl, reduced nematode damage, as did abamectin and dazomet.

P NEM 43

Morphological, Molecular and Pathotype identification of the cereal cyst nematodes (*Heterodera* spp) in Turkey

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The global distribution of cereal cyst nematode (CCNs) is considered a major economic restriction to rainfed wheat production systems, especially where monoculture system exist. The genus *Heterodera* includes 12 species that adversely affect the roots of cereals and grasses. The CCN species were identified using using both sequence analysis of the Internal Transcribed Spacer region of the ribosomal DNA (ITS-rDNA) phylogenetic analysis using ITS-rDNA sequences, and classical methods in Turkey. The

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populations of CCNs can be differentiated by differences in hatching cycles (ecotypes) and in virulence to the hosts (pathotypes). CCNs have a complex of 12 species and 11 intraspecific pathotypes that invade roots of cereals and grasses. CCN Virulence groups (pathotypes) are differentiated by testing unknown populations against a matrix of cereals in "The International Cereal Test Assortment for Defining Cereal Cyst Nematode Pathotypes". Three main CCN species which are attacking cereal crops and considered economically important: *Heterodera avenae* (Wollenweber) and *Heterodera filipjevi* (Madzhidov) Shelter and *H. latipons* (Franklin) and have been reported from wheat-growing areas of different parts of Turkey. Moreover, the pathotype of *H. avenae* and *H. filipjevi* were studied. To determine the pathotype of *H. avenae*, Karlik (Adana-Saricam), Imece (Hatay-Kirikhan) and Besaslan (Hatay-Reyhanlı) populations were used whereas Afsin (Merkez), Elbistan (Büyük Yapalak) and Yozgat (Çiçek Dağı) populations were used to determine the pathotype of *H. filipjevi* in the Eastern Mediterranean and Central Anatolian Regions of Turkey. According to the results, all populations for each species demonstrated similar reactions and the *H. avenae* populations were consistent with reactions for the Ha21 pathotype of the Ha1 group, whereas *H. filipjevi* populations were determined as Ha 33 pathotype. However, comprehensive studies should be conducted with more populations to check *H. avenae* and *H. filipjevi* pathogenic and biochemical characteristics. East Anatolian nematod project (Tubitak, 1120565) will be identified rest of population of CCN species in Turkey.

P NEM 44

Efficacies of some plant essential oils on root-knot Nematod *Meloidogyne incognita*

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This study was conducted to determine the toxicity of 10 essential oils (*Artemisia absinthium*, *Citrus bergamia*, *Eucalyptus citriodora*, *Hypericum perforatum*, *Lavandula officinalis*, *Mentha arvensis*, *Ocimum basilicum*, *Piper nigrum*, *Thymus serpyllum*, *Zingiber officinale*) against second stage juveniles of the root-knot nematode *Meloidogyne incognita* under laboratory conditions. The aqueous solutions of these essential oils have been applied to the second stage juveniles in three different concentrations (1, 3 and 5%), at four different application times (12, 24, 48 and 72 hours). The interactions between the variables have been examined both at each time point with one way ANOVA and over time with repeated measures ANOVA. According to the results of the one way ANOVA, *A. absinthium*, *L. officinalis*, *P. nigrum*, *C. bergamia* and *M. arvensis* have the most toxic effect in all concentrations and times. The repeated measures ANOVA showed that the interactions of essential oil-time and essential oil-concentration were statistically significant, and it is concluded that *L. officinalis*, *A. absinthium*, *P. nigrum*, *C. bergamia* and *M. arvensis* have the most toxic effect in all concentrations and times, respectively.

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#Determining the efficacies of some plant essential oils on root knot Nematode *Meloidogyne incognita* (Kofoid & White, 1919) (Nemata: Meloidogynidae)

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In the first part of this study, the toxic effects of 10 essential oils (*Artemisia absinthium*, *Citrus bergamia*, *Eucalyptus citriodora*, *Hypericum perforatum*, *Lavandula officinalis*, *Mentha arvensis*, *Ocimum basilicum*, *Piper nigrum*, *Thymus serpyllum*, *Zingiber officinale*) have been tested under the laboratory conditions in three different application doses (1%, 3% ve 5%) and at four different application intervals (12, 24, 48 and 72 hours) against the second stage juveniles of the root-knot nematode *Meloidogyne incognita* (Kofoid & White, 1919). In the second part of the study, the effects of above mentioned essential oils against the rate of root-knot in the plant roots, the number of *M. incognita* egg masses around the root and the length of the tomato plants, which were infected with the root-knot nematode larvae and grown in pots in a climate chamber, have been examined. As a result of the laboratory trials, *A. absinthium* has the most toxic effect in its 5% application dose at the 72nd hour with a 96.92±0.68 mortality rate. It is also observed that the essential oils *L. officinalis*, *P. nigrum* ve *C. bergamia* have quite similar effects with *A. absinthium*. These essential oils were followed by *E. citriodora*, *M. arvensis* ve *T. serpyllum* with a mortality rate over 70%. As a result of the pot trials, it was also examined that the same essential oils have been the most efficient ones according to the all observed features.

P NEM 46

Effects of some indigenous plant extracts on mortality of root lesion nematode (*Pratylenchus thornei* Sher & Allen) *in vitro*

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Root lesion nematodes of genus *Pratylenchus* (RLN) feed and reproduce in the root cortex of many plant species. They migrate through root tissue cause extensive root damage, and result severe reductions in growth and yield in crops. Root-lesion nematode *Pratylenchus thornei* Sher & Allen, a polyphagous, migratory endoparasitic nematode, is an important pathogen of cereals, vegetable and ornamental plants in Europe, Africa, North America, Asia, the Middle East and Australia. Our objective was to determine the virulence of plant extract against RLN (major wheat root-lesion nematode, *P. thornei*) under *in vitro* condition. RLN was produced on carrot culture using pure culture from Biological Control Research Station in Adana (Turkey). *In vitro* tests were evaluated the effects of plant extracts from three different indigenous plants: henbane, *Hyoscyamus niger* (*Hn*); bead-tree, *Melia azedarah* (*Ma*) and common cocklebur, *Xanthium strumarium* (*Xs*) on *P. thornei*. The nematode juveniles exposed 12, 24, 48, 72 and 96 hours in three concentration of plant extracts. Effect of plant extract on *P. thornei* mortality; suspensions of *P. thornei* (100 nematodes ml⁻¹) in distilled water. One ml of nematodes suspensions and 0.5, 1 or 1,5 ml of extract in 4 ml distilled water were transferred in sterilized Petri dishes in five replicates while, distilled water served as a check and all dishes were kept at 28±2 °C. *P. thornei* were considered dead if they did not move when probed with a fine needle. The plant extracts of *Hn*, *Ma* and *Xs* exhibited highly promising mortality 100% after 72 hours exposure. All plant extracts were found to be effective in reducing *P. thornei*.

Acknowledgments: We thank to Plant Protection Central Research Institute, Ankara, Turkey which supplied the plant extracts.

P NEM 47

Effects of some indigenous plant extracts of root-knot nematode (*Meloidogyne incognita* in tomato natural grown greenhouse conditions

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Plant parasitic nematodes such as root-knot nematodes (RKNs) are major threat to vegetables, fruits and ornamental plants which caused loss of production by causing knots in the roots. Control of root-knot nematode like *Meloidogyne incognita* is not easy in field and greenhouse conditions. Using commercial pesticides are negative side effect to environment and human health. Researches focus on alternative control methods. One of the best alternative methods of nematode control is to use plant extracts. The aim of this study was to determine four different indigenous plant extracts of *Juglans regia* (*Jr*), *Rhododendron sp.*(*R*), *Hypericum perforatum* (*Hp*) and *Helichrysum arenarium* (*Ha*) were evaluated for effect on *M. incognita* in greenhouse (Greenhouse soil was heavily infected by RKNs. RKNs are big problem in vegetable growing area in Mediterranean zone) from July 2014 to January 2015 in Fethiye, Muğla, Turkey. Ilgin F1-Tomatoes (*Lycopersicon lycopersicum*) variety was used for the experiment. Plant extracts were applied 4 times (early planting, with planting, 15th day after planting, 30th day after planting). QI Agri 35 SL(BASF) and Vydate L (DUPONT) nematicide were used as a positive control. Negative control was only applied water by drip irrigation. The experiment contained 3 dose (2.5, 3.75 and 5 L da⁻¹) and four replicates for each treatment and arranged in a randomized block design. At the end of growing season the efficiency of the applications were determined according to the gall index of the root based on 0-10 Zeck scale and yield of tomatoes were observed. The results showed that *Jr* and *Hp* were found more effective than the other plant extracts. All doses of *Jr* aqueous extracts could significantly reduce the nematode infection, the number of galls in root and showed promising nematicidal activity on root-knot nematode.

Acknowledgments: We thank to Plant Protection Central Research Institute, Ankara, Turkey which supplied the plant extracts.

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P NEM 48

Effects of some indigenous plant extracts on the inhibition of root-knot nematode (*Meloidogyne javanica*) eggs hatching and the survival of juveniles on tomatoes in greenhouse pot experiments

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One of the major pests of the vegetables is root knot nematodes (*Meloidogyne* spp.) (RKNs) which cause loss of production due to galling and reduction root development and shoot growth. Herein, the effects of plant extracts from five different plants: pepper (*Capsicum frutescens*) (Cf), henbane (*Hyoscyamus niger*) (Hn) (Solanaceae), bead-tree (*Melia azedarah*) (Ma) (Meliaceae), Common Cocklebur (*Xanthium strumarium*L.) (Xs) and yarrow (*Achillea wilhelmsii*) (Aw) (Asteraceae) were evaluated on eggs and juvenile of RKN [*Meloidogyne javanica* (Treb.) Chitwood]. Experiments were carried out on greenhouse conditions from March to May 2013. The experiments contained five replicates (pots) for each treatment. Suspensions of concentrations of 3, 6 and 12% were prepared. According to treatment, *M. javanica* 3000 eggs (egg hatching test) and 1000 J2s (mortality test) were applied to the root of tomatoes. Both experiments were tested 9 weeks. Each experiment was arranged in a randomized block design. All of the plant extracts showed different level of anti-nematode activity. The results were indicated by 12% concentration of Hn and Xs plant extracts were found more efficient than the same concentration of Ma, Cf and Aw extracts against the egg hatching. J2 penetration and number of the galls were significantly reduced when applied % 12 concentration of Ma and Xs extracts. Results indicated that Hn, Xs and Ma have potential to be used biopesticide in the control of *M.javanica*.

Acknowledgments: We thank to Plant Protection Central Research Institute, Ankara, Turkey which supplied the plant extracts. This study was supported by the Scientific and Technical Research Council of Turkey (TÜBİTAK 1110784).

P NEM 49

Nematode assemblages as bio-indicators of soil quality in conservation and conventional agricultural regimes in South Africa

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Conservation agriculture (CA) relies on interventions that promote soil quality by increasing population and/or diversity levels of beneficial organisms, while still aiming to optimise crop production. The trend is increasing in South Africa but of importance is how CA impact on parasitic and beneficial nematode assemblages and how soil quality could be defined in terms of the ratio between nematode trophic groups. The objective of the study was to monitor nematode assemblages over time in CA conventional crop production systems by determining whether and how such organisms can be used as indicators of soil health. Two rain-fed trial sites, one in the North-West and the other in the Free State provinces, were monitored where distinctly different maize production conditions are practised. Conventional tillage and CA practices with different maize-based crop rotations (monoculture maize, maize-sunflower, maize-cowpea, maize-babala-sunflower and maize-babala-cowpea) were compared in terms of various physical and biotic parameters. For nematode analyses, soil was annually sampled from all plots in both trials before planting, while soil and root samples were also obtained at (60 and 100 days) post-plant. Beneficial nematodes are identified to trophic and plant-parasitic ones to species level. Results indicated that *Meloidogyne*, *Pratylenchus*, *Rotylenchulus* spp. and Hoplolaimidae generally were the predominant nematode pests that parasitize crops in both crop systems. High population levels of these genera were particularly present in monoculture maize and maize-babala-sunflower/cowpea rotations. An interesting trend emanating from this research is the presence of high *Rotylenchulus* sp. population levels in root and soil samples from particularly CA plots at one of the sites. This is the first record of this phenomenon and warrants further investigation. In terms of beneficial nematodes, Rhabditidae and particularly Cephalobidae, generally dominated in terms of their numbers and diversity in the majority of soil samples. Fungivore populations followed, with *Aphelenchus* and *Aphelenchoides* being predominant. Predators were present in low numbers and diversity. Also, strong associations for crops and cultivation practices existed with regard to nematode population levels and diversity. For example, at the one site maize and babala maintained higher *Rotylenchulus* spp. and Hoplolaimidae numbers, whereas at the same crops maintained higher *Meloidogyne*, *Pratylenchus* and *Tylenchorhynchus* spp. numbers at the other site.

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P NEM 50

The influence of minimum tillage, subsidiary crops, and compost amendments on plant-parasitic nematodes in organic agriculture

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Introduction: Plant-parasitic nematodes are a major constraint to organically grown crops. Factors such as long term crop rotations and at high weed pressure seem to particular support nematode taxa with a broad host spectrum such as *Pratylenchus* spp. and *Meloidogyne hapla*. With minimum tillage increasingly applied in organic farming, the question raises, if those conditions will foster plant-parasitic nematodes.

Objectives: To study the effect of minimum tillage, cover crops, living mulches and compost applications on the population dynamics of plant-parasitic nematodes.

Materials and methods: Two-year field experiments were conducted to monitor the population dynamics of plant-parasitic nematode genera throughout a crop rotation of winter wheat and potatoes. The winter wheat was either undersown with clover living mulches or followed by winter cover crops (oilseed radish/ lopsided (black) oat mixture or summer vetch). Compost was applied prior to sowing winter wheat at 5 t DM/ha and prior to planting potatoes at 10 t DM/ha. Soil samples for nematode assessments were taken in Month (M) 1 prior to breaking down the clover grass pre-crop, after wheat (M11), prior to breaking down cover crops and living mulches (M18), and after potato (M24).

Results: Initial plant-parasitic nematode numbers decreased over the two year period from 1416 nematodes per 100 ml soil to 527 nematodes per 100 ml soil. Population densities of plant-parasitic nematodes were slightly higher under minimum tillage compared with ploughing, whereas compost as well as cover crops and living mulches did not have a major effect on nematode densities. In contrast, there was an effect of the main crop on nematode population dynamics. *Helicotylenchus* spp. and *Pratylenchus* spp. increased under winter wheat while *Tylenchorhynchus dubius* and *Paratylenchus projectus* decreased slightly. Quite interestingly, all plant-parasitic nematode genera decreased under potatoes although potatoes are a host for most of the nematode taxa found in the soil.

Conclusions: Although plant-parasitic nematode numbers decreased over the experimental period, minimum tillage seemed to support slightly higher numbers compared with ploughing.

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P NEM 51

Biofumigation using mustard for nematode disease control - Canadian contributions

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Biofumigation *sensu stricto* refers to the suppression of pests, weeds, pathogens and nematodes by volatile biocidal compounds mainly isothiocyanates released from Brassicaceous plants when glucosinolates in their tissues are hydrolysed. Biofumigation for soil borne diseases, and pest control, results have not been consistent. In Canada, recent research using Canadian oriental mustard (*Brassica juncea* L.) has shown great potential for nematode control. Allyl isothiocyanate (AITC) is the most toxic isothiocyanates on several nematode pests such as root-lesion nematodes (*Pratylenchus* spp.), cyst nematodes (*Heterodera* spp.) and root-knot nematodes (*Meloidogyne* spp.), and the toxicities are species selective. Canadian variety Forge has the highest concentration of AITC, reaching up to 1% in it seeds. Different materials such as bran, seed meal and defatted seed meal have significant different concentrations of AITC, therefore were shown different nematicidal activities. Physical properties such as particle size; and environmental factors such as amount of water applied after application of materials also had effect on the efficacy.

P NEM 52

A survey of nematode-parasitic fungi for biocontrol of the cereal cyst nematode *Heterodera filipjevi*

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Parasitism caused by cereal cyst nematodes (CCNs) is a major limiting biotic factor in cereal cropping systems. CCNs are classified in the *Heterodera avenae* group among which *H. avenae*, *H. filipjevi* and *H. latipons* have been reported to cause economically significant yield losses. Integrated management strategies including biological control are necessary for achieving long term effective control of this group of plant parasitic nematodes.

In field trials on the experimental fields of CIMMYT at Yozgat, Turkey, a sharp decline of the final population of *H. filipjevi* has been demonstrated. This suggested the presence of soil suppressiveness against *H. filipjevi* hypothetically caused by nematode-antagonistic fungi or bacteria. Therefore, the present study was conducted to: i) isolate and identify naturally occurring fungi associated with *H. filipjevi*; ii) evaluate the antagonistic effects of the fungal isolates against *H. filipjevi*.

In the first screening, 121 fungal isolates were obtained from *H. filipjevi* cysts or wheat root samples. A green house screening was then carried out to evaluate the biocontrol potential of the obtained fungal isolates against *H. filipjevi*. All fungal isolates were identified using light microscopy and molecular phylogenetic analyses. The ten isolates showing the strongest biocontrol potential reduced the final nematode populations by up to 50%. These isolates belong to the following species: *Pochonia chlamydosporia*, *Paecilomyces fumosoroseus*, *Acremonium persicinum*, *Gliomastix murorum* and *Fusarium acuminatum*. The first three species belong to the Clavicipitaceae, which comprise the ergot fungi and many invertebrate pathogens.

In further screenings molecular characterisation of nearly 50 additional fungal isolates of field-collected cyst samples indicated a variety of fungal species belonging to the genera of *Embellisia*, *Ophiosphaerella*, *Pleospora*, *Ilyonectria*, *Periconia*, *Arthrobotrys*, *Lecanicillium* and also a potentially new fungal species belonging to the family of Bionectriaceae. The presence of this bionectriaceous species in cysts and single eggs could be repeatedly demonstrated using both culture dependent and independent methods. The fungus is slow growing and could be isolated from the eggs of old cysts and young gravid females. This suggests that egg colonisation can be initiated in the early stages of cyst formation and the fungus is capable of long-term surviving. Some of the here reported fungus-nematode associations might be exploited for the biological control of CCNs in future.

P NEM 53

Occurrence and distribution of root-knot nematodes (*Meloidogyne* spp.) in kiwifruit (*Actinidia deliciosa* A. Chev) orchards in Black Sea Region of Turkey

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Kiwi fruit (*Actinidia deliciosa* A. Chev.) has become one of the popular commercial fruit crop in Turkey. The root-knot nematodes (*Meloidogyne* spp.) is one of the major pathogen of kiwi fruit worldwide and limited fruit production. In this study, a survey of root-knot nematodes (*Meloidogyne* spp.) was conducted in various kiwi fruit growing areas in the Black Sea region of Turkey. Root and soil samples were collected from 57 orchards representing five provinces (Artvin, Giresun, Ordu, Rize, Trabzon) of Black Sea region of Turkey during autumn season. The infected roots were observed under a stereomicroscope at 40x magnification. Second stage juveniles (J2) were extracted from soil samples by using modified Bearman Funnel Technique. Out of 57 samples, 46 showed the root-knot nematodes infection with an overall incidence of 80.7%. The percent incidences of disease ranged between 33 and 100% in five provinces. All samples collected from Ordu province identified as *Meloidogyne incognita*.

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Parasitism of *Zonocerus variegatus* (Linnaeus, 1758) (Orthoptera: Pyrgomorphidae) by *Mermis* sp. (Nematoda: Mermithidae) in the agro-systems of Mbankomo and Zamakoé (Cameroon)

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Introduction and objective: *Zonocerus variegatus* (Linnaeus, 1758) (Orthoptera: Pyrgomorphidae) is known as an agricultural pest in West and Central Africa (Kekeunou et al. 2006).

The search for a strategy to protect food crops against this pest led us to study its parasitism by *Mermis* sp. (Nematoda: Mermithidae).

Materials and methods: For this purpose, two batch of *Z. variegatus* were collected once a month in Mbankomo and Zamakoé, from September 2012 to August 2013. The individuals of the first batch were dissected and those of the second batch were raised in the laboratory.

Results: The results showed that, *Mermis* sp. is an endoparasitic that lives in the body cavity of *Z. variegatus*. *Mermis* sp. parasitized stages 3, 4, 5 and 6 larvae and adults of *Z. variegatus*. Its size and weight increased with the grasshopper's stage of development. The prevalence was 6 times higher in the field (4.77 ± 1.47) than dissection (0.71 ± 0.52). The parasite abundance was about 7 times higher in the field (0.11 ± 0.02) than in the dissection (0.016 ± 0.016). The emergence of the nematode has always resulted in the death of the grasshopper. The death of *Z. variegatus* would be linked to the loss of tissue fluid and dehydration related to perforations of the body through the emergence of the parasite; either depleted food reserves or related diseases induced by microbial infections (Toye, 1982).

Conclusion: These results contribute to a better understanding of the biology and ecology of *Mermis* sp., species potentially useful in biological control against *Z. variegatus*.

Reference:

Kekeunou S, Weise S, Messi J, Tamo M (2006) Farmers' perception on the importance of *Zonocerus variegatus* (Orthoptera: Pyrgomorphidae) in Humid Forest Zone of Southern Cameroon. *Journal of Ethnobiology and Ethnomedicine* 2 (17), www.biomedcentral.com.

Toye A S(1982) Studies on the biology of the grasshoppers *Zonocerus variegatus* (L) (Orthoptera: Pyrgomorphidae) in Nigeria. University of Ibadan Ibadan. Nigeria. *Insect Sciences and its Application* 3 (1), 1-7.

P NEM 56

Biological Management of *Meloidogyne incognita* Using Entomopathogenic Bacterial Cell Suspensions with other Bio-products in Eggplant

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In vitro assessment was performed with bacterial cell suspensions of *Xenorhabdus* spp. and *Photorhabdus* spp. with bio-products (abamectin, emamectin and azadirachtin) against *Meloidogyne incognita* on egg plants (*Solanum melongena* L.). Bacteria were isolated from *Steinernema asiaticum* and *Heterorhabditis bacteriophora*, respectively. Maximum control for number of females was observed in combine treatment of *Xenorhabdus* spp.+ abamectin i.e. 63.6%, while 60.7% control in case of *Photorhabdus* spp.+ abamectin treatment and 57% in abamectin treatment, whereas minimum control was observed in azadirachtin treatment i.e. 45% as compared to control treatment. In case of reproduction factor, *Photorhabdus* spp. + abamectin treatment proved the most effective against *M. incognita*, whereas azadirachtin and emamectin treatments were the least effective. The results clearly showed synergistic effect of bacterial cell suspensions and abamectin in controlling *M. incognita* population. Compatibility and the combined action of bacteria (*Xenorhabdus* spp. and *Photorhabdus* spp.) with bio-products is the first time study in Pakistan

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P NEM 57

Root Knot Nematode *Meloidogyne* spp. on Pepper in Indonesia and its Control

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Root knot nematode (RKN) *Meloidogyne* sp. cause damage and losses significantly to black pepper in Indonesia. The objective of this study was to identify *Meloidogyne* species that cause yellow disease on pepper in Bangka, Indonesia using Polymerase Chain Reaction (PCR) based molecular character and tracing nucleotides and to evaluate the effect of endophytic bacteria and organic material for controlling the nematodes. Endophytic bacteria that used in this study were isolated from roots of pepper plants and some of selected endophytic bacteria collection. DNA extraction was obtained from adult female nematodes. PCR was performed using specific primers for *Meloidogyne incognita*, *Meloidogyne arenaria*, *Meloidogyne javanica*, and Multiplex PCR for (*Meloidogyne chitwoodi*, *Meloidogyne fallax*, *Meloidogyne Hapla*). *M. incognita* and *M. Arenaria* was found in Bangka. The result indicates that *M. incognita* and *M. arenaria* from Bangka are similar with species of *M. incognita* and *M. arenaria* from other countries according to the Genbank. The results showed that application of endophytic bacteria and organic materials were able to suppress the number root galls caused by *M. incognita* and the juveniles of nematode in the soil and also to increase the plant growth of pepper in the greenhouse.

P NEM 58

Effects of the biological control agents (*Trichoderma album* and *Bacillus megatrium*) against citrus nematode (*Tylenchulus semipenetrans*) on Baladi Orange and Lime cultivars

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In vivo test, there were found significant differences between the two highest rates (25.0 and 30.0 g/l) of both bioagents. *T. album* at 30 g/l was more effective in reducing the nematode population J₂ in soil and females in roots on Baladi Orange and Lime varieties, and gave the highest percentage of reduction on lime (89.2%) followed by *B. megatrium* (88.4%), whereas *T. album* gave reduction on Baladi Orange (88.3%) followed by *B. megatrium* (87.4%). Nemathorin G10% as nematicide was used for comparison recommended dose (12.5kg/ feddan), Result gave the highest value of nematode reduction percentage (94.9%) on Orange and (94.7%) on lime. The biocontrol agents also increased the plant growth in Baladi Orange (22.5%) and Lime (17.8%).

P NEM 59

Different citrus juices and oils as soil amendments used in control of *Meloidogyne incognita* on tomato.

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Glasshouses experiments were conducted to evaluate the effect of soil amendments with extracted juices and oils of different citrus plants (grapefruit, lemon, lime, naartjie and sweet orange) for the control of *Meloidogyne incognita*. Soil was inoculated with 3000 root-knot nematode eggs after which the treatments were applied and 3 days later tomato seedlings were transplanted in each bag. The height of the tomato plants was measured bi-weekly and root weight, plant mass, root system, gall index and nematode numbers were determined at 56 days after transplanting. The organic amendment consisting of orange juice gave persistently the best improvement in growth as well as best reduction of nematodes. Citrus oils consistently performed weaker than the juice and a clear difference was observed between the different juices with orange followed by naartjie, grapefruit, lime and lemon. Lime and lemon were similar to the control. Further studies are required to confirm the potential of these organic amendments in the control of *Meloidogyne incognita* in the field.

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P NEM 60

An annexin-like protein from the Cereal Cyst Nematode *Heterodera Avenae* suppresses plant defense

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Heterodera avenae is one of the most important cereal cyst nematodes (CCNs) in the world. Parasitism genes encoding secreted effector proteins of plant-parasitic nematodes play important roles in facilitating parasitism. An annexin-like gene was isolated from the cereal cyst nematode *H. avenae* (termed *Ha-annexin*) and had high similarity to *annexin 2*, which encodes a secreted protein of *Globodera pallida*. *Ha-annexin* encodes a predicted 326 amino acid protein containing four conserved annexin domains. Southern blotting revealed that there are at least two homologies in the *H. avenae* genome. *Ha-annexin* transcripts were expressed within the subventral gland cells of the pre-parasitic second-stage juveniles by *in situ* hybridization. Additionally, expression of these transcripts were relatively higher in the parasitic second-stage juveniles by quantitative real-time RT-PCR analysis, coinciding with the time when feeding cell formation is initiated. Knockdown of *Ha-annexin* by method of barley stripe mosaic virus-based host-induced gene silencing (BSMV-HIGS) caused impaired nematode infections at 7 dpi and reduced females at 40 dpi, indicating important roles of the gene in parasitism at least in early stage *in vivo*. Transiently expression of Ha-ANNEXIN in onion epidermal cells and *Nicotiana benthamiana* leaf cells showed the whole cell-localization. Using transient expression assays in *N. benthamiana*, we found that Ha-ANNEXIN could suppress programmed cell death triggered by the pro-apoptotic mouse protein BAX and the induction of marker genes of PAMP-triggered immunity (PTI) in *N. benthamiana*. In addition, Ha-ANNEXIN targeted a point in the mitogen-activated protein kinase (MAPK) signaling pathway downstream of two kinases MKK1 and NPK1 in *N. benthamiana*. In conclusion, Ha-annexin from the cereal cyst nematode suppresses plant defense to facilitate parasitism.

P NEM 61

The New 'Kit' in Town for Nematode Diagnostics

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Plant pathogenic nematodes pose huge problems to farmers around the world. Some nematode species have a dubious reputation as parasites of major food crops such as potato, sugar beet and soybean. Among the most notorious ones are cyst (*e.g. Globodera rostochiensis* and *G. pallida*), root knot (*e.g. Meloidogyne chitwoodi*, *M. fallax* and *M. minor*), stem and bulb (*e.g. Ditylenchus dipsaci* and *D. destructor*), and foliar nematodes (various *Aphelenchoides* species). Annually, the global damage to agricultural crops caused by nematodes amounts to \$80 billion. The use of agrochemicals to control these pathogens is under pressure and sustainable disease management becomes of utmost importance, requiring fast, affordable and reliable pathogen detection methods. However, current nematode inspections are based on microscopic examinations, which are laborious, expensive and lack the required resolution. An adequate solution would be a radical switch to new identification technologies that do not depend on (subtle) external characteristics.

Molecular diagnostics solve this problem: Real-time PCR based assays make use of unique DNA motifs in each nematode species or genus, and can detect and quantify individual nematode species within complex DNA backgrounds (Holterman *et al.*, 2006). Molecular based detection of nematodes has a much higher resolution than microscopic detection and therefore allows for more specific identification and detection.

On the basis of a proprietary, phylum-wide, small subunit ribosomal DNA (SSU rDNA)-based framework consisting of ~ 2,800 full-length nematode sequences, we have developed over 25 diagnostic Real-time PCR assays for plant pathogenic nematode species. These Real-time PCR nematode assays are offered as all-inclusive diagnostic kits to agricultural service and inspection laboratories worldwide, allowing these laboratories to perform nematode analyses that are fast, sensitive, specific, objective and independent of nematode life stage and sample size. A number of examples will be presented during this meeting. ClearDetections offers agricultural (inspection) laboratories a turn-key solution that could completely replace the current routine detection through microscopic examination, enabling large scale, sensitive and accurate detection of plant pathogenic nematodes.

References: Holterman M; Van der Wurff A; Van den Elsen S; Van Megen H; Bongers T; Holovachov O; Bakker J; Helder J (2006) Phylum-wide analysis of SSU rDNA reveals deep phylogenetic relationships among nematodes and accelerated evolution toward crown clades. *Molecular Biology and Evolution*; 23 (9): 1792-1800.

Poster Presentations

Nematodes

P NEM 62

Efficiency Of The Entomoparasitic Nematodes On The Peach Fruit Fly, *Bactrocera Zonata* (Saunders) And The Cucurbit Fruit Fly, *Dacus Ciliatus* (Loew) (Diptera : Tephritidae)

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Laboratory experiments were performed to evaluate the parasitic and lethal effects of the entomoparasitic nematodes Hb (*Heterorhabditis bacteriophora* Poinar) and Sc (*Steinernema carpocapsae* All strain) on the full grown larvae, newly formed pupae and seven days old adults of the peach fruit fly, *Bactrocera zonata* and the cucurbit fly, *Dacus ciliatus*. Mortality rates ranged from 9.3 to 42.7%, 12.7 to 52.3 % for the full grown larvae of both *B. zonata* and *D. ciliatus* treated by Sc nematode, respectively, and from 67.3 to 100%, from 46.3 to 100% for the full grown larvae of both *B. zonata* and *D. ciliatus* treated by Hb nematode, respectively, whereas mortality rates of pupae ranged from 2.7 to 32.7% for the pupae of *B. zonata* treated by Sc nematode, from 1.7 to 23.3% for the pupae of *D. ciliatus* treated by Sc nematode, from 12.7 to 51.7 % for the pupae of *B. zonata* treated by Hb nematode and from 6.3 to 39.3 % for the pupae of *B. zonata* treated by Hb nematode. Furthermore, the mortality rates varied from 35.0 to 78.7%, from 7.7 to 50.3% for 7 days old adults of both *B. zonata* and *D. ciliatus* treated by Sc nematode, respectively, from 41.7 to 90.3 and from 17.0 to 67.7 % for 7 days old adults both *B. zonata* and *D. ciliatus* treated by Hb nematode, respectively. LC₅₀ and LC₉₀ values were 325.3, 286.2, 1718.6 and 1650.0 IJs/ cm² for larvae of *B. zonata* and *D. ciliatus* treated with Sc nematode, 28.8, 56.7, 167.2 and 156.1 IJs/cm² for larvae of *B. zonata* and *D. ciliatus* treated with Hb nematode, 540.2, 447.4, 1785.4 and 2009.8 IJs/ cm² for pupae of *B. zonata* and *D. ciliatus* treated with Sc nematode, 235.0, 420.8, 1167.0 and 1941.5 IJs/ cm² for pupae of *B. zonata* and *D. ciliatus* treated with Hb nematode, 116.8, 261.6, 319.3 and 375.0 IJs/ cm² for adults of *B. zonata* and *D. ciliatus* treated with Sc nematode and 77.3, 196.7, 290.8 and 253.7 IJs/ cm² for adults of *B. zonata* and *D. ciliatus* treated with Hb nematode, respectively. From the obtained results we can conclude that the entomoparasitic nematodes: Hb (*Heterorhabditis bacteriophora* Poinar) and Sc (*Steinernema carpocapsae* All strain) were effective on the different stages of *B. zonata* and *D. ciliatus*, Hb nematode was more virulent than Sc nematode and the larvae and adults of *B. zonata* and *D. ciliatus* were more susceptible to the nematodes infection than the pupae as effective and virulent parasites.

P NEM 63

Pathogenic Nematode Identification on Angelica Disease and Its rDNA-ITS Sequence

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The diseased angelicas were surveyed from the main producing areas in Minxian, Zhangxian, Dangchang and Huichuan, Gansu, China infested with nematode. No obvious symptoms were found above ground parts of diseased plant, which main occurred roots. During early infected stage, no symptoms demonstrated on root appearance. Infection site of brown and rot like chaff was seen at longitudinal section. Then the root epidermis became yellow longitudinal crack, its deep reached about 1 ~ 2 mm, and many root hairs were obtained around them. When the disease index became serious, cortex tissue of the whole root showed stem rot. Rotted parts were not reached cambium zone, but individual root rot reached vascular tissue.

Female, Body 789~1131µm long. Body 26~35µm wide. Stylet 10~11.5 µm long. Tail 51~75 µm long. Male, Body 930~1248 µm long. Body 28~30 µm wide. Stylet 9.5~11.3 µm long. Tail 59~87 µm long. Body was slightly to the ventral surface of bend, ring pattern clearly. Lip flat, slightly constricted or head slightly narrower than its connection body width. The lateral zone width about body width 1/5, lateral line 6. Stylet base ball was small and round. Oesophageal bulb like spindle, and front end after esophageal gland from the back covered intestine. Vent located esophageal gland position, crescent in the excretory pore former. The female single was ovary and protrusive. Vulva was clear, which located at the rear of body. Uterine sac obviously, the length was about anal vulva distance 2/3. Tail was conical, slightly to the ventral bending, thin end round. Males and females were similar to the front. Spicule on dichotomy, to the ventral bending, its wide like big two digitations. To the horizontal position and the umbrella from the spicular apex began extending back to the end of tail. Gubernaculum was short and simple. According to the morphological characteristics, 4 different areas of entomopathogenic nematodes was *Ditylenchus destructor*.

rDNA-ITS-PCR amplification of 4 different nematode samples with ITS universal primer rDNA1 (5'-TTGATTACGTCCTGCCCTTT-3') and rDNA2 (5'-TTTCACTCGCCGTTACTAAGG-3') obtained sequence length about 940 bp. Compared with the region of rDNA-ITS sequence and GenBank (<http://www.ncbi.nlm.nih.gov>) database, the Nucleotide sequence of BLASTN reported at home and abroad of *D. destructor* ITS region sequence homology reached 99%. Morphological and molecular biological feature data showed that 4 areas of our province Minxian, Zhangxian, Dangchang and Huichuan angelica disease pathogenic nematode was *D. destructor*.

Poster Presentations

Nematodes

P NEM 64

Nematocidal properties of *Thymus* sp. plants - results of the model study on *Ditylenchus dipsaci*

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Restriction of pesticide usage in recent years leads to the lack of chemicals available for crop protection against plant parasitic nematodes. In the case of Stem and Bulb Nematode (*Ditylenchus dipsaci*) especially preventive treatment of seed garlic and bulbous ornamental plants is of crucial importance. Currently the only official method utilizes hot water treatment however this technique could result in damage of the seed material and its reliability is questionable. With the need of obtaining new methods of seed material protection against *D. dipsaci* nematode pilot study was conducted to evaluate nematocidal effects of essential oil from *Thymus serpyllum*, *Thymus vulgaris* and pure Thymol on mortality of *D. dipsaci* under in vitro conditions. Results show overall good efficiency of the all tested chemicals on *D. dipsaci* mortality with the best effect of the oil from *Thymus vulgaris*. Next research would focus mainly formulation of this oil for practical utilization and detailed study of its phototoxic properties.

The research was supported by the Czech Ministry of Agriculture, project number QJ1310226.

P NEM 65

Morphological characterization and comparison of the two populations of Sugar Beet Nematode (*Heterodera schachtii*) from the Czech Republic

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Sugar beet production constitutes important and traditional segment of agriculture business in the Czech Republic. Considering pests of sugar beet the *H. schachtii* still presents problem. Even if resistant cultivars are widely available research of alternative methods of sugar beet protection against this pest should be investigated. Monogenically based resistance of current sugar beet cultivars could be overcome in the future so designing of functional antiresistant strategy with direct protection measures would be great benefit for sugar beet producers. As the first step of research the new management techniques for *H. schachtii* two infested plots were selected for future field experiments, sampled and present *H. schachtii* cysts were characterised using classical morphological approach. Obtained data were compared both mutually and with data available for this nematode species from other countries. Results show that both characterised populations possess morphological characteristics typical for *H. schachtii* species.

The study was supported by the Technology Agency of the Czech Republic, project number TA04021117.

P NEM 66

Occurrence and pathogenicity of *Meloidogyne enterolobii* on vegetables in Nigeria

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Resistant tomato and pepper varieties were evaluated for their reaction to a local population of root-knot nematodes at the University of Ibadan, Nigeria. The pot experiment was laid out in a completely randomised design with five replications. Varieties used included three *M. incognita*-resistant tomato and pepper varieties each, one local susceptible tomato variety and two local pepper varieties. The local *Meloidogyne* population was obtained from the root-knot nematode (*M. incognita*) inoculum plot of the Nigerian Institute of Horticulture (NIHORT). Twelve weeks after planting, the crops were evaluated for galling, nematode populations per plant and reproductive factor (RF). All the plants were heavily galled (GI 2.6-4.5) and were moderate to good hosts (RF 2.7 - 7.1 in tomato and 2.2 - 4.4 in peppers) to the local population. Ten egg masses were thereafter selected from each plant and cultured on local susceptible tomato cv *kerewa*. Single egg masses picked from the individual cultures were hatched at room temperature, individually hand-picked, killed and preserved in 70% ethanol. Samples were identified using SCAR primers in PCR reactions. Seventy percent of the populations were identified as *M. enterolobii*, 23% as *M. incognita* and 7% as *M. erenaria*. This refutes the original reports that *M. incognita* is the most prominent root-knot nematode in south-western Nigeria and is the first report of *M. enterolobii* on vegetables in Nigeria.

Poster Presentations
Nematodes

Figure 1: Gallings index and reproductive factor of local population of *Meloidogyne incognita* on resistant varieties of pepper and tomatoes.

No attachment submitted

Figure 2: Pathogenicity of *Meloidogyne* spp. on *M. incognita*-susceptible and resistant varieties

No attachment submitted

P NEM 67

Reproduction and Biology of *Scutellonema Bradys* in roots of tropical cover crops

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One of the options for the management of *Scutellonema bradys* is the use of non-host crops such as cover crops. This study evaluated the ability of *S. bradys* to reproduce in the roots of selected cover crops. Two pot trials were set up with 10 cover crops and compared to a known susceptible control in a completely randomized design with 5 replicates. Plants were inoculated with 2000 *S. bradys* two weeks after planting. Fresh shoot and root weight, number of nematodes in roots and soil, and reproductive factor (RF) of the nematode were taken at harvest, 10 weeks after planting. A second experiment was set up with the same crops and inoculated with 500 adult nematodes. Plants were harvested daily for 45 days. The roots from each harvested plant was stained in lactoglycerol and observed for stages of nematodes present. *Tagetes erecta*, *Stylosanthes guianensis*, *Centrosema pubescens*, *Pueraria phaseoloides*, *Aeschynomene histrix* and *M. pruriens* were designated as poor hosts based on significant reduction of *S. bradys* populations in their roots and RF less than 1. Also these cover crops lengthened or terminated the life cycle of *S. bradys*. *Cajanus cajan* was regarded as a trap crop because it supported initial nematode penetration but hindered reproduction of *S. bradys*. On the other hand, *Lalab purpureus*, *Crotalaria ochroleuca* and *Crotalaria juncea* were good hosts and similar to the susceptible *Vigna unguiculata* in their reaction to *S. bradys*. Cover crops that negatively affect the development and life cycle of *S. bradys* have the potential to manage the nematode in yam based cropping systems.

Table 1: Number of *S. bradys* in roots and soil associated with inoculated cover crops and the Reproductive factors (RF)

No attachment submitted

Figure 1: Root lesion index on roots of cover crops inoculated with *S. bradys*.

No attachment submitted

P NEM 68

Morphological and molecular identification of potato cyst nematodes populations from Ain Defla region of Algeria

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Potato cyst nematodes PCNs are the most economically damaging pathogens of potato crop worldwide. The fast and correct species identification and diagnosis is the key element for the management of those pests. A survey was conducted in potato growing areas of Ain Defla region of Algeria in 2013. Sixteen PCNs populations belonging to five localities were characterized by morphological techniques. Then six representatives PCNs populations were analyzed by PCR- RFLP and sequencing ITS rdna. With this approach, we identified for the first time, the species of potato cyst nematodes in this region. Both species *G. rostochiensis* and *G. pallida* are present either separately or in mixed populations. We have shown the existence of intraspecific genetic variability within *G. pallida* and *G. rostochiensis* populations. Concerning the origin of populations present in Ain Defla, phylogenetic study supported the hypothesis of a multi-introduction for *G. pallida* and that a country from Europe or Canada was the "transition host" for *G. rostochiensis*. Other surveys can be done to complete the information on the distributions of PCNs in this area.

P NEM 69

Incidence of plant-parasitic nematode infections and aflatoxin production in groundnut kernels

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Groundnut (*Arachis hypogaea*) is a nutritious cash crop for subsistence farmers. Such crops are often damaged by plant-parasitic nematodes and infected by *Aspergillus flavus*, hence impacting adversely on groundnut quality and yield. Consumption of aflatoxin-contaminated groundnut pods/kernels can lead to acute or chronic aflatoxicosis in humans and animals. The aims of this study were to i) identify nematode pests that parasitise groundnut hulls and kernels and ii) quantify aflatoxin production in groundnut kernels. Groundnut pod samples were collected at harvesting from the Jozini, Manguzi and Mbazwana districts in the KwaZulu-Natal Province of South Africa during the 2012/13 and 2013/14 growing seasons. Plant-parasitic nematodes were extracted from groundnut hulls and kernels by soaking such plant parts in tap water for 24 h at 25 °C. Nematodes obtained were subsequently counted and identified using a stereomicroscope. The LC-MS/MS technique was used to quantify aflatoxin levels in groundnut kernels. *Ditylenchus africanus* (peanut-pod nematode) as well as individuals belonging to the genera *Pratylenchus* (lesion nematode), *Helicotylenchus* (spiral nematode) and *Meloidogyne* (root-knot nematode) spp. were identified from hulls and kernel samples. Their population levels were generally higher in the hull than in kernel samples for both seasons. The peanut-pod nematode was, however, the predominant nematode pest in both hulls and kernel samples. During the 2012/13 season, none of the groundnut-kernel samples obtained from all three districts were contaminated with aflatoxins at harvest. However, relatively low levels of aflatoxins were detected in samples that were stored. There was significant aflatoxin contamination (above 500 ppb per 1 g sample) in kernels at harvest and at storage from Manguzi and Mbazwana during the 2013/14 season. Groundnut kernels from Jozini had less nematode numbers and were least contaminated with aflatoxins, whereas those from Manguzi had high nematode numbers and the highest aflatoxin contamination. The extent of variation in this study necessitates further investigations on nematode survival under stress conditions in a controlled environment and application of an atoxigenic *A. flavus* strain for potential biocontrol of the aflatoxin-producing strains.

P PLANT 1

Effect of temperature on bionomics of invasive pest and its native parasitoid

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The present study used a mealybug-parasitoid cotton system to evaluate the effects of global warming on bionomics of invasive pest (*Phenacoccus solenopsis* Tinsley (Sterrhorrhycha: Pseudococcidae)) and its native parasitoid (*Aenasius bambawalei* Hayat (Hymenoptera: Encyrtidae)) under a range of temperatures i.e. 20°C, 25°C, 30°C, 35°C and 40°C. High temperatures (30°C, 35°C, 40°C) significantly reduced developmental duration of immature longevity and also reduced the overall longevity for both male and female *P. solenopsis*, whereas, the survival percentage of all immature stages was higher at 30°C. The reproductive potential of *P. solenopsis* was recorded higher at 30°C. Pre-oviposition duration was significantly shorter at 30°C and post-oviposition period was longer at same temperature, whereas, the oviposition duration was shorter at higher temperatures (35°C and 40°C). Percentage parasitism by *A. bambawalei* was significantly greater both at 30°C and 35°C, whereas, percentage parasitoid emergence and number of female parasitoids were significantly higher at 30°C compared with other temperature. The present study demonstrated that higher temperatures had negative impacts on development and survival of invasive pest and also had a negative impact on the efficacy of its native parasitoid which may have profound effects on the community composition of terrestrial ecosystem.

Figure 1

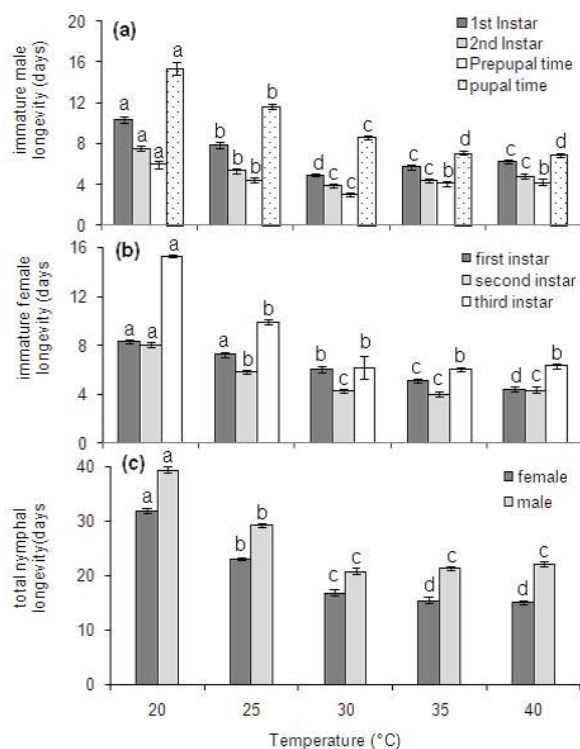
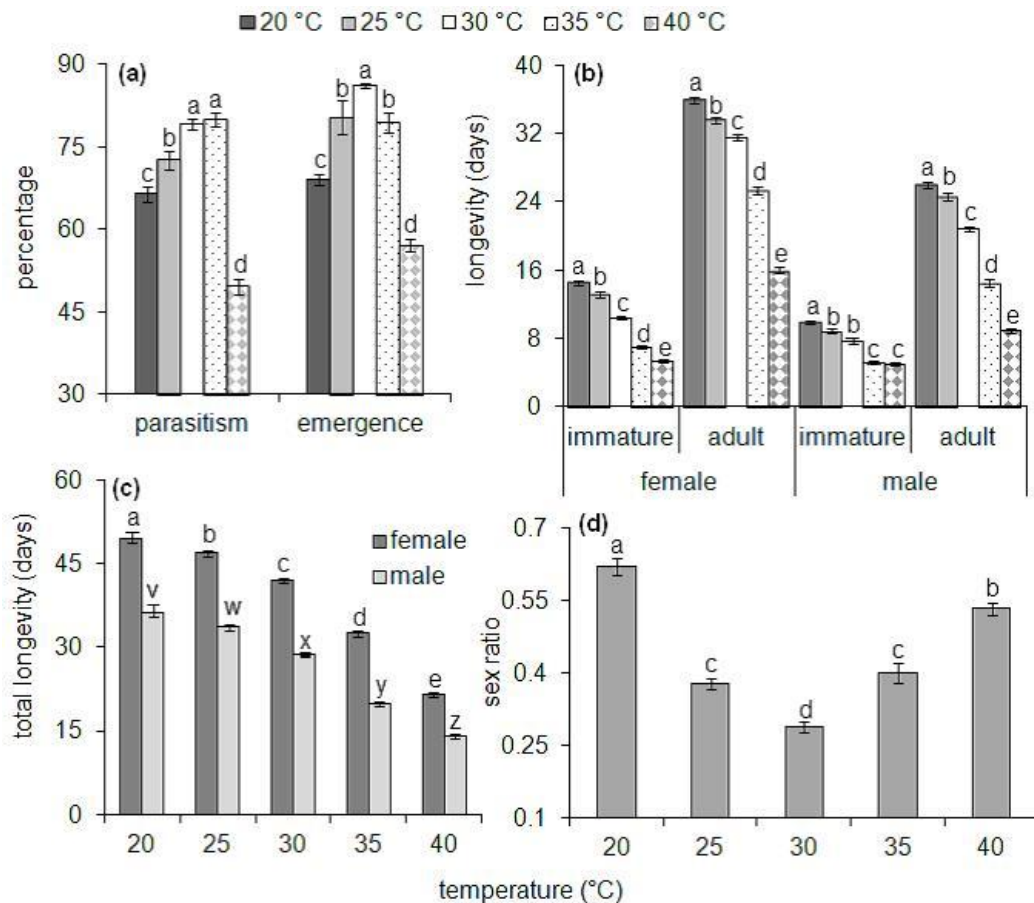


Figure 2

Poster Presentations
Plant Protection in a Changing Climate



P PLANT 2

Rice Pollen Characteristics as Influenced by High Temperature and Exogenously Applied Plant Growth Regulators

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Increasing temperature due to global warming has emerged a gravest threat to rice production. Present study was conducted to determine the influence of high temperature and exogenously applied plant growth regulators on pollen fertility, anther dehiscence, pollen germination, antioxidant activities and metabolites in pollens of two rice cultivars (IR-64, Huanghuazhan). Plants were subjected to high day temperature (HDT: 35°C ± 2 during day time), high night temperature (HNT: 32°C ± 2 during night time), and ambient temperature (AT: 28°C ± 2 throughout the day) in controlled environment growth chambers. We used four different combinations of ascorbic acid (Vc), alpha-tocopherol (Ve), brassinosteroids (Br), methyl jasmonates (MeJA), and triazoles (Tr) in this study. A nothing applied control was also maintained for comparison. Our results depicted that high temperature severely reduced the pollen fertility, anther dehiscence, pollen retention and germination, metabolites synthesis and significantly altered the antioxidant activities in pollens of both rice cultivars. Nonetheless, exogenous application of various PGRs assuaged the adverse effects of high temperature and Vc+Ve+MeJA+Br was found the best combination than rest of treatments in terms of all studied attributes. Overall, the damaging effects of HNT were more than those of HDT. Contradictions were also apparent between cultivars regarding their response to high temperature stress. Higher pollen fertility, better anther dehiscence, higher pollen retention and germination were observed in Huanghuazhan compared with IR-64 under high temperature, which were possibly due to increased activation of antioxidants and higher synthesis of metabolites, thus more stress resistance of this cultivar.

P PLANT 3

Population dynamics of white fly (*Bemisia tabaci* Genn.) on ladyfinger (*Abelmoschus esculentus* L.) in the sub-Himalayan region of north-east India and their sustainable management by using biopesticides

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Ladyfinger (*Abelmoschus esculentus* L.) is susceptible to various pests of which white fly (*Bemisia tabaci* Genn.) causes heavy damage. The study on incidence revealed that the pest was active throughout the growing period with a peak population (3.98 white fly /leaf) and (4.33 /leaf) during 20th SMW (May) in the pre-kharif crop and during 42nd -43rd SMW (October) in the post kharif crop respectively. Sudden fall of population was found during July because of heavy rains. White fly showed non-significant positive correlation ($p=0.05$) with temperature and relative humidity and significant negative correlation with weekly total rainfall. This study evaluated the efficacy of extracts from *Polygonum hydropiper* L. flower (locally available weed), microbial insecticides like spinosad 45 SC (*Saccharopolyspora spinosa* Mertz) and *Beauveria bassiana* Vuillemin against *B. tabaci* infesting ladyfinger. Better white fly control (> 50% population suppression) was achieved with spinosad and extract of *Polygonum* flower. The *Polygonum* extract was very effective against the white fly, achieving more than 60 % mortality at 3 and 7 days after spraying. Spectrophotometric scanning of crude methanolic extract of *Polygonum* flower showed strong absorbance wave length between 645-675 nm. Considering the level of peaks of wave length the flower extract contain some important chemicals like Spirilloxanthin, Quercetin diglycoside, Quercetin 3-O-rutinoside, Procyanidin B₁ and Isorhamnetin 3-O-rutinoside. These chemicals are responsible for pest control. Plant extracts and microbial insecticides have less or no hazardous effects on human health and the environment, therefore they can be incorporated in IPM programmes and organic farming in vegetable cultivation.

Figure 1

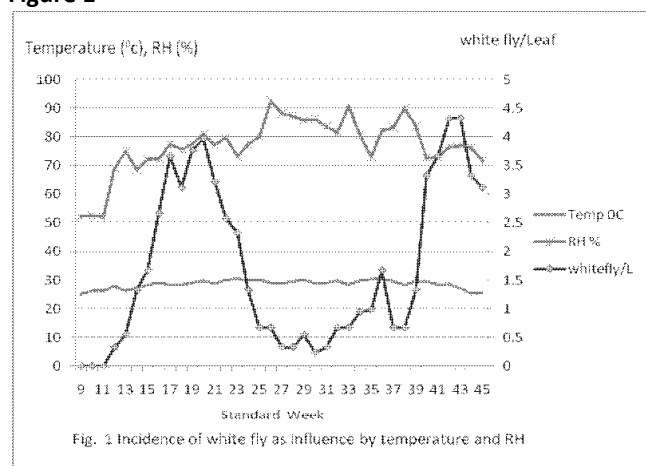


Figure 2

Table – Overall efficacy of plant extracts and microbial insecticides against *Bemisia tabaci*, and the fruit yield of ladyfinger

Treatments	Dose ml./litre (%)	Pre treatment observation of w.fly/Leaf	Overall efficacy (% reduction)				Fruit Yield (q/ha)
			Days after treatment			Mean	
			3	7	11		
<i>S. sponisa</i> (Spinosad 45 SC) (T ₁)	1 ml/3 L	0.83	80.19 (63.62)	70.50 (57.13)	58.71 (50.03)	69.80 (56.92)	40.20
Imidacloprid (Confidor 17.8 S.L.) (T ₂)	1 ml/5 L	0.72	85.95 (68.28)	73.36 (58.95)	71.69 (57.89)	77.00 (61.70)	41.17
<i>Pongamia</i> (1.0%) (T ₃)	10.00 (1.0%)	0.69	43.34 (41.16)	38.48 (38.29)	25.81 (30.50)	35.76 (36.58)	32.90
<i>Pongamia</i> (5.0%) (T ₄)	50.00 (5.0%)	0.77	54.28 (47.47)	48.36 (44.05)	34.48 (35.93)	45.70 (42.48)	36.13
<i>Polygonum</i> (1.0%) (T ₅)	10.00 (1.0%)	0.63	49.94 (44.96)	47.39 (43.67)	30.23 (33.50)	42.50 (40.59)	31.49
<i>Polygonum</i> (5.0%) (T ₆)	50.00 (5.0%)	0.77	62.44 (52.16)	60.07 (50.34)	40.41 (39.46)	54.31 (47.47)	36.53
<i>B.bassiana</i> (T ₇) (Biorin 10 ⁷ conidia/ml)	1 ml/L	0.75	46.80 (43.16)	42.30 (40.56)	29.60 (32.85)	39.56 (38.85)	32.51
Untreated Control (T ₈)	-	0.83	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	0.00 (4.05)	27.08
SE m (±)	-	-	2.14	2.81	1.75	-	1.14
CD at 5%	-	NS	6.36	8.35	5.21	-	3.87

Figures in parentheses are angular transformed values, NS = Not significant

P PLANT 4

Drought alters the expression of a candidate *Zea mays* p-coumarate 3-hydroxylase gene and caffeic acid biosynthesis

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The enzymatic activity of p-coumarate 3-hydroxylase (C3H) synthesizes caffeic acid from p-coumaric acid. We recently showed that exogenously applied caffeic acid confers salinity tolerance in soybean (*Glycine max*) by inducing antioxidant enzymatic activity to promote enhanced scavenging of reactive oxygen species, thus limiting salinity-induced oxidative stress. Recent evidence also establishes that pre-treatment of plants with exogenously supplied caffeic acid improves plant tolerance to osmotic stress by improving plant antioxidant capacity and enhancing biosynthesis of compatible solutes. We aimed to identify a C3H in maize (*Zea mays*) and evaluate the effect of drought on the spatial and temporal expression of the gene encoding the candidate maize C3H (*ZmC3H*). Primary sequence analysis shows that *ZmC3H* shares 71% identity with an *Arabidopsis thaliana* C3H that is implicated in the control of *Arabidopsis* cell expansion, growth and responses to stress. *In silico* *ZmC3H* promoter analysis reveals the presence of *cis*-acting elements that interact with transcription factors implicated in plant responses to drought. Spatial expression analysis by semi-quantitative RT-PCR shows that *ZmC3H* is expressed in both leaves and roots under normal conditions. However, drought represses the expression of *ZmC3H* in leaves whereas it up-regulates its expression in roots. These changes in *ZmC3H* expression correlate with the changes in the content of caffeic acid in maize in response to drought. We illustrate the implications of these changes in the expression of the gene in relation to maize responses to drought and discuss the potential of regulating caffeic acid biosynthesis towards genetic improvement of maize tolerance to drought stress. These findings have implications for food security because of the potential of the implications of the study for drought tolerance in maize.

P PLANT 5

Application of biological and chemical agents as alternative fungicides for management of brown spot disease on rice (*Oryza sativa* L.)

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Brown spot is one of the important foliar diseases on rice plants (*Oryza sativa* L.) in the world as well as in Egypt causing a considerable yield loss. *Bipolaris oryzae* fungal is the causal brown leaf and grain spot disease of rice plant during growing season. *In vitro*, antifungal activity of benzoic acid, hydroquinone and antagonistic potential of biocide rhizo- N (*Bacillus subtilis*) were tested against *B. oryzae* growth and sporulation. Soaking rice seeds (Cv. Giza 101) before sowing for 24 hours of each suspension of benzoic acid, hydroquinone, and biocide rhizo- N (*Bacillus subtilis*) at 5g/L led to a significant reduction of fungal flora associated with germination seeds and significantly increased seed germination % than the untreated seeds. Under natural field conditions, all biotic and abiotic agents were significantly decreased percentage of brown spot disease of rice plants and disease severity on leaves and grains in addition, significant increase grain yield. Benzoic acid was the best agent significantly reduced brown spot disease incidence on rice plants and grain yield.

P PLANT 6

Thrips pest of vegetables in Biskra an arid province of Algeria

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Thrips of Algeria have been rarely studied. A survey of thrips on vegetable plants was conducted during 2010-2011 in three sites of Biskra an arid province in Algeria. Scouting for thrips was carried out on plants and flowers from ten locations: several thrips species belonging to 6 genera were recorded. *Melanthrips fuscus*, *Aeolothrips intermedius*, *Rhipidothrips gratusus*, *Frankliniella occidentalis*, *thrips physapus*, *thrips angusticeps*, *Thrips tabaci* and *Odontothrips loti*. The two last species were the most abundant. However, majority of the thrips are cosmopolitan in distribution and recorded as pest species, they can harm crops. These include *Frankliniella occidentalis* that may transmit *Tospovirus*

P PLANT 7

Quantification of climate change impacts on agricultural pests

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Temperature is the dominant abiotic factor determining development rates, reproduction and migration of many insects. Climate change will therefore alter population abundance, geographical distribution and seasonal phenology of important agricultural pests. Scenarios concerning possible impacts of climate change on pests are necessary to identify adapted plant protection strategies and sustainable plant management options. Model-based studies are a valuable method for estimating the impacts of climate change on insect pests. Such projections are not easy to develop, because impact models often require a high temporal and spatial resolution of future climate data and long-term field observations are necessary for model calibration and validation.

Over the last decades impact studies have been aided by advances in climate modelling. For instance, the Swiss climate research network produced regional and localized climate change scenario information that provide an unprecedented opportunity for the impact modelling community in terms of spatial resolution. Nevertheless, the question of how to estimate the impact of climate change on pests across spatial and temporal scales remains open. In this contribution, we will discuss different issues related to impact modelling and its application to climate change adaptation at the example of a key apple fruit pest, the codling moth (*Cydia pomonella* L.).

Finally we consider research needs to improve assessments of climate change impacts on agricultural pests. Among other issues we address the needs for: (i) information about relevant species traits increasing the sensitivity of the species to climate change; (ii) improved knowledge on non-seasonal mortality, indirect effects of climate change and interactions (i.e. drought and pest); (iii) improved knowledge concerning the effectiveness of predators and parasitoids under changing climate to model complex trophic interactions; (iv) coupling pest models with crop suitability models and ecological niche models, a step required to obtain a comprehensive risk analysis.

P PLANT 8

Analysis of Genetic Diversity of *Glycine soja* Germplasm Resources in Shaanxi Province of China

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Wild soybean (*Glycine soja*) is commonly accepted as the progenitor species of the cultivated soybean (*Glycine max*). Wild soybean has its distribution in central and northern parts of East Asia, including China, Korean peninsula and Japan, as well as the far east of Russia. China is the origin and diversification centers of the Wild soybean that is found in all provinces of China. Except for Xinjiang, Qinghai, and Hainan. Wild soybean possesses agronomically beneficial traits, such as high protein and nitrogen fixation, adaptation to severe condition, and resistance to insects and diseases. As valuable genetic resources, wild soybean comprises an extraordinarily important gene pool for soybean breeding, particularly when the genetic background of cultivated soybean becomes narrow under its extensive modern agricultural practices. Therefore it is urgently important to conserve Wild soybean genetic resources, given that a great number of Wild soybean populations have gone extinct or been significantly reduced due to the deterioration of their natural habitats under human influence, including urbanization, road construction and change of farming systems. Yet little research has been done on genetic diversity in natural populations of wild soybean in China.

In order to evaluate the genetic diversity of the wild soybean in the different region of Shaanxi Province, 13 SSR primers were used to analyze genetic diversity and genetic structure among 6 natural populations of Wild Soybean and 1 population of cultivated Soybean as comparison. We detected 113 alleles in 13 microsatellite loci; the mean number of alleles per locus (A) was 8.69; the effective number of alleles per locus (N_e) was 5.632; the mean expected heterozygosity (H_e) was 0.482; and the mean observed heterozygosities per locus (H_o) was 0.080. The Shannon diversity index (I) was 0.657, with the proportion of genetic differentiation among populations (F_{ST}) 46.5%. The research shows that the genetic diversity of wild soybean with a higher level in Shaanxi Province, The genetic diversity of wild soybean generally higher than cultivated soybean. Along with the altitude increasing, the genetic diversity of wild soybean variation is low. The wild soybean germplasm resource is rich in central. South of Shaanxi province, and this population has high genetic diversity speculated that the region is the center of genetic diversity of wild soybean in Shaanxi province.

P PLANT 9

Changes in the transcriptome of rice infected with *Magnaporthe oryzae* in response to elevated temperature

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It is currently hypothesized that climate change may lead to global warming, and temperatures might increase by 2-5°C at the end of the twenty-first century. However, the consequences of the expected temperature elevation on plant pathogen interaction have continued to provide conflicting results due to several interacting factors. The rice-*M. oryzae* pathosystem is well-studied at both the phenotypic and genomic level, and is suitable for understanding the mechanisms underlying plant pathogen interaction at high temperature.

We used RNA-seq to test whether an increase in temperature from 28°C to 35°C (HT, high temperature) has an influence on rice transcriptome in the interaction of *Magnaporthe oryzae* (Mo) with rice.

Two rice genetic backgrounds, Li-Jiang-Xin-Tuan-He-Gu (LT) and Co39 (CO), carrying the resistance gene *Pi54*, were compared after exposure to HT for 7 days and Mo for 48 hours. We analyzed the phenotypic and transcriptional changes in response to HT and Mo as single stress factors as well as their combination.

Our data revealed that high temperature, despite reducing the expression of *Pi54* at 35°C compared to 28°C, induced resistance to *M. oryzae* in both LT and CO. In contrast, CO had a more severe disease phenotype compared to LT at 28°C. The transcriptome data revealed that 81% and 74% of the transcripts were shared between double stress (HT+Mo) and single stress (Mo), whereas 35% and 15% were shared between HT+Mo and HT in LT and CO, respectively. From the shared transcripts between HT+Mo and HT, 42% and 65% were down-regulated in LT and CO, respectively. In both backgrounds, up-regulated genes common to pathogen and HT stresses were related to transferase, oxidoreductase and nucleic acid binding, whereas hydrolase activity was more related to down-regulated genes. In response to HT, heat acclimation and pseudouridine synthesis were higher enriched in LT, whereas starch biosynthesis and sucrose transport were higher enriched in CO.

Our data suggest that R gene mediated resistance is more dependent on the genetic background compared to temperature. High temperature appears to play an additive role in improving resistance against *M. oryzae*, but the effect likely varies between rice genotypes. Cellular processes related to HT response are apparently more affected in LT compared to CO.

P PLANT 10

Contribution of chemical crop protection to carbon footprints of crops

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Introduction: Agriculture is responsible for approximately 14% of global anthropogenic greenhouse gas (GHG) emissions and thus contributes significantly to global climate change (GCC). At the same time GCC constitutes a serious threat to satisfying the increasing demand for food. In this respect chemical crop protection, which in public debates often has a negative connotation with regard to its environmental impact, obviously contributes to securing crop yields and may thus potentially help to mitigate climate change.

Objectives: Therefore the current study aimed at assessing GHG emission and respective product carbon footprint (PCF) of major agricultural crops under different chemical plant protection intensities.

Materials and methods: Building on detailed crop production data of a long-term (11 year) field experiment conducted at Dahnsdorf experimental station in northeast Germany, GHG emissions per hectare and PCF per unit of harvest product were evaluated for major crops. Comparative assessment was conducted for three plant protection intensities, namely situation-related application (100%), application rates reduced by half (50%), and untreated control (0%).

Results: It was shown that under the 100% treatment the contribution of chemical crop protection to total GHG emission was less than 2%. At the same time the crops under intensive crop protection realized in average 20% to 60% higher yields compared to the 0% treatment. Over all experiment years, this resulted in reduced PCFs of between -14% for rapeseed and -26% for wheat in the 100% compared to the 0% treatment. Comparing the 100% and 50% treatments lower PCF need to be noted in the 50% treatment for first experiment years. However, for the second period of the experiment a steadily increasing advantage in PCF could be observed for the 100% treatment. There is strong evidence that this was caused by an increasing weed infestation and related competition, resulting in significant yield reductions in the 50% treatment.

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Conclusion: It can be concluded that chemical crop protection exerts a strongly positive effect on crops' PCFs under conventional crop production. Reductions in chemical crop protection doses need to be conducted with great care to avoid potentially negative long-term effects on field conditions and crops' PCFs.

P PLANT 11

Wild Plants as Sources of the Permanency of Viruses Infecting Cultivated Plants: Case of Cassava Begomoviruses in Togo

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A study was carried out on some wild plants in order to understand the permanency of Cassava Begomoviruses diseases in Togo. Leaves were collected in cassava fields from cassava and some wild plants (*Albizia zygia* (DC) J.F. Mabry, *Senna hirsuta* (L.) H.S. Irvin and Barneby, *S. obtusifolia* (L.) H.S. Irvin and Barneby, *S. occidentalis* (L.) Link., *Manihot glaziovii* Müll. Arg., *Pupalia lappacea* (L.) Juss., *Strophanthus hispidus* DC) that exhibiting leaf curling, distorting and chlorotic lesions near the veins and stunting which were similar to the symptoms of Begomovirus infection and were analyzed by PCR assays with degenerate primers. Symptomatic leaf tissues from some of these infected plants were ground in 0.1 M phosphate buffer (1:1, w/v pH7.0) and squeezed through double layered muslin cloth and the filtrate was mechanically inoculated to several indicator species. Consistent amplification of DNA fragment was obtained (770 bp fragments) from symptomatic plants. The PCR analysis revealed the association of Cassava mosaic begomovirus in *Albizia zygia*, *Senna obtusifolia*, *Manihot glaziovii*, *Pupalia lappacea*, *Strophanthus hispidus*. The presence of the virus was confirmed in host inoculated plants. The presence of the virus was confirmed in inoculated host plants analysed by PCR.

P PLANT 12

Drought stress and its effect on parameters associated with soft rot resistance of potatoes

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Introduction: Drought stress will become a growing risk for crop production worldwide. Potatoes are highly susceptible to drought, which may not only affect yield but also the resistance of tubers to pathogens such as *Pectobacterium carotovorum* (Pc) causing soft rot diseases. It is important thus to get a better knowledge on drought stress responses and their impact on resistance determining factors.

Objective: Therefore, the effect of drought stress on anthocyanins (Ac), antioxidants, soluble phenols, proteins, free amino acids (AAS), fatty acids (FA), peroxidase (POD) and lipid acyl hydrolase (LAH) enzyme activities expressed in tuber tissue was evaluated.

Methods: Studies were carried out on three potato genotypes grown in the glasshouse under control (sufficient water supply) and drought stress conditions. The tubers of the two variants were tested for antioxidants measured on a Photochem Instrument. Amounts of Ac, soluble phenols and proteins as well as POD and LAH were analysed on a UV photometer. AAS were assayed by HPLC and FA by GC.

Results: Drought stress had no significant effect on Ac, antioxidants, phenols and POD. But, drought stress significantly increased the level of soluble proteins ($P \leq 0.0001$) and LAH ($P \leq 0.001$), lipolytic enzymes involved in changes of membrane lipids. Also AAS were elevated in the drought stressed tubers as well as the portion of α -linolenic acid (ALA) in total lipids.

Conclusions: These results highlight the role of AAS, soluble proteins, LAH and ALA in drought stress responses. Enhanced α -linolenic acid levels may also be beneficial for the soft rot resistance which was found to be enhanced in the drought stressed tubers. ALA functions as a precursor for jasmonic acid and oxylipins associated both with plant resistance.

P PLANT 13

Climate changes lead to changes in precipitation - how does this influence the black dot disease of potatoes?

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Introduction: *Colletotrichum coccodes* is a pathogenic fungus that causes the black dot disease of potato (*Solanum tuberosum*). The phytopathogen is able to colonise and affect all vegetative plant organs [1], e.g. leaves, stolons and tubers, especially on

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senescent or stressed plants [2]. During the last few years the pathogen's importance has increased internationally [3], [4]. In Austria (experimental station Lambach/Stadl-Paura, Upper Austria) significant yield losses were observed since 2007 [5].

Objectives: The aim of this study is to investigate how different precipitation amounts influence the black dot disease concerning its symptoms as well as crop failures.

Materials and methods: Different cultivars of *S. tuberosum* were grown in climate chambers as well as field-grown. The impact of different precipitation amounts and water management, respectively, on pathology and harvest results was investigated. These analyses were done by inter alia resistance rating, determining the weight of tubers and analysing the influence of black dot disease on the plants' photosynthesis power.

Results: The amount of precipitation during the growing season influences the potato black dot disease significantly. This is particularly valid with regard to hot-dry summers such as in 2013.

Conclusion: Further investigations concerning the effects of climate change (especially precipitation and temperature) on black dot disease should be made to increase the understanding of this weakness parasite.

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P PLANT 14

Association Among Virulence, Temperature Tolerance and Triadimefon Resistance of *Blumeria graminis* f. sp. *tritici*

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Evolution of plant pathogen populations was affected by many factors, such as host resistance, fungicide applying, and environment factors. To explicit the association among virulence, temperature sensitivity, and azole resistant of *Blumeria graminis* f. sp. *tritici* (*Bgt*), 129 isolates collected from nine provinces/cities in China was tested. Furthermore, the relationships among them were figured out.

Virulence gene diversity showed that index was highest in Sichuan Province (0.2241) and lowest in Zhejiang province (0.0968). Triadimefon sensitivity showed that median EC_{50} was 109.97 mg/L with the coefficient 107.2, and the mean resistance factor (RF) was 52.62. The resistant frequency of all isolates to triadimefon was up to 99.21%. Temperature sensitivity showed that the range of ET_{50} was 21.34-24.46 °C and the mean ET_{50} was 23.14°C. Among those isolates, ET_{50} s of 58.76% isolates were within 23 - 24 °C range, and ET_{50} s of 3 isolates were above 24 °C, which was defined high temperature tolerance.

Fitness of high temperature tolerant and sensitive isolates were characterized. The latent period of temperature tolerant isolates in 23°C was as same as in 18 °C, and was lower in comparison with sensitive isolates. Wheat infection and conidiation of temperature tolerance was higher compare to sensitive isolates. Take all data together, the fitness of high temperature tolerance in higher temperature (23°C) were higher compare with temperature sensitive isolates.

The association among triadimefon-sensitivity, temperature sensitivity and virulence diversity of *Bgt* isolates showed that there was a logarithmic function ($r=0.2404$, $P=0.0096$) relationship between EC_{50} and numbers of virulence genes of *Bgt* isolates, and a negative correlation between temperature sensitivity and virulence diversity.

Those data suggested that high temperature tolerance and azole resistant isolates appeared in the fields. The fitness of temperature tolerance in high temperature was higher in comparison with sensitive isolates. The more virulence of the populations, the more resistant to triadimefon, but the less sensitive to temperature. These data may provide a reference for reasonable utilization of resistance varieties, as well as the use of triazole fungicides.

P PLANT 15

The Interaction of Temperature and Light on the Vegetative and Reproductive Growth of *Vitis vinifera* cv. Shiraz in the field condition

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Introduction: Heat events are common occurrences during the summer months in many parts of the warm regions in Australia. One of the ameliorative practices to cope with the negative effects of high temperatures is using artificial cover over the vines and grapes in the vineyard. However, an alteration of the vine microclimate can affect vine growth and development and grape berry traits which in turn can effect the wine quality.

Objectives: This study provides an understanding of the impact of artificial shade on the vegetative and reproductive growth of the Shiraz vines over the three years in the field conditions including summer heat events.

Material and Methods: In the three growing seasons (2011/12, 2012/13 and 2013/14), open canopy vines were compared with vines exposed to three different light intensities. These were obtained by using artificial shade cloth of differing densities notably, no light reduction (open canopy), hereafter referred to as 'Control'; 10% light reduction, hereafter referred to as 'light shade'; 30% light reduction, referred to as 'medium shade', and 50% light reduction hereafter referred to as 'heavy shade'.

Results: The vegetative and reproductive growths were affected by the different shade treatments. With respect to the control berries, the light shade berries had markedly higher sugar content and yield during the summer heat events. In addition, anthocyanin and berry bio mass also increased which are important quality characters for the wine industry. The medium shade berries enriched with the higher sugar content even subjected to high temperature events and had lower pH and higher acidity. Moreover the berry growth, bio mass accumulation and yield were not affected under the characteristic high temperature during the summer. Similarly, dynamics of berry expansion and bio mass accumulation were not penalised. The heaviest shade berries had lower pH and higher acidity under the warm climate conditions and higher dynamics of berry expansion under the cool climate conditions.

Conclusion: Shade treatments were highly effective in sugar accumulation, anthocyanin biosynthesis, higher acidity, berry growth and yield during the summer high temperature events. Unequivocally, this will be a potential risk reduction mechanism of summer heat events in Australian viticulture.

P PLANT 16

Studies of the uprising disease *Ramularia* leaf spot for the improvement of an established Integrated Pest Management system to match challenges of a changing climate

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Introduction: Long term surveys show a shift in pathogen population in Bavaria. As a consequence the efficiency of established control strategies has been reduced. Unsatisfying barley yields concerning quantity and quality have been dominantly attributed to the occurrence of heavy leaf spotting caused by environmental factors and *Ramularia* leaf spotting (RLS). *Ramularia collo cygni* (Rcc), the biotic cause of RLS, has been detected in most barley growing regions in the world and although it was not included in established Integrated Pest Management (IPM) systems it had a strong impact on fungicide use.

Objectives: Improvement of an established IPM strategy, Gerstenmodell Bayern, by integrating the control of RLS. The investigation of the pathogen biology is the basis for optimized control.

Material and methods: In a joint project between the Technische Universität München and the Bavarian State Research Center different sites in Bavaria were monitored and specific fungicide trials conducted. The project has the advantage of intense observations in a region with high incidence and high agricultural and climatic variability. An improved strategy was developed and evaluated based on the experience of the fungicide trials.

Seed- and airborne inoculum, systemic infection and the impact of weather data were studied for a further improvement of the strategy.

A high number of isolates were collected for fungicide sensitivity and population genetic studies.

Results: The fungicide trials proved the high impact of the disease complex on yield quantity and quality. The monitoring showed a broad and regular occurrence on all sites. Results with an improved strategy generally gave a positive yield benefit under different environmental conditions.

Although Rcc showed high presence on seed and seed transfer is likely, epidemics were dominantly influenced by seasonal effects. A new seed treatment gave good control.

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Conclusions: Including RLS into IPM showed an important improvement. This points out the necessity of continuous adaptation to match the challenges of economic, political and climate changes. Fungicides prove good control for the moment. Still there remain open questions in the evolution of Rcc becoming a dominant pathogen. Advances in our understanding of the fungus could lead to longer term solutions.

P PLANT 17

Effects of Climatic Changes on Fire Blight Disease

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Plant health is predicted to generally suffer under climate change through a variety of mechanisms, due to mismatches between ecosystems and their climate and the more frequent occurrence of extreme weather events. There is potential for an overall increase in the number of outbreaks and northward migration of a wide variety of pathogens in the northern hemisphere as a result of global warming. Climate change affects disease management with regard to timing, preference and efficacy of chemical, physical and biological measures of control and their utilization within IPM strategies. Fire blight disease caused by *Erwinia amylovora* is the most serious current and long-term threat to pome fruits and unfortunately, it is still spreading geographically into new apple and pear growing areas. Warm and damp weather during flowering promotes spread of the disease. Data indicated that warmer springs will increase the rate of cell division within the immature fruit and more, smaller cells will lead to a crisper apple, better able to use the water available to it. The warmer springs could simply encourage the trees to grow more, rather than putting their energies into developing better fruit. Climate change and global warming will promote such condition in the future. With increasing temperatures and humidity due to global warming, fire blight is likely to reach devastating proportions in several countries with a similar climate. Most models use daily temperature and rainfall records, but there is now increasing use of surface wetness records to supplement grower observations of dewfall. The incidence of damaging storms varies widely among different climatic areas, but records of unusually heavy rain, hail, and strong gusty winds are important throughout the growing season. Evaluations of Maryblyt, Cougarblight, and Billing's Integrated System have been made over periods of 10 to 15 years in Europe, Asia, Australia and the United States. Research is needed in warmer climates on the effects of high daily temperatures (above 30°C) on fire blight epidemiology. There is a need for more sophisticated 'big picture', modeling studies that establish where climate change is likely to result in damaging fire blight epidemics.

P PLANT 18

Evaluation of different cultivars of *Olea europae* L. to attack of *Bactrocera olea* in Mediterranean climate.

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The olive fly (*Bactrocera oleae* Gmel.) is considered the pest that most adversely affects the olive grove, across the Mediterranean basin. Knowledge of resistance / susceptibility of germplasm *Olea Europeae* L. in Mediterranean countries is important to face the challenge of climate change and know how to drive a breeding program of the olive tree, in this issues. The use of resistant / tolerant cultivars may also be a resource to a significant reduction in the use of insecticides to control this pest, to protect subsidiary species and increase of organic olive oil production. To this goal, several olive cultivars (Spanish, Portuguese and Greek) were tested at Elvas (Portugal) in three consecutive years. The results showed significant differences among cultivars in susceptibility-tolerance sense. Among the olive cultivars studied were found resistant (R), moderately resistant (MR) and susceptible (S) cultivars. In addition to the differences observed between cultivars climate conditions / year appears to influence the fly attack.

P PLANT 19

Possible impact of climate change on the epidemic development and the fungicidal protection treatment of *Cercospora* leaf spot disease (*Cercospora beticola* sacc.) in sugar beets for Rhineland-Palatinate and the southern part of Hesse

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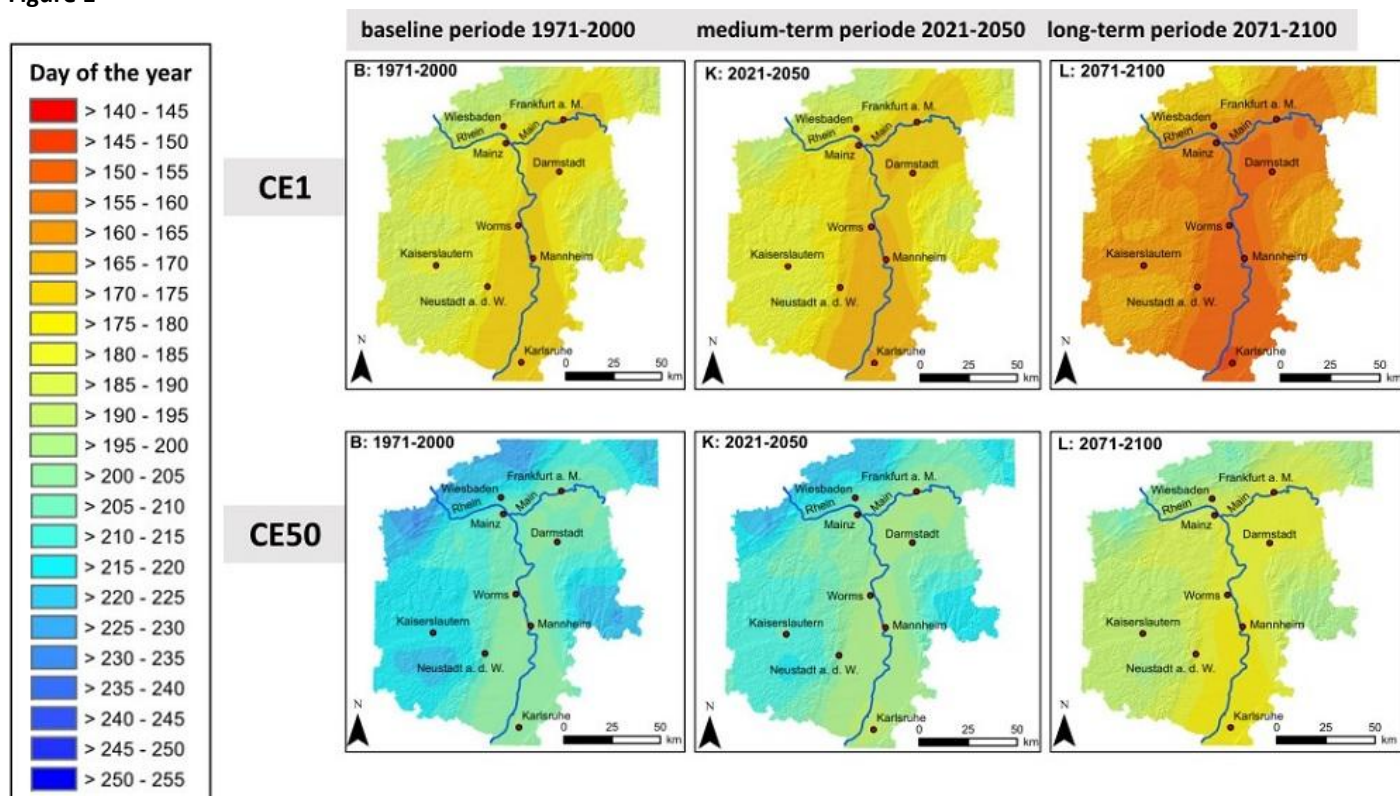
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The possible impact of climate change on the plant protection of the leaf spot disease *Cercospora beticola* sacc. (CLS) in sugar beets was analyzed by means of the forecasting models CERCbet1 and CERCbet3. In practical use, CERCbet1 projects the day of the year when 1 % (CE1) and 50 % (CE50) of the fields in a region are potentially infested by CLS. If CERCbet1 projects the attainment of CE50, the fungicidal strategy is being simulated and recommended by CERCbet3 on the basis of three-day weather forecast data of the German weather service. Besides these weather data, the model needs site-specific farmer input data such as attached variety, site characterization and the already conducted fungicidal treatment.

Other than that, CERCbet1 is usable as a model on the impact of climate change. The model was used in this context with REMO (REgional MOdel) climate projection data as input. The possible impact of climate change on the occurrence of CLS was studied in three time windows: a baseline period 'B' (1971-2000), a medium-term period 'K' (2021-2050) and a long-term period 'L' (2071-2100). Also Moreover, the ontogenesis of the sugar beet plants was simulated with the aid of a leaf-growth model simulating the leaf formation in early growth stages. The simulation results of CERCbet1 and the leaf-growth model were compared in order to draw conclusions on whether CLS would potentially occur in a different leaf stage. The date of completion of the 20- and 40-leaf stages (B20 and B40) was examined.

The comparison of the time windows B and K indicates that CLS1 has an earlier occurrence of 4 days, CLS50 of 5.7 days, and CLS100 of 7 days. In period L, CLS1 is achieved 20.9 days, CLS50 23.9 days, and CLS100 27.5 days earlier than in period B. The leaf-growth-stages shift slightly less forward than the CLS occurrence. For period L, B20 is projected 9.5 days and B40, 14 days earlier than in period B. An increasing number of fungicide applications could be one consequence.

Figure 1



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P PLANT 20

Malacological Diversity on Four Lamiaceae in the Region of Tlemcen (Northwest Algeria)

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The region of Tlemcen is located in the northwestern part of Algeria. Its arid climate leads to the degradation of vegetation in open formation, where can be found the doum, the diss and broom. Other aromatic species such as rosemary, thyme, lavender and horehound are considered as well. The four previous aromatic species belong to the family Labiatae for their morphological and botanical characters. The authors propose to design an approach to identify the diversity of malacofauna found on these different Lamiaceae. These are certainly a nutritional source for this malacological fauna. So, a survey was performed in various stations. The malacological richness is estimated to be 19 for thyme, 18 for rosemary, 14 for lavender, and finally 7 for horehound. It includes four families, namely Milacidae, Sphincterochilidae, Helicidae and Subulinidae. Milacidae are present only in horehound and lavender stations. On the other hand, the Sphincterochilidae, namely *Sphincterochila candidissima*, is absent on horehound and lavender. *Rumina decollata* is the only species in the family Subulinidae. As for the family Helicidae, it is the richest and includes two specific subfamilies: Helicinae and Helicellinae. The first subfamily consists of 12 species of lavender, 11 species of thyme, and 10 species of rosemary. The second subfamily includes 7 species of lavender, 6 species of thyme, and rosemary respectively. In addition, the author tries to look for the malacological species specific to each of these plants and those who are common to them as well. Finally, the vertical distribution of gastropods is given.

P PLANT 21

Crop protection prioritization to diseases of chickpea and pigeonpea in present climatic variations

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Increase in the frequency of climate extremes is likely to influence the distribution, establishment, survival and spread of pathogens. We initiated investigations to study changing scenario of diseases in two largest cultivated pulses - chickpea and pigeonpea to prioritize the crop protection measures. These pulses are largely grown in rain-fed environments that are most vulnerable to climate change. Changes in the disease spectrum in chickpea and pigeonpea for the past one decade were monitored through extensive surveys and analysis of disease and weather data indicated shift in the occurrence and distribution of diseases as well as emergence of new diseases. In chickpea, dry root rot (*Rhizoctonia bataticola*) is becoming more intense in typically tropical-humid areas, while viruses and rusts dominate in warm but dry zones. Rise in temperature coupled with reducing soil moisture was found responsible for increasing incidence of dry root rot. Extension in the range of *Ascochyta* blight and *Botrytis* gray mold in chickpea to new niches have been recorded due to the extended winters. Sporadic occurrence of diseases like rust, anthracnose, stunt and powdery mildew has also been observed. In pigeonpea, increased incidence of *Phytophthora* blight (*Phytophthora cajani*), is witnessed and a high correlation between rainfall, humidity and disease has been observed. Incidence of *Alternaria* blight, *Macrophomina phaseolina* and *Fusarium acuminatum* was also found in some regions. Expression of disease resistance in relation to climate change variables temperature and elevated CO₂ levels (550 and 700ppm) maintained under Open top chambers (OTC) and Free Air CO₂ Enrichment (FACE) indicated varied responses. Advancement in the incubation period in wilt and increased incidence of sterility mosaic disease in pigeonpea was found. Important challenges and opportunities will be highlighted for long-term planning to prioritize crop protection strategy to address future needs in these two legumes. Published and unpublished findings will be used to illustrate approaches and strategies to generate new and useful knowledge.

P PLANT 23

Management of Verticillium wilt with selective bacterial biological control agents in cotton

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Biological control agents (BCAs) show promise as safe and sustainable tools for integrated plant disease management. The objective of the present study was to determine the potential of BCAs acting alone or in combination with safe chemicals against *Verticillium* wilt in cotton under *in vitro* and greenhouse conditions. Nine BCAs tested by dual culture and volatile metabolite

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assays showed significantly varied responses. *Bacillus subtilis* was the most effective, followed by *B. megaterium*, *B. amyloliquefaciens*, *Pseudomonas aeruginosa*, and *P. fluorescens*, and their efficacies were further enhanced by their use in conjunction with safe chemicals. Under greenhouse test conditions, *B. subtilis* provided the greatest reduction in Verticillium wilt severity when used alone or in combination with safe chemicals, followed by *B. amyloliquefaciens* and then *P. aeruginosa*; similar results were obtained for the increase in plant height, fresh weight, and number of leaves. The findings of current study offer insights about the biological basis for managing Verticillium wilt in cotton crop and should ultimately assist growers and managers.

P PLANT 24

Wheat and barley production and protection in Morocco during 2013-14 growing season

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Septoria leaf blotch, yellow rust and leaf rust are the most damaging diseases on wheat whilst net blotch is so on barley. The objective of this survey was to assess the prevalence, incidence and severity of wheat and barley diseases across Morocco. The survey was carried out from mid-April to mid-June 2014 and the growth stage ranged from heading to physiological maturity. The data recorded were host species and its growth stage, visual assessment of grain yield and incidence and severity of the main diseases. A total of 84, 35 and 26 fields of bread wheat, durum and barley were inspected respectively.

The survey revealed that the most prevalent diseases on both bread and durum wheats were Septoria-like diseases (SLD), leaf rust, yellow rust and root rot complex. The same trend was observed during previous seasons except for yellow rust. The latter disease was, in previous seasons, limited to areas near mountains whilst during this season, it was observed on bread wheat in all inspected areas and on durum wheat in 5 out of the 7 inspected areas. Common bunt and Powdery mildew were less prevalent and were observed on both bread and durum wheats whilst Loose smut was observed on only one bread wheat field. In contrast, stem rust was not detected at all.

On barley, the observed diseases were Net blotch, Scald, Barley stripe, Leaf rust, Root Rot, Powdery mildew, Loose smut and Covered smut with the former one being the most devastating disease. Both Spot-type and Net-type net blotch diseases induced respectively by *Drechslera teres f.sp. maculata* and *D. teres f.sp. teres* were simultaneously detected even on the same leaf.

Crop growth conditions ranged from poor to excellent conditions and grain yield ranged from 2 to 60q/ha. Moreover, the estimated average grain yield across regions was 21.6, 19.9 and 14.0 q/ha for bread, durum and barley respectively. Almost all inspected fields of bread, durum and barley were under irrigated regime at Doukkala and Middle Alas.

P PLANT 25

Mycorrhization in Urban Tree Species Tested for Future Climate Conditions: Microanalyses, Enzyme Profiling, and Sequencing

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Urban trees grow in an unnatural environment. Limited tree pits, degraded, compacted soil with disturbed water and air balance, multiple pollutants, as well as frequent mechanical damage affect their vitality. Additionally, climate change causing rising temperatures with increased drought stress, and the appearance of new pests have exacerbated this stressful condition. Under such adverse circumstances mycorrhizal associations may play an even more beneficial role for plant health.

In an urban trees selecting program diverse tree species, representing different mycorrhizal preferences, were planted at three sites with differing climates in 2010. At planting half of the trees were inoculated with a mycorrhiza product (INOQ).

Fine roots of selected trees have been sampled from the root balls and since 2011 twice a year. Besides microanalysis of mycorrhization, activities of extracellular enzymes relevant to tree nutrition and connected to mycorrhizal colonization were profiled in a microplate multiple test (Courty et al. 2005). Fungal species in the rhizosphere were identified by molecular biological analyses, including 454 deep amplicon-sequencings (SYMPLANTA) for root samples taken in 2012.

In all trees tested mycorrhizal fungi could be detected microscopically. The frequency was always higher than 50% and displayed seasonal changes. Intensity of endomycorrhizal colonization averaged 15%.

Activity profiles of extracellular enzymes exhibited a high diversity. The patterns were tree species specific. In addition, seasonality could be detected with higher activities in spring compared to summer and autumn.

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Sequence analyses revealed a complex species assemblage of saprophytic, parasitic, ecto and endo- mycorrhizal fungi with individual patterns. Preferential associations between tree and fungal species could be detected.

Neither method revealed a difference between inoculated and non-inoculated trees.

In samples from the urban tree selecting program microanalysis, extraradicular enzyme profiling and sequencing of rhizosphere fungi exhibited individual patterns with tree species specificity. Seasonal changes could be observed in the degree and intensity of mycorrhization as well as in activity profiles of extraradicular enzymes. An effect of additional inoculation at planting could not be detected.

P PLANT 26

Predicting the effects of climate change on the plant/pest interaction of Strawberry and the Two Spotted Spider Mite

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Introduction: The two spotted spider mite (*Tetranychus urticae*) is a very successful polyphagous pest that can infest >1100 different plant species including diverse crops such as strawberry, apple, maize, tomato and grape. They favour hot and dry conditions, growing particularly well in the warmth of greenhouses. This means that the problem of *T. urticae* infestation for the agricultural industry is likely to become worse in areas of Europe which are predicted to become hotter and drier in the future (eg Southern Britain and Western Europe)

Objectives: Model population dynamics of spider mite infestation on strawberry plants under climate change conditions.

Study the transcriptomic changes in spider mites (*T. urticae*) and strawberry plants (*Fragaria ananassa*) during infestation under climate change conditions (high temperature, deficit irrigation).

Materials and methods: For modelling, strawberry plants will undergo either irrigation deficit or normal irrigation and be infested with adult female strawberry-adapted mites which were previously cultured on plants undergoing the relevant irrigation regime. The mites will be removed after 24 hours, leaving the eggs. The mite populations will be observed over 3 weeks and the data will be used for predicting mite population dynamics under irrigation deficit conditions.

For plant/pest interactions, strawberry plants will undergo either irrigation deficit or normal irrigation and be infested with adult female strawberry-adapted mites which were previously cultured on plants undergoing the relevant irrigation regime. Plant and mite samples will be taken at frequent intervals in the first 12 hours post-infestation and also after one and two days. The samples will be used to determine changes in the transcriptome using Next Generation Sequencing.

Conclusion: Experiments are currently ongoing so our initial conclusions will be presented for the first time at the IPPC.

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P PLANT 27

Environmental Factors and Management Practices Related to the Epidemics of Black Pod Disease of Cacao in Sulawesi, Indonesia

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To obtain a comprehensive understanding of factors related to the epidemic of black pod disease (BPD) of cacao, the development of the disease, environment parameters (altitude, distance to pristine forest, land use history), management practices (weeding frequency, degrees of shading, fertilizer application, canopy cover), and the age of host trees were investigated in 86 plots in two subdistricts adjacent to Lore Lindu National Park, Sulawesi, Indonesia. The study was conducted from November 2007 to August 2008. A simple correspondence analysis was performed to examine the association between the epidemic and the studied parameters. The result show that weeding frequency, altitude, preceding cropping practices, and land use history before establishing cacao plantation were significantly associated with the epidemic of black pod disease of cacao, while application of nitrogen fertilizer, distance to pristine forests, plant age, canopy cover and degree of shading by shade trees were not associated with the disease development. The highest incidence of BPD was significantly associated with an altitude above 600 m asl, in plots with preceding intensive inputs of pesticides and fertilizers, with high frequency of weeding, and in a plots with previously pristine forest or perennial plants.

P PLANT 28

Morphological and Molecular Characterization of *Bemisia tabaci* Genn. Spread in Syrian Coast

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Introduction: *Bemisia tabaci* (Gennadius) is one of the most devastating tropical and subtropical agricultural pest. It has been known in Syria since 1966. The Syrian coast is a suitable environment for whitefly populations where they are able to multiply rapidly during spring, summer and autumn and complete 8 generations in a year.

Objectives: To detect genetic variations among the whitefly populations which spread on the Syrian coast by using RAPD-PCR; and to determine the biotypes of this insect via mtCOI and the possibility of finding morphological differences that can be relied upon to distinguish these biotypes of whitefly populations.

Materials and methods: In 2011 about 44 insect samples were collected from the Syrian coast and reared for eight successive generations. DNA was extracted according to the protocol described by Barro & Driver (1997). The 18 primers were used for RAPD-PCR and the universal COI primers. The method used for preparation of slide mounted specimens is similar to that used by Martin (1987) and Wilkey (1962).

Results: 11 out of 18 specific primers for RAPD technique showed polymorphism with 169 polymorphic fragments and polymorphism percentage reaching 86.39 %. OPA-13 primer gave the highest number of amplified fragments with 29 bands and 93.1 % polymorphism percentage, whilst the least number of amplified fragments was four bands with 50 % polymorphism percentage gained by OPF-02 primer (Table 1).

The phylogenetic tree (Fig. 1), resultant from nucleotides sequencing of cytochrome c oxidase I (COI) gene, showed the existence of four biotypes common at the Syrian Coast, **nonB**, **Q**, **B** and **M** biotypes. Bootstrap value obtained by mtCOI technique was between 59 to 100 %. The four sequences have been submitted to the GeneBank databases under the following accession numbers: syMB-25 (KP202968), syMB-1 (KP342512), syMB-20 (KP342513) and syMB-41 (KP636539).

Common biotypes in the Syrian Coast were characterized morphologically, depending on some of morphometric parameters and morphology. Local biotypes were arranged according to morphometric parameters as follows; **M > Q > B = nonB**.

Conclusion: This paper is the first report of the geographic distribution of *B. tabaci* biotypes and their morphological in the Syrian Coast. The cluster analysis by using RAPD-PCR showed that the phylogenetic tree was divided into three classes. Four samples were identified using whitefly mitochondrial cytochrome oxidase I sequence analysis. They were the Q, B, nonB and M biotypes of *Bemisia tabaci*. Local biotypes were arranged according to morphometric parameters as follows; M > Q > B = nonB.

Figure 1

Table 1: Primers used in RAPD-PCR and number of amplified fragments.

Primer (Code)	Sequence 5' to 3'	Amplified fragments	Polymorphic fragments	Polymorphism%
OPF-02	GAGGATCCCT	4	2	50
OPF-12	ACGGTACCAG	19	19	100
OPH-09	TGTAGCTGGG	9	9	100
OPH-16	TCTCAGCTGG	15	12	80
OPA-04	AATCGGCTG	16	11	68.75
OPA-10	GTGATCGCAG	12	10	83.30
OPA-11	CAATCGCCGT	21	20	95.24
OPA-13	CAGCACCAC	29	27	93.10
OPA-15	TTCGAACCC	9	5	55.56
OPA-20	GTTGCGATCC	15	13	86.67
OPR-07	ACTGGCCTGA	20	18	90
Total		169	146	86.39
Average		15.36	13.27	

Figure 2

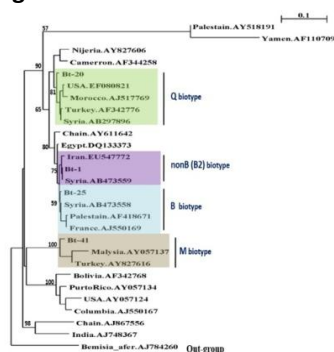


Fig. 1. Maximum likelihood phylogenetic tree for *Bemisia tabaci* reconstructed using the whitefly mitochondrial cytochrome oxidase I (mtCOI) sequence.

P PLANT 29

Evaluation of fungicides against anthracnose of chilli caused by *Colletotrichum capsici* under field conditions

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Anthraco disease is one of the major economic constraints to chilli production worldwide, especially in tropical and subtropical regions. Anthracnose of chilli is caused by more than one *Colletotrichum* species including *C. acuatum*, *C. capsici*, *C. gloeosporioides* and *C. coccodes*. *Colletotrichum* is one of the important plant pathogens worldwide causing diseases in a wide range of hosts including cereals, legumes, vegetables, perennial crops and tree fruits. Among these hosts, chilli (*Capsicum* spp.) an important economic crop worldwide is severely infected by anthracnose which may cause yield losses of up to 50%. Typical anthracnose symptoms on red chilli fruit include sunken necrotic tissues, with concentric rings of acervuli. Fruits showing blemishes have reduced marketability. Fungicides play an important role in management of the disease. This experiment was conducted in field area of Plant Pathology at CCS HAU Hisar on cv Pusa Jawala to find out efficacy of the new fungicides against the diseases. Among the different chemicals tested for the management of the disease three sprays of Propiconazole @0.1% at an interval of 12-15 days efficiently superior to manage / reduce the disease to the extent of 2.6% with increase in 85% yield followed by Hexaconazole @0.1%, Mancozeb @0.3% and Mancozeb @0.2% in comparison to control (20 % disease intensity).

P PLANT 30

Oxidative damage and photoprotectives roles of phenolic compounds in leaves of *Acacia arabica* (Lam) Willd submitted to drought stress.

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Introduction: Drought is a major environmental stress that has many threats on the growth and development of plants, especially in arid and semi-arid areas prone to desertification, mainly due to climate change. *Acacia arabica* (Lam) Willd. (Mimosoideae) is a fast growing tree native to Africa, frequently recommended to rehabilitate and restore degraded habitats due to natural events or human activities. This species has a high agroforestry potential in semi-arid regions of Africa.

Objectives: The aim of this work was to study how water deficit affected the physiological and biochemical behaviors of *A. arabica* seedlings, and determine the intervention of phenolic compounds in the oxidative stress caused by water stress.

Materials and methods: *A. arabica* plants of 6 week-old maintained under greenhouse conditions are submitted to water deficit by irrigation suppression for 32 days. Control and stressed leaves were analysed 7, 15, 22 and 32 days. RWC was evaluated according method of Barrs and Weatherley [1]. *Fv/Fm* measurements were carried out using chlorophyll fluorimeter. MDA was quantified by method of Heath and Packer [2]. Extraction and determination of total phenolics and anthocyanins were performed using method described respectively by Singleton et Rossi [3] and Mancinelli [4]. PAL activity was measured according to Olsen et al. [5].

Results: Water stress caused a decrease of RWC and a decline of *Fv/Fm* which was more prominent on day 22 and 32 of stress duration. Drought induced oxidative stress at the end of treatment by the accumulation of MDA. An increase in MDA concentration occurred only at high stress intensities, suggesting lipid peroxydation as a consequence of oxidative stress. In contrast, the severity of water stress (32 days after stress), causes an increase in phenylalanine ammonia-lyase activity (PAL; EC 4. 3. 1. 5), crucial enzyme in phenylpropanoid metabolism and consequently, produced important content of total phenolics and anthocyanins.

Conclusion: Increased PAL activity can be considered an important mechanism contributing to increasing accumulation of phenolic compounds in response to water stress and was expected to contribute to enhancing the antioxidant capacity of the cell and to the physiological adaptation of the *Acacia arabica* plants to water stress.

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P PLANT 31

Efficacy of bacterial isolates from five soils against *Aspergillus flavus* and *Fusarium verticillioides*

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Bacterial species, including the Actinomycetes were isolated from five soil samples from Akua and Ibaka areas of Akungba Akoko, Ondo State, Nigeria and were screened for their ability to reduce radial growth of mycotoxigenic fungi: *Aspergillus flavus* and *Fusarium verticillioides*. The isolates include *Pseudomonas fluorescens*, *P. aeruginosa*, *Bacillus subtilis*, *B. cereus*, *B. megaterium*, *B. globosporos*, *B. thurigiensis*, *Actinomyces* spp, *Mycobacterium* spp, *Rhodococcus* spp, *Norcadia* spp, *Streptomyces* spp and *Streptomonospora* spp. The results indicate that all the bacterial species exhibited varying degrees of biological control potential against the tested fungi. Out of all the bacterial species, *P. fluorescens* and *B. subtilis* showed more effective biocontrol potential against the tested fungi with the reduction of radial growth by 2.33cm and 0.59cm, while *B. globosporos* was the least effective with reduction of radial growth by 0.03cm for *Aspergillus flavus*. Among the Actinomycetes, *Streptomyces* spp had the highest inhibition zones of 1.60 cm against *Aspergillus flavus* and 2.10 cm against *Fusarium verticillioides* respectively, while *Streptomonospora* spp had the least. The results showed the antimicrobial activity of the bacterial species, and thus indicate the potential of using them as biofungicidal agents in reducing the damages and threat caused by *Aspergillus flavus* and *Fusarium verticillioides* in crop production.

P PLANT 32

Microtubules serves as „Thermometer“ under cold stress in grapevine

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Plant microtubules, in addition to their role in cell division and axial cell expansion, may function as alternative sensor for gravity, temperature fluctuation, osmolarity alteration as well as pathogen attack. The disassembly and reassembly of MTs in response to cold stress indicates its role as a „Thermometer“ by which the plants can sense and adapt to low temperature. This study aims to understand the relationship between MT and cold sensing as well as other partners participate in cold signaling in grapevine and facilitate the development of strategies to improve cold tolerance of grapevine.

Questions:

- 1) How MTs respond to cold stress?
- 2) What is the response of other stress/adaptation related signals/events during cold stress?
- 3) What is the optimal conditions for inducing efficient cold hardening.
- 4) Developing approaches (adjusting the changes of MTs) to engineer cold resistance through acceleration of cold hardening or increase the amplitude of cold resistance.

Materials and methods: The *Vitis* suspension cell cultures of *V. Rupestris* _GFP-AtTUB6 were used in this experiment. Different signals involved in cold stress were tested through different inhibitors and activators. For instance, calcium ions inhibitor $GdCl_3$, blocking the calcium ion channel on the membrane; Membrane fluidity inhibitor DMSO and activator benzyl alcohol (BA); and others like Diphenyleneiodonium(DPI), Sodium nitroprusside (SNP), Pertussis toxin and Aluminum Tetrafluoride.

Results (parts):

Result 1: MTs depolymerize within 30 mins after being exposed to cold stress. (Figure.1)

Result 2: Blocking of Calcium fluxes through $GdCl_3$ and chelation of Ca^{2+} via EGTA inhibit the depolymerization of MTs in response to cold stress (Figure.2)

Result 3: Membrane perforation using DMSO as well as $[Ca^{2+}_{cyt}]$ increase through could induce MTs depolymerization at 27°C and increasing membrane fluidity through Benzyl alcohol could restrain the depolymerization of MTs under cold stress. (Figure.3)

Result4: Blocking of ROS production through DPI treatment could inhibit the depolymerization of central and cortical MTs in response to cold stress. (Figure.4)

Conclusions: In this study, cold-activated calcium channels and the changes of membrane fluidity working together act as modulators to adjust the changes of MTs under cold stress, During this pathway, reactive oxygen species, Jasmonic acid, phospholipase D and G proteins were all activated and synthesized a complicate signal transduction system and MTs are in the core position, i.e. they would function as “thermometers”. The efficiency of this “thermometer” function would depend on the degree of microtubule dynamics. Within taxol and oryzalin treatment could further demonstrated that the initial microtubule disassembly is sufficient to trigger cold acclimation. This could be a new method to engineer cold resistance through acceleration of cold hardening or increase the amplitude of cold resistance.

Figure 1

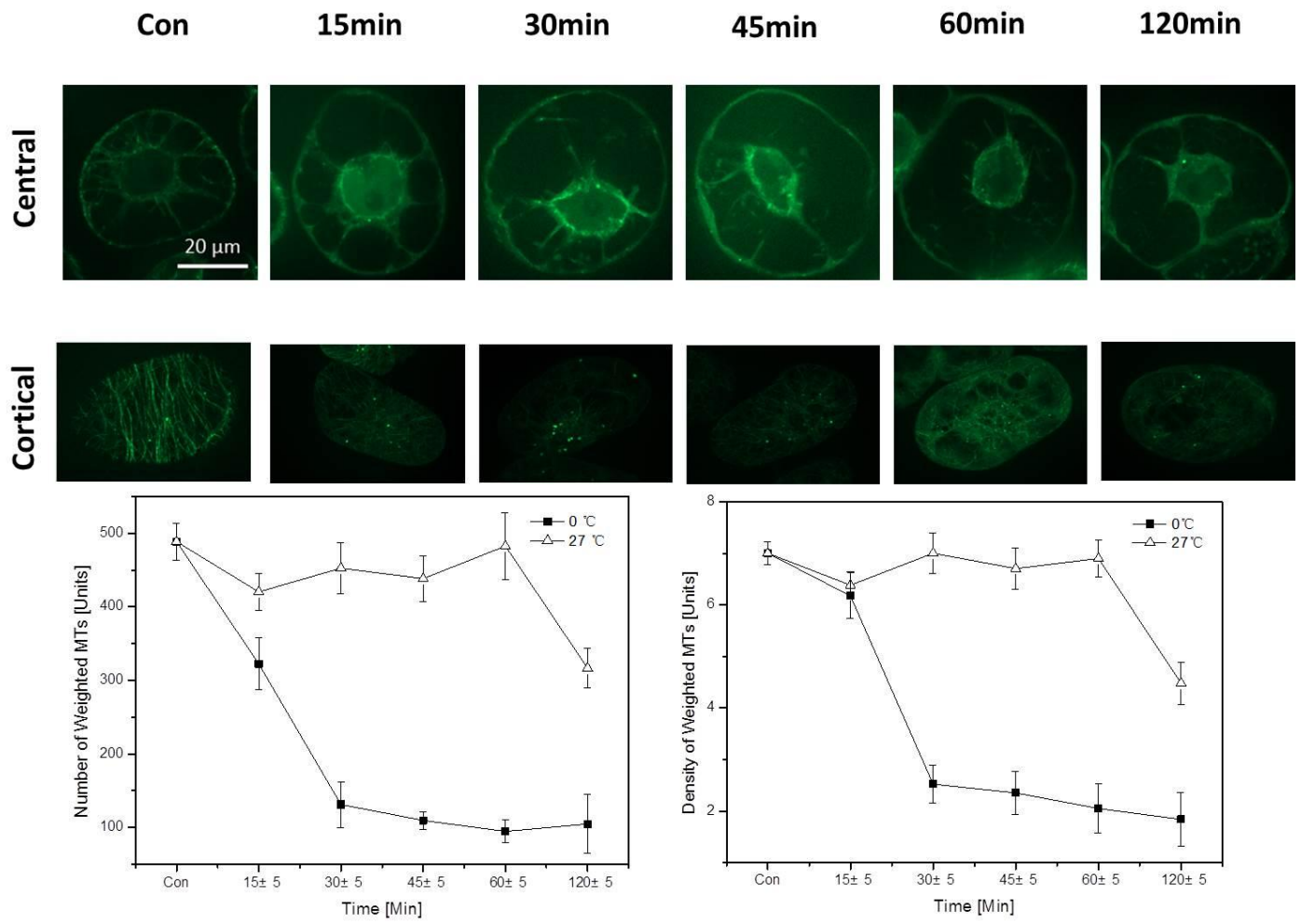
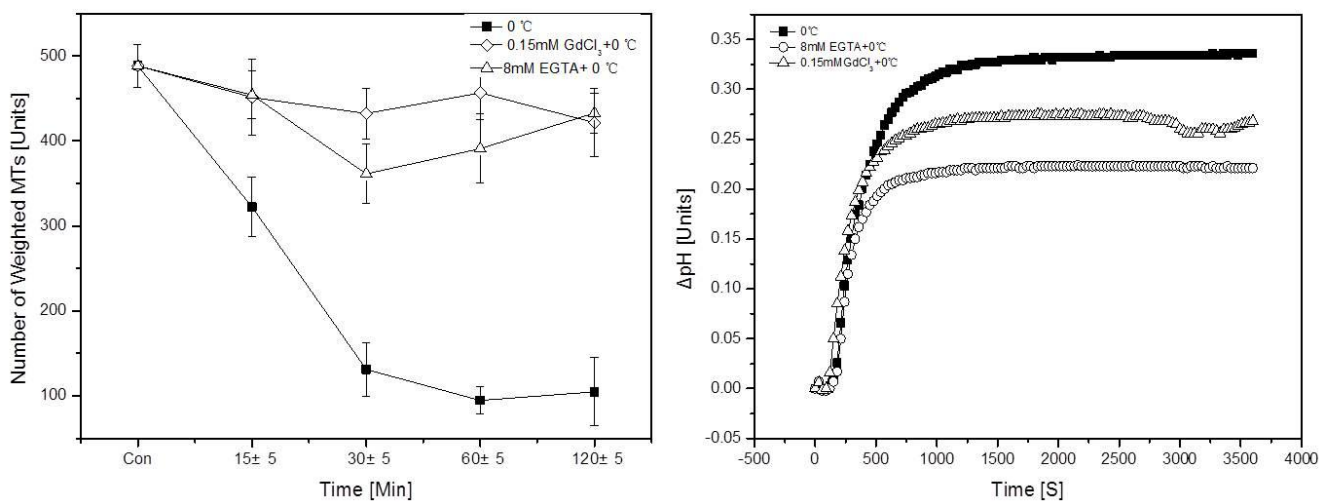


Figure 2



P PLANT 33

Population genetic analyses of South African *Venturia inaequalis* isolates from four apple growing regions

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Introduction: Apple scab caused by *Venturia inaequalis*, is one of the most important diseases of apple (*Malus pumila*), in terms of economic crop losses, in South Africa and world-wide. The disease is mainly controlled by fungicides, and up to when 100% crop losses will occur when no fungicides are sprayed.

Objective: The aim was to characterise the *V. inaequalis* populations from four climatically different apple growing regions of South Africa at the molecular level to determine if there are any differences between them.

Material and methods: In the 2013/14 apple growing seasons, apple scab isolates were collected during October to December 2013 (summer months) from lesions on leaves and fruit from the four apple growing regions in South Africa, namely Lower and Upper Langkloof, Elgin and Ceres apple growing regions. DNA was extracted from 250 (2013/14) isolates and species identification was done and confirmed by using ITS sequencing. Eight microsatellite markers were used for genotyping the South African *V. inaequalis* population.

Results: Five ITS haplotypes were identified. Results from microsatellite genotype analyses, indicate that the South African *V. inaequalis* populations are highly variable and sexually outcrossing ($V_o/V_e = 1.18$; $P = 0.55$). Each region's population was found to have 'private' alleles indicating moderate differences between them ($F_{st} = 0.15$; $P = 0.001$). Little population differentiation was found between the Ceres and the two Langkloof populations although these were more than 500 km apart. There were moderate differences between the other populations.

Conclusion: The differences between the populations, may have implications on the apple scab control and that different management practices will be needed to control the disease in these different areas. The disease may also have adapted to the different climatic conditions in these areas.

P PLANT 34

Evaluation of bioagents for management of downy mildew of pearl millet caused by *Sclerospora graminicola* (Sacc.) Schroet

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Downy mildew of pearl millet incited by *Sclerospora graminicola* (Sacc.) Schroet is the most widespread and destructive disease of pearl millet in India and other pearl millet growing area of the world. This disease is a major factor limiting full exploitation of high yield potential hybrids in India. The field trials were conducted in Department of Plant Pathology research area of CCS Haryana Agricultural University Hisar with the objective to evaluate different biological agents for the management of pearl millet downy mildew with an attempt to develop an ecofriendly management strategy through seed treatment. Pearl millet seeds of moderately resistant hybrid B 2301 were treated with *Bacillus pumulis* (INR 7 @10 g/Kg), Chitosan @ 2.5 g/Kg seed, Chitosan + *Bacillus pumulis* @ 10 g/Kg, *Trichoderma viride* @ 4g/Kg, *Trichoderma harzianum* @ 4g/Kg, *Pseudomonas fluorescens* (Pf1 @ 10g /Kg), chemical treatment with Apron SD 35 @ 6 g/Kg seed, normal control and then sown under sick plot and observation on field emergence, disease incidence at 30 and 60 DAS were recorded. Among the different treatment field emergence was maximum in treatment *Bacillus pumulis* (INR 7) + Chitosan and the disease incidence was minimum among the different biological agents and significantly better than control and almost equivalent to the chemical treatment of Apron SD 35 @ 6g/Kg seed.

P PLANT 35

Host feeding resistance of *Sitobion avenae* (F.) harbouring bacterial secondary symbionts (BSS) against *Aphelinus abdominalis* (Dalman) at different temperatures

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Parasitoids are important components of almost all terrestrial ecosystems. The interactions between host and parasitoid species is largely depending upon host genotype and climatic conditions. Host feeding benefit parasitoids to enhance their longevity and fecundity. However, abiotic stresses like temperature significantly affects host feeding. Aphid resistance against environmental stress depends on extracellular bacterial secondary symbiont (BSS). The interactions between *Sitobion avenae* (F.) and *Aphelinus*

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abdominalis host feeding behaviour were tested with and without bacterial secondary symbionts (*Hamiltonella defensa* and *Regiella insecticola*) at variable temperatures. Genetically identical wheat aphid clones possessing BSS (+ve) or lacking BSS (-ve) were used in these experiments. Choice and no choice experiments revealed that BSS increased resistance in wheat aphids against *A. abdominalis* host feeding at all tested temperatures, thus reducing aphid mortality. *A. abdominalis* host feeding efficiency directly correlated with increasing temperatures. In choice experiments *A. abdominalis* consumed more *S. avenae* without BSS as compared to those harboring BSS. Variation in host feeding resistance was also observed in genetically different *S. avenae* clones. Our results explain the role of endosymbiotic bacteria in improving the fitness in *S. avenae* clones, a key to their successful spread among many insect populations, particularly in aphids.

P PLANT 36

Efforts to reduce degradation of land by cultivation of *Vetiveria* in Alahan Panjang Solok Regency, West Sumatra Indonesia

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Agricultural land in Alahan Panjang Batu Bagirik district, Lembah Gumanti, Solok Regency West Sumatera for crop development is intensive agricultural land used by farmers. The agricultural land in Alahan Panjang, Batu Bagirik district. Lembah Gumanti, Solok had potential for the development of the wheat crop, with the result of the weight of 1000 seeds g^{-1} from 47.90 to 49.88. Although the opportunity for agricultural cultivation, the land is prone to landslides and erosion, due to the level of the slope, rainfall is relatively higher, and the ground is unstable. The bulk density of the soil at Alahan Panjang Batu Bagirik district was 0.40 - 0.45 gcm^{-3} . The application of cultivation techniques using plant fence hall vetiver (*Vetiveria zizanioides* L.) on agricultural land is expected to reduce land degradation in Alahan Panjang Solok, West Sumatra Province. The main benefit of the expected results of the activities is the planting of vetiver as the fencing hall can increase the organic matter content and reduce erosion after several years of growing wheat, and ultimately obtained an increase in the fertility of physics, chemistry and biology as well as wheat crop yield and income local farmers, both of crops of wheat and vetiver, so it will be beneficial to the welfare of the life of local farmers, especially farmers' groups who follow a program of planting wheat in a wheat-based industrial village. Also the officers and field extension and Indonesian society in general.

P PLANT 37

Influence of the climate changes from spring period concerning maize leaf weevil (*Tanymecus dilaticollis* Gyll) attack at maize crops in south-east of the Romania

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Maize leaf weevil (*Tanymecus dilaticollis* Gyll) is one of the most dangerous pest of maize crops in south and south-east of the Romania. The pest attack occurs when maize is in first vegetation stages, between plants emergence (BBCH 10) and four leaf stages (BBCH 14). Every year, approximate 1 million hectares with maize are attacked by *T. dilaticollis*. In some cases, maize plants emerged from the untreated seeds can be destroyed. The insect activity is favored by high temperatures and draught from spring period, especially last decade of April and first two decades of May. In this paper, there were presented effect of the climatic conditions from spring period concerning attack of the maize leaf weevil, both at maize untreated and treated plants. Field experiences were carried out at NARDI Fundulea, Calarasi County, Romania, between 2011 and 2014. Attack intensity is evaluated when maize plants are in four leaf stage (BBCH 14), according a scale from 1 to 9, elaborated by Paulian (1972), where 1 represented an unattacked plant and 9 completely destroyed plant. At maize untreated plants, higher attack intensity of *T. dilaticollis* was registered in 2012 ($I=6.7$) and 2013 ($I=6.3$). In 2014 attack intensity of maize leaf weevil at untreated plants was 5.9 while in 2011 was 5.2. From four years taken in study, the most favorable for the pest attack was 2012. Rainfalls amount and average air temperatures registered in last decade of April and first two decades of May in 2012 were over multiyear averages. However more then 60 % of the precipitations registered in second decade of May, 2012, occur in one day. As result, even if the total amount of rainfalls registered in first two decades of May, 2012, were over multiyear average, maize leaf weevil attack was higher. Rainfalls distribution from period when maize plants are in first vegetation stages can favor *T. dilaticollis* attack. In different climatic conditions from spring period, between 2011 and 2014, seed treatment with clothianidin, imidacloprid and thiametoxam active ingredients provide effective protection of the maize plants, in first vegetation stages, against maize leaf weevil attack. After EU directive 485/2013 no insecticides remain available for maize seed treatment against *T. dilaticollis* in Romania.

P PLANT 38

Comparative efficacy of certain plant extracts alone and combination with profenofos against *Spodoptera littoralis* (Boisd.) (Lepidoptera: Noctuidae)

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This study was to evaluate the efficacy of some plant extracts for controlling cotton leaf worm. Ethyl alcohol, acetone and petroleum ether extracts of three plant species belonging to three different botanical families [*Strychnos nux-vomica* L. (Loganiaceae), *Euphorbia lathyris* L. (Euphorbiaceae) and *Datura stramonium* L. (Solanaceae)], a chemical insecticide; profenofos and their combinations were tested against second and fourth instars of *Spodoptera littoralis* under laboratory conditions. Obtained results revealed that, the ethanol extract of *S. nux-vomica* was the highest effective among all plant extracts, where the corrected mortality percentages were 92.31, 80.77, 57.69 and 26.92% to 2nd instar and 88.68, 73.58, 33.96 and 11.32% to 4th instar at concentrations 0.5, 0.25, 0.125 and 0.0625% respectively. Calculated LC₅₀'s were 0.109, 0.22 and 0.34% to 2nd instar and 0.168, 0.374 and 0.518% to 4th instar for ethanol, petroleum ether and acetone extracts respectively. Acetone extracts of all plants were of lower effect. The chemical insecticide profenofos proved of higher efficacy than plant extracts (LC₅₀'s = 0.0018 and 0.0027% for *S. littoralis* 2nd and 4th larval instars, respectively). The Co-toxicity factor reached 76 and 60 when mixing *S. nux-vomica* + Profenofos and *D. stramonium* + Profenofos at ratio 1:1 against *S. littoralis* 2nd instar larvae, thus indicating a potentiative effect. While treatment of the 4th instar larvae by the same mixtures resulted a co-toxicity factor below 20 at all mixing ratios indicating, only, an additive effect against this instar. This study observed that the ethanol extract of *S. nux-vomica* had the highest efficacy on *S. littoralis* 2nd and 4th instar larvae than all the remaining extracts.

P PLANT 39

Destruction of antioxidant property in *Hibiscus sabdariffa* leaves by signals from GSM Antennae

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Introduction: *Hibiscus sabdariffa* L. is a popular vegetable whose leaves and calyces have been widely used, with claims to have the potentials for treatment of several ailments such as hypertension, pyrexia, liver damages, leukaemia and a host of other diseases. These feats are attributed to its high content of antioxidants, the purpose for which it is cultivated and consumed.

Objective: This is to assess the impact of the electromagnetic radiation from 900 MHz GSM antennae on the antioxidant property of the leaf of *H. sabdariffa*.

Materials and methods: Seeds of *H. sabdariffa* were raised in eleven liters' sized containers filled with garden soil and placed at 100 m, 200 m, 300 m and 400 m from the GSM mast. Four locations in Niger State, Nigeria were used including a control site, without GSM signals. Signals distribution were determined with Model AM-10 RF meter (Acoustimeter) designed by EMFields, United Kingdom.

The fresh leaves were harvested after 24 weeks of the plant growth in the aforementioned locations and were subjected to assay for the free radical scavenging activity using 3.96 mg DPPH (2,2-diphenyl-1-picrylhydrazyl).

Results: The control plants gave a value of 71.62%, while all samples exposed to GSM ray emissions gave much lower values. For instance, leaf samples in location A 100 m gave 50.5 %, 200 m (30.5 %). 300 m (25.0 %) and 400 m (42.0 %). In location B 100 m, the value was 45 %, 200 m (26.8 %), 300 m (27.3 %) and 400 m (40.2 %). Samples from location C 100 m gave a value of 47.0 %, 200 m (38.5 %). 300 m (33.2 %) and 400 m (39.0 %). The results revealed a general decrease in the antioxidant potentials in the exposed plants compared with that of the control, indicating depleting impact of the ray emissions from the 900 MHz antennae on this property of the plant.

Conclusion: The study showed that the seemingly weak electromagnetic radiation from the 900 MHz GSM antennae destroyed the antioxidant potentials of *Hibiscus sabdariffa*, which is also a measure of its medicinal value for which the crop is grown and consumed.

This study therefore is very important in assessing the impact of the ray emissions from the GSM antennae on the sustenance of plant biodiversities, more so that the world at large is facing the great challenges of food security. The study also throws more light on the assessment of the environmental impact of the radiations from the GSM antennae on living things and the earnest need to address this issue in order to protect man, his crops and other life forms.

P PLANT 40

Occupational Pesticide Exposure in Southwestern Nigeria

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In Southwestern Nigeria, pesticide use is one of the most significant occupational health exposure risks for agricultural workers, especially those involved in producing arable crops. Pesticides are used extensively based on the idea of preventive spraying to protect the crop to ensure good yields and are often handled and applied irresponsibly and incorrectly. Farmers, labourers, and rural communities are jeopardised by the high dependence on pesticide in arable production and are exposed to pesticides on a daily basis. Farmers often do not use effective Personal Protective Equipment (PPE) and labourers, often women, are present in the field during spray activities. This leads to unacceptable levels of risks due to pesticide exposure and related health impact. At the moment there is limited awareness about the chronic negative health effects of exposure to pesticides such as cancer, infertility and miscarriages and especially women receive no or limited information on pesticides. A case study of World Bank intervention training on safe and effective use of pesticides in Ekiti State, Nigeria in order to curb non-compliance resulting in injuries and deaths is a positive development. The approach on safe and effective use of pesticides involves collaboration with local stakeholders like e.g. local government, agriculture and health extension, health clinics, farmers and female labourers, and the local rural community. Intervention activities consist of among others training, workshops, coaching activities, organising dialogues, development and distribution of awareness raising material and implementation of mitigation measures. Hence, integrated pest management offers the best approach to mitigating occupational pesticide exposure in southwestern Nigeria.

P PLANT 41

Entomological situation on native grapevine cultivars in Albania

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Introduction: Cultivation of grape in Albania has an ancient tradition and it is advantaged by suitable climatic conditions for a quality grape production. This fact is testified by its extension over whole territory of the country, as well as, by the existence of a great number of native cultivars, which are distinguished for quantitative productivity and they are well accepted by market. Albanian farmer knows well enough traditional technique of grape cultivation, based on generation experience and area's specificities. Recently, farmers are going gradually through from agro technique traditional means at cultivating systems based on modern and rational criteria. Those objectives can be achieved using integrated protection which consists in well recognition of phytosanitary problems of grape, in rational evaluation of every chemical intervention.

Methods: The experiment was carried out in Durres area. The varieties selected for experiment was a native cultivar "Shesh I zi". To identify the infected vegetative plant parts the analyzed plants were taken randomly in experimental field. The samples were analyzed in lab conditions under microscope for identification of pest species. In the same time the dynamic population of *L. botrana* monitoring was another goal of study. The adult flight of *Lobesia botrana* was monitored by setting up four pheromone traps. Captured adults were counted every week the population estimates of second and third generations were always carried out by periodical sampling of larval stages on bunches. Damage evaluation of 2-th and 3-rd is the basis of the economic thresholds.

Results: There are many species of insects which attacks native cultivars (Shesh I zi) of viticulture in Albania. They are as follow: *Derpanothrips reuteri* Uzel; *Frankliniella occidentalis* (Pergande); *Thrips* spp.; *Empoasca decipiens* Paoli; *Empoasca vitis* (Goethe); *Scaphaideus titanus* Ball; *Zygina flammigerra* Geoffroy; *Zygina rhamni* Ferrari; ***Viteus vitifoliae* (Fitch)**; *Planococcus* spp.; *Pseudococcus* spp.; *Parthenolecanium* spp.; ***Lobesia botrana* (Denis et Schiffermuller)**; *Otiorrhynchus* spp.

Conclusions: Only some of the abovementioned species could be considered as main pests of grapevine, which could infect plants during the year in a high level, with such a population density that could caused damages continuously and primary treatments are needed. We consider the main pests of grapevine (native cultivars) in Albania, ***Lobesia botrana*** and ***Viteus vitifoliae***.

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P PLANT 42

Evaluation of the Toxicity and Developmental Effects of New Plant Essential Oil Formulations against the Eggs of *Bemisia tabaci* B Biotype

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Bio-pesticides are one of the main alternatives to conventional insecticides. Several plant based products have been tested against the silverleaf whitefly (SLW). From literature, some results showed that plant extracts can be used as potential effective method to control *Bemisia tabaci* B biotype. Essential oil formulations are one of the alternatives that could manage this pest. After several preliminary tests, some botanical products showed ovicidal, nymphicidal and/or adulticidal effects against SLW developmental stages. Formulations prepared from those effective products and tested against SLW developmental stages. This study assessed the insecticidal effects of three formulations (F1, F2 and F3) that contain essential oil and surfactants under laboratory conditions. These formulations were tested at different concentrations (0.25%, 0.44%, 0.69%, 1%, and 1.23%) comparing with the control against the egg stages of SLW using a spraying method. Additionally, LD₅₀ and LD₉₀ values were also estimated and then LD₉₀ values were tested for phytotoxicity effects. At 1.23% mortality rates of the F1, F2 and F3 formulations were 85%, 70.8% and 69.2%, respectively. The LD₅₀ and LD₉₀ of the formulations were (0.73% and 1.59%), (1.02% and 3.43%) and (1.05% and 2.69%), respectively. The LD₉₀ values of F2 and F3 showed severe phytotoxicity effects on leaves whereas a slight phytotoxicity effect on the edge of the leaves when the LD₉₀ value of F1 was sprayed. The symptoms of unhatched eggs were, shriveled and dark brown egg cases. However, in some eggs, embryos managed to hatch but they died after hatching without leaving egg shell. From these results, those formulations that showed an insecticidal effect could be used as potential biopesticide against the eggs of silverleaf whitefly.

P POST 1

Post Harvest Treatment of Potato Tubers for protection against *Phthorimaea operculella* (Zeller) Infestation using Extract of *Agrotis ipsilon* (Hufn.) Larval Frass

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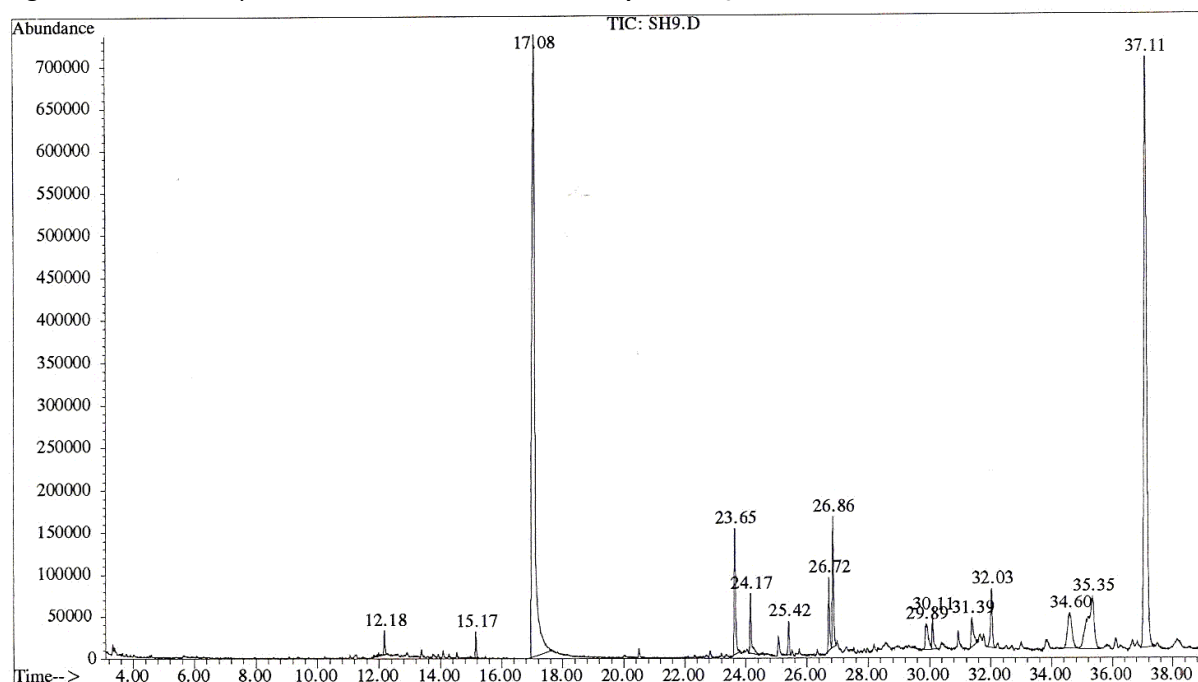
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The oviposition deterrent of ethanolic extract of *Agrotis ipsilon* (Hufn.) larval frass to *Phthorimaea operculella* (Zeller) adult females was evaluated under laboratory conditions. High concentrations of frass extracts were more effective than low at all treatments. Frass of young larvae (from 1st to 3rd instars) fed on natural host obtained more deterrent effect than others fed on semi-artificial diet while the opposite was found at treatments with older larval instars (from 4th to 6th) of the tested noctuid insect. The difference between the mean numbers of laid eggs was almost insignificant when the larvae fed on natural host as well as fed on semi-artificial diet. Ester form of several fatty acids (Palmitic acid, Myristic acid, Linolenic acid, Octadecanoic acid, Oleic acid, Ethyl9-Hexadecenoate and 7,10,13-Hexadecatrienoic acid) were identified during fractionation of the extracts using GC/MS technique. Palmitic acid and Myristic acid were mainly the most effective fractions.

Figure 1: GC/MS analysis of hexane extract of frass of *A. ipsilon* L₁₋₃ instars larvae fed on castor oil leaves.



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Poster Presentations
Post Harvest Treatments

P POST 2

Postharvest decay of persimmon fruit in Spain

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Introduction: In recent years, the cultivated area of persimmon (*Diospyros kaki* Thunb.) in Spain has increased to more than 13,000 ha, mainly located in Valencia province (90%). Most of this area is planted with cv. 'Rojo Brillante', an autochthonous cultivar of very high quality subjected to a growing demand by export European markets. This is basically due to the general adoption by Spanish growers of new postharvest technology (application of CO₂ at very high concentration) to efficiently remove the natural astringency of the fruit, which allows persimmons to be commercialized with a crisp texture and considerably extends fruit postharvest life. However, the incidence of postharvest diseases is an important factor limiting such extension of fruit storability.

Objectives: The aim of this study was to identify the most important pathogens causing postharvest decay on 'Rojo Brillante' persimmons grown in Spanish environmental conditions.

Materials and methods: For two consecutive seasons, 'Rojo Brillante' persimmons from two different orchards in the area of L'Alcudia (Valencia, Spain) were used to determine the etiology of postharvest diseases on both intact and artificially wounded fruits that were kept in humid chambers at 25°C for up to 9 weeks. Diseases were also determined in persimmons commercially-handled in a local packinghouse (no postharvest fungicide treatments are currently allowed in Spain) and stored at 1°C for up to 5 months. Fungal isolates were incubated in potato dextrose agar (PDA) medium at 25°C for purification and identification. In some cases pathogenicity tests were performed.

Results and conclusion: The main fungus causing disease on intact fruits was *Alternaria alternata* (black spot), for which a specific disease severity index (ponderate score from 0 to 5) was established for 'Rojo Brillante' persimmons. Other pathogenic fungi isolated from intact or cold-stored fruits were *Pestalotiopsis clavispora*, *Lasiodiplodia theobromae*, *Neofusicoccum mediterraneum*, *N. luteum* (stem-end rots), *Botrytis cinerea* (gray mold) and *Colletotrichum gloeosporioides* (anthracnose). Black spot, blue mold caused by *Penicillium expansum* and, to a much lesser extent, rots caused by *Rhizopus stolonifer*, *Cladosporium* spp. and *Trichoderma* spp. were the most common diseases on artificially wounded fruits.

P POST 3

Quantification of pesticide residues in grains from four major markets in Akure, Ondo State, Nigeria.

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Use of pesticides in crop protection against destructive pests often leads to the accumulation of residues of these chemicals in foods. Pesticide residues found in food stuffs are commonly associated with direct application of pesticides on crops to attack pests on farmland as well as protect produce during storage. This study focused on the determination of the concentrations of organophosphate and organochlorine pesticides in grain samples from major markets in Akure, Ondo State comparing it with established safety values.

Grain samples of beans, local rice, wheat and maize were purchased from different markets in Akure and analysed for residues of organophosphate and organochlorine pesticides. Extraction of grains was done using standard methods. Clean-up to remove co-extractives was done using Solid Phase Extraction with silica and sodium sulphate. Chemical analysis was done using gas chromatography with mass spectrometric detector (GC-MS) and Electron Capture Detector (GC-ECD) after careful extraction and clean-up.

The results of the study showed that all the samples contained residues of more than one organophosphate and organochlorine pesticides. Mean concentrations ranged from 0.01 to 5.99ppm. Maximum residue limits (MRL) of some organochlorine pesticide were exceeded in up to 80% of samples, while the organophosphate pesticides were all below the maximum residue limit described by FAO. Organochlorine pesticides were the most detected pesticides in all grains.

Due to the results of this research, it is important for regulatory agencies to monitor the type and concentration of pesticides used as grain protectants in storage. It will also be necessary to sensitize farmers and traders on the type of pesticide and best method of pesticide application that may prevent direct contacts with grains meant for consumption.

P POST 4

Inoculum sources of the post-harvest pathogens *Neofabraea* spp. and *Cadophora* spp. in Dutch apple and pear orchards

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Post-harvest diseases of apple and pear cause significant economic losses during long storage. Quiescent infections by fungal pathogens such as *Neofabraea alba*, *N. perennans*, *Neonectria galligena*, *Phytophthora* spp., *Alternaria* spp., *Fusarium* spp., *Cadophora* spp. and *Stemphylium vesicarium* can occur in Dutch orchards and lead to post-harvest fruit rots in storage. Knowledge on the occurrence of the different post-harvest diseases and their epidemiology is very limited. The objective of the study was to identify the inoculum sources of main post-harvest pathogens and to gain insight into their population dynamics.

Samples of various necrotic residues and tree parts were collected in 10 apple and 10 pear orchards during the growing season 2012 monthly from May until September and in December from 4 replicate plots in each orchard. Species-specific primers and probes were developed for *N. alba*, *N. perennans* and *C. luteo-olivacea*. TaqMan-PCR assays were used to quantify the amount of DNA of each of the three pathogens in the environmental samples from the orchards.

N. perennans was found only in few samples whereas *N. alba* and *C. luteo-olivacea* were abundantly present on necrotic tissues of apple and pear such as mummies, cankers and dead leaves. Interestingly, these pathogens were also found in varying amounts on necrotic tissues of other plant species present on the orchard floors such as various weeds and grasses. The concentration of pathogen DNA in the various substrate types varied during the growing season. Population dynamics also differed between individual orchards indicating that orchard characteristics and management strategies may influence the development of the pathogen populations.

The new knowledge on the major inoculum sources of fruit rot pathogens in apple and pear orchards is essential for the development of preventative sanitation measures which reduce the risk of pre-harvest infections by the pathogens followed by post-harvest losses. The inoculum load can be reduced by physical removal of the sources, enhancing decomposition or application of competitive biological control agents. The new knowledge on the variation of populations dynamics between but also within orchards can be used to identify major factors affecting pathogen survival and multiplication during the growing season.

P POST 5

Postharvest biocontrol of brown rot of peach with a chitinase produced by *Metschnikowia fructicola*

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Brown rot caused mainly by *Monilinia laxa* and *Monilinia fructicola* is considered the main postharvest disease of stone fruit. Biological control using antagonistic yeasts has been explored as one of several promising alternatives to chemical fungicides. Under semi-commercial conditions, *Metschnikowia fructicola* strain AP47 showed a high efficacy in controlling brown rot caused by *Monilinia* spp. on stone fruits. The yeast was able to produce chitinase enzymes in the presence of pathogen cell wall. A novel chitinase gene *MfChi* was amplified from the genomic DNA of *M. fructicola* AP47. *MfChi* was highly induced in *Metschnikowia fructicola* after interaction with *Monilinia fructicola* cell wall, suggesting a primary role of *MfChi* chitinase in the antagonistic activity of the yeast. *MfChi* gene was overexpressed in the heterologous expression system of *Pichia pastoris* and the recombinant chitinase showed high endochitinase activity. The antifungal activity of the recombinant chitinase was investigated against *Monilinia fructicola* and *Monilinia laxa* *in vitro* and on peaches. The chitinase significantly controlled the spore germination and the germ tube length of the tested pathogens in Potato Dextrose Broth medium and the mycelium diameter in Potato Dextrose Agar. The enzyme, when applied on peaches cv. Redhaven, successfully reduced brown rot severity. This work shows that the chitinase *MfChi* could be developed as a postharvest treatment with antimicrobial activity for fruit undergoing a short shelf life.

P POST 6

Use of gamma radiation to disinfestation of *Chamomilla recutita* from *Sphaericus gibboides* (Coleoptera: Ptinidae).

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This specie infests dried products of plant origin, grain and flour, dehydrated herbs and spices. The present study aimed to using different doses of gamma radiation with the aim of disinfestation of dehydrated *Chamomilla recutita* and to determine the lethal dose of gamma radiation on *Sphaericus gibboides*. The *C. recutita* material was infested with adults of *S. gibboides*, divided into 5 portions within of 30g with 20 individuals each. The last one month after infestation, insects adult were removed and using Radiator Multipropósito Cobalt-60 dose rate during the tests of 6 kGy/h, the plots were subjected to increasing doses of gamma radiation of 0 (control), 0.25; 0.50; 0.75; 1.00; 1.25; 1.50; 1.75 and 2.00 kGy. The determination of the lethal dose for adults *S. gibboides*, proceeded radiating 4 replicates per treatment each containing 25 insects adults. The doses used were: 0.6, 0.8, 1.1, 1.2, 1.4, 1.6; 1.8; 2.0, 2.2, 2.4, 2.6, 2.8 and 3.0 kGy. The lowest dose at no emergence of adult *S. gibboides* in *C. recutita* material tested was to 0,25 kGy. The dose of 2,2 kGy reached 100% mortality for adults of *S. gibboides*. The LD50 and LD90 was 1,55 and 2,36kGy, respectively.

P POST 7

New non-thermal postharvest technologies reducing strawberry fruit contamination

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New anti-microbial technologies are innovative and effective measure for reduce postharvest losses. *Botrytis cinerea* Pers.:Fr. infections caused seriously reduce yield and post-harvest quality. High-power pulsed light is an emerging non-thermal food technology that influences decontamination of objects by broad spectrum light. β -Na-chlorophyllinogen (Na-Chl II) is known as photosensitizer in photodynamic therapy, it is known as water-soluble food additive (E140) which is used as food toner. This study aimed to evaluate the efficacy of non-thermal measures for reducing *B. cinerea* in postharvest strawberry fruit.

The postharvest of Na-Chl II photosensitization effect on strawberry cv. 'Dar Select' were investigated at the LRCAF Institute of Horticulture in 2013. Experimental treatments included 1) control + not illuminated untreated fruit, 2) Na-Chl II + illuminated visible light and 3) sterile water + illuminated visible light. Strawberry fruits were incubated with photosensitizer Na-Chl II and then illuminated 30 min. with visible light ($\lambda=400$ nm with energy density of 20 mW/cm²). Fruits assessments were after 6, 8, 12 days on stimulated storage at 5-7°C.

Mycological analysis of fruits showed that fungi of the genus *Botrytis* showed a high relative density and were detected in all treatments. The decontamination of strawberries by Na-Chl II was effective compared to control and water. Na-Chl II reduced the spread of grey mould in strawberries after 6, 8 days on stimulated storage, compared with control there was less 20% and 15% rotten fruits accordingly. Evaluating strawberry we find out that using Na-Chl II after 8 days of storage there was 5 points more healthy fruits comparing with control and 4 points with water.

The photosensitizer Na-Chl II prolongs strawberry fruits shell life.

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P POST 8

Effect of inorganic salts on *Colletotrichum musae* and *Fusarium solani* - causal organisms of crown rot disease of banana

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Eight inorganic salts viz. Boric acid, Calcium carbonate, Calcium chloride, Calcium nitrate, Sodium bi carbonate, Sodium carbonate, Sodium chloride, Zinc sulphate were tested *in vitro* at five concentrations ranging from 0.1 to 0.5 per cent against *Colletotrichum musae* and *Fusarium solani* - the causal organisms of crown rot disease of banana. The conidial germination of both the fungi was completely inhibited by Boric acid (0.4%), Calcium carbonate (0.5%), Calcium chloride (0.5%), Sodium bi carbonate (0.4%) and Sodium chloride (0.5%). The other three salts viz. Calcium nitrate, Zinc sulphate, Sodium carbonate could not inhibit the conidial germination up to 100 per cent at the concentrations tested. The promising five inorganic salts, effective to inhibit the conidial germination of *C. musae* and *F. solani* were tested against crown rot disease of banana. Of the salts, Boric acid (0.4%) was found to be the most effective against the disease resulting highest crown rot reduction per cent.

The studies on textural and visual qualities and also sensory evaluation indicate that the maximum shelf life (14 days) was recorded under Boric acid (0.4%) followed by Calcium chloride at 0.5 per cent (12.5 days) against 9.5 days under control.

P POST 9

Evaluation of hardening and darkening of common beans during storage by HR-MAS NMR

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Introduction: Most foods can have easily deteriorate, especially in plants it starts immediately after harvest. Thus, conservation methods are important to ensure the quality of food products.

The beans storage at relatively high temperature conditions cause the development of Hard-to-Cook and Hard-to-Shell phenomena, reducing the grain's ability to absorb water and increasing cooking time¹. Several conditions can be used for storage, such as different temperatures and modified atmospheres. Understanding the phenomena that occur during the grain storage is important to reduce post-harvest losses.

Objectives: Use the NMR to analyze the variation in the metabolic profile of bean cultivars stored at controlled temperatures and modified atmosphere. Establishing the correlation between the metabolic variation with darkening and hardening grains proposing the best storage condition.

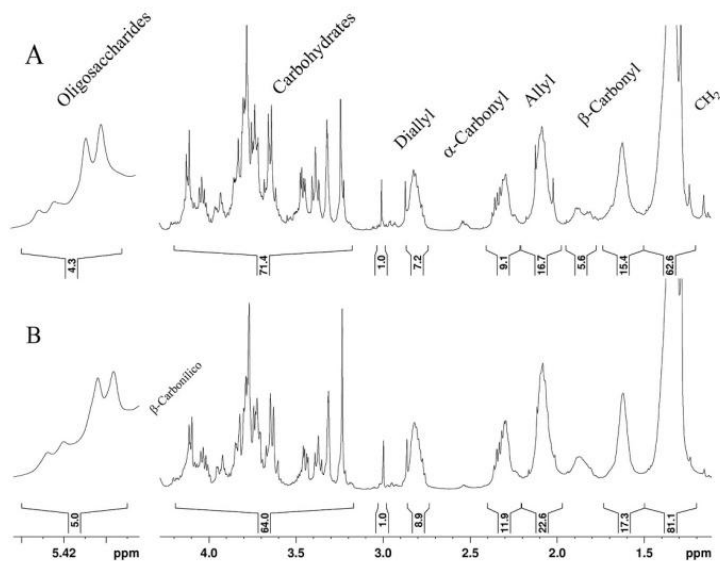
Materials and methods: Four recently collected bean cultivars were stored at controlled temperatures (-20, 15, 21 and 37 °C) and modified atmosphere (vacuum and nitrogen), and analyzed by ¹H HR-MAS NMR. The spectra were obtained in triplicate, 5 kHz spinning speed, 28 °C and 256 scans, on a Bruker Avance III 500 spectrometer.

Results: The major change in the grain metabolic profiles occurred in carbohydrates, fatty acids and oligosaccharides levels, as observed in Figure 1. It is remarkable that fatty acids increases the hydrophobicity of the grain contributing with the hardening of beans.

There was also observed variation in the phenolic compounds content, especially anthocyanins, whose oxidation minimized their content and were related to the darkening of the grain.

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Figure 1: ^1H HR-MAS NMR spectra highlighting the bean metabolic profiles variation during storage. A) recently harvested and B) storage during 108 days at 21 °C.



The lower storage temperature presents good grain quality maintenance. On the hand, better results were observed storing beans at nitrogen atmosphere.

Conclusion: The most efficient and economical way in the common bean storage, ensuring better grain quality, is to use modified atmosphere by nitrogen, preventing loss post-harvest and consequent decrease in the rejection by consumers.

References: ¹ GARCIA, E. *et al.* Journal of Agriculture and Food Chemistry, 46, 2110-2116, 1998

Acknowledgments: CNPq and FAPEG

P POST 10

Taxonomic identification of antifungal strain ZLZ261 and its activity assay on the control against *Monilinia fructicola*

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Introduction: Antifungal strain ZLZ261 was isolated from a tundra at the altitude of 4530 metres in Xizang, China, and presented a distinct inhibitory activity *in vitro* to *Monilinia fructicola*, the main pathogen of peach brown rot in China. Based on 16S rRNA gene sequences, the strain should belong to the genus *Collimonas*.

Objectives: The study was aimed at confirming the taxonomic status in species level of ZLZ261 and its control effect against peach brown rot, one of the major fruit diseases during post harvest and storage in China.

Materials and methods: Strain ZLZ261 and the pathogen *M. fructicola* were isolated by ourselves. Reference strains *C. fungivorans* CCUG 48868^T, *C. arenae* CCUG 54727^T and *C. pratensis* CCUG 54728^T were provided by CCUG, Sweden. For the taxonomy, the DNA homology analysis by DNA-DNA hybridization from renaturation rates in liquid phase and the carbon sources utilization test with Biolog were launched. The bioassay method to determine the inhibition zone diameters and the inoculation test on peach fruits were used for antifungal activity and control effect assay.

Results: The hybridization experiment showed that the DNA homologous hybrid rates between strains ZLZ261, CCUG 48868^T, CCUG 54727^T and CCUG 54728^T were 45.32%, 32.52% and 83.99% respectively, in which the DNA homology of ZLZ261 with *C. pratensis* CCUG 54728^T was within the species bound approved by International Committee on Systematic Bacteriology. Biolog test resulted in the same utilization of 51 carbon sources and the difference utilization of 11 carbon sources between the 4 strains of *Collimonas*. Especially, ZLZ261 was different from *C. pratensis* CCUG 54728^T on the utilization of succinic acid, urocanic acid and glucose-6-phosphate. The 23 mm diameter of inhibition zones against *M. fructicola* and the 86.21% control effect on the brown rot of peach fruits were presented by the fermentation broth of ZLZ261 in average.

Conclusion: The antifungal strain ZLZ261 was identified as the species *C. pratensis*, and possessed of good control effect on brown rot of peach fruits caused by *M. fructicola*.

P POST 11

Postharvest management of Monilinia rot of peach by DA-meter, a non-destructive technique

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Monilinia rot occurring in the postharvest phase can be a serious cause of fruit losses. Therefore, rapid, sensitive and reliable methods for the precise assessment of peach fruit ripening and, consequently, a probable incidence of Monilinia rot in asymptomatic fruit become crucial for effective fruit management. In the last few years, the development of non-destructive techniques to evaluate fruit ripening and assess internal quality attributes has increased. Among these, non-destructive approaches seems particularly promising, as the DA-Meter, that measures the new ripening Index of Absorbance Difference (I_{AD}) correlating with the actual flesh chlorophyll- α content and fruit ethylene production. The relationship between I_{AD} with quality parameters, ethylene emission and brown rot incidence in artificially infected peaches were investigated on "Springbelle", "Redhaven" and "Royal Summer" peach. Peach fruit of each variety were initially analyzed by a portable DA-Meter for the ripening I_{AD} . In order to define the ripening classes, ethylene emission of five to ten fruit per I_{AD} class was assessed before storage. After ethylene measurement, each varieties was divided in two I_{AD} classes: specifically, class I: 0.2 -0.4 (climacteric peak) and class II: 0.5-0.9 (onset of climacteric) for 'Springbelle', class I: 0.0-0.3 (climacteric peak) and class II: 0.4 -0.6 (onset of climacteric) for 'Redhaven' and class I: 0.5-1.0 (climacteric peak) and class II: 1.1-1.5 (onset of climacteric) for 'Royal Summer' peaches. Fruits, previously classified in two I_{AD} classes, as reported above, were wounded with a sterile nail (2x2x2mm) and inoculated with 20 μ L of the *M. fructicola* conidia suspension adjusted to 10^3 mL⁻¹. Fruits were stored at 20°C for 3 d and evaluated for lesion diameter. Fruit classified in two different classes of I_{AD} , class I more ripe than class II, revealed a diverse susceptibility to the pathogen. The severity of disease was significantly higher in peaches of class I than those of class II; the lesion diameters ranged from 9.3 mm ('Springbelle') to 21 mm ('Redhaven) in class I, and from 3.8 mm ('Springbelle') to 15.8 mm ('Redhaven') in class II. The results obtained in our study indicated, for the first time that the I_{AD} can be an "easy to use" parameter to improve the final destination of the product at harvest.

P POST 12

Freeze-dried *Lens Culinaris*: Analysis of texture applying non-destructive techniques.

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Introduction: One of the most important quality parameters in lentils is colour and texture; changes in any of these parameters affect customer's acceptance.

Objectives: The aim of the present research was to compare soaked-cooked and soaked-cooked-freeze dried (rehydrated) lentils in order to increase shelf life applying image analysis as a nondestructive, economic and rapid method to estimate mechanical texture property.

Material and methods: Colour and texture image, scanning electron microscopy (SEM) and mechanical texture were analyzed.

Results and discussion: SEM image showed that freeze-dried samples had similar structure to soaked-cooked samples. Significant differences ($P < 0.05$) within samples were observed for colour (lightness). Freeze-dried (rehydrated) samples showed higher lightness associated with the freezing process before drying samples. Differences in colour also depend on the dehydration and rehydration kinetics of the drying method. In order to evaluate image texture features for estimation of mechanical texture of lentils, linear regressions were conducted with texture image (contrast, homogeneity, entropy, correlation and energy) with mechanical texture (adhesiveness, chewiness, cohesiveness, gumminess and hardness). Texture images showed good correlations with hardness, adhesiveness, gumminess and chewiness. Multiple Linear Regression between mechanical texture and image texture features could predict high correlation of coefficient for hardness ($R^2 = 0.994$), chewiness ($R^2 = 0.791$), adhesiveness ($R^2 = 0.873$) and gumminess ($R^2 = 0.862$).

Conclusions: Results suggested that freeze-dried lentils showed difference among colour (lightness), but showed similar microstructure, image and mechanical texture when it was compared to soaked-cooked samples. Freeze-drying treatment is optimal to increase shelf life without losing quality factors. On the other hand image texture can be applied as a nondestructive, economic and rapid method for estimation of quality.

P POST 13

Rapid assessment on quality of fresh *Daucus carota* L. grown under organic and conventional farming systems

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Introduction: Carrot (*Daucus carota* L.) is an important vegetable crop worldwide, consumed by humans during the whole-year period in different forms. The most important quality attributes of carrot roots delivered for fresh market are root size, shape, uniformity, colour, texture and internal quality aspects.

Objectives: The aim of this research was to develop a rapid assessment on quality of fresh carrots grown under organic and conventional farming systems using odour pattern, colour and falcarindiol content as quality factors.

Material and methods: Carrots of different cultivars were harvested under two different farming systems (conventional and organic). In order to evaluate quality changes odour pattern was performed with an electronic nose composed by metal oxide sensors. Falcarindiol content was analyzed with a HPLC. Colour parameters were studied using a Spectrocolourimeter.

Results and discussion: Significant differences ($P < 0.05$) were observed in colour and falcarindiol content within cultivar and farming system. Linear Discriminant Analysis (LDA) with stepwise procedure was applied in each farming system to evaluate odour pattern. *Organic system* showed two discriminant functions (DF), explaining 95.7% and 3.0% of the total variance (98.7%), with a success rate of correct classification of each sample in their respective group of 98.1% and 91.0% of the original cases and after cross validation. *Conventional system* showed two DF, explaining 93.5% and 4.1% of the total variance (97.6%), with a success rate of correct classification of 96.1% and 91.5% (respectively). LDA applied to electronic nose data, colour and falcarindiol showed that in both farming systems, DF_1 was associated with metal oxide sensors type LY and falcarindiol content, being DF_2 related to colour. Results revealed that *conventional* carrots can be differentiated by colour values L^* , a^* , falcarindiol content and LY sensors; *organic* carrots by colour value a^* , falcarindiol content and LY sensors. L^* parameter was very remarkable to differentiate organic carrots from conventional system.

Conclusions: Colour, falcarindiol content and metal oxide LY type sensors showed to be a good match to approach a quick quality in field. With this approach to quality, this will be a useful tool to incorporate in horticulture field

P POST 14

First study on causal agents of post-harvest soft and dry rots in vegetables store and cold-room of Erbil province, Iraq

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Post-harvest soft and dry rot are considered as destructive diseases of vegetables and occur worldwide. In north of Iraq also especially Erbil region a huge proportion of harvest are discarded because of wrong disease management. However, no research is available on this problem so the present study gives inclusive information regarding the main pathogens to suggest proper disease prevention and control strategies. During six months, several stores were surveyed in order to etiological study of potato, tomato and capsicum that exhibited either soft or dry rot symptoms. The fungal and bacterial pathogens separated from infected samples and identified through special identification methods. Pathogenicity tests carried out on health samples which were same cultivars by common plant pathological methods. According to results, *Alternaria solani*, *Fusarium solani*, *Ralstonia solanacearum* and *Bacillus* sp. identified as major fungal and bacterial diseases agents of vegetables. In order to reach a successful disease control it is necessary to be familiar with all potential pathogens. For the first time, this study provided important information about post-harvest soft and dry rot pathogens on the most popular vegetables in Erbil, Iraq and the result could be useful to issue correct control methods.

P POST 15

Novel Biodegradable Coating to control postharvest Anthracnose and maintain quality of fresh fruits and vegetables

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Fresh fruits and vegetables have relatively short postharvest life. Their storage life is limited by several factors including transpiration, postharvest diseases, increased ripening and senescence. Many storage techniques including low temperature, controlled atmosphere and modified atmosphere storage have been used for retention of freshness of fruits and vegetables. But these processes are capital intensive and costly to run. Synthetic fungicides have also been used for controlling postharvest diseases. However, persistent use of these fungicides has resulted in the emergence of resistant strains and also posed more risks to human beings and environment. Therefore, more emphasis has been given to discover sustainable, non-chemical alternative techniques. A novel approach is the use of edible coatings obtained from waste materials which are biodegradable and environmentally friendly in nature. These can generate a modified atmosphere around the fruit by providing a semi-permeable barrier to gaseous exchange, reduce respiration rate and water loss. Gum arabic is a dried gummy exudate from the stems or branches of *Acacia* species. It is the most extensively used hydrocolloid in industrial sector because of its emulsification, film forming and encapsulation properties. Chitosan is another polysaccharide obtained from the exoskeleton of crustaceans, such as shrimps and crabs. It has become a potent alternative treatment for extending storage life and to control decay of fruits and vegetables due to its natural antimicrobial effects and elicitation activities in plant tissues. Propolis is a resinous substance, obtained by honey bee from different parts of plants. It has several antimicrobial and antifungal properties. It can also be used as an edible coating to control anthracnose of fresh fruits and vegetables. Therefore, edible coatings based on gum arabic, chitosan and propolis was developed which presents a simple, inexpensive and effective alternative for controlling anthracnose, enhancing quality and maintaining shelf-life of fresh fruits and vegetables, particularly in cold storage.

P POST 16

1. Effect of Postharvest Nitric oxide and Chitosan Treatments on Quality Attributes and Control of Fungal Decays of Peach

(*Prunus persica* cv. zaferani)

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The effects of postharvest nitric oxide and chitosan treatments on quality attributes of peach (*Prunus persica* cv. zaferani) fruits were evaluated. This experiment was carried out as a factorial experiment with a complete randomized design. Treatments were nitric oxide (at concentrations of 0, 5 and 10 $\mu\text{mol L}^{-1}$) with 4 replications, chitosan (at concentrations of 0, 0.5 and 1% w/v) and storage time (10, 20 and 30 days after treatments). Fruits after treatment with chitosan and nitric oxide were kept at 4 °C and 85-95% RH. Fruit quality attributes, including weight loss, firmness, total soluble solids (TSS), titrable acidity (TA), pH, ascorbic acid, decay index and browning percentages were evaluated. Results showed that the fruits treated with 5 and 10 $\mu\text{mol L}^{-1}$ nitric oxide had the less amount of pH in compared to control. During 10 days of storage, 1% chitosan+ 10 $\mu\text{mol L}^{-1}$ nitric oxide had the highest amount of ascorbic acid but in 20th day of storage 5 $\mu\text{mol L}^{-1}$ nitric oxide was the highest. Also, fruits treated with 5 $\mu\text{mol L}^{-1}$ nitric oxide had the highest amount of TA and fruits treated with 1% chitosan had low changes in TA amount. In 20th day 5 $\mu\text{mol L}^{-1}$ nitric oxide and 1% chitosan showed the lowest amount of TSS. At the end of storage period, fruits treated with 10 $\mu\text{mol L}^{-1}$ nitric oxide had the lowest amount of TSS. During the storage period, the percentage of weight loss were highest in the control treatment. In 30th day of storage period, fruits treated with 1% chitosan showed the lowest amount of weight loss, but there was no significant differences with 0.5% chitosan and fruits treated with both nitric oxide+ chitosan. At the end of storage period, 0.5% chitosan+ 5 $\mu\text{mol L}^{-1}$ nitric oxide treatment had the most effect on decay index of peach fruits and 1% chitosan+ 5 $\mu\text{mol L}^{-1}$ nitric oxide had the most effect on browning of peach fruits. By the way, in the control the highest amount of decay were observed in comparison with the other treatments.

P POST 17

The use of essential oils in combination with controlled atmosphere to control postharvest decay caused by *Botrytis cinerea* and *Penicillium expansum* on apples

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Fungal infections are the main source of postharvest rots of fruit during storage and transport, and cause significant economic losses. Increasing consumer concern regarding food safety and demand for organically produced fruit makes it necessary to search for natural environmentally friendly alternative methods for disease control. Preventative application of essential oils through thermofogging and dipping were tested *in vivo* for their potential to inhibit postharvest decay caused by *Botrytis cinerea* and *Penicillium expansum* on 'Granny Smith', 'Golden Delicious' and 'Pink Lady' apples. Treated fruit were stored at controlled atmosphere ('Granny Smith' and 'Pink Lady': 1.5% O₂ + 1% CO₂, -0.5°C and 'Golden Delicious': 1.5% O₂ + 2.5% CO₂, -0.5°C) for 28 days followed by 7 days at 20°C. After storage, lesion diameter was measured and expressed as percentage inhibition relative to control treatment. Application of essential oils (alone and mixtures) through thermofogging significantly ($P < 0.0001$) inhibited *B. cinerea* and *P. expansum* on all three cultivars compared to Rovral™, ethanol and control treatments. Essential oil mixtures showed the highest inhibition of *B. cinerea* and *P. expansum* on all three cultivars compared to single application of essential oils. In case of dipping of fruit, Rovral™ and essential oils provided the best inhibition of *B. cinerea* and *P. expansum* on each apple cultivar compared to ethanol and control treatments. The results suggest the possibility of using essential oils in combination with controlled atmosphere as natural fumigants for controlling postharvest diseases of apples.

P POST 18

Residues and effects on the aroma profile of apples after phosphine fumigation

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The fumigation of stored products is common practice to prevent the infestation and spread of pests through international trade. The fumigants most frequently used for quarantine and pre-shipment (QPS) purpose are methyl bromide, phosphine and sulfuryl fluoride. However, little is known on the effects caused by these substances on the fumigated goods. Based on the Montreal Protocol, the non-QPS use of methyl bromide was phased out by 2005 in developed countries and will be banned by 2015 in developing countries. One of the substitutes used is phosphine. Apples are fumigated prior to export to control eggs of pest insects like the codling moth (*Cydia pomonella*). In this study we addressed the question whether phosphine fumigation affects the aroma profile of apples (*Malus domestica* 'Royal Gala'). For this purpose a headspace solid-phase microextraction (HS-SPME) technique was developed and coupled to subsequent gas chromatography-mass spectrometry (GC-MS). The apples were fumigated for 48 h with a phosphine concentration of 2000 ppm. Following the fumigation procedure apples were aired and stored under controlled conditions. Samples for the analysis of the aroma profile were taken at day 1, 5, 9, 14, and 35 after fumigation has ended. The apples were processed and subsequently analyzed by HS-SPME-GC-MS. The results obtained for the fumigated samples were compared to untreated control samples using multivariate statistics (discriminant analysis). A second question addressed concerns the adsorption and desorption behavior of phosphine from apples under different conditions as well as the chemical residues. The impact of the initial fumigation concentration and of the storage temperature was analyzed. The phosphine concentration was thereby monitored using GC-MS instrumentation. This study shows that fumigation can affect the aroma profile of the goods fumigated and that further research is needed. Significant data are being obtained and will be presented.

P POST 19

The mode of antifungal action of lemongrass (*Cymbopogon citratus* (DC.) Stapf) essential oil on *Botrytis cinerea*

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The essential oil of lemongrass (*Cymbopogon citratus* (DC.) Stapf) was demonstrated in our previous studies as a potential source of an eco-friendly antifungal agent however, the antifungal effects of this essential oil on the morphology and biochemistry of *Botrytis cinerea* has not been documented. To elucidate the mechanism of the antifungal action, the effect of the essential oil was investigated using light microscopy, scanning electron microscopy and fluorescence microscopy. Moreover, light and scanning electron microscopy observations on hyphae exposed to lemongrass essential oil showed morphological alterations in hyphae, such as damaged cytoplasmic membrane, clear separation of cytoplasm from cell wall, vacuolation, and shriveling. *B. cinerea* hyphae treated with lemongrass essential oil showed strong propidium iodide fluorescence in the cytosol. Lemongrass essential oil increased the concentration of potassium ion and cellular materials in the medium. The glucose-induced reduction in external pH of *B. cinerea* was inhibited by lemongrass essential oil in a time and concentration dependent manner. These observations indicate that the antifungal activity of lemongrass oil results from its ability to disrupt permeability barrier of the plasma membrane, attack on the cell wall integrity and retraction of cytoplasm in the hyphae, and ultimately death of the mycelium.

P POST 20

Determining the efficacy of ozone technology against red flour beetle, *Tribolium castaneum* (Herbst), under high temperatures

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Ozone gas (O₃) as a fumigant substitute has shown potential for controlling insect pests of stored products. Additionally, extreme temperatures have been used to control insects in food processing structures. However, both have disadvantages and it is thought that combining both control methods could reduce the negative effects of both strategies, when used separately. In this study, efficacy of ozone for controlling adults, one of the most important insect pests in storage facilities, was evaluated at high temperatures. Insects were exposed to a continuous stream of 100ppm ozone for 6, 12, 18, 24, 30 and 36 hours at 35 and 40°C. Results indicate that there is a direct relationship between these two factors i.e temperature & exposure time with the mortality of *T. castaneum*. Mortality increased with increasing temperature and exposure time. 100 % mortality was achieved at 36 and 30 hr with 35 and 40°C.

P POST 21

Study of the potential use of essential oil from *Syzygium aromaticum* in *Dioscorea rotundata* rot control

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The post harvest rots due to fungi cause enormous losses of crops and the mycotoxins they produce are involved in many human and animals diseases. The essential oil of *S. aromaticum* fruits was investigated for its antifungal activity on yam pathogenic fungi in order to identify natural products for plant disease control. The chemical composition analysis of the essential oil was carried out using gas chromatography (GC) and gas chromatography coupled to mass spectrometry (GC/SM). Fungi species were isolated and identified through macroscopic and microscopic identification methods. The antifungal parameters of the essential oil were determined in vitro using solid and liquid dilution methods and in situ by direct inoculation method. Many compounds were identified amongst which Eugenol (79.4 %) and Eugenol acetate (9.2 %) were the main components accounting for 99.7 % of the total oil. The fungi isolated and identified were *Fusarium solani* and *Rhizopus stolonifer*. Pathogenicity test was carried out to confirm these organisms as the pathological agents of the *Dioscorea rotundata* post-harvest rot. The results of the present study showed that *S. aromaticum* essential oil portrayed MIC values of 200 and 300 ppm

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respectively on the mycelium growth of *R. stolonifer* and *F. solani*, 31.25 and 250 ppm on their spore's germination respectively. The results of in situ studies showed that *S. aromaticum* essential oil decreased the disease severity compared to the control (inoculated and not untreated).

These results show that the essential oil of *S. aromaticum* fruits might be used for the post-harvest protection of *Dioscorea rotundata*.

P POST 22

Combination of Fludioxonil and LI-F type antibiotics produced by *Paenibacillus polymyxa* for controlling against citrus green mold

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Green mold, caused by *Penicillium digitatum*, is one of the most important postharvest decays of citrus fruit. The conventional synthetic fungicides are very effective against this pathogen while intense application of synthetic fungicides undoubtedly causes concerns over the environment and human health. The use of biological agents in postharvest decay control as alternative to synthetic fungicides has drawn public attention in the last two decades.

The extraction process of secondary metabolites LI-F type antibiotics (LI-Fs) produced by *Paenibacillus polymyxa* SG-6 was improved and synergistic effects of LI-Fs mixed with fungicides were investigated in this study.

Inhibitory effect of citrus green mold by LI-Fs, fludioxonil, imazalil, prochloraz, pyraclostrobin, propiconazole were measured and the six fungicides had strong inhibitory activity on mycelial growth of citrus green mold. Growth rate of mycelia of citrus green mold was determined to screen the efficiency ratio of LI-Fs and fungicides. The optimal mixed ratio of LI-Fs and fludioxonil against mycelia growth of citrus green mold was demonstrated to be 8:2 with the synergism ratio of 2.22. However, there were no significant synergistic effects of other fungicides when mixed. Preservation effects of LI-Fs- fludioxonil mixture at different concentrations were studied with citrus fruit as the test materials. The mixtures could significantly control the natural incidences of citrus green mold comparing with control in storage conditions of 25 °C for 4 weeks and at 6 °C for 4 weeks followed by 25 °C for 2 weeks. In especial, when mixed concentration was 500 mg.L⁻¹, the natural incidences of citrus green mold in both storage conditions were 17.78% and 10.18%, which were significantly higher than the single agent.

According to the test results, it was the first time that LI-Fs mixed with chemicals as a new kind of fruits preservation agent was proposed, which laid the foundation for the further research and development.

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P SOIL 1

Mass production, formulation and application of *Trichoderma* for soil borne disease management of vegetable crops

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Trichoderma, a bio-control agent has gained maximum attention due to its effectiveness to control a large number of soil-borne plant pathogenic fungi (*Fusarium*, *Sclerotium*, *Rhizoctonia*, *Pythium* and *Phytophthora*) and root-knot nematodes without adversely affecting beneficial microbes. There are two major methods of inoculum (spore) production of *Trichoderma* spp. viz., solid state form and liquid state form. In solid form, the fungus is grown on various barley grain, maize bran, grass pea bran, rice husk and sawdust. The solid state productions are used mainly for direct soil application nurseries or greenhouses (small scale production) to suppress the soil-borne diseases. In liquid form, *Trichoderma* is grown large scale in yeast enriched Richrds solution or yeast enriched potato dextrose broth. Biomass (spore and mycelium) from the liquid form can be directly used for decomposing organic materials in composting house, where the final product is considered as bio-fertilizer (Tricho-compost) or preserved them by making different formulations such as talc base. Shelf life of talc base *Trichoderma* can be six months or one year depending on temperature and moisture contained in formulated products. Tricho-compost can effectively control soil-borne diseases of vegetables and some field crops. However, as Tricho-compost is heavily volumetric in nature, it is expensive and laborious to take long distance. On the other hand, talc base *Trichoderma* is easy to handle and carry in long distance. Talc base *Trichoderma* may be used for production of Tricho-compost or for spraying on foliage to control air borne diseases or drenching in root zone to control soil borne diseases. After seven months the spore of *Trichoderma* was found alive in talc formulation. However, apart from the counts of live spore, bio-efficacy was examined against diseases of vegetable crops to ensure effectiveness of the products.

P SOIL 3

Nursery evaluation of indigenous and exotic apple cultivars against alternaria blight in kumaun region of Uttarakhand

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Alternaria leaf spot (caused by *Alternaria* sp.) is an emerging disease of apple nursery field. Alternaria leaf spot causes unsightly necrotic spots or blotches on the leaves which when viewed under magnification often have concentric rings of darker brown within the lesion. The symptoms first appeared minutely in the month of June and continuously grow more in number and size in July, August and September months. Alternaria also causes leaf yellowing followed by leaf drop. By the end of season the apple plants appear very thin with foliage concentrated at the drop. There is speculation that the very cool and wet spring and rainy summer of 2012, 13-14 provided ideal environmental conditions for the growth of this pathogen. A study was conducted during 2012, 13-14 to evaluate 31 variety of apple (*Malus domestica* Borkh.) propagated in MM-rootstock series and seedling rootstock for alternaria blight disease in farmer's field and CITH RS Mukteshwar. The apple variety CITH Apple Lodh-1 in seedling rootstock, CITH-Apple Lodh-1, Chaubatiya Princess on MM-106, MM111 have been found 20-25% susceptible for alternaria blight respectively. The Skyline Supreme, Oregon Spur, Red Spur, Red Delicious, Vance Delicious have been observed the 10-15 % susceptibility upon seedling rootstock. The variety Gala Mast, Mollis Delicious, Golden Delicious, Chaubatiya Anupam on MM111 and Prima, Mayan on seedling rootstock have been found resistance to alternaria blight. The present study showed that the cultivars Gala Mast, Mollis Delicious, Golden Delicious, Chaubatiya Anupam were showed highly resistance to alternaria blight disease.

P SOIL 4

Utilization of Rice Straw, Uncomposted and Composted Swine Manure to Suppress Soil-Borne Pathogens in Selected Cruciferous Vegetable in the Philippines

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This study was conducted in a field plots to determine the effects of rice straw, composted and uncomposted swine manure on the incidence of damping off caused by *Sclerotium rolfsii* and *Fusarium* sp. on pechay, mustard and radish in Nueva Ecija. Treatments were based on the presence or absence of rice straw in combination with composted and uncomposted swine manure. Plots were artificially infested with *Sclerotium rolfsii* and *Fusarium* sp. Disease incidences as well as yields were

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assessed at intervals. In pechay, mustard and radish, lowest incidence of damping off occurred in plots incorporated with rice straw + composted swine manure. Highest marketable yield was obtained in plants grown in plots incorporated with rice straw + composted swine manure. Other than the negative control, the incidence was highest in mustard and pechay plots where only rice straw was amended.

P SOIL 5

Study of aggressivity of *Fusarium culmorum* isolates associated with root rot and head blight of wheat in Algeria

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Root rot and *Fusarium* head blight of wheat are considered among the most serious and widespread diseases in the world. They are leading cause of economic losses which may reach 50 % and also of mycotoxins accumulation in wheat seeds. In Algeria these last year the most predominant species is *F. culmorum* which can be associated with root and collar rot and head blight. This investigation focused on the study of the pathogenicity of a collection of *F. culmorum* isolates obtained from the ear, the collar and root of samples of different variety of wheat harvested in the Central North region of Algeria. Pathogenicity tests were assessed by using two methods: soil inoculation (to evaluate disease severity on the collar and the root of wheat seedlings) and ear infection during flowering stage (to evaluate disease severity on the spikes). The aggressiveness of *Fusarium* isolates evaluated by soil inoculation and estimated by a disease scale ranging from 0 to 3 showed that all *F. culmorum* isolates were able to induce disease on the collar and root. Data recorded showed that disease index varied between 0.5 and 1.8, the most aggressive isolates (FC 09-11) was obtained from the ear, and the less aggressive one FC 10-11 was also obtained from the ear. Spikes inoculation showed also a variability of disease index which varied from 2.25 to 7.88 following a disease scale ranging from 1 to 9. The most aggressive isolate was FC 09-11 with an index of severity of 7.88 and the weakest one was FC 10-11 (2.25). Results obtained in this study showed that *F. culmorum* isolates obtained from roots and diseased collar were able to induce symptoms on the ear and also those obtained from the spikes were aggressive on the collar and root. Significant correlation ($r=0.5$) between disease index on the collar and on the ear was observed for *F. culmorum* isolates.

P SOIL 6

Cloning, characterization and expression of a novel laccase gene Pclac6 from *Phytophthora capsici*

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Phytophthora capsici is an aggressive plant pathogen that affects solanaceous and cucurbitaceous hosts. A novel laccase gene pclac6 and its corresponding full-length cDNA were cloned and characterized from *Phytophthora capsici* for the first time. The 1683 bp full-length cDNA of pclac6 encoded a mature laccase protein containing 560 amino acids preceded by a signal peptide of 23 amino acids. The deduced protein sequence of PCLAC2 showed high similarity with other known fungal laccases and contained four copper-binding conserved domains of typical laccase protein. In order to achieve a high level secretion and full activity expression of PCLAC6, expression vector pPIC9K with the *Pichia pastoris* expression system was used. The recombinant PCLAC6 protein was purified and showed on SDS-PAGE as a single band with an apparent molecular weight ca. 69.5 kDa. The high activity of purified PCLAC6, 76 U/mL, at the seventh day induced with methanol, was observed with 2,2'-azino-di-(3-ethylbenzothiazolin-6-sulfonic acid) (ABTS) as substrate. The optimum pH and temperature for ABTS were 4.0 and 30 °C, respectively. The reported data add a new piece to the knowledge about *P. capsici* laccase multigene family and shed light on potential function about biotechnological and industrial applications of the individual laccase isoforms in oomycetes.

P SOIL 7

Minimum Tillage to prevent Soil Erosion - control of Wetsern Corn borer and *Fusarium* disease by Insecticide - and Fungicide spraying

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In Austria more than 400.000 ha arable land are seriously endangered by soil erosion. Soil loss, nutrient loss, water runoff and pesticide loss are environmental risks and also danger for settlements. Minimum tillage systems can significantly reduce soil erosion and all the negative consequences. In combination with effective cover crops we can introduce the system of permanent

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covered arable land with a maximum protection of soil against soil erosion, surface runoff, nutrient - and pesticide loss. The technical requirement and the farmers know how are necessary. These advantages are combined with the risk of Fusarium sp. caused by straw residues of wheat and corn on the soil surface and occur of the European corn borer, if straw residues of corn are not shredded and incorporated consequently.

In that case minimum tillage trials and measurements of soil erosion were done for 20 years in Lower Austria. In this period the interest more and more focused to plant diseases and Fusarium sp. was examined more in detail. New fungicides were introduces in the last two years so that researches became possible. 22 Variants could be tested:

The results demonstrate a significant reduction of Mycotoxines ZEA and DON in the year 2013 and 2014. 2013 DON was reduced by Fungicides from 1812 ppb in the untreated control to less than 1000. 2014 the DON - value in the control was 4014. With Fungicide spraying in EC 59 this value could be reduced to 665 - 1023 ppb.

The insecticide trials for the control of European Cornborer showed also good options. These trials were applied with assistance of light traps catches in comparison to the forecast model proPlant. A yield - increase from 9 - 26 % appeared and a reduction of DON 2013 was investigated. The lodging of corn could be reduced significantly and the quality of the harvest ensured.

Figure 1

number of variant		ZEA ppb		DON ppb	
1	seeddressing Maxim XL (Metalaxyl M + Fludioxinil) - untreated control pre	542	1038	1812	4014
2	Retengo plus (Opera) Pyraclostrobin + Epoxiconazol 1.5 EC 31	315	430	813	2273
3	Retengo plus (Opera) Pyraclostrobin + Epoxiconazol 1.5 EC 51	148	247	580	2379
4	Retengo plus (Opera) Pyraclostrobin + Epoxiconazol 1.5 EC 59	132	209	526	1023
5	Retengo plus (Opera) Pyraclostrobin + Epoxiconazol 1.5 EC 65	139	142	610	448
6	Prosaro Prothioconazol + Tebuconazol 1 EC 31	105	450	454	2764
7	Prosaro Prothioconazol + Tebuconazol 1 EC 51	233	350	1076	2283
8	Prosaro Prothioconazol + Tebuconazol 1 EC 59	114	99	592	870
9	Prosaro Prothioconazol + Tebuconazol 1 EC 65	141	198	430	400
10	Propulse (Fluopyram 125 g + 125 g Prothioconazole) 1 EC 31	133	504	442	2961
11	Propulse (Fluopyram 125 g + 125 g Prothioconazole) 1 EC 51	108	588	413	1776
12	Propulse (Fluopyram 125 g + 125 g Prothioconazole) 1 EC 59	62	164	209	1350
13	Propulse (Fluopyram 125 g + 125 g Prothioconazole) 1 EC 65	86	80	245	1023
14	Quilt Xcel (Azoxytrobin 141,4 g/l + 122,4 g/l Propiconazol 1 EC 31		335		3082
15	Quilt Xcel (Azoxytrobin 141,4 g/l + 122,4 g/l Propiconazol 1 EC 51		255		1026
16	Quilt Xcel (Azoxytrobin 141,4 g/l + 122,4 g/l Propiconazol 1 EC 59		114		665
17	Quilt Xcel (Azoxytrobin 141,4 g/l + 122,4 g/l Propiconazol 1 EC 65		192		866
18	Seeddressing Prothioconazole + Retengo plus (Opera) 1.5 EC 59	51	307	209	1807
19	Seeddressing Prothioconazole + Propulse 1.0 EC 59	64	254	263	1452
20	Seeddressing r Prothioconazole+ Retengo plus (Opera) 1.5 + Prosaro 1.0 EC 31 + EC	32	163	138	404
21	Seeddressing Prothioconazole + Quilt Xcel 1.0 EC 59		240		941
22	Seeddressing Prothioconazole + Retengo plus 1.5 + Quilt Xcel 1.0 EC 31 + EC 65		160		846

P SOIL 8

Assessment of Inoculum Decline in Ex Basal Stem Rot (*Ganoderma boninense*) Hole in Replanting Area

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Basal Stem Rot is caused by *Ganoderma boninense* is the most important soil borne disease in palm oil plantation, which the most infection through root contact. *Ganoderma* brackets usually develop on the cut roots in sanitation holes, especially in ex BSR hole. Finding out how much inoculum exist in these holes, the depth at which the inoculum will develop and how long it survive in the hole, will be useful information to be correlated with applying *Trichoderma* to sanitation hole to suppress of *Ganoderma* mycelium growth. The aims of this trial was to investigate decline periode of inoculum remaining in ex BSR hole. This trial was conducted in palm oil plantation, Bah Lias Estae, London Sumatera. This trial used randomized block design with 8 treatments and 20 replicates. The treatments were dig out the BSR palms and dig a hole 1.5 x 1.5 x 1.5 m deep, count *Ganoderma* brackets developing on cut root on the surface of sanitation hole (0 cm) and depth from the surface of sanitation hole (20 cm, 40 cm, 60 cm, 80 cm, 1 m, 1.2 m and 1.5 m). This trial was started in December 2010. Observation of the number of *Ganoderma* brackets growing inside the sanitation hole and the survival time of the basidiocarps growing inside the hole was

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recorded every two weeks. First observations were conducted in February 2011. The results showed that after 44 months observation different numbers of *Ganoderma* brackets grew at different levels in the sanitation hole. No *Ganoderma* bracket grew on the surface of sanitation hole. The highest number of *Ganoderma* brackets growing was observed at a depth of 60 cm. 54 brackets were observed in 20 holes at this depth so a mean of approximately 3 bracket/hole at this depth. Binomial analysis also conducted and the results showed that there was no significant difference ($p > 0.01$) between mean *Ganoderma* bracket growing at each depth. *Ganoderma* brackets developing on cut roots on the inside of the sanitation hole did not develop every month. The highest number developed in the first months after sanitation hole was made. After that period, brackets only developed rarely. *Ganoderma* brackets growing on cut roots inside the sanitation holes can survive from 1 day to 284 days but most basidiocarps growth survive around 22 days. From this observation, it is recommended to estate to apply directly *Trichoderma* after the sanitation holes are dug.

Figure 1



Figure 2

Table of Number of *Ganoderma* brackets growing in the sanitation holes (1.5 x 1.5 x 1.5m depth) at all depths over time.

Treat	Feb-11	May-11	Aug-11	Nov-11	Feb-12	May-12	Aug-12	Nov-12	Feb-13	May-13	Aug-13	Nov-13	Feb-14	May-14	Aug-14
A. 0 cm	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
B. 20 cm	1	0	0	0	0	0	0	0	0	0	0	0	0	0	0
C. 40 cm	10	1	0	2	2	2	0	0	2	0	0	0	0	0	0
D. 60 cm	29	4	0	7	3	5	0	0	1	1	0	1	0	0	0
E. 80 cm	22	2	0	2	2	0	0	0	0	0	0	0	0	0	0
F. 1m	15	6	0	2	1	2	0	0	0	0	0	0	0	0	0
G. 1.2 m	12	2	0	0	0	0	0	0	0	0	0	0	0	0	0
H. 1.5m	3	2	0	0	0	0	0	0	0	0	0	0	0	0	0

P SOIL 9

The cereal nematodes; *Heterodera avenae* and *Pratylenchus thornei* associated with wheat yield reduction in Eastern Mediterranean Region of Turkey

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Wheat (*Triticum* spp.) is one of the most important field crops in Turkey where both bread and durum wheat types are cultivated under irrigated and non-irrigated conditions. Wheat yield is affected by biotic and abiotic factors. Among the biotic factors, plant parasitic nematodes attack wheat crop and cause serious economic losses worldwide. Especially, the cereal cyst nematode *Heterodera avenae* (Wollenweber) and root-lesion nematode *Pratylenchus thornei* (Sher et Allen) are found predominantly in spring wheat-growing areas in Adana province in the Eastern Mediterranean region of Turkey. It has been reported that these nematode species are associated with loss of grain yield under field conditions in this region of Turkey. According to the results, *H. avenae* resulted in a reduction in wheat grain yield of up to 25.7% in the susceptible variety (Seri 82) and *P. thornei* caused a reduction of 19.85% in the susceptible wheat variety (Gatcher). So far the most promising strategy to control cereal nematodes is by using the resistant varieties. Therefore, the International Maize and Wheat Improvement Center (CIMMYT) and Turkey breeding programs are collaborating and developed hundreds of wheat lines with high levels of resistance to both CCN and RLN. More research is being carried out to investigate the background of the resistance sourced by the use of association mapping technique.

P SOIL 10

Occurrence and Distribution of Entomopathogenic Nematodes (Steinernematidae and Heterorhabditidae) in Kayseri Province, Turkey

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Entomopathogenic nematodes (EPNs) have potential for biological control of insect pests. A total of 82 species of entomopathogenic nematodes have been identified worldwide belonging to Steinernema (65 species), Neosteinerema (1 species) and Heterorhabditis (16 species). Seven and four species of Steinernema and Heterorhabditis respectively were identified in Turkey. Particularly here we sought to determine the species of EPN in agricultural areas as well as in natural habitats in Kayseri, Turkey during 2010-2011. Nematode isolates were identified based on morphology and sequence analysis using ITS regions of ribosomal DNA. Sixty one entomopathogenic nematode isolates were obtained from 174 soil samples from 5 different habitats. Positive soil sample ratio was 35%. Forty-one of these isolates were identified as *Steinernema feltiae*. Two of them were *S. carpocapsae*, one of them was *S. bicornutum*, three of them were *Steinernema* sp., and fourteen of them were *Heterorhabditis bacteriophora*. *S. bicornutum* is the first report from Turkey. Based on morphology and sequence analysis, three isolates correspond to genus *Steinernema* were not identified as a species. More detailed studies are indispensable to decide whether these isolates represent a new species or not.

P SOIL 11

Aggressiveness of *Fusarium oxysporum* f. sp. *medicaginis* on Alfalfa

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Alfalfa (*Medicago sativa* L.) is a perennial forage legume with a worldwide distribution consisting one of the world's most valuable forages. Diseases can reduce the production and life cycle of alfalfa stand *Fusarium* spp. that cause crown and root rot

as well as *Fusarium* wilt, has been implicated as the cause of alfalfa plant death and decreased yield quantity and quality. *Fusarium oxysporum* Schlechtend.:Fr. causes vascular wilt diseases on many different crops, and over 120 formae speciales. *F. oxysporum* f. sp. *medicaginis* (Weimer) Snyder and Hans. (FOM) is the usual incitant of Fusarium wilt of alfalfa. The typical symptoms of Fusarium wilt, which include chlorosis, wilting, stunting and death of plant, increase in severity and incidence during subsequent growing season. Alfalfa plants showing symptoms were sampled in different localities from Lakes Region in Turkey. Symptomatic crown and root samples were cut about 5 mm and surface disinfected with 70% ethanol for 1 min. Then the cuttings were rinsed quickly sterile distilled water, blotted dry on filter paper and placed on Potato Dextrose Agar (PDA) in Petri dishes and incubated at $25 \pm 1^\circ\text{C}$ for 7 days. Mycelium developed from the tissue cuttings was sub-cultured onto PDA and single spore technique was used to purify the *Fusarium* isolates. FOM was consistently isolated from diseased plants. Pathogenicity tests with twenty four isolates were conducted with agar plate assay. There were significant differences among aggressiveness of isolates of FOM from alfalfa. In pathogenicity tests on alfalfa (cv. Gea), the FOM isolate IA-29 was the most aggressive among 24 isolates from alfalfa. Some isolates (IA-10 and IA-18) were non-pathogenic. *Fusarium oxysporum* consists of pathogenic and non-pathogenic strains that are morphologically indistinguishable.

P SOIL 12

Development of a Co-encapsulation of baker's yeast, maize starch and *Beauveria bassiana* attractive towards western corn rootworm

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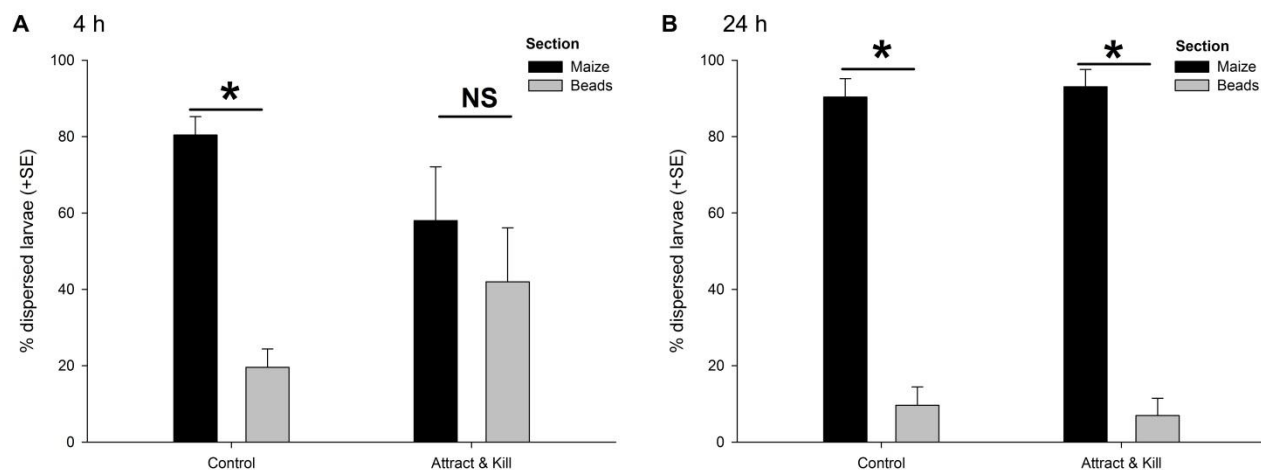
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Soil-dwelling larvae of pest insects like western corn rootworm (*Diabrotica virgifera virgifera*) cause considerable economic damage in agricultural production systems. As CO₂ is known as an attractant for western corn rootworm larvae there is a high interest in CO₂ emitting formulations for an "attract-and-kill" strategy. Ca-alginate encapsulation of baker's yeast has previously been shown to result in a slow release of CO₂ in soils. The objective of this research project was to develop a formulation which releases CO₂ during at least four weeks by providing a co-formulated nutrient source. The challenge was to identify a nutrient source which can be encapsulated in a biocompatible material and utilized by the baker's yeast.

Addition of starch extended and increased CO₂ release resulting in the formation of significant CO₂ gradients in a defined soil during several weeks. The missing starch degrading activity of baker's yeast was compensated by random soil microorganisms or co-encapsulated *Beauveria bassiana* producing exoenzymes with amylase activity. In a non-destructive observation device we further demonstrated that the co-formulation with baker's yeast, maize starch and *B. bassiana* was attracting western corn rootworm larvae within the first two hours after their insertion, but was neither able to keep the larvae at the target point nor to affect their vitality (Fig. 1). We speculate that incubation time of six days was too short for fungal sporulation, which is needed for a successful infection. Additionally, we assume that attractiveness needs to be increased by other stimuli to prolong contact between larvae and the entomopathogenic fungus in order to increase the chance for a infection by the conidia.

Figure 1: Percentage of dispersed larvae observed in section "Maize" (black) and "Beads" (grey) 4 hours (A) and 24 hours (B) after insertion of western corn rootworm larvae. (Wilcoxon test between each section; * = P *S. cerevisiae* H 203, maize starch and *B. bassiana*).



P SOIL 13

Selection of microorganisms from suppressive compost to control soil-borne pathogens on potted vegetables

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Introduction: Soil-borne diseases are the cause of severe losses of economically important crops and the use of organic amendments and compost is an alternative approach for their control. Compost suppressiveness has been widely studied, suggesting an important role for the microbial component of compost and the possibility to isolate antagonists from high quality composts.

Objectives: The objective of the present work was to isolate microorganisms from a suppressive compost and to test them for their activity against soil-borne pathogens on vegetable crops.

Materials and methods: A compost from green wastes and municipal biowastes that showed a good suppressive activity in previous trials was used as source of microorganisms. Serial diluted suspensions of compost samples were plated on different media: selective for *Fusarium* sp., selective for *Trichoderma* sp., potato dextrose agar (PDA) for isolation of fungi, lysogeny broth (LB) for isolation of bacteria. Colonies were isolated from plates and tested in greenhouse on potted plants against *Fusarium oxysporum* f. sp. *basilici*/basil, *Pythium ultimum*/cucumber and *Rhizoctonia solani*/bean. Antagonistic microorganisms were blended into a peat substrate at 10 g L⁻¹ or 10 ml L⁻¹ 14 days before seeding. Pathogens were mixed into the substrate at 1 g of wheat kernels L⁻¹ 7 days before seeding. Seeds of basil, cucumber and bean were sown into 2 L pots in greenhouse. The number of alive plants and above ground biomass were measured 20-30 days after seeding.

Results: Three fungi were able to significantly control *F. oxysporum* f. sp. *basilici* on basil and one of them was effective also against *R. solani*. Other 8 microorganisms, including 6 bacterial strains and 2 fungi, significantly increased the number of alive plants in the pathosystem *P. ultimum*/cucumber.

Conclusions: Among all isolated microorganisms, bacterial strains showed to significantly control the pathogens better than fungi. However none of the microorganisms was able to control the three soil-borne pathogens together.

P SOIL 14

Evaluating systemic semi-selective chemicals for the management of apple replant disease in fumigated and non-fumigated orchards systems

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Apple Replant Disease (ARD) is a phenomenon where apple trees are stunted when replanted onto old apple soil. The main soilborne organisms that cause ARD include oomycetes, fungi and nematodes. Preplant soil fumigation with broad spectrum fumigants, mainly mixtures of chloropicrin (Chl) and 1,3-dichloropropene (1,3-D), has been the standard method for control of ARD. In South Africa, oomycetes (certain *Pythium* spp. and *Phytophthora cactorum*) and *Pratylenchus* spp. have been identified as the most virulent ARD pathogens, along with *Cylindrocarpon* spp. and binucleate *Rhizoctonia* spp. that are generally less virulent. Semi-selective chemicals that target oomycetes and nematodes might thus have potential for suppressing ARD in South Africa. The aims of the study were to (i) evaluate in two orchard trials the efficacy of two fumigants varying in Chl and 1,3-D content (33.3% Chl:60.8% 1,3-D vs. 57% Chl:38% 1,3-D), and semi-selective chemicals (fenamiphos, metalaxyl, potassium phosphonate) applied to fumigated and non-fumigated soil and (ii) to elucidate the ARD etiology of the two orchard soils under glasshouse conditions. Evaluation of tree growth responses (increases in trunk diameter, total shoot length and leader length) after the first growing season showed similar trends for both trials. All fumigant treatments had significantly better growth than the untreated controls. The semi-selective chemicals applied to non-fumigated soil did not significantly increase tree growth. However, in both trials there was a trend for this treatment to contribute towards improved growth. The semi-selective chemicals in combination with a fumigant also did not significantly enhance growth relative to the fumigant treatment alone. Fumigants containing different Chl and 1,3-D contents performed equally well. The glasshouse trial has been completed for the one orchard soil, which showed that the orchard soil had a severe ARD status. This was evident since soil pasteurization significantly enhanced seedling growth and soil dilution of 15% (v/v) non-treated soil into pasteurized soil caused pathogen re-infestation and significant seedling growth reductions. The etiological agents identified included *Cylindrocarpon* spp., *Pythium vexans*, *Pythium irregulare*, *Phytophthora cactorum* and *Pratylenchus* spp.

P SOIL 15

Clubroot of oilseed rape epidemics, virulence of *Plasmodiophora brassicae* and possible management strategies

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Clubroot, caused by *Plasmodiophora brassicae*, has been one of the most destructive diseases of oilseed rape in Germany and recently has become a more frequent problem worldwide. Our previous field studies showed the number of very virulent pathotypes has increased over the past years and subsequently commercial resistant cultivars become susceptible (Zamani Noor, unpublished data). Therefore, understanding the type of the pathotype is useful for developing better strategies to study the disease epidemiology, which should lead to more effective control of the disease. The pathotype classification of 45 new populations of *P. brassicae* has been determined during 2013 to 2014 from infected root samples of oilseed rape collected in different states of Germany on the differential hosts of Somé. In total, the collected isolates were classified to 4 different pathotypes, among them eight isolates were highly virulent on the resistant oilseed rape cultivar Mendel.

Additionally, it was described that when lime or calcium cyanamide are applied to the infected soil, the soil became suppressive to clubroot (Hwang et al. 2011; Dixon 2012). Therefore, field experiments with natural infection on three different locations in Germany were carried out in 2014 to evaluate control strategies for improving resistance in susceptible and resistant cultivars by investigating the effect of different fertilizers application (Calcium cyanamide and burnt lime) at different times during the growing season. The preliminary results indicated well variations between the treatments. Changing the time of application had significant effect on the final severity of the disease ($P \leq 0.05$). Clubroot incidence and severity, relative to untreated control, were significantly lowered by application of fertilizer at later growth stages. In compare to calcium cyanamide, burnt lime application has lower effect.

References:

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P SOIL 16

Using conditional probability to predict inoculum level of *Verticillium dahliae* from commercial potato fields in Michigan, USA

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Introduction: A recent survey of potato (*Solanum tuberosum*) growers in the U.S. state of Michigan identified that soilborne pathogens were a concern to potato grower's ability to continue to meet the high demands for marketable potatoes for both the processing and fresh market. Of these soilborne pathogens, *Verticillium dahliae* is one of the most economically important pathogens from a marketability standpoint due to direct correlation with potato early die (PED) and its persistence in the soil.

Objectives: A statewide soil evaluation was conducted, following the distribution of a grower survey, to study soilborne pathogens and their interactions with multiple abiotic and biotic factors. The use of geostatistics and geographical information systems (GIS) were incorporated into this study to assess the spatial distribution of colony forming units (CFUs) of *V. dahliae* across fields and to use geostatistical methods to determine *V. dahliae* inoculum levels throughout entire fields from 20 soil samples/field.

Materials and methods: Three fields were used in this study to illustrate this technique. Furthermore, the research team incorporated the use of a nonlinear Kriging method (indicator Kriging) to create conditional probability maps of soilborne pathogen inoculum levels and predict where inoculum levels would result in infection and significant disease incidence and severity.

Results: The methods presented in this study evaluated mapping of soilborne plant pathogens as potential practical disease management tool for commercial potato crop production. Cross-validation statistics analysis of data for the three fields with a low-, high- and variable-risk based on spatial distribution of CFUs. A conditional probability map was generated of a low-risk field, a high-risk field and variable-risk field. Field maps spatially represented the probability of PED incidence based on a 5 CFU/10 g of soil threshold.

Conclusion: The procedures discussed, when incorporated into commercial integrated disease management, will allow for more accurate prediction and treatment of potato diseases, such as PED, associated with *V. dahliae*. The ability to sample a relatively few data points to predict values for an entire field could greatly influence how integrated disease management is conducted in the future.

P SOIL 17

The Epidemiology of Bacterial Fruit Blotch Disease of Watermelon in Eastern Mediterranean Region

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Bacterial fruit blotch of watermelon caused by *Acidovorax citrulli* is an economically important disease in Turkey. Even though all cucurbit species (cucumber, watermelon, squash and melon) are the potential hosts of this seed borne pathogen, the most common host of the disease is watermelon. After the first occurrence of the disease determined in 2005, the major outbreaks were occurred in 2009 and 2010 in the Eastern Mediterranean region. The present status of the disease in Adana, Mersin and Osmaniye provinces during 2011 and 2012 was determined in this study. Totally 51 strains were isolated from diseased watermelon fruits in the region since 2009. The strains were identified by traditional methods and species specific PCR. Diseased plants and fruits had been eradicated and infected fields in the region have been banned during for four years for cucurbit cultivation by Republic of Turkey Ministry of Food, Agriculture and Livestock. Additionally, in the study, survival of the pathogen in non-vegetation period on/in seed and soil was investigated. A 100 mg/l rifampicin resistant regional strain was used in the study of overwintering of the pathogen on/in seed and soil. It's determined that the pathogen can survive in the soil samples taken from Osmaniye and Adana provinces for 180 and 150 days, respectively and in the watermelon seeds stored in room temperature during 9 months

P SOIL 18

***Aphanomyces trifolii*, an Evolving, Causal Agent of Severe Root Disease in Annual Clovers in Australia**

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Introduction: Subterranean clover (*Trifolium subterraneum*) is grown extensively as a pasture legume in southern Australia (29 M ha) and elsewhere and is the most important pasture legume in Australia. However, various soil-borne pathogens severely curtail its establishment, productivity and long term persistence, with severe reductions in livestock carrying capacity and whole farm profitability. This decline in pasture production has been positively associated with several root rot diseases, including *Aphanomyces*, misidentified for decades as *A. euteiches* until recently confirmed as the new species *A. trifolii*.

Objectives: To identify host resistance to *A. trifolii* in subterranean clover and to compare virulence and phylogeny of collected isolates from southern Australia.

Materials and methods: A series of controlled environment experiments were undertaken to define virulence and physiological variation of isolates of *A. trifolii* and to identify appropriate host resistances.

Results: Cultivars Dalkeith, Bacchus Marsh, Riverina and Yarloop are the first host resistances identified against *A. trifolii* in subterranean clover. Investigations of virulence and the population phylogeny across isolates of *A. trifolii* confirmed extensive variation in virulence and physiological specialisation, with three distinct clades, two of which were distinct from isolates collected previously, signalling greater genetic diversity present in current compared with historical populations. Host resistance(s) against a mixture of 20 *A. trifolii* isolates representing the current pathogen population have now been identified for the first time.

Conclusion: This is the first study to show physiological specialisation in *A. trifolii* in subterranean clover and the first to identify host resistances against these different pathotypes. Our study confirms *A. trifolii* as a significant but largely unknown contributor to severe root disease of subterranean clover in southern Australia.

P SOIL 19

Efficacy of dimethyl disulfide against Fusarium wilt and weeds on lettuce.

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The management of soil-borne pathogens is complicated by the limited number of registered chemicals and by restrictions in the use of pre-plant fumigants.

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Three experimental trials were carried out in Northern Italy in order to evaluate the efficacy of soil disinfestation treatments based on dimethyl disulfide (DMDS) applied by shank injection under virtually impermeable transparent films for 14 days, under open field conditions. The effectiveness of DMDS at 30, 40, 60 g/m² alone or combined with metham sodium (DMDS 40 g/m² + Metham 14.1 ml/m²) was compared with that of dazomet against *Fusarium oxysporum* f. sp. *lactucae* race 1 on lettuce. The efficacy of DMDS in controlling monocot and dicot weeds was also evaluated. Trials have been carried out both in a sandy loamy soil naturally infested by *Fusarium oxysporum* f. sp. *lactucae*, and in artificially infested soil. In the presence of a disease incidence ranging from 23.4 to 78%, DMDS at 40 and 60 g/m² showed the best effectiveness in *Fusarium oxysporum* f. sp. *lactucae* control. DMDS, at the dosage of 30 g/m², did not always ensure satisfactory reduction of Fusarium wilt on lettuce compared to dazomet. DMDS at 40 and 60 g/m², significantly reduced the total weed population compared with the untreated control, showing a good effectiveness (from 70% to 87% of control) against *Portulaca oleracea*, the prevalent species in the experimental sites. Also the combination of DMDS and metham sodium provided a consistent effect in Fusarium wilt reduction, as well as weed control. The improved plant health provided by the tested soil disinfestation treatments constantly caused significant yield increases.

P SOIL 20

No evidence of stem bleeding transmission by ambrosia beetles to coconut palms in Brazil

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The coconut palm (*Cocos nucifera*) is a valuable source of food and material throughout the tropics. Coconut plantations are attacked by several diseases, such as the stem bleeding, caused by the fungus *Thielaviopsis paradoxa* (syn. *Chalara paradoxa*, *Ceratocystis paradoxa*). Infections can initiate from *T. paradoxa* spores or mycelium that survive on decaying plant litter and are spread from soils to coconut stems by splashing rain or irrigation water. It is known that the coconut stem borers *Rhynchophorus palmarum* and *Metamasius hemipterus* (Coleoptera: Curculionidae) can carry viable spores of *T. paradoxa*, being able to disseminate the disease. However, little is known about stem bleeding transmission by other stem borers, such as ambrosia beetles (Coleoptera: Curculionidae: Scolytinae). Here, we aimed at investigating whether coconut palm-infesting ambrosia beetles could disseminate stem bleeding since this knowledge could be of great importance when designing disease control and prevention strategies. Adult ambrosia beetles were collected directly from stems presenting stem bleeding symptoms (black stains coming from a hole or wound and seeping down the stem). In order to verify if *T. paradoxa* could be carried either on the insect cuticle or in its digestive tract, ambrosia beetles were straightly inoculated in Petri dishes containing PDA (potato-dextrose-agar) culture medium (treatment 1); and externally disinfested with 70% alcohol solution (2 seconds) and sodium hypochlorite (60 seconds), washed with distilled water, macerated, and subsequently inoculated in Petri dishes with PDA medium (treatment 2). Petri dishes were kept under an incubation chamber at 25±1°C and daily evaluated, during seven days. The experiment consisted of a completely randomized design with 60 replications (insects) per treatment. Results showed no *T. paradoxa* germination from either cuticle or digestive tract of insects evaluated. The fungus *Aspergillus* sp. grew in PDA medium but it was considered a contaminant. We found no evidence that ambrosia beetles could carry viable spores of *T. paradoxa* that could infect healthy coconut palms and disseminate stem bleeding disease.

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P SOIL 21

Predatory mites of the superfamily Bdelloidea (Acari: Trombidiformes: Prostigmata) from Iran

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Members of the superfamily Bdelloidea have an important role in the biological control of small insects, scales, mites and nematodes in agricultural ecosystems. These mites found in a variety of habitats including leaf litter and soil, agriculture fields and stored products. During 2013-2014 a faunistic survey of Bdelloid mites associated with crop fields and orchards was conducted in Mazandaran province, Iran. Different samples were transferred to the laboratory and put into the Berlese-Tullgren funnel. The extracted mites were cleared by Nesbitt's fluid and mounted in Hoyer's medium. During this study, the genus

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Neobiscirus Gomelauri, 1963 and *Trachymolgus purpureus* Fisher & Dowling, 2011 are recorded for the first time from Iran and the specimens collected were identified as: **Bdellidae**: *Neobiscirus* sp. nov.; *Bdella muscorum* Ewing, 1909; *Bdella longicornis* (Linnaeus), Latreille, 1795; *Biscirus silvaticus* Kramer, 1881; *Cyta coerulipes* Duges, 1834; *C. lattirostris* Hermann, 1804; *Hexabdella persiaensis* Paktinat Saeed & Bagheri, 2014; *Spinibdella cronini* (Baker & Balock, 1944); *Trachymolgus purpureus* Fisher & Dowling, 2011; **Cunaxidae**: *Cunaxa setirostris* (Hermann, 1804); *Cunaxoides croceus* (Koch, 1838); *Pulaeus* sp.; *Lupaeus* sp.

P SOIL 22

Development of a substrate for the production of seedlings optimized with regard to plant nutrition and suppressiveness to soil borne diseases.

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Introduction: The soil-borne pathogen *Pythium ultimum* causing damping-off disease is responsible for high yield losses in organic vegetable production and is difficult to control. It has been shown before that composts have the potential to control soil-borne diseases. Yet, quality of composts is variable, depending on factors such as the starting material, the composting process and the maturity of the compost and can change during storage.

Objectives: The aims of the present study were to evaluate (i) whether suppressiveness of a high quality green compost can be improved by adding selected nitrogen fertilizers and/or BCOs, and (ii) whether the addition of BCOs to standardized substrates results in suppression levels comparable to a high quality compost.

Material and methods: Cress-*P. ultimum* and cucumber-*P. ultimum* were used as model bioassays. Substrate mixes based on 70% peat and 30% compost or coco fibers were amended with different organic nitrogen fertilizers and/or different commercially available biocontrol organisms and were tested for their suppressiveness to *P. ultimum*.

Results: The results showed that compost was essential for suppressiveness against *P. ultimum* and a chitin-containing fertilizer strongly improved this effect. In contrast, none of the five tested BCOs could improve the suppressiveness of the compost or a standardized substrate.

Discussion: Compared to single BCO, the complex microbial community of a compost amended with an adequate fertilizer was highly suppressive against the soil-borne pathogen *P. ultimum*.

Conclusion: By using an appropriate compost, production of healthy and robust seedlings is feasible even in the presence of high levels of pathogens.

P SOIL 23

Evaluation of biological control properties and characterization of three Brazilian Plant Growth Promoting Bacteria

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Fungal diseases are an important source of plant stress and are responsible for big amounts of annual crop loss. The use of microorganisms to control plant diseases is a promising and eco-friendly alternative to chemical pesticides. Thus, the aim of this work is to characterize and evaluate the biocontrol properties of three Brazilian Plant Growth Promoting Bacteria. *Bacillus mycoides* B38V, *Burkholderia cepacia* 89, and *Paenibacillus riograndensis* SBR5^T were characterized for enzyme production; biofilm formation; oil emulsification; and soft-rotting of potato. Antagonistic activities were evaluated by seeding 10⁴ spores of 20 phytopathogenic fungi in King B and PDA agar plates followed by inoculation of 20 µL of 10⁸ UFC mL⁻¹ of each bacterium. Similarly, antagonistic tests were done against phytopathogenic and Gram positive bacteria. Inhibitory effect was verified through halo formation. Greenhouse assays were carried out to investigate plant growth promotion of four cultivars of wheat and disease protection of strain 89 against *Bipolaris cynodontis*, *Drechslera tritici-repentis* and *Fusarium graminearum*. All bacteria were proteolytic and none were able to produce urease or chitinase, which could be related to antifungal activities. No bacteria provoked soft-rotting of potato tubers. *B. cepacia* 89 and *P. riograndensis* SBR5^T solubilized at least 4 of the substrates tested, suggesting important nutritional abilities to compete in the rhizosphere. Strains B38V and SBR5^T did not form any halo in the antagonistic assays, although the former presented a type of competition as it at least was not overgrown by the fungi. *B. cepacia* 89 showed a broad spectrum of antifungal activity, inhibiting almost all fungi tested. No activity against bacteria was detected. Despite being previously characterized by our group as having plant growth promoting abilities, these bacteria had presented no effect on wheat. Besides, *B. cepacia* 89 had not protected wheat from fungal diseases in the conditions tested. *B. cepacia* 89 has potential to be used as plant inoculant and biocontrol agent, although the conditions of inoculation might be

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optimized so the bacterium could be able to express its beneficial activities shown *in vitro* also *in vivo*. Further experiments are underway to identify the antagonistic metabolite(s) being produced.

P SOIL 24

Distribution of physiological races of *Fusarium oxysporum* f.sp. *pisi* in western Algeria

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Wilt caused by *Fusarium oxysporum* f.sp. *pisi* (FOP) is a serious constraint to peas production in Algeria. It is a major yields limiting factor in the production zones. The disease could cause appreciable yield losses under favorable environmental conditions. The objective of this study is to identify and evaluate the importance and frequency of the wilt disease, characterization of physiological races of FOP and their geographical distribution in western Algeria.

A surveys were conducted in four different agro-climatical zones in western Algeria (coastal plains, interior plains, High plateaus and Sahara). Several pea fields were surveyed during the period from 2007 to 2011 at different stages of the plant growth (seedling stage, flowering and pod-formation). Wilt affected plant were collected from all regions. Isolations were made from the rhizosphere, root and stem pieces (five per plant). The pathogens associated with pea wilt samples were isolated and cultured on PDA, and incubated at 25°C for 7 days. Fungi were purified by single spore and identified.

The pathogenicity of 52 isolates of FOP and one nonpathogenic isolate was determined by inoculating each of the differential lines. The root prune and dip technique it's using. Plants were visually assessed at the 10-12 nodes stage 28 days after inoculation using the following 0-5 rating system.

The wilt was prevalent in all pea growing areas prospected. The percentage of wilt was from 7.30% to 33.98% for the entire western region of Algeria. An isolates collection of Fop was investigated. Five- two isolates were assigned to races 1, 2, 5 or 6 by virulence tests on a series of differentials sets. Races 1and 2 were more common in all areas with 61.47% and 19.2% respectively, race 6 was present at 11.52%, it is absent in the Sahara. For race 5, only two isolates were identified in the coastal plains. In addition to the 4 races, two new or unknown pathovars of Fop were identified in the interior plains. Variability in cultural characteristics was observed among isolates. This study is the first report of peas *Fusarium* wilt races distribution in western Algeria.

This study allowed us to put in evidence the presence of pea wilt in all regions surveyed, we have established a geographic distribution map of four races of the FOP at the western Algerian regions. Races of the fungus and the population types present in the environment area need to be evaluated for disease control management. Specifically this information assists when examining newly bred cultivars and for the development of new resistant cultivars.

P SOIL 25

Comparative studies of structural and functional diversity of soil microbiomes in the rhizosphere and bulk soil in an energy crop rotation using denaturing gradient gel electrophoresis (DGGE)

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Introduction: The demand for high yielding energy crops such as maize, oil seed rape (OSR) and wheat has enormously increased worldwide. From previous studies, the influence of cultivars and cropping practices on the soil microbial communities is known. However, there is limited knowledge about the interactions between crop species and the soil microbial community in long term energy crop rotation systems. The better understanding of the changes in the diversity and abundance of soil microbial community is indispensable for efficient control of soil borne diseases.

Objectives: The aim of this project was to study the effect of crop rotation on major soil borne root diseases of wheat and to analyze the changes in the soil microbial communities under different energy crop rotations based on maize, OSR and wheat.

Materials and methods: A field experiment consisting of four crop rotations (maize monoculture (FF1), OSR - Wheat (FF2), OSR - Maize - Wheat (FF3), and OSR - wheat- maize - wheat (FF4)) was conducted for six consecutive years. After six years of rotation (spring 2014), bulk and rhizosphere soil samples were collected from wheat plots at growth stage BBCH 34-37 and the diversity of the soil microbial community was analyzed using DGGE. The identity of the microbial community in the bulk soil is being analyzed by pyrosequencing.

Results: Results of the rhizosphere samples revealed a significant impact of plant species on the diversity of the microbial community. In contrast, analysis of the bulk soil showed a significant difference between crop rotation FF1 and FF2, FF1 and FF3, FF2 and FF3, FF2 and FF4 in the fungal community. Similarly, analysis of the DGGE profiles of bacterial taxa showed significant

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difference between maize monoculture and crop rotation FF3 (OSR - maize - wheat), and between crop rotation FF2 (OSR - wheat) and FF3 (OSR - maize - wheat).

Conclusions: The crop species had the strongest effect on the rhizosphere microbial community regardless of crop rotations. Regarding to the bulk soil the bacterial and fungal communities were mainly affected by the differences in crop rotations. A future analysis of the microbial community in the bulk soil by pyrosequencing will provide a more in-depth understanding of the effects of crop species and energy crop rotations.

P SOIL 26

Characterization of *Macrophomina phaseolina* from sugar beet using SSR markers

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Introduction: Soil-borne fungus *Macrophomina phaseolina* (Tassi) Goid. is a plant pathogen that causes charcoal rot by infecting the root and lower stem of over 500 plant species. This polyphagous plant pathogen attacks many cultivated plants, including sugar beet. Although charcoal root rot, caused by *Macrophomina phaseolina*, appears regularly in Serbia, it is most destructive sugar beet pathogen during extremely warm and dry seasons. Isolates of *M. phaseolina* have shown variation in morphological and physiological characteristics, as well as in the pathogenicity or host specificity. Genetic variation has also been detected using DNA markers, such as RAPD and SSR.

The objective of our study was to use SSR in assessing the genetic diversity of *M. phaseolina* isolates from sugar beet within local geographical area.

Material and methods: In the experiment were used 58 *M. phaseolina* isolates, 56 from sugar beet, 1 from maize and 1 from soybean, which were screened using 24 SSR primer pairs. The optimization of PCR reaction for each primer was done by using two MgCl₂ concentrations (1.5 mM and 2.5mM), and two types of amplification protocols. At the moment, the work is in the progress; the optimizations is done and until the submission of the abstract isolates were screened by 10 SSR primer pairs.

Estimation of genetic variation will be carried out by using the POPGENE software package for calculation of the following parameters: number of polymorphic loci and their percentage, effective number of alleles per loci, expected heterozygosity and Shannon's information index. For estimation of variance components among the isolates, analysis of molecular variance (AMOVA) will be performed. Cluster analysis of SSR marker results for *M. phaseolina* isolates will be done using the unweighted paired group method and arithmetic averages (UPGMA) algorithm implemented in the SAHN program of NTSYSpc. Robustness of the clustering pattern will be tested by bootstrap analysis.

Results: The results will be correlated with already known isolate traits, such as culture characteristics and pathogenicity.

P SOIL 27

Biodiscovery of compounds from Plant Growth-Promoting Rhizobacteria (PGPR) and their role in stimulating Pseudo-Chemical responses in *Phytophthora Cinnamomi*

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Several advances have been made in our understanding of how soil-borne pathogens interact with plants and of how we may provide resistance in natural systems, but our ability to control these diseases is still very much limited. *Phytophthora cinnamomi* continues to cause devastating disease in major agricultural crops and native vegetations worldwide and consequently threatening our global biodiversity. In this study, we screened approximately 400 bacterial isolates from our rhizosphere soil microbial collection and selected 28 isolates based on their biocontrol activity against *P. cinnamomi*. We then carried out chemical extractions of compounds from six bacterial isolates with high anti-oomycete activity and discovered four bioactive diketopiperazines (DKP) isomers, namely cyclo(Phe-4-hydroxy-Pro) (A), cyclo(Phe-Pro) (B), cyclo(Phe-Phe) (C) and cyclo(Trp-Pro) (D). One of the DKPs (compound D) significantly stimulated the production of nitric oxide (NO) in *P. cinnamomi* under fluorescence microscopy (figure 1). This may suggest that the bacterial DKP induced pseudo-chemical defence responses in the pathogen, allowing the pathogen to produce NO as a defence signal and making it detectable for plants to respond. We also discovered that while NO production is induced, superoxide dismutase (SOD) activity is greatly inhibited (figure 2), confirming the defence-signalling role of NO in *P. cinnamomi* due to the loss of the primary defence strategy in combatting superoxide radicals by SOD. This work provides us with a good background on the discovery of potential biocontrol agents

against *P. cinnamomi* diseases. The isolated strains and their bioactive compounds may provide a promising strategy to control soil-borne pathogens, in particular *P. cinnamomi*.

Reference: 1.Khalil Z., Kalansuriya P., Capon R. 2014.Lipopolysaccharide (LPS) stimulation of fungal secondary metabolism. Mycology: An international journal of fungal biology. Vol. 5, 3, pp. 168-178.

Figure 1

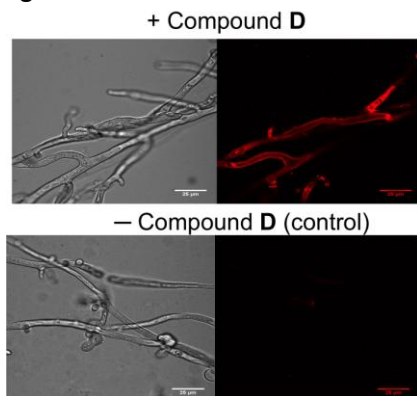
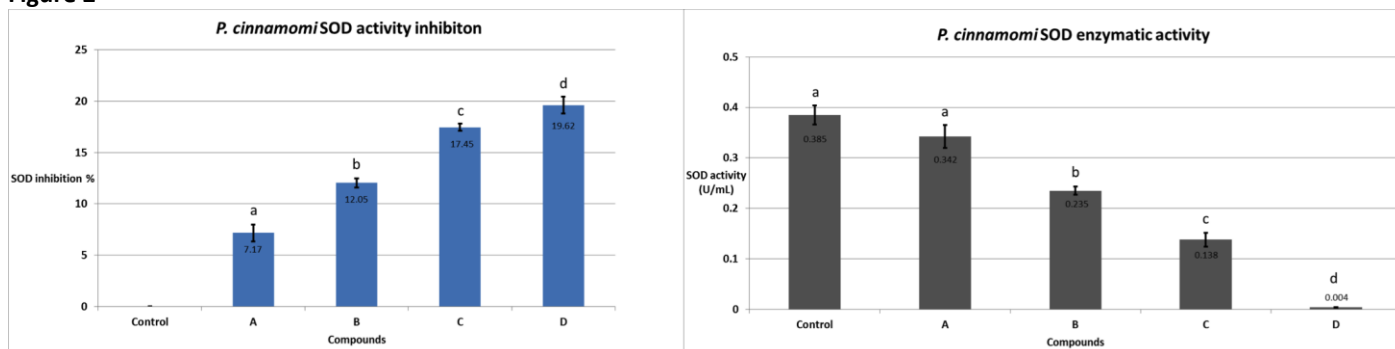


Figure 2



P SOIL 28

Fusarium solani causing lemon verbena root rot in Iran

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Introduction: Lemon verbena (*Lippa citriodora*) is a medicinal plant with a recently increased plantation in south of Iran. Leaf chlorosis and necrosis associated with root rot symptoms were observed in visits to lemon verbena farms and greenhouses of Kerman province (southeast of Iran) in November 2012. More than 45% of 140 randomly examined plants were symptomatic.

Objectives: This research was performed to study the etiology of lemon verbena root rot in Kerman.

Materials and methods: The infected root tissues were surface sterilized, cultured onto PDA and incubated in the dark at 25°C for 7 days. Pathogenicity tests for the fungal isolats were performed using inoculated wheat seeds. Fungal DNA was extracted from seven day old mycelium using CTAB buffer. Universal fungal primers were used to amplify the internal transcribed spacer (ITS) region of the rRNA gene complex, incorporating ITS1, the 5.8S gene, and ITS2.

Results: Cream colored colonies developed from all samples, with thin-walled, hyaline, ovoid, 1-2 celled microconidia. Macroconidia were 3-5 septate, thick walled, hyaline and slightly curved. Chlamyospores were rounded, thick-walled, single, double or several in chains. Based on the morphological features, the isolated fungi were identified as *Fusarium solani*. Amplification of the internal spacer (ITS) region of rDNA of a representative isolate using the universal primers ITS1F and ITS4 resulted in a 531bp fragment which showed 99% identity with other corresponding *F. solani* sequences and clustered with other *F. solani* isolates in phylogenetic analysis.

Conclusion: To our knowledge, is the first molecularly evidenced report of *F. solani* on lemon verbena in the world.

P SOIL 29

Possible utilization of organic and bio-organics with inorganic fertilizers on growth and yield attributes of okra in relation to the management of plant pathogens

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A field experiment was conducted for three summer seasons of 2011-2014 at Agricultural Research Farm of Aligarh Muslim University to assess the nutritional potential of soil application of sesamum cake, bio-organics such as *Azotobacter chroococcum* and *Glomus fasciculatum* individually and concomitantly along with different recommended doses of inorganic fertilizer on growth and yield attributes of okra (*Abelmoschus esculentus*) in relation to the eco-friendly management of plant pathogens like plant-parasitic nematodes and soil-inhabiting fungi. Significant reduction was observed in the multiplication of plant-parasitic nematodes in all the treated beds with sesamum cake and bio-organics along with recommended doses of inorganic fertilizers when applied individually as well as concomitantly. The different treatments also brought significant inhibition in the frequency occurrence of pathogenic fungi such as *Fusarium oxysporum*, *Rhizoctonia solani*, *Macrophomina phaseolina* etc. however, frequency occurrence of saprophytic fungi was increased significantly as compared to untreated control. Number of root-galls, root-rot as well as index greatly reduced due to the incorporation of such organic and bio-organics along with different doses of inorganic fertilizers. The highest improvement was recorded in the growth and yield attributes of okra such as fresh as well as dry weights, fruit weights/plant, number of total fruits/plant, ascorbic acid content and chlorophyll content when these bio-organics added concomitantly along with sesamum cake and 100% recommended dose of nitrogenous fertilizers. *Glomus* was found to be less effective than *Azotobacter* in singly as well as in other combinations. Agronomic parameters like N, P and K contents in plants as well as in soil considerably increased in all most all the treatments irrespective of combinations but more prominently in dual inoculation of both these bio-organics this type of investigation will be beneficial in organic agricultural system to enhance the organic production of crop without addition of pesticides and synthetic chemicals which constantly altered the ecological harmony among microorganisms existing in soil environment.

P SOIL 30

Biological control of Fusarium root rot of beans by *Trichoderma hamatum* and silicon treatments

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Introduction: Root rot caused by *Fusarium solani* f. sp. *phaseoli* (*Fsp*) is one of the most common diseases of beans. *Trichoderma* spp. are well known as parasites of fungal plant pathogens; however, certain strains can also induce systemic resistance in plants against several pathogens. Silicon (Si) is the second most abundant element in the lithosphere and plays an important role in the biota.

Objectives: This research was performed to study the inhibitory effect of simultaneous application of of *T. hamatum* (*Th*) and silicon amendment against *Fsp* both *in vitro* and *in vivo*.

Materials and methods: *In vitro* experiments were conducted either by examination of dual cultures or exposing *Fsp* to volatile compounds of *Th*. *Fsp* was grown on PDA amended with eight concentrations (0, 1, 2, 3, 4, 5, 6 and 7 mM) of silicon dioxide (SiO_2). To perform *in vivo* experiments, bean seeds were surface sterilized with 0.5% sodium hypochlorite, washed with sterile distilled water and cultured into the soil. Simultaneously, *Fsp* and/or *Th* grown on autoclaved wheat seeds were added as inoculums (30g/kg soil). SiO_2 solution (2mM) was added to the soil at a ratio of 1:10 (v/w). Plants were incubated under greenhouse conditions for five weeks and examined for root rot symptoms and growth parameters such as plant height, fresh and dry weight of roots and foliage.

Results: Results showed that the highest inhibition of *Fsp* mycelial growth was observed in dual culture plates and plates exposed to volatile exudates of *Th* in the presence of 2 mM SiO_2 , so this concentration was selected for *in vivo* experiments. In greenhouse experiments, treatments with either *Th* or SiO_2 showed reduced disease symptoms; however, the most significant disease reduction and improvement of plant growth and yield parameters was observed in simultaneous treatments with *Th* and SiO_2 .

Conclusion: This finding highlights the role of integrated application of a biological control agent and silicon in management of Fusarium root rot of beans.

P SOIL 31

The importance of the low temperature threshold for clubroot development on canola

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Background: Many studies have been conducted to understand the relationship between temperature, infection by *Plasmodiophora brassicae*, and the development of clubroot. The focus has been on optimum temperatures, but research has also shown that seeding into cool soils can reduce symptoms, and cool temperatures prior to harvest suppress symptoms. A greater understanding of the low temperature threshold that prevents or inhibits disease development will improve disease forecasting and timing of seeding of crops and research trials.

Objective: To determine the relationship between temperature and clubroot symptom development with emphasis on identifying the low temperature threshold.

Methods: Canola was seeded in to soil, naturally infested with *P. brassicae*, at 2 week intervals from 2011- 2014, to provide a range of temperature and soil moisture conditions. Plants were harvested from each rep 4 and 6 weeks after seeding and clubroot severity was assessed. Mean soil and air temperatures were recorded, as was daily rainfall. Correlation analysis and stepwise regression was used to determine the relationship between clubroot severity at 6 weeks after seeding, and soil and air temperature, rainfall and day degrees with a base of 12, 14 and 17 °C.

Results: In field trials, day degree accumulation (base 14 °C) in the first two or three weeks after seeding was the best indicator of clubroot incidence or severity at 6 weeks after seeding, especially where soil moisture was not limiting or excessive. Day degree calculations with a base threshold of 12.5 and 17 had lower correlation coefficients than day degrees with a 14 °C base. Air temperature was more closely correlated to clubroot development than soil temperature.

Conclusions: Day degree calculations with a minimum threshold of 14 °C, were best for predicting clubroot development. The number of days when mean temperatures are above this threshold may be a better indicator of clubroot development, than mean temperature or day degrees. These results are consistent with controlled environment studies which showed infection of root hairs at 10 °C, but very low rates of cortical infection at 15 °C.

P SOIL 32

Spatial behavior of fusariosis in black pepper

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For the plant epidemiology, the spatial behavior study is an important tool used to understand and support the management of plant diseases. Black pepper is the third item of agricultural exports from Espírito Santo State, Brazil. Plant death caused by the fungus *Fusarium solani* f. sp. *piperis* is a major disease of black pepper. This disease limits the expansion and economic performance of the crop. The present study look for describe the spatial behavior of fusariosis during the beginning of the disease in a field. The spatial spread of the disease was monitoring in a field with 500 plants, with three years old and spacing of 3x2m. The position of each plant was identified geographically. The spatial pattern of the diseased was analyzed using the Taylor's law and the technique of *Kriging*. The disease intensity was assessed during six months at 60 days intervals. For the disease evaluation was used a scale of one to nine, were one means no symptoms of the fusariosis and nine means died plant. The percentage of diseased plants observed in each evaluation was 2, 11.2 and 14.2%. The indices of spreading the infection (observed sample variance/binomial variance) was equal to 0.90 in the first evaluation, 1.94 and 1.98 in the second evaluation in the third respectively. Using the *Kriging*, at the first evaluation was observed the nugget effect, indicating the absence of spatial dependence between diseased plants. The spatial dependence index increased from the second to the third evaluation, ranging from 1.78 to 5.00. The results obtained utilizing the both technic of analysis showed that disease begin with a random distribution and at the second and third evaluation showed a spatial dependence and begin the define groups of diseased plants. The random initial distribution is an indication that the disease could be introduced through infected seedlings or woodpiles. The aggregate distribution with spatial dependence structure is one of the characteristics of the soil-borne pathogen, spreading plant-to-plant. The *Kriging* improved the study of the behavior of the disease by providing the index of spatial dependence and estimate the map with the spatial distribution of the disease.

P SOIL 33

Biological control of charcoal rot of mungbean by *Trichoderma harzianum* and shoot dry biomass of *Sisymbrium irio*

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A pot experiment was carried out for biological control of charcoal rot of mungbean [*Vigna radiata* (L.) Wilzeak] caused by *Macrophomina phaseolina* (Tassi) Goid. Pot soil was made sick with inoculum of *M. phaseolina*. Dried powdered leaves of *Sisymbrium irio* L., a weed of family Brassicaceae, were mixed in soil at 1, 2 and 3% (w/w) with and without application of *Trichoderma harzianum* Rifai, a fungal biological control agent. The highest grain yield (3.62 g pot⁻¹) was recorded in combined application of *T. harzianum* and 1% *S. irio* leaves amendment that was 62% and 805% higher than negative and positive control treatments, respectively. Application of *S. irio* leaf amendment and *T. harzianum* generally enhanced leaf protein, sugar and chlorophyll content, and catalase activity. The present study concludes that *T. harzianum* in combination with 1% dry leaves of *S. irio* as soil amendment can be used to achieve maximum grain yield under biotic stress of *M. phaseolina*.

P SOIL 34

Integrated solution proposals for nematode control based on the current and upcoming Bayer CropScience portfolio

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Many broad-spectrum fumigants for soil disinfection or highly effective chemical nematicides of the organophosphate (OP) or carbamate class are of toxicological or environmental concern, and will disappear or be severely limited in their use. Thus, the risk of crop damage is increasing, and easy and effective nematode control is becoming more difficult for growers. Today there are rather few alternatives available to manage serious nematode pressure. A number of new chemical and biological products are developed by the crop protection industries which have better safety profiles, but are not quite as powerful as the old solutions. Thus, it is advisable to combine the use of the new products in an integrated approach, providing diversity by making use of their specific properties and strength. We will discuss examples for such integrated solution proposals based on the current and upcoming Bayer CropScience portfolio for nematode control.

P SOIL 35

Races of *Phytophthora sojae* in Ontario during 2010 - 2012

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Phytophthora root rot (PRR), caused by the oomycete *Phytophthora sojae*, is a devastating disease of soybean worldwide, and has been present in Canada since the 1950s. Major resistance to *P. sojae* (*Rps*) genes have been deployed by soybean breeders to mitigate yield losses, which has caused shifts in the pathogen population to more complex pathotypes and the emergence of new races. To determine the occurrence and frequency of races of *P. sojae* in Ontario, where most Canadian soybean is grown, a total of 359 single-zoospore *P. sojae* isolates were obtained from plant and soil samples collected from 203 soybean fields and two PRR nurseries during 2010-2012. Twenty-four races and two intermediate reaction types (IRT) of *P. sojae* were identified from the 359 isolates on a set of eight soybean differentials, each containing a single resistance *Rps* gene. Race 25 was the predominant race, representing 16.4% of the pathogen population in commercial fields. Races 3, 4, 5, 6, 7, 9, 28, and 45 were commonly detected, each of these races represented 5 to 11% of the pathogen population. Twelve races and one IRT were identified from 44 *P. sojae* isolates obtained from the PRR nursery in Ottawa, and 12 races and one IRT identified from 52 isolates from the PRR nursery in Woodlee. Races 3, 5, 6, 7, 8, 9, 14, 22, 25, and 28 were commonly detected in these nurseries. Of the 24 races, 18, including the predominant race 25, were identified for the first time in Ontario. These results suggest that the race profile of *P. sojae* in Ontario has changed and new sources of resistance are needed for the development of resistant cultivars. The common races in the two PRR nurseries were similar to what were found in commercial soybean fields, suggesting that both PRR nurseries are appropriate and effective for screening soybean germplasm for cultivar development for Ontario.

P SOIL 36

Identification of compatibility factor genes involved in the plant-fungus interaction and their potential use in breeding for *Verticillium longisporum* resistance in oilseed rape (*Brassica napus*)

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The hemibiotrophic soilborne fungus *Verticillium longisporum* represents one of the important pathogenic fungi in oilseed rape (*Brassica napus*). So far, only minor genetic variation in resistance to the fungus could be identified in oilseed rape germplasm. To develop resistance against the fungus in oilseed rape, we followed a strategy based on the molecular understanding of plant-fungus interaction and the identification of genes, which are required for a compatible plant-fungus interaction. By comparative transcriptome analyses and subsequent loss of function characterization in *Arabidopsis*, three genes were identified as putative compatibility factors. These genes were highly upregulated at early infection stages in oilseed rape after infection with *V. longisporum*. Knock-out of the candidates resulted in a significantly reduced susceptibility to the fungal infection, suggesting their crucial role in the compatible plant-fungus interaction. To identify corresponding oilseed rape mutants, an EMS-mutant TILLING population has been screened, giving rise to a set of mutants for each gene. Here, we report recent results of molecular and functional characterization of the three putative compatibility factor genes in respect of their role in the modulation of a compatible plant-fungus interaction as well as their potential use for breeding of *V. longisporum* resistance in oilseed rape.

P SOIL 38

Effect of *Brassica* pellet on the survival and pathogenicity of *Phytophthora nicotianae*, the causal agent of root and crown rot of red pepper in western Spain

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Phytophthora nicotianae is the principal causal agent of root and crown rot disease of red pepper plants in Extremadura (western Spain). Biofumigation with *Brassica* pellet in spring, before the pepper crop establishment was assayed as control method of the disease, since the hydrolysis products of GSL in tissues of *Brassica* species are potential fungicides. The experiment was conducted at the Agricultural Research Centre Finca La Orden (Southwestern Spain) and the treatments were: *Brassica* pellet, *Brassica* pellet and plastic cover, *Brassica* pellet and *Brassica* cover crop (*B. nigra* and *S. alba*), *Brassica* pellet and *Brassica* cover crop and plastic cover. Controls were fallow plots with or without plastic cover. The experimental design was a randomized complete block design with four replications. Cover crops were seeded in autumn and incorporated into the soil (mean *Brassica* fresh biomass: 27.6 t·ha⁻¹) with a disk together with the *Brassica* pellet (3 t·ha⁻¹) on 27 March. Biological probes were prepared with 100 g of disinfected soil inoculated with chlamydospores of *P. nicotianae* (5000 chlamydospores/100 g of soil) and wrapped in agryl cloth. Biological probes were buried at 15 and 30 cm depth. Sprinkler irrigation was run and the corresponding plots were covered with plastic (PE 0.05 mm). On 10 May plastics were removed, the biological probes were dug out and a bioassay was established. One pepper seedling at the 2 to 4 true-leaves stage was transplanted in the soil of each biological probe. Plants were grown in a climatic chamber and disease symptoms were recorded every week during 2 months. Fragments of roots and crown of symptomatic plants were analysed on PDA and PARP media and soil from rizospheres was analysed using carnation petals as baits. Data were analysed by Mann-Whitney tests. *P. nicotianae* inoculum survived in all treatments. *Brassica* pellet+*Brassica* cover crop with plastic treatment achieved the lowest pathogenicity and it was the only treatment significantly different from the control with plastic. However, no significant differences were found when it was compared with *Brassica* pellet or control with plastic treatments. The decrease in pathogenicity of *P. nicotianae* may be related to liberation of isothiocyanates from GSL and to factors associated with the application of plastic, like anaerobiosis.

P SOIL 39

Control of *Phytophthora nicotianae* using *Brassica* pellet and Chicken Poultry pellet incorporated into the soil under controlled conditions.

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This work was carried out to test, in laboratory conditions, the effectiveness of *Brassica* seed meal (Biofence[®]) and chicken poultry (CP), both in pelletized forms, against *Phytophthora nicotianae*, the main causal agent of root and crown rot disease of red pepper plants in Extremadura (western Spain). The treatments were: Biofence[®] (1.5 and 3 g·l⁻¹ of soil, which are equivalent

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to a rate of 3 and 6 t·ha⁻¹ in the field) and CP (7.5 and 15 g·l⁻¹ of soil, which are equivalent to a rate of 15 and 30 t·ha⁻¹ in the field). Disinfected soil was inoculated with *P. nicotianae* V8-vermiculite inoculum in order to obtain a concentration of 37 CFU·ml⁻¹ of soil. Besides these treatments, a control without *P. nicotianae* inoculation was also included. These materials were thoroughly mixed with soil in a 10l container, wetted to water-holding capacity and the containers were sealed with plastic (PE 0.05 mm). The containers were kept in climatic chamber for a period of 4 weeks and controlled temperature (14 °C to 19 °C). Then, plastic covers were removed and soil from each container was analysed using carnation petals as baits to detect *P. nicotianae*. After a week of aeration, one pepper seedling at the 2 to 4 true-leaves stage was transplanted per pot with 1l of treated soil + vermiculite (1:1, v:v). The experiment was performed in a completely randomized design with 4 repetitions and 10 plants (pots) per repetition. Plants were grown in a climatic chamber and disease symptoms were recorded every week during 2 months. Fragments of roots and crown of plants were analysed on PARP medium and soil from rizosphere of each plant was analysed using carnation petals as baits. Data of incidence (percentage of diseased plants), infectivity (percentage of plants with infested roots or crown) and viability (percentage of plants with positive isolation with baits) of the inoculum were calculated. After the experiment, inoculum was not detected in the two treatments with Biofence® and any diseased plant was recorded in these cases. However, CP treatments were not different from control, although the disease incidence in plants was very low compared to control. Results suggest that *Brassica* pellet has potential for being evaluated in field conditions under moderate soil temperatures.

P SOIL 40

Evaluation of phytotoxicity of Brassica pellet and Chicken poultry pellet

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Phytotoxicity of *Brassica* seed meal “Biofence®” (BSM) and chicken poultry (CP), both in pelletized forms, has been determined by germination bioassays using sensitive species to phytotoxic metabolites. It was evaluated using mustard (*Sinapis alba*) and radish (*Rhapanus sativus*) seeds. A sterile filter paper was placed inside a 55-mm Petri plate and ten disinfected seeds were placed on it. Dilutions of pellets were prepared using distilled water in different w:v ratios, 1:5 for BSM and 1:8 for the extract of CP. From these dilutions the following concentrations were prepared: 100%, 50%, 25%, 10%, 5% and 0%. One millilitre of each dilution was used to moisten the filter paper and the plates were closed. Ten replicates per dilution and species were prepared. Seeds were incubated at 25 °C in darkness and germination and root length were recorded after three days. If primary root was ³ 1 mm, seed germination was considered positive. The Relative Germination Percentage (RGP), Relative Root Growth (RRG) and Germination Index (GI) were calculated for each dilution and species. Furthermore, phytotoxicity was also determined by germination of the species above mentioned incorporating into a disinfected substrate (peat+vermiculite 3:1, v:v) different dose of BSM (1.5 and 3 g·l⁻¹ of substrate, which are equivalent to a rate of 3 and 6 t·ha⁻¹ in the field) and CP (7.5 and 15 g·l⁻¹ of substrate, which are equivalent to a rate of 15 and 30 t·ha⁻¹ in the field). The substrate and pellets were thoroughly mixed in a 1l pot and ten seeds were sown per species and dose. Control without pellets was included. Ten replicates per dose and specie were prepared. Pots were kept in a climatic chamber and percentage of germination was recorded after one month. Data were analysed by ANOVA followed by a Duncan test. In the first experiment, the GI with CP was gradually decreasing for increasing concentration higher than 10% and no germination was recorded with 100% dilution in mustard seeds. Similar results were found with BSM, where no germination was observed either with 100% concentration in radish seeds or with 50% and 100% concentrations in mustard. Mustard seeds were more sensitive. No phytotoxicity was observed in seeds germinated in pots with any dose or species. These results suggest that it is unlikely that BSM and CP produce phytotoxic effects using field dose.

P SOIL 41

Evaluation of vertical and horizontal resistance to Phytophthora root and stem rot in Canadian short-season soybean cultivars

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Introduction: Phytophthora root and stem rot (PRSR) is a destructive disease of soybean throughout the world. The causal agent, the soil borne oomycete *Phytophthora sojae*, causes seed rot, seedling damping-off and root rot in adult plants. There are two forms of genetic resistance to this pathogen: (1) vertical or complete resistance, mediated by *Rps* genes, of which at least 14 are known to be located at eight loci in the soybean genome, and which provide protection against specific races of *P. sojae* in a gene-for-gene model; and (2) horizontal or partial resistance, identified by QTLs, which is effective against most isolates of *P. sojae*. Resistance has been deployed by soybean breeders in order to mitigate yield losses caused by *P. sojae*, and new germplasm is routinely screened for resistance in *P. sojae* nurseries.

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Objectives: The objectives of this study were (1) to determine vertical resistance to six races of *P. sojae* and corresponding Rps genes; and (2) to determine horizontal resistance to *P. sojae* in a set of Canadian short-season soybean cultivars.

Materials and methods: Approximately 100 Canadian soybean cultivars and breeder lines were tested for resistance to *P. sojae* under controlled conditions in the greenhouse. The hypocotyl wounding method was used to screen for vertical resistance using six different races, and the root layer inoculum method was used to screen for horizontal resistance. Pedigree analysis was also performed using available information on parents and ancestors and compared with the experimental results.

Results: Various patterns of resistance to the six races of *P. sojae* used to test for vertical resistance revealed the presence of at least three Rps genes in the soybean lines tested: Rps1a, Rps1c and Rps6. This corroborated with expectations based on pedigree analysis. Degrees of horizontal resistance varying from low to high were observed in the set of soybean lines tested. Lines displaying a moderate to high degree of horizontal resistance were identified.

Conclusion: Both vertical and horizontal resistance exists in Canadian short-season soybean germplasm. The lines identified as sources of both types of resistance can be used by soybean breeding programs for the development of short-season soybean cultivars with improved resistance to PRSR.

P SOIL 42

Impact of pollination on smut infection in maize cobs

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Ustilago maydis (DC) Corda, causal agent of corn smut, can form enormous swellings called as galls on maize cobs. The infection process of maize ear by *U. maydis* is similar to its natural pollination. In other words, in case of reaching of infective hyphae of the fungus into ovaries of ears through silk, instead of pollen, ear infection occurs. As such, pollination may affect disease development in maize ovaries. Thus, a 2-year study under ecological conditions of Antalya was conducted to determine effect of pollination on *U. maydis* infection in cobs. In the field experiments, 8-maize-cultivar belonging to various maize variety groups including dent corn, flint corn, sweet corn and popcorn were used as host plants. Inoculations were performed by injecting inoculum into ear silk of each cob of the plants in inoculated plots. This application was separately executed both 3 days later in silk emergence (before pollination) and 3 days later at the onset of natural pollination of the hosts, during silk browning (after pollination). For each treatment, control plots were also set up. In conclusion, mean disease severity and incidence among the varieties in the before pollination treatments varied from 0.9 to 7.3 and from 9.3 to 31.8% whereas in the after pollination treatments, those were 0.2-2.2 and 5.8-24.5% respectively. However, mean disease severity of all the hosts in the before and after pollination treatments were at the rates of 3.8 and 0.9 but mean disease incidence of those were 20.7 and 15.7% respectively.

P SOIL 43

Molecular characterization of *Fusarium* spp. causing peanut brown root rot and strategies for their biocontrol

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Argentina is a major peanut-producing country. During the 2011/12 season, peanut production exceeded 680 000 tons. Most of the peanuts are exported to the European Union and the USA. Diseases caused by soil pathogenic fungi limit peanut production. Peanut brown root rot (PBRR) was first described in Argentina in 1992. The etiological agent responsible was reported as *Fusarium solani* (Mart.) Appel and Wollenweber, but the pathogen was not well described. Koch's postulates were completed with strains from this group to show that these strains are responsible for this disease. We used a combination of morphological and molecular markers, amplified fragment length polymorphism (AFLPs), and molecular sequence analysis of internal transcribed spacer region (ITS) of the translation elongation factor 1-alpha gene (TEF-1 α), and the α -tubulin gene in 15 *Fusarium* isolates obtained from peanut plants exhibiting symptoms of root rot, to characterize strains of this pathogen in the *Fusarium solani* species complex (FSSC). Also, evaluation of the efficacy of bioagents application against PBRR disease incidence was carried out under greenhouse conditions. The evaluated bioagents were *Trichoderma harzianum* ITEM 3636 and/or *Pseudomonas* sp. RC-93 alone and in co-inoculation. The disease was significantly reduced at all treatments comparing with untreated plants. Based on unique pathogenic capabilities, the genetically close relationship identified with the AFLPs, and the evidence of a monophyletic clade in the phylogenetic analysis, we conclude that *Fusarium solani* strains causing PBRR formed part of a distinct lineage previously known to be associated with soils, FSSC 3+4, to which the name *F. falciforme* has been associated. Recently, and due to the fact that these two groups are not well supported individually in phylogenetic analyses,

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they have been combined provisionally into a single phylogenetic species, FSSC 3+4, in the absence of strong support for their respective distinctiveness. Since both *T. harzianum* ITEM 3636 and *Pseudomonas* sp. RC-93 exhibited several traits beneficial to the plant host and showed promising results when applied as bioinoculants, they may be used to develop new, safer and effective formulations as an alternative or supplement to chemical fungicides and/or fertilizers.

P SOIL 44

Sources of Resistance to Ashy Stem Blight Caused by *Macrophomina phaseolina* in the Cowpea Major Gene Pool Two

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Cowpea (*Vigna unguiculata* L. Walp) is an important source of inexpensive protein and income in sub-Saharan Africa and elsewhere. However, in agro-ecological zones with arid climate where *Macrophomina phaseolina* is prevalent, the economic and nutritional benefits of cowpea are reduced by yield loss from ashy-stem-blight. Host-plant resistance is the most effective strategy to mitigate *M. phaseolina* damage to cowpea; however, only limited sources of resistance to this pathogen have been identified. Forty-eight cowpea genotypes, from the southern East Africa germplasm which comprises cowpea gene pool 2, were evaluated for *M. phaseolina* resistance in a 3-year (2012-2014) series of field experiments conducted under natural infestation at UC-Riverside. Based on ANOVA for cumulative plant mortality (CM) and correlations of percent plant mortality (%M) with environmental stress index (ESI, $r = 0.53$, $P < 0.0001$) and plant senescence score (SSc) 6 genotypes were consistently highly resistant to ashy-stem-blight disease with %M < 5, while 20 resistant genotypes had % M in a range of 6-12 %. In addition, 17 (%M 12.1-18) and 5 (%M 20-26) genotypes were classified as susceptible and highly susceptible, respectively. Plant senescence was significantly correlated with susceptibility to the disease ($r = 0.56$, $P < 0.0001$); however, one early maturing resistant genotype showed delayed senescence. These new sources of *M. phaseolina* resistance provide an important resource to cowpea breeders for variety improvement in marginal production areas.

P SOIL 45

Virulence and molecular polymorphism in *P. brassicae* isolates from Germany

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As revealed out by using differential host tests *Plasmodiophora brassicae* field isolates in Europe display great pathogenic variation. However, little is known about the genetic and molecular basis of pathogenicity. The objective of this study was to differentiate *Plasmodiophora* field isolates from different regions in Germany according to genotype and virulence phenotype, and to detect genetic polymorphisms directly related to pathotype classification. In total, 28 isolates of *Plasmodiophora* were collected from regions that differ in oilseed rape cropping history, oilseed rape acreage and incidence of clubroot. Using AFLP analyses every isolate displayed a unique genotype pattern indicating that *P. brassicae* is a genetically diverse species. Regarding the differential host test, out of 8 *Brassica* lines tested, six reacted differentially to the isolates. Three isolates were virulent against 'Mendelson' and originated from the same region. Correspondence analysis (CA) grouped the AFLP genotypes as well as the virulence phenotypes into the same two clusters based on the geographic origin. Procrustes test showed that the genotype and phenotype pattern were significantly correlated. Hypotheses about association of genotypes and virulence phenotypes with different spatial scales were tested with generalized linear model (GLM): The region, reflecting the cropping history, had a significant effect on genotypes and virulence phenotypes. We propose that geographic differentiation results from low levels of gene flow due to the limited dispersal of this soil-borne pathogen and from localized selection pressure as unifying force. Random forest identified DNA fragments related to pathotype classification. Further research has to clarify if these DNA fragments can be used for marker assisted identification of pathotypes. Markers for virulent pathotypes against 'Mendelson' could be an important tool in the monitoring for these pathotypes provided by the breeding company of 'Mendelson'.

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The Impact of Wilt Disease Caused by *Fusarium oxysporum* f. sp. *lycopersici-radicis* on the Tomato (*Solanum lycopersicum* Mill.)

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The tomato (*Solanum lycopersicum* Mill.) is world's most produced, consumed and trade issues of agricultural products and also is considered as indispensable human nutrition products and due to its wide variety of uses in food industry such as canned tomato paste, ketchup, pickles, the tomato is one of the very important vegetable. This study aimed to determine the given response of three different tomato varieties against the *Fusarium oxysporum* f. sp. *lycopersici-radicis*. The study was carried out in 2009 in air-conditioned rooms that belonging to Adana Directorate of Biological Control Research Station. In this experiment, 3 different tomato varieties (Malike F1, F1 and Diamond Izmir F1) were infected with *Fusarium* before planting and after a period of 0-35 day, the ratio of the diseases was determined by dismantling the tomato varieties. In the addition, symptoms formation during the growth of the infected plants has been observed. In the study results, growth retardation in the diseased tomato plants, wilt and death were observed. From these three different tomato varieties, the resistant and non-resistant varieties against the disease were identified.

P SOIL 47

The Research of the Comprehensive Control Technology to *Fusarium* Wilt Disease

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With the selective pressure of the crude toxin in *Fusarium oxysporum* f.sp.*cubense*, the new banana cultivar showing good comprehensive characters, named 'Min jiao NO.6' was screened by 'Taiwan' tissue cultured seedling of banana. The wilting index of 'Min jiao NO.6' was 12.75 in the crude toxin of *Fusarium oxysporum* f.sp.*cubense*, and the contrast wilting index of 'Tianbao Rod' that was the susceptible cultivar reached 48.89. The result showed that 'Min jiao NO.6' has strong resistance ability to fungi toxin. The 'Min jiao NO.6' was planted in Tianbao town, Pantuo town, Longshan town, Chenxiang town and Tingxi town in the year of 2010 and 2012. It showed there were no occurrence of *Fusarium* wilt of banana in five areas. In contrast of 'Taiwan' banana, 'Minjiao NO.6' had excellent and stable growth characters. In addition, the *Bacillus subtilis* T122F strain was gained in health banana plant in banana wilt disease area. Biocontrol of *Bacillus subtilis* T122F strain and its metabolite to *Fusarium oxysporum* f.sp. *cubense* were detected through bioassay. The results showed the inhibitory rates to mycelia growth were 87.11% and 85.96%, it was also found that the inhibit rate of metabolite to spore germination was up to 79.52%. The result of pot experiment showed that the control effect of 20000000000/g *Bacillus subtilis* biocontrol agent (T122F) produced by Pucheng green shell biology company was 63.44% in 100 fold diluted solution, nearly by 1000 fold diluted solution of 45% prochloraz emulsifiable. Based on the above research, the comprehensive control technology to *Fusarium* wilt disease, mainly on planting resistant cultivar 'Min jiao NO.6' and biocontrol agent (T122F) was proposed. The experiment of this control technology was tested in the five banana producing areas, about 14 hectare in Xiangchen and Changtai town. The results showed that the control effect to *Fusarium* wilt of banana were 99.5 to 100% in two years, and the yield was as much as other main cultivars in the location. The prevention technology could control the damage and extension of *Fusarium* wilt disease, improve the quality of banana product, and protect agricultural ecology.

P SOIL 48

Soil-borne Fungi of the Harran Plain in Sanliurfa-Turkey and Salinity Relations

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Not only plants and also soil microorganisms live and reproduce in soil environment. Factors such as compositions in the soil and the amount of detritus ambient conditions, soil pH, temperature and humidity impact number of microorganisms, and their activities. This research was conducted in agricultural areas of the Harran Plain in the province of Şanlıurfa. In this study, fungal flora of the areas under cultivation was investigated and their relationships with soil properties were determined. Soil-borne

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fungal pathogens were determined in 9 soils from 240 soil samples collected in different soil series at 0-30 cm depth in the Harran Plain of Sanliurfa province of Turkey during 2012-2013 years. These are *Aspergillus* sp., (12×10^5 cfu/g soil), *Trichoderma harzianum* (5×10^5 cfu/g soil), *Penicillium* sp., (5×10^5 cfu/g soil), *Fusarium* spp., (21×10^5 cfu/g soil) and *Verticillium* sp., (1×10^5 cfu/g soil). In order to investigate the relationships between soil variables and the existence of fungi, soil samples from the Harran Plain were grouped based on soil chemical, physical and biological properties using K-means non-hierarchical clustering method. In addition, Principal Component Analyses method was used. According to the results obtained, there have been found relationships between soil-borne fungal pathogens and soil quality parameters such as soil electrical conductivity, soil organic matter, K and Katalaz enzyme activity and soil salinity supports the existence and living environment of fungi. This study is a part of project supported by TUBITAK-TOVAG (Project Number: 111O706).

P SOIL 49

Inhibitory Influence of Organic and Inorganic Sodium Salts and Synthetic Fungicides Against Bean Root Rot Pathogens

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The efficacy of 20 organic and inorganic sodium salts, and two synthetic fungicides against eight bean root rot pathogens (*Fusarium equiseti*, *F. proliferatum*, *F. semitectum*, *F. solani* f. sp. *phaseoli*, *F. verticillioides*, *Rhizoctonia solani* AG4-HG I, *Macrophomina phaseolina* and *Sclerotium rolfsii*) were evaluated in this study. Accordingly to preliminary in vitro tests, only captan, benzoate and metabisulfite (2 %) were able to completely inhibit mycelial growth of all eight fungi. Moreover, no significant differences were observed among the inhibitory effect of these three compounds and EDTA ($P \leq 0.05$). With few exceptions, the ED₅₀ values indicated captan to have a greater effect against fungi than benzoate, EDTA and metabisulfite. However, captan, benzoate and EDTA all had MIC values that varied greatly from that of metabisulfite. Whereas captan, benzoate and EDTA showed fungitoxic activity against all fungi tested at concentrations greater than 0.1 %, metabisulfite showed fungitoxic activity against all fungi tested at concentrations of 0.025-0.25 %. Soil bioassays showed 0.25 % metabisulfite to completely inhibit mycelial growth of *F. proliferatum*, *F. semitectum*, *R. solani* AG-4 HG I, *M. phaseolina* and *S. rolfsii*, but not *F. equiseti*, *F. solani* f. sp. *phaseoli* and *F. verticillioides*. Higher concentrations of captan and benzoate were required to achieve total inhibition in soil bioassays when compared to metabisulfite, whereas EDTA was not able to completely inhibit growth of any of the fungi tested, even at the highest concentration. Moreover, the application of 1.0-2.0 % EDTA was found to be phytotoxic to bean seeds in terms of both seed germination and root elongation, whereas 0.1-0.75 % captan, 0.1-0.75 % benzoate and 0.1 % metabisulfite did not exhibit any phytotoxicity in terms of germination; 0.5 % captan, 0.1 % benzoate and 0.1 % metabisulfite did, however, have a negative effect on root elongation. The results of pH studies also demonstrated all eight fungi tested to be capable of growth in both acidic and basic environments, although the growth of some species was inhibited at the lowest value tested (pH 2), and the growth of all species was totally inhibited at the highest value tested (pH 12).

P SOIL 50

Varied root exudates and enriched rhizospheric biodiversity by grafting can contribute to resist the watermelon fusarium disease

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Question: Grafting watermelon onto bottle gourd rootstock is commonly used method to generate resistance to *Fusarium oxysporum* f. sp. *niveum* (FON), but knowledge of the effect of the root exudates and rhizosphere microbiome of grafted watermelon on this soil-borne disease remains limited. We hypothesize that root exudates of watermelon after grafting, especially the compositions and contents of the allelochemicals, may shift, the shifted root exudate profiles may result in variations in their rhizospheric microbiome, and in these changes there would be the existence of an indirect positive soil feedback effect in pathogen-resistance potential.

Methods: A recirculating hydroponic culture system was developed to continuously trap root exudates, and the root exudate profiles of the bottle gourd, grafted-root watermelon and own-root watermelon were determined by HPLC. A field experiment with fusarium-infested soil was conducted, and the bacterial communities in the plant rhizospheres were investigated by 454 pyrosequencing.

Results: The composition of the root exudates released by the grafted watermelon differed from the un-grafted watermelon. We identified chlorogenic acid and caffeic acid presented in root exudates from grafted-root watermelon but not from un-grafted watermelon. Both the phenolic acids could inhibit FON conidial germination and growth in a dose-dependent manner,

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and FON was much more susceptible to chlorogenic acid than to caffeic acid. Moreover, clear distinctions in microbial activity and bacterial community between grafted and un-grafted watermelon rhizosphere were obtained. Compared with grafted-root watermelon, ungrafted watermelon recruited significantly higher beneficial bacterial genera, such as *Bacillus* spp. and *Paenibacillus* spp., suggesting the grafted watermelon root could not have the ability to harbor highly beneficial bacteria to exert soil-borne disease resistance. However, a significantly higher Shannon-Wiener index at any reads level was found in the rhizosphere of grafted watermelon compared with ungrafted watermelon.

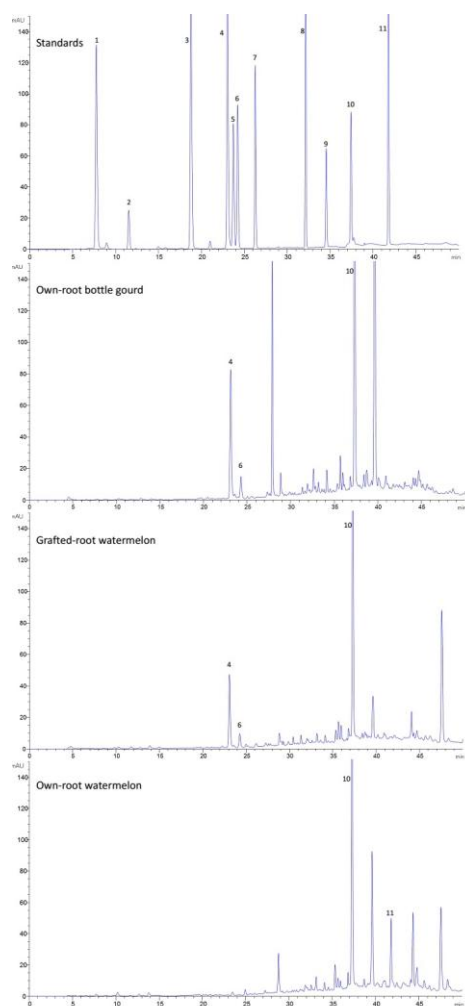
Conclusion: The root exudates profile showed some contribution both in inhibiting FON and in assembling the rhizosphere microbiome. Root-associated bacteria of grafted watermelon possess a broader niche overlap which would provide the potential to exclude the pathogen challenge. We proposed that the grafted watermelon might exert soil-borne disease resistance by secreting the pathogen-suppressive phenolic acids and by maximizing the niche occupancy of rhizosphere rather than by recruiting more beneficial bacteria.

Reference:

Ling et al., 2015. The response of root-associated bacterial community to the grafting of watermelon. *Plant and Soil*. DOI: 10.1007/s11104-015-2399-3

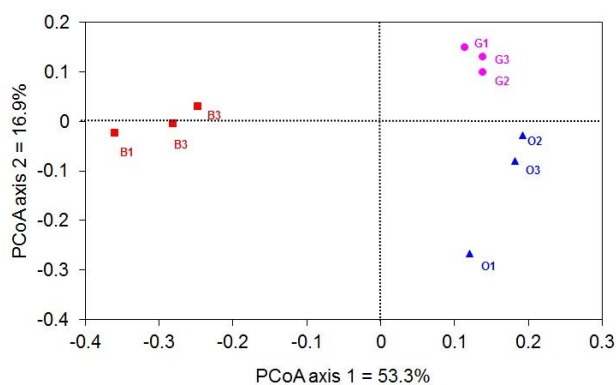
Ling et al., 2013. Root Exudates from Grafted-Root Watermelon Showed a Certain Contribution in Inhibiting *Fusarium oxysporum* f.sp. *niveum*. *PLoS ONE* 8(5): e63383.

Figure 1



The chromatograms detected by HPLC both in standard chemicals and root exudates. The peaks from left to right in the Standards represent the following standard compounds: 1, gallic acid; 2, coumaric acid; 3, β -hydroxybenzoic acid; 4, chlorogenic acid; 5, vanillic acid; 6, caffeic acid; 7, syringic acid; 8, ferulic acid; 9, benzoic acid; 10, salicylic acid; 11, cinnamic acid. The peaks identified by HPLC in the root exudates collected from bottle gourd, grafted-root watermelon and own-root watermelon were showed by the corresponding numbers.

Figure 2



Principal coordinate analyses (PCoA) of bacterial communities based on the phylogenetic distance of 16S rDNA gene sequencing for individual samples. B, plant own-root bottle gourd; O, plant own-root watermelon; G, plant grafted-root watermelon. The number after B, O and G indicates the replicates.

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Temperature Responses, Pathogenicity and Genetic Diversity of *Macrophomina phaseolina* Isolates from Melon in Turkey

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Temperature responses, pathogenicity and genetic diversity of *Macrophomina phaseolina* isolates from melon were studied using RAPD and SSR markers in this study. Fifty isolates of *Macrophomina phaseolina* from melon plants collected from 5 different locations in Eastern Mediterranean Region of Turkey. All the isolates were subjected to growth rate tests at 15, 20, 25, 30, 35 and 40°C. Optimum growing temperature was found to be 30°C but there was no correlation between location and growth rate at optimum growing temperature. Chlorate phenotype of each isolate was determined after growing on a minimal medium containing 120 mM potassium chlorate. Three chlorate phenotypes; Feathery, Dense and Restricted; were differentiated by their chlorate sensitivity. Among the isolates, 46% were Feathery, 44% were Dense and 10% were Restricted. In the other words, 90% were tolerant but 10% were chlorate sensitive. In the pathogenicity tests, the isolates were very pathogenic on melon and watermelon, moderately pathogenic on sunflower and soybean, mildly pathogenic on maize and not pathogenic on sugar beet. Dense isolates were more virulent on melon and watermelon but there was no correlation between virulence and phenotype on the other hosts. Polymorphic bands with the RAPD primers were 35% but 55% with SSR primers. RAPD primers were not able to differentiate isolates in at least two clusters but SSR primers differentiate the isolates in three clusters. Two clusters were combined of mostly chlorate tolerant (Dense and Feathery) with 90% and the last cluster included mostly chlorate sensitive isolates. We found no correlation between location and SSR groups but there was a high correlation between chlorate sensitivity and SSR groups. It seems to be SSR technique was more efficient than RAPD for the molecular tests.

P SOIL 52

Studies on integrated and ecofriendly management of Fusarium wilt in tomato (*Lycopersicon esculentum*)

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Tomato subjected to attack by several pathogens, which cause serious diseases as foliar diseases, soil rots, blights and wilt. Among them wilt is major constrain to the production of tomatoes. Wilt diseases are caused by pathogens that invade the vascular system (xylem tissue) and disrupt water flow through the plant. Fusarium wilt is the major wilt disease of tomato. strong Fusarium is known as soil borne fungi. These fungi are facultative parasite. They produce dormant structures, mostly in the form of chlamydospores. Micro- and macro conidia are produced on branched and unbranched monophialids. The fungi have caused plant diseases such as crown rots, head blights, scabs, vascular wilts, root rots, and cankers. Fusarium spp. generally produces symptoms such as wilting, chlorosis, necrosis, premature leaf drop, browning of vascular system, stunting, and damping-off. Fungicides are main tool for controlling fungal diseases nevertheless; fungicides have many undesirable attributes (Bastos, 1996). In All the three isolates of Trichoderma viz. T. harzianum, T. viride and T. virens were observed readily interacting with F. solani. The mycoparasite grow towards host, ran parallel and coiled around host hyphae leading to lysis. Similar findings were observed by Goswami and Islam, 2000; Shabir-U-Rehman et al., 2013, Geetha and Bhadraiah 2012, Most Trichoderma strains produce volatile and non-volatile toxic metabolites. In the present study all the selected isolates of Trichoderma appears to produce volatile and non-volatile antibiotics inhibitory to the growth of F. solani. Similar findings has been demonstrated by Devi et al., 2013; Hutchinson and Cowan, 1972; Pandey and Upadhyay 1997; Karunanithi and Usman, 1999. Evaluation of fungicides against pathogens showed that the fungicides inhibited growth of the test fungi F. solani to varying extent at different concentration. 15% Bavistin gives complete inhibition while 10.23% and 19.09% inhibition of F. solani was observed at 15% amendment of media by ridomil and Copper-oxychloride respectively. Similar studies has been demonstrated by Maitlo et al., 2013; Nasreen and Ghaffar; Shabir-U-Rehman et al., 2013; Abed et al., 2013; Sumana et al., 2012; and Arunodhayam et al., 2014. For field application of a potential fungal biocontrol agent, an inert immobilizing substrate is essentially required which could carry maximum number of propagules of the biocontrol agent with minimum volume and necessarily maintain integrity of the organism. Bentonite based bioformulation > neem oilseed cake> Bavistin >ridomil > talc based bioformulation. Similar findings observed by Arunodhayam et al. (2014)., Sultana and Ghaffar (2013)., Shabir-U-Rehman et al. (2013). From the present study it is concluded that Trichoderma species have the potential to suppress the colony growth of Fusarium solani, which is cause of wilt disease of tomato. Further studies are required to develop biopesticides based on above biocontrol Trichoderma isolates for the management of tomato wilt and to enhance overall yield, to decrease the economic crises, food shortage and mostly minimize the use of chemicals which have deleterious effect on environment.

P SOIL 53

A new approach to fungal and Oomycete soilborne pathogen identification in common bean grown in eastern and southern Africa

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Introduction: Common bean (*Phaseolus vulgaris*) is among the most important crops for nutrition, food security and fighting poverty and hunger in eastern and southern Africa. Root and crown rot diseases cause severe yield losses and are especially problematic in areas with deficit or excess water. A number of soilborne pathogens within different species of fungi and Oomycetes can be the primary causes of root rots, but little is known about specific pathogens in Africa.

The objective is to compare two methods to identify and characterize soilborne pathogens associated with plants showing root/crown rot symptoms in initial diverse germplasm screening nurseries in two representative dry bean production areas each in Zambia and Mozambique.

Materials and methods: Using DNA analysis on tissue samples taken at the root disease symptom and healthy root or crown tissue interface facilitates identification of fungal pathogens. Specifically, plant tissue with a small amount of water is crushed in a plastic bag, pipetted onto FTA cards and shipped to a laboratory where DNA is sequenced by 454 pyrosequencing. Specific primers identify fungal genera and species in the sample. A part of the same tissue sample is extracted with a scalpel, plated on agar media and allowed to grow while transferring fungal hyphal tips until the culture is purified. DNA is isolated from hyphae of each fungal culture, sequenced and probed with primers to identify genus and species.

Results: *Fusarium oxysporum* and *Fusarium solani* were the most frequently isolated pathogens in both Zambia and Mozambique bean fields. The DNA 454 pyrosequencing confirmed the frequency of *Fusarium* species, but other fungi were also identified. Pathogenicity information is available for other *Fusarium* spp and other fungi found in cultures from root rot symptom isolations.

Conclusion: Further research is required to be certain that use of pyrosequencing for DNA identification of fungal pathogens does not compromise pathogenicity. Any common fungal root rot pathogen will need to have pathogenic isolates available to use in screening for resistance in beans.

P SOIL 54

Fungi associated with stem borer frass in maize stems

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Busseola fusca stem borers are one of the major pests of maize in South Africa (RSA). Larvae feed in whorl tissue, eventually tunnelling into stems where they cause damage of great economic importance. Feeding of larvae inside maize tissue often takes place in close proximity to its frass, which could eventually lead to the development of maize diseases. The aim of this study, therefore, was to determine fungal pathogens associated with *B. fusca* frass in maize stems. Non-transgenic maize plants with visible *B. fusca* damage were randomly collected in *B. fusca*-endemic localities at Buffelsvlei (11 plants), Sannieshof A (30), Coligny (30) and Sannieshof B (26) during the V3-V4 growth stage. The stems were cut open and *B. fusca* frass was collected into sterile tubes. Debris isolation and dilution plating techniques were applied on the frass, followed by plating-out on PDA and incubation for 4 days at 25°C. Developing colonies were purified and grouped according to morphology. Fungal species were identified based on BLAST results from *ITS*, *TEF1* or *RPB2* gene regions on MycoBank. Results indicated that *Acremonium zeae*, *Aspergillus flavus*, *A. niger*, *Fusarium chlamydosporum*, *F. incarnatum*/*F. equiseti* complex, *F. oxysporum*, *F. subglutinans*, *F. verticillioides*, *Mucor circinelloides*, *Rhizopus oryzae*, and *Talaromyces flavus* were associated with the *B. fusca* frass. A potential new fungal species was also discovered and is currently being studied and described, thus increasing knowledge on fungal biodiversity in maize production in RSA. *Fusarium* spp. and *Aspergillus* spp. produce mycotoxins harmful to human and animal health. Their presence in the frass suggests that larvae are vectors of fungal spores. In addition, wounds produced during larval feeding, and the associated nutrient exudates, provide an environment conducive for fungal growth. From this, it proved that the control of stem borers is an important element in the integrated management of maize stem and ear rot diseases.

P SOIL 55

Characterization and Pathogenicity of three subgroups of *Rhizoctonia solani* AG 4 isolated from winter squash in the Black Sea region of Turkey

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During the growing season of 2011, a total of twenty four *Rhizoctonia solani* isolates were isolated from diseased winter squash (*Cucurbita maxima* Duch.) plants, collected from Amasya, Ordu, Samsun and Sinop provinces in the Black Sea region, Turkey. The isolates of *R. solani* associated with root rot of winter squash were examined for their cultural characteristics, anastomosis groups, nucleotide sequence variations in the internal transcribed spacer (ITS) regions including the 5.8S gene of nuclear ribosomal DNA (rDNA) and pathogenicity. The ITS 5.8S region was amplified using a pair of primers, ITS-4 and ITS-5. Sequences of these isolates were aligned with other known *Rhizoctonia* sequences from the NCBI GenBank and phylogenetic analysis were used to determine phylogenetic relationships. Twenty four isolates of *R. solani* were further divided into three subgroups, AG 4 HG-I (13), AG 4 HG-II (6) and AG 4 HG-III (5), belonging to AG 4 based on the similarity of the nucleotide sequence of the ITS-5.8S rDNA. Thirteen of the isolates belonged to AG 4 HG-I which was the most frequent group (54.2%) in all fields surveyed. Morphological characteristics of three subgroups of *R. solani* AG 4 were recorded on potato dextrose agar (PDA; Oxoid Ltd, Basingstoke, UK). After 3 weeks of incubation in the dark at 25 °C, *R. solani* AG 4 colonies became brown or grayed-brown. The sclerotia diameters were changed from 0.19 to 1.20 mm, generally occurred grey-brown coloured at first, but became dark brown with age. All *Rhizoctonia* isolates tested for growth rates grew at temperatures of 10, 15, 20, 25, 30 and 35 °C, whereas they were completely inhibited at both 5 and 40 °C. The results of pathogenicity tests showed that the differences in virulence among three subgroup isolates of *R. solani* AG 4 were statistically significant ($P < 0.05$). The disease severity index (DSI) of the *R. solani* AG 4 isolates ranged from 2.0 to 5.0. Especially, AG 4 HG-I and AG 4 HG-III isolates had the highest virulence (DSI: 3.8-5.0) on winter squash seedlings. To our knowledge, this is the first report of AG 4 HG-I, AG 4 HG-II and AG 4 HG-III occurring on winter squash, one of the most important cucurbit species in Turkey.

P SOIL 56

Different forms of green manures to control *Verticillium dahliae*

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Introduction: Incorporating green manure plants can reduce the number of microsclerotia (MS) of *V. dahliae* in the soil. Part of this effect is caused by the release of volatile molecules.

Objectives: Investigate the influence of the form of green manures i.e., as fresh or dried plants, or as silage, through the release of volatile substances on the viability of *V. dahliae* MS or mycelium.

Materials and methods: Two brown mustard (*Brassica juncea*) cultivars (a high and a low glucosinolate content type), a rye (*Secale cereale*) and a Sudangrass (*Sorghum bicolor* x *S. sudanense*) cultivar grown in a greenhouse were used to produce fresh, dried and silage plant material. The plant material was crushed and then placed in an airtight jar. Two additional treatments were a chemical fumigation using dazomet and a control treatment with no amendment. After adding water, four petri dishes, two inoculated with *V. dahliae* MS and two with mycelium plugs, were placed in the jar, which was immediately closed hermetically. After 24 h incubation at 19°C in the dark, petri dishes were removed from the jar and incubated at 24°C for 2 wks. Germination of MS and growth of mycelium was measured after 1 and 2 wks incubation.

Results: The fresh and dried plant material of the high-glucosinolate brown mustard cultivar ISCI-99 had a fungitoxic effect on both the MS and the mycelium of *V. dahliae*. Even after 2 wks, no MS germinated and the mycelium did not grow. The dried form of Sudangrass cultivar Susu and the chemical fumigant dazomet had a fungistatic effect. MS germination and mycelium growth were significantly reduced after 1 wk of incubation but no more after 2 wks. None of the other treatments had an effect on *V. dahliae*.

Conclusions: The effect of the release of toxic compounds by a brown mustard cultivar with a high glucosinolate content is known as biofumigation. This effect was not affected by the drying procedure, but when transformed to silage, the plants lost the ability to produce toxic volatiles. Another case is Sudangrass where drying the plant enhanced the effect of the plant compared to the fresh or silage form.

P SOIL 57

Soil borne pathogens associated to *Trifolium subterraneum* and *T. alexandrinum* used as subsidiary crops in different cropping systems in two climatic regions in Morocco

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The use of legumes as cover crop (CC) or living mulch (LM) in cropping systems can provide ecological and agronomical benefits including protecting soils from erosion, improving soil fertility (especially nitrogen availability), increasing microbial diversity and suppressing weeds. However, the benefits of legumes crop can only become effective if they are healthy. Legumes can be affected by several soil-borne pathogens that not only reduce productivity but also Nitrogen fixation. Experiments were conducted in two different climatic regions in Morocco (semi-arid and sub-humid regions) to test three cropping systems (C: wheat only, Cc: pure wheat followed by cover crop of *Trifolium alexandrinum* and LM: intercropped wheat with *T. subterraneum* as living mulch) and two Nitrogen rates effect (N1: 50 kg/ha and N2: 100 kg/ha of nitrogen fertilizer) followed by maize. Root and foot diseases were important in sub-humid conditions on both clover species. Dominating pathogens isolated were Fusarium species. Only 5% of the isolated species belonged to the Ascochyta complex (*Mycosphaarella pinodes* and *Phoma medicaginis*). These were associated to *T. alexandrinum*. Several species of Fusarium could be identified from each crop. In the semi arid experiment site, *F. redolens* and *F. culmorum* dominated in both species, in the sub- humid experimental site, *F. equiseti*, *F. culmorum* and *F. avenaceum* dominated. The presence of some of these Fusarium species (e.g. *F. culmorum*, *F. avenaceum*, *F. graminearum*) in legumes has to be carefully considered as they can be also pathogenic on cereals and maize where they can produce several carcinogenic mycotoxins. To take full advantage of legume species in any cropping system, their potential as hosts as well as their epidemiological role need to be fully understood.

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Vegetative compatibility groups in *Verticillium dahliae* isolates from olive in Lebanon

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Introduction: Verticillium wilt of olive (VWO), caused by *Verticillium dahliae* Kleb., is one of the most serious biotic threats to olive in Lebanon. Genetic diversity in *V. dahliae* populations has traditionally been studied by means of vegetative compatibility grouping. Four vegetative compatibility groups (VCGs) have so far been identified among isolates infecting olive worldwide: VCG1A including highly-virulent isolates of the Defoliating pathotype, VCG2A, VCG2B, and VCG4B.

Objectives: The study aimed at i) assessing the genetic diversity of a representative collection of *V. dahliae* isolates infecting olive in Lebanon, and ii) identifying differences in virulence among isolates belonging to different VCGs.

Materials and methods: A collection of 57 isolates from 39 olive orchards distributed in the main olive growing areas in Lebanon was tested using nitrate-non-utilizing (*Nit*) mutants. *Nit* mutants were generated from all isolates on water agar chlorate medium, phenotyped into *Nit* M and *Nit* 1/3 mutants, and then used in complementation tests with international testers. Virulence of representative isolates for each VCGs was assessed by biological assays on cotton seedlings, in order to determine the relative area under the disease progress curve (RAUDPC).

Results: From 570 chlorate-resistant sectors, 248 *Nit* mutants were generated from all tested isolates, of which 7% were characterized as *Nit* M. The most prevalent was VCG2A (31.6%), followed by VCG4B (21.1%) and VCG2B (7.0%); none of the isolates was assigned to VCG1A. Twenty isolates did not complement with any tester and three were self-incompatible. VCG2A was spread in all the Lebanese regions, whereas VCG2B was restricted to Bekaa region. In general, isolates assigned to VCG4B were the most virulent on cotton plants with an average RAUDPC (39.1%), significantly ($P < 0.05$) higher than that of VCG2A and VCG2B (29.6% and 28.3%, respectively).

Conclusion: Despite VCG assignment provides useful information on the diversity of *V. dahliae* populations, it would be useful to determine the pathotypes of the tested isolates using molecular tools and to characterize the different VCGs by using molecular approaches.

P SPP 1

Weevil Feeding Site Preference on Maize Grain: Implications and Clues for Successful Breeding Programmes against *Sitophilus zeamais* Motschulsky

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The possibility of *Sitophilus zeamais* Motschulsky to show feeding preference for a particular grain side and side area, its implications and value in resistance maize breeding programmes were investigated in the laboratory at ambient temperature (32.7 °C) and relative humidity (70.4 %) using standard techniques. Twenty elite maize varieties were used and these are: TZBRCOMP.2C1F1, TzBRELD3C5, PVASYN3F₂, PVASYN6F₂, DTSYN-11-W, BR9943DMRSR, IWDC3SNY-W, WHITEDTSTRSYN, 2008DTMA-YSTR, 2000SYNEE-WSTR, ILE-1-OB, IFEMAIZEHYBRID-1, IFEMAIZEHYBRID-2, IFEMAIZEHYBRID-5, IFEMAIZEHYBRID-6, ARTCOMPOSITE-A-Y, ARTCOMPOSITE-B-Y, ART/98/SW1-Y, ART/98/SW4-OB and ART/98/SW5-OB. The first ten were obtained from the Maize Breeding Units of International Institute for Tropical Agriculture (IITA), Ibadan, Nigeria while the rest were obtained from Institute of Agricultural Research and Training (IAR&T), Moor Plantation, Ibadan, Nigeria. The flat side of a maize grain at the point of penetration was significantly preferred by the weevil ($P \leq 0.05$) because it possibly provided the weevil with much needed space for anchorage and feeding. The South locus of the flattened side of a maize grain was significantly preferred by the weevil ($P \leq 0.05$) possibly because of its closeness to the nutrient - rich embryo and the soft nature of the locus in question. However, it was established that the preference shown to the South area of the grain - flat side has implications for seed viability. The findings of the study provided useful clues in breeding for resistance to weevil infestation in stored maize.

P SPP 2

Comparative lethality of three plant powders, a diatomaceous earth and their mixes to adults of four storage beetles

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Introduction: There is a dearth of research directly comparing the lethality of diatomaceous earths, insecticidal botanical powders and their combinations to storage insect pests. The study was carried out to compare the lethality of powders of *Oryza sativa* husk, *Eugenia aromatica* dry flower buds, dry fruits of *Piper guineense*, a diatomaceous earth (Silico Sec[®]) and their mixtures to adults of *Callosobruchus maculatus*, *Sitophilus zeamais*, *S. granarius* and *Lasioderma serricornis*.

Methods: The lethality of powders made from rice husk (RHP), dry fruits of *P. guineense* (PGP), dry flower buds of *E. aromatica* (EAP) and a diatomaceous earth (SilicoSec) (DE) to adults of *S. zeamais*, *S. granarius*, *L. serricornis* and *C. maculatus* was investigated under controlled conditions of 25° C and 70% r. h. The materials were tested singly and combinations with DE (1:1, 3:1 and 1:3 ratios) at 2% of grain weight in glass Petri dishes against 20 adults of each beetle. Adult mortality was observed up to 10 days post treatment.

Results: DE produced 100% mortality in *L. serricornis* and *C. maculatus* at 1 day post treatment followed by EAP, 90.0% for *L. serricornis* and for *C. maculatus* 86.7%. DE and EAP caused 100% mortality of *S. zeamais* and *S. granarius* at 10 days post treatment. The adult storage beetles irrespective of species and at all times of observation suffered significantly lower mortality when exposed to grain treated with RHP and PGP. The combinations of EAP and DE (ratios 1:1, 3:1 or 1:3) produced generally higher mortality in *S. zeamais* and *S. granarius* than other combinations 3, 5 and 7 days post treatment; producing 100% mortality of the beetles by 10 days post treatment. One day post treatment EAP/DE combinations produced significant mortality (100%) in *C. maculatus* and *L. serricornis*. DE combined with PGP and RHP enhanced their lethality to the beetles. EAP and DE applied at 0.01, 0.02, 0.03 and 0.04 g/20 g of grain produced 100% mortality of *C. maculatus* adults at 4 days post treatment.

Conclusions: DE and EAP applied at 2% of grain weight are lethal to adults of *C. maculatus*, *S. zeamais*, *S. granarius* and *L. serricornis* and may effectively prevent grain damage by these insects. DE and EAP applied at 0.05% of grain weight are also sufficiently lethal to *C. maculatus*. The lethality of DE against adults of the four storage beetles was not mitigated by mixing with EAP, RHP or PGP. DE enhanced the lethality of PGP and RHP to the adult beetles. There is great promise in combining DE and botanicals for stored products protection against insect depredations.

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P SPP 3

Efficacy of *Azadirachta Indica* and *Cymbopogon Citratur* as storage grain protectants against *Callosbruchus Maculatus* and *Sitophilus Zeamais*

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The agrochemical industry is faced with increasing demand for the development of new crop protection agents that are safe for the environment and consumers. Presently, over 40% of crops is lost as a result of application of non-selective crop protection agents. This research aimed at contributing to the baseline data on the status of natural insect-repelling and insecticidal agents; that is bioactive compounds extracted from plants that can serve these purposes especially for cowpea and maize plants. It also aimed at developing storage crop protection agents that would do no harm to crops, farmers and consumers but would preferentially get rid of pests. The need to develop pest control measures as alternative to chemicals is a priority for scientists worldwide. Therefore, it is critical to find a cost effective and non-toxic method to prevent pest attack and increase market value of the target crops. In this study, *Azadirachta indica* (Neem), was used as pest control agent for storage insect pests of maize and cowpea. The pest repelling property of *Cymbopogon citratur* (Lemon grass) was also investigated and results were analysed statistically. This led to the development of technique adoptable for small holder farmers and food crop store keepers thereby increasing food supply at a reduced cost.

P SPP 4

Monitoring the activity of the egg parasitoid *Trichogramma evanescens* (Hymenoptera, Trichogrammatidae) on an industrial Big Bag after release

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The control of stored-product moths within bagged commodities is difficult because the developmental stages of the moths are protected by the bagging material from the application of contact insecticides. Moreover, only a limited number of contact insecticides are registered for storage of organic products, and insecticide residues should not be present on packages. An alternative approach is the release of egg parasitoids outside the bags for biological control of moth eggs and consequently the prevention of moth infestation. Semi-field trials were carried out with the aim to identify a method to monitor the host finding efficiency of the parasitic wasp *Trichogramma evanescens* on industrial Big Bags, which are used by the food processing industry to store products prior to processing. The Big Bag used in the experiments had the standard size of 177 cm height x 100 cm width x 90 cm length. Eight sticky surfaces (5 x 3.5 cm) were placed onto the Big Bag at different distances from the *T. evanescens* release point. A total of about 500 to 6000 *Trichogramma* adults were released. The *T. evanescens* release units were not removed for the three weeks experimental period but the sticky surfaces were removed weekly and the number of *T. evanescens* that were trapped was counted and sexed. In a second experiment, the sticky surfaces were replaced by moth eggs glued on paper to monitor parasitism. Parasitoids were captured at all positions on the Big Bag, showing the small wasps (body length: 0.3 mm) are capable of foraging on the whole surface. There was a significant difference in the mean the number of *T. evanescens* trapped on the sticky patches depending on the number of parasitoids released. The use of sticky traps versus moth eggs for monitoring the activity of *T. evanescens* is discussed.

P SPP 5

Evaluation of the efficacy of some insecticidal plant materials against dry wood termite infestation

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Laboratory study was conducted to determine the efficacy of six insecticidal plant extracts *Gmelina arborea leaf*, *Afromanum melegueta*, *Zingiber officinale*, *Morinda citrifolia*, *Moringa oleifera* (seed and leaf) and *Garcinia kola* on the management of African wood termite under ambient conditions of temperature and relative humidity (30^oc and 72%). Wood termites were collected from the wood workshop of the Department of Forestry and Wildlife Resources Management, University of Calabar,

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Nigeria. Twenty termites were introduced into a covered container and treated with three levels (0.0g, 0.5g, 1.0g,) of the various plant powders. The experiment was laid out in a completely randomised design (CRD) and replicated four times with a factorial arrangement of treatments. Mortality rate was recorded at 12, 24, 36, 48, 60, 72, 84 and 96 hours exposure periods respectively. *A melegueta* and *G kola* were effective at 60 hours of exposure for 0.5g rate of application recording 95% mortality rate while *Z officinale*, *M oleifera* recorded 100% mortality after 84 hours of exposure. At 1.0 g rate of application, *M oleifera* seed recorded 95% mortality after 60 hours and 100 % at 72 hours followed by *M oleifera* leaf and *A melegueta* recording 100 and 95 % mortality at 96 hours of exposure. No mortality was recorded in the untreated experiment throughout the experiment. Conclusively, *A melegueta*, *G kola*, *Z. officinale* and *M. oleifera* can be used on the control of wood termite due to higher percent mortality recorded on application and as a substitute for synthetic insecticides.

P SPP 6

Study on olive oil characteristics in infected and uninfected fruits by olive fruit fly

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Olive Fruit fly is one of the important and major olive pests in the world .It became widespread in olive growing regions of our country in 2004 and damaged economical injury to the olive orchards of these regions. This pest larvae causes olive oil qualitative and quantitative reduction because of entering pathogenic fungus and quality reduction in olive oil specially after harvesting and in fruit storing time until oil extracting as well as fruit falling before harvesting. This research purpose is study the effect of infected and uninfected olive fruits by olive fruit fly larvae storage duration on some qualitative and quantitative characteristics of olive oil. This experiment was conducted in RCBD with six treatments and three replications on Zard olive cultivar, included:

- 1 control: (uninfected fruits, immediately after harvesting)
- 2 infected fruits immediately after harvesting.
- 3 infected fruits, a week after storing
- 4 infected fruits, two weeks after storing
- 5 infected fruits, three weeks after storing
- 6 infected fruits, four weeks after storing

Oil was extracted centrifuge after sampling and splitting flash from fruit stone and was sent to oil seed laboratory for measuring qualitative characteristics. Obtained data were analyzed by MSTATC statistical programs and LSD method used for means comparing. Acidity mean comparison showed that there wasn't any significant difference between control and second and third treatments and it had low acidity and good quality. Highest oil acidity was observed in fifth and sixth treatments (5.070%, 7.627% respectively), that shows sever reduction in oil quality after three or four weeks of storing fruits.

P SPP 7

Characterization of inorganic and organic-clays modified materials: an approach for adsorption of an insecticidal terpenic compound

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Two organic molecules and two inorganic polymers have been used to modify the structure of bentonites as to obtain a high adsorption capacity of thymol, an insecticidal terpenic compound. The selected organic molecules were cetyl and phenyl trimethyl ammonium chlorides (CTMA and PTMA); the inorganic compounds were aluminum hydroxyl and iron hydroxyl polymers. Organic bentonites were obtained after treatment with CTMA and PTMA solutions. Inorganic bentonites were obtained after treatment with polymeric solutions of $Al_x(OH)_y$ and $Fe_x(OH)_y$. Materials were characterized by several physicochemical techniques including X-ray diffraction (XRD), thermal gravimetric and differential analysis (TGA, TDA), infrared spectroscopy (FTIR) and nitrogen adsorption-desorption. Adsorption capacities of modified and unmodified bentonites for terpenic compounds were also carried out. Results show that the organic cations have been intercalated successfully into the bentonite interlayer space. XRD measurements show that PTMA and CTMA induce an interlayer space expansion of 4.1 Å and 10.5Å, respectively. The increase of specific surface areas of materials treated with metallic polycations attests the occurrence of exfoliation. The highest adsorption capacity of thymol is obtained with bentonites modified by alkylammonium, which proves

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that the modification of the interlayer space is an interesting way to improve adsorption properties of clays usable as support for natural insecticides.

P SPP 8

Estimation of losses in some advanced sorghum genotypes caused by red flour beetle, *Tribolium castaneum* (Herbst) (Tenebrionidae: Coleoptera)

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Red flour beetle, *Tribolium castaneum* (Herbst), is a cosmopolitan stored grains insect pest posing severe threat to post-harvest storage of grain foods such as sorghum (*Sorghum bicolor* L. Moench), which is a major staple crop of Pakistan. This study was undertaken to investigate the quantitative and qualitative losses induced by *T. castaneum* to some advanced sorghum genotypes viz; Hegari, F-114, J.S-2002, M.R.Sorghum-2011 and P.C-1 and to screen out the most resistant genotype against this insect pest. Quantitative (insect pest adult emergence, damaged grains percentage, frass weight and grains weight loss) and qualitative (crude protein, fat, ash and fiber contents) were determined after a 90 days incubation of sorghum grains in dark at three different temperatures i.e. 28, 32 and 35 °C. M.R.Sorghum-2011 was found to be the most susceptible genotype while the genotype Hegari was found the most resistant one against *T. castaneum*. On an average basis, maximum adult emergence and frass weight were recorded in M.R.Sorghum-2011, while those were found minimum in Hegari genotype. Regarding qualitative losses, minimum losses induced by *T. castaneum* infestation were recorded in Hegari followed by J.S-2002, F-114 and P.C-1, while high nutritive losses were observed in M.R.Sorghum-2011. Furthermore, qualitative losses to sorghum grains were high for all genotypes at 32 °C, followed by 35 °C and were least at 28 °C. The results will aid in future breeding programs for finding promising sorghum genotypes (such as Hegari) and for better assessment of storage losses due to *T. castaneum*.

P SPP 9

Insecticidal activity of *Cleistopholis patens* (Benth) against *Plodia interpunctella* (Hübner) and its toxicological effect on albino rat

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The moth, *Plodia interpunctella* (Hübner) is a major pest of stored grains in which it causes marked economic losses. This study investigated the insecticidal activity of parts of *Cleistopholis patens* against the developmental stages of the moth in stored maize grains. Powders and oils from the root bark, stem bark and leaves of *C. patens* were tested as both contact and fumigant insecticides in the laboratory.

The efficacies of the powders and oils as contact insecticides were evaluated by admixing different concentrations (0.5, 1.0, 1.5, 2.0 and 2.5g) to maize grains containing developmental stages of the moth. Fumigant toxicity was evaluated by hanging the powder contained in a muslin sack and oils impregnated in cardboards in containers containing the maize grains and developmental stages of the moth. Egg hatchability, adult emergence, larvae mortality and adult mortality of the moth were used as indices of the insecticidal activities at 24hrs, 48hrs, 72hrs, and 96hrs post-treatment period. The toxicological effects of the powders and oil extracts from the plant were evaluated on the liver and kidney of albino rat. Liver function indices such as Aspartate aminotransferase (AST), Alanine aminotransferase (ALT), and Alkaline phosphatase (ALP) were determined. Kidney function indices such as Urea, Total bilirubin, direct bilirubin, and creatinine were also evaluated.

Results showed that powders and oils from the root bark and stem bark of *C. patens* completely inhibited egg hatching and adult emergence as both contact and fumigant insecticide. Also, they evoked 100% mortality at 2.5% protectant concentration at 96hrs post-treatment period against larvae and adult *P. interpunctella*. The leaf powder was the least effective as both contact and fumigant insecticide as it achieved

No significant difference was observed in the biochemical parameters of the liver and kidney of animals fed with basal diet containing the powders and oils of different concentrations when compared with the control. It is thus suggested that *C. patens* powders and oils were not toxic to the organs and can be recommended as protectant against *P. interpunctella* in stored maize grains.

P SPP 10

Residual toxicity of pirimiphos-methyl on concrete surface to the rusty grain beetle, *Cryptolestes ferrugineus* (Stephens) (Coleoptera: Laemophloeidae)

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Cryptolestes ferrugineus (Stephens) is a cosmopolitan insect pest of stored products including wheat, barley, flour, peanuts, sorghum, oilseeds, cassava root, dried fruits, chillies and packaged and processed foods. Concrete is a typical surface in Brazilian warehouses and food processing plants. The aim of this work was to evaluate the residual toxicity of pirimiphos-methyl emulsifiable concentrate (50% EC) on concrete surface to *C. ferrugineus*. Concrete surfaces were treated with nine different concentrations (from 50 to 250 mg of a.i. per m²) of pirimiphos-methyl, using a compression sprayer at working pressure of 45 psi and cone spray nozzle. Five round concrete surfaces (0.0061m² by 0.017m) infested with 20 adult insects were used for each treatment. The insects were exposed to pirimiphos-methyl residue (on a concrete surface area of 0.0033m²) four hours after treatment. The mortality was assessed at 1, 2, 3, 4 and 24 hours after exposing the beetles to the insecticide residues. Additional bioassays were carried to observe the effects of 30-day and 60-day-aged pirimiphos-methyl residues on the mortality of *C. ferrugineus*. For the bioassays of 30 and 60-day-aged residues, the mortality was assessed up to 72 hours after exposing the insects to the treated surfaces. The residue of pirimiphos-methyl on concrete was effective to control *C. ferrugineus*. The lethal concentration (LC₅₀ and LC₉₀) values for the first day pirimiphos-methyl residue were less than 50 mg a.i. per m². The LC₅₀ value increased significantly for the 60-day-aged residue (98.6 mg a.i. per m²). The lethal time (LT₅₀) value of the highest concentration (250 mg a.i. per m²) of pirimiphos-methyl was less than 1.0 hour for the first day residue. For the 60-day-aged residue, this value (LT₅₀) increased to 8.2 hours.

P SPP 11

Ovicidal Efficacy of *Xylopiia aethiopica* and *Parinari macrophylla* extracts against *Sitotroga cerealella* (Olivier) Infesting Stored Paddy Rice.

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Introduction: Powders, extracts and essential oils from different plant species have been reported to possess ovicidal, larvicidal, antifeedant and repellent properties against various insect pests and are regarded as environmentally compatible pesticides (Isman 2000; Cetin *et al.*, 2004). Notably amongst them is *Sitotroga cerealella*.

Objectives: The work is focused on investigating seed extracts of *Parinari macrophylla* and *Xylopiia aethiopica* in the control of *Sitotroga cerealella* in paddy rice.

Materials and methods: Oils extracted from the seeds of *Parinari macrophylla* and *Xylopiia aethiopica* according to the methods of Ashamo and Akinnowonu (2012) were tested against *Sitotroga cerealella* at 10000 - 30000 ppm/ 20g of paddy rice. Parameters tested includes adult emergence of moth, developmental period (days), % weight loss in treated paddy rice.

Results: Petroleum ether extract significantly (PP. *macrophylla* had the highest insecticidal activity at 30000ppm compared to *X. aethiopica* at that same concentration. Developmental period was highest at 20000 ppm (44.00%) and lowest in the controls (30.00, 30.33 and 30.67%). Phytochemical investigations of the plants revealed the presence of terpenoids, saponins, tannins, cardiac glycosides, alkaloids, steroids, anthraquinones and flavonoids.

Conclusion: It is quite obvious that biopesticides have a potential in modern stored product protection. The use of plant products especially oil is an aspect of biological control utilizable as a component of integrated pest management.

Reference:

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Green Botanical Powders as Affordable Insect Pest Protectant Against Post- Harvest Losses Associated With Grains

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Introduction: One of the most promising ways to reduce dependence on synthetic pesticides in agriculture is to cultivate insect resistant crops. However, since multiple resistances to all insects is not likely, it is important that resistance can be combined with eco-friendly methods such as use of plant powders in the management of stored product insect pests such as *Sitotroga cerealella* in paddy.

Objectives: The work aimed at determining the efficacy of powders (leaves and seeds) of three plants (*Ricinus communis*, *Aframomum melegueta* and *Helianthus annuus*) in controlling *S. cerealella* in stored paddy rice.

Materials and methods: Studies were carried out to determine the efficacy of three plant materials (*A. melegueta*, *R. communis* and *H. annuus* against *Sitotroga cerealella*, an insect infesting paddy rice (*Oryzae sativa* (L)). Powders of the tree indigenous plants were applied at 0.1- 0.8g/ 20g of paddy rice previously screened moderately and most susceptible rice varieties under laboratory conditions (28 -32°C and 75-80% relative humidity).

Result: Among the plant materials tested, *A. melegueta* leaf and seed powders were able to achieve high moth mortality at various concentrations and exposure period. Both leaf and seed powders of this plant were able to evoke 100% at high concentrations in variety that was most susceptible in the earlier experiment. The seed powder completely prevented adult *S. cerealella* emergence. Efficacy of plant powders is dose dependent.

Conclusion: In the light of the findings of this study, it is therefore recommended that *R. communis*, *A. melegueta* leaf and seed powders and extracts which had multiple insecticidal effects in the management of Angoumois grain moth *S. cerealella* infestation on harvested paddy rice.

P SPP 13

Chemical composition and in vitro biological activities of essential and vegetable oils from four Cameroonian spices as potential protectants of stored grain against insects and microorganisms infestations

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Introduction: Once the crops are mature, they become the food for insect pests. Feeding by these insects favors the development of microorganisms such fungi which leads to the production of allergenic compounds and mycotoxins which do not only result in a significant loss of seeds quality in both nutritional and economical values, but, are also associated with carcinogenicity in humans and animals. Facing this problem of public health, researchers are engaged in the development of both synthetic and bio-pesticides to preserve the quality of grains. Because of the side effects of synthetic pesticides, our research team is committed for a number of years in the search for strategies to preserve seeds quality and health of the consumers through the use of some Cameroonian spices.

Objectives: The objective of our work is:

- to obtain essential and vegetable oils from six select Cameroonian spices and carry on their chemical characterization.
- to evaluate the efficacy of these oils against some major grain pests found in Cameroon- to find out the most appropriate formulation of these oils as those generally possessing this potential also present some limits;- to identify microorganisms present on stored grains and evaluate the antimicrobial properties of these formulations;- to preserve the consumers health through the reduction of free radicals produced by insects when feeding and the inhibition of proliferation of cancer cells which can be observed after eating grains containing high amount of mycotoxins.

Material and methods: Oils obtained by steam extraction and hydro distillation from seeds and barks of six Cameroonian spices were analyzed for their chemical composition by GC-FID and GC-MS, and for *in vitro* biological activities namely insecticidal, antioxidant, cytotoxicity and antimicrobial properties.

Results: The oils were highly toxic to adult insects and the mortalities generated by the aromatized powders based on clay and essential oils were in general significantly high, and both oils greatly reduced the F₁ progeny insect production and grain weight loss. Moreover the essential oils exhibited a strong inhibitory effect on some human cancer cells (T98G cells, MDA-MB 231 cells, A375 cells and HCT116 cells), and a good scavenging activity against DPPH and ABTS⁺ radicals.

Conclusion: The significant activities detected make these oils worthy of further investigation as promising chemopreventive agents to be exploited in the African pharmaceutical market.

P SPP 14

Evaluation of the resistance of Bt and non Bt maize varieties against *Sitophilus zeamais* (Coleoptera: Curculionidae)

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The weevil *Sitophilus zeamais* (Coleoptera: Curculionidae) is a serious maize pest worldwide that infests grains in the field and under storage conditions, leading to great economic losses. Host plant resistance is an environmentally safe control method compatible with most others. *Bacillus thuringiensis* (Bt) proteins have been used to confer resistance to maize against caterpillars and cross resistance to weevils would be desirable. We aimed at evaluating the resistance of Bt and non Bt maize varieties against *S. zeamais*. Samples (20g) of each variety were infested with 20 unsexed adult weevils in plastic vials which were kept in laboratory (25°C). Insects were allowed to mate and oviposit during 4 days before being removed. The number of insects emerged was registered daily and the total number of adults emerged and the duration of developmental time were calculated. The experiment consisted of a completely randomized design with 11 treatments (maize varieties), with five replications (vials) per treatment. Data were submitted to Anova followed by Scott-Knott test ($P \leq 0.05$). Significant differences among treatments were observed for both variables evaluated. A low number of insects emerged and the highest developmental time (desirable traits) were observed for the non Bt variety SAX 790, which was considered the most resistant among the varieties evaluated. Varieties P 4285 H, BIOZ 2535, 2B 707 PW and DKB 285 PRO had intermediate values and the remaining were considered susceptible to *S. zeamais*. Our results indicate that maize varieties expressing Bt proteins on leaves do not affect the grain-feeding *S. zeamais*, and cross resistance against caterpillars and weevils is not expected to occur.

Table 1: Means of *Sitophilus zeamais* adults emerged and developmental time (days) in different maize varieties. Means followed by the same letter within columns do not differ based on Scott-Knott test ($P \leq 0.05$).

Maize varieties	Bt proteins	Adults emerged		Developmental time	
		(total)			
4M 50	-	107.40	a	35.68	c
RB 9005 PRO	Cry1A.105 + Cry2Ab2	80.00	a	35.95	c
CD 316	-	74.00	a	36.25	c
AG 4051	-	73.80	a	36.92	c
AG 9045 PRO	Cry1A.105 + Cry2Ab2	70.60	a	35.17	c
P 4285 H	Cry1F	57.40	b	37.76	b
BIOZ 2535	-	52.40	b	39.09	b
2B 707 PW	Cry1A.105 + Cry2Ab2 + Cry1F	46.20	b	38.00	b
SAX 790	-	38.60	b	41.72	a
DKB 285 PRO	Cry1A.105 + Cry2Ab2	29.60	b	38.47	b
RB 9110 YG	Cry1Ab	16.60	b	37.11	c

P SPP 15

GC-MS analysis of *Clerodendrum capitatum* as fumigant against stored grain insect pest

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Natural sources for novel bioactive molecules hold promise to mitigate the short comings associated with the use of synthetic insecticides in prevention of post-harvest grains loss. In this study natural bioactive molecules were isolated from the leaves of *Clerodendrum capitatum* and analyzed by gas chromatography-mass spectrometry (GC-MS). Tetraneurin - a - diol (8.87%), Octadecanoic acid, 2-hydroxy-1,3-propanediyl ester (34.26%), Octadecanoic acid (32.96%), Hexadecanoic acid, 1-(hydroxymethyl)-1,2-ethanediyl ester (31.91%), 1-Isopropyl-4,8-dimethylspiro[4.5]dec-8-en-7-one (14.94%), Tricyclo

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[20.8.0.0(7,16)] triacon tane, 1(22),7(16)-diepoxy- (14.80%), E,E,Z-1,3,12-Nonadecatriene-5,14-diol (19.28%) and E-8-Octadecacen-1-ol acetate (9.41%). The fumigant activity of *C. capitatum* bioactive molecules exerted significant adult mortality against *Tribolium castaneum* and *Sitophilus oryzae*. These findings suggested that the volatile molecule of *C. capitatum* has potential to be developed as a new natural fumigant for the control of stored grains insect pests.

P SPP 16

Study of the biological activity of the entomopathogenic *Beauveria bassiana* (Vuil., 1912) on the biochemistry and structure of the cuticle of *Schistocerca gregaria* (Forskål, 1775) .

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This work was undertaken in order to help identify the biological activity of the entomopathogenic *Beauveria bassiana* AGAINST the cuticle of adult individuals and 5th stage larvae of *Schistocerca gregaria* viewpoint biochemistry and structure.

The application of entomopathogenic *B.bassiana* with the dose 1.69×10^5 spores / ml on the cuticle of L5 and adults of *S.gregaria* showed a significant reduction in the dry weight of the cuticle from 36 , 50mg to 32.93 mg in L5 and 46.49 mg to 42.31 mg in adults. The same is true for the chitin content is increased from 7.38 mg to 6.44 mg in L5 and 7.41 mg to 4.20 mg in adults. For proteins, the amount dropped to 29.12 mg 26.48 mg in L5 and 39.08 mg to 37.88 mg in adults. The larval cuticle of the fifth stage *S.gregaria* highlighted by differential staining with Heidenhain azan, shows the presence of the epicuticle, the exocuticle and endocuticle.

The epicuticle colored red is as a thin layer on the surface of the cuticle and L5 *S.gregaria* exocuticulaire least orange colored thinner than the first part of a colorless and a greater thickness corresponds to the endocuticle .

The Sensibility of larvae cuticle was dependent of an apparent disturbance on the structure of the cuticle. Indeed the third day of treatment it has been observed a complete disappearance of the epicuticle and a sharp decline in the thickness of the exo and endocuticle and that compared to the control larvae cuticle or three layers are distinct and exposed with a visibly thicker.

The breakdown of cuticular layers larvae treated is probably due to degradation by proteolytic enzymes and chitinolytic the infectious unit of our fungal strain, spore which was spotted a few cells in parts of the cuticle digested.

P SPP 17

Relative susceptibility of four coleopteran stored product insect species to diatomaceous earth SilicoSec®

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Laboratory bioassays were conducted to evaluate the insecticidal effect of diatomaceous earth SilicoSec against four stored-product beetle species, *Callosobruchus maculatus* (F.), *Rhyzopertha dominica* (F.), *Sitophilus zeamais* (Motschulsky.), and *Tribolium castaneum* (Herbst) in cowpea, wheat and maize. SilicoSec was applied at four dose rates: 250, 500, 750 and 1000 mg/kg of commodity. Adult mortality in the treated commodities was measured after 3 and 5 days for *C. maculatus* and 3, 7 and 14 days of exposure for *R. dominica*, *S. zeamais* and *T. castaneum*. Progeny production was assessed after 40 days in case of *C. maculatus* and 56 days in the case of the other species. Results showed that insect species varied in their sensitivity to SilicoSec, with *S. zeamais* being most susceptible, but no significant differences in mortality levels observed between *R. dominica*, *C. maculatus* or *T. castaneum* after three days of exposure. After 5 days of exposure all *C. maculatus* dies on cowpea treated at 1000 mg/kg. Similarly, after 14 days of exposure adults of all the three other insects species died on grains treated at 1000 mg/kg. These were ranked in decreasing order of susceptibility to SilicoSec *S. zeamais* > *T. castaneum* > *R. dominica*. DE treatment also substantially (30 to 100%) suppressed progeny production in all the insect species. The external feeder *T. castaneum* was severely affected in terms of progeny suppression. The implications of these findings to DE-based control strategy are discussed.

Figure 1

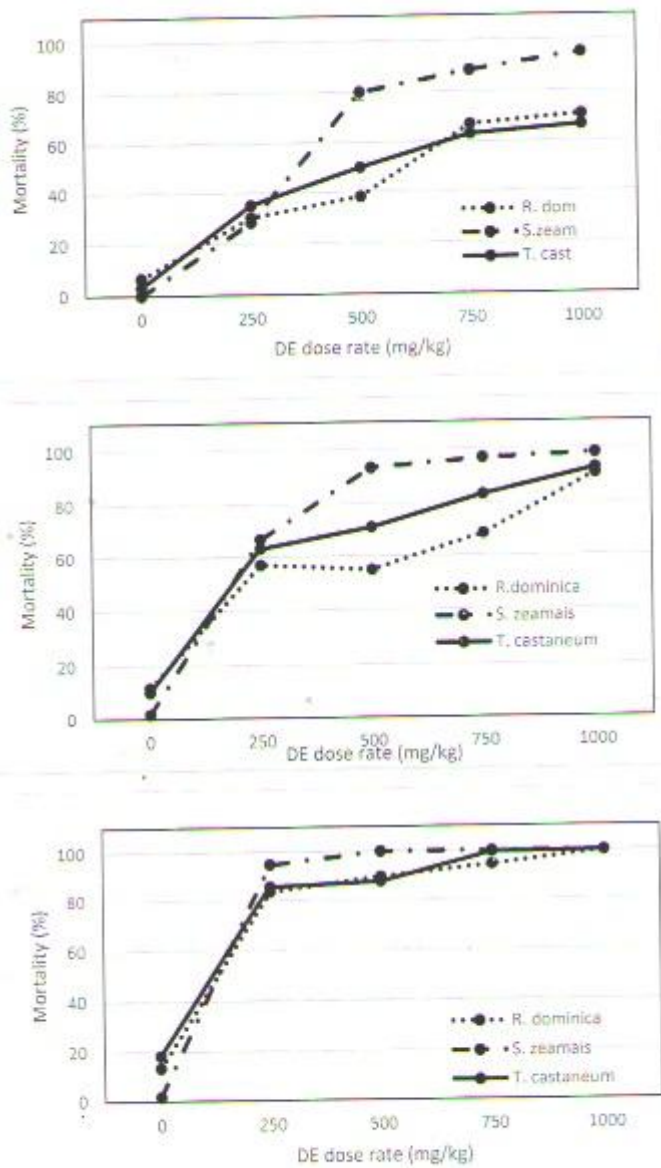


Fig. 1. Percentage mortality of *R. dominica*, *S. zeamais* and *T. castaneum* adults after three (A), seven(B) and 14 (C) days of exposure to different dose rates of SilicoSec

Figure 2

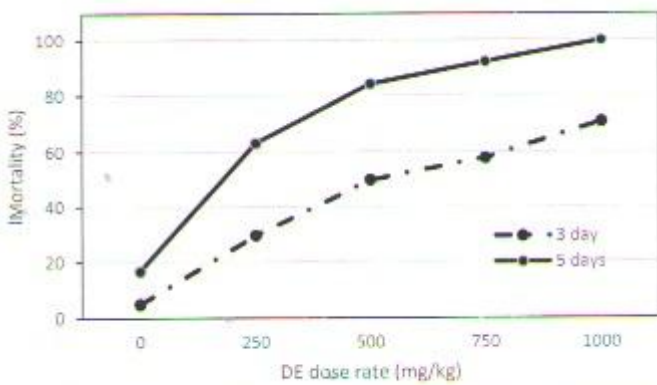


Fig. 2. Mortality of *C. maculatus* adults after three and five days of exposure to different dose rates of SilicoSec

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P SPP 18

Bioactivity of binary mixture of NeemAzal, *Azadirachta indica* seed powder and *Plectranthus glandulosus* leaf powder against *Sitophilus zeamais*

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Sitophilus zeamais Motschulsky (Coleoptera: Curculionidae) are very serious storage pests of maize. Chemical synthetic residual insecticides, which degrade the environment, are widely used for the control of these pests. Alternative control methods are required to minimize the hazardous effects of such insecticides. Botanical insecticides are more biodegradable and could be a source of more environmental-friendly insecticides. At various proportions (100 + 0, 75 + 25, 50 + 50, 25 + 75 and 0 + 100%, the mixture of *P. glandulosus* + NeemAzal powders and *P. glandulosus* + *A. indica* powders were tested on adult mortality, inhibition of offspring production, grain damage and their persistence on maize. Generally, the binary combinations of *P. glandulosus* and *A. indica* seed powder on the one hand, and *P. glandulosus* and NeemAzal in the other hand, were antagonistic, regarding their toxicity to *S. zeamais*. The mixed 75% NeemAzal + 25% *P. glandulosus* of powder led to a higher mortality (100%) of *S. zeamais* 3-d post exposure at the dose of 5 g/kg while the mixture of 25% *P. glandulosus* and 75% *A. indica* powder caused 50% mortality at the highest tested rate (20 g/kg) 14-d post exposure which was far less than that when the botanicals were applied alone. As they were applied alone, *P. glandulosus* and *A. indica* recorded respectively 100% mortality within the same exposure time (14-d) and dose (20 g/kg). The binary combination of the powders produced more progeny and incurred more grain damage, compared to the cases with the individual powders as *P. glandulosus* and *A. indica* were mixed. The opposing effect was observed with the mixture of *P. glandulosus* and NeemAzal. The 75% *P. glandulosus* + 25% NeemAzal mixture persisted on grains up to 180-d at all dose levels. Mixing these products could not be advantageous in adult mortality since the binary mixture gave similar result as when they were applied alone. And powder from NeemAzal and *P. glandulosus* leaves stand as good candidates to protect maize against the emergence of *S. zeamais* during storage.

P SPP 19

Post harvest problems in North Cameroon

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North Cameroon is confronted in its great majority with the problem of food insecurity which strikes the rural populations in a recurring way. The income of more than 70% of population relies in a large extent on the agriculture which is unfortunately less productive. The uncertain climatic conditions pose a real problem of the post harvest management. The inappropriate structures of storage lead to the insect attacks which damage the stored products. Without any protection up to 60% of the stored products are lost. More so, the unavailability and the misuse of the chemicals remain a challenge. Some NGOs are promoting community attics with an aim of setting up a local device of management of stored products deficit. Also the use of local botanicals is to support since some farmers mix their stored products with plant parts. One of known insecticidal plant *Azadirachta indica* and some aromatic plants which are present locally are subject of our interest and could be integrated to the management of stored products protection.

P SPP 20

Efficacy of Essential Oils to Control Maize Grain Weevil (*Sitophilus zeamais* Motschulsky)

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The efficacy of essential oils from peel of *Metha cordifolia* Opiz ex Fresen, peel of *Citrus aurantifolia* (Christm.) Swingle, fruit of *Dacydium elatum* (Roxb.) Wall. ex Hook, leave of *Lantana camara* L., rhizome of *Zingiber officinale* Roscoe, stem of *Litsea cubeba* (Lour.) Persoon, flower of *Illicium verum* Hook. f., peel of *Citrus hystrix* DC., seed of *Foeniculum vulgare* Mill., bark of *Styrax apricus* Fletcher, flower of *Melaleuca quinquenervia* (Cav.) S.T. Blake, bark of *Syzygium aromaticum* (L.) Merrill & Perry, leaves of *Ocimum basilicum* L., peel of *Citrus maxima* (Burm.f.) Merr. and seed of *Myristica fragrans* Houtt. were tested against Maize

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grain weevil, *Sitophilus zeamais* Motschulsky for repellent, fumigant, contact and antifeedant activities. The maximum repellency action, 84 and 80 was found when *Z. officinale* oil, at the concentrations of 1.5 and 2.0% were applied for after 3 and 5 h, respectively. The fumigant test of *O. basilicum* oli resulted in 100% mortality, at volume 55, 70 and 85 µL at 24 h after treatment. *Myristica fragrans* oil showed the highest rate (56%) at the concentration of 2.5% at 72 h by contact test. *Dacydium elatum* oil demonstrated 46% feeding deterrence index, at the concentrations of 2.0% at 72 h after treatment.

P SPP 21

Exploitation of *Origanum dictamnus* oil vapour to control *Botrytis cinerea* postharvest development in key horticultural products of Crete

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The degree of postharvest losses of fruit and vegetables due to attack by microorganisms worldwide is significant. In addition, the use of chemicals increases consumer concerns regarding food safety. Recent studies indicate that exploitation of natural compounds such as essential oils (EOs) may provide an alternative and friendlier way for the preservation of fresh produce. In this research project the efficacy of dittany (*Origanum dictamnus* L.) essential oil for the control of *Botrytis cinerea*, a common postharvest pathogen of three economically important Cretan vegetables, tomato, pepper and eggplant was examined. Pathogen development (vegetative or reproductive phase) in culture medium or in fruits was evaluated after treatment with dittany EO (0, 50, 100, 250 ppm) in vitro and in situ when stored at 12°C and 95% RH during or following exposure to EO volatiles. In vitro, fungal development was completely inhibited by the application of 100 or 250 ppm of EO volatiles. In inoculated fruits, the application of 50 ppm EO resulted in suppressed disease development by reduced lesion growth and fungal sporulation, where increasing EO concentration led to greater effects. Pre-exposure of the three fruits to volatiles, before fungal inoculation, revealed reduced lesion growth, indicating that dittany EO probably caused induced resistance of fruits against the pathogen. Moreover, EO application did not affect quality-related characteristics of fruits in general, while skin lightness and pulp lightness of eggplant fruits was improved under the presence of dittany EO volatiles. Overall, the results suggest that dittany EO volatiles may be considered as an alternative food preservative treatment, significantly reducing or eliminating *B. cinerea* infection during fruit storage.

This research has been co-financed by the European Union (European Social Fund - ESF) and Greek national funds through the Operational Program "Education and Lifelong Learning" of the National Strategic Reference Framework (NSRF) - Research Funding Program: ARCHIMEDES III. Investing in knowledge society through the European Social Fund.

P SPP 22

Evaluation of Anti-fungal Activities of the Leaf Extract of Two Endemic *Aloe* Species Against *Aspergillus* species that Affect Groundnut (*Arachis hypogaea* L.)

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Medicinal plants are a rich source of antimicrobial agents by producing many secondary metabolites which constitute an important source of microbiocides, pesticides and many pharmaceutical drugs. These bioactive compounds from plants are naturally produced in the plants as secondary metabolites; tannins, flavonoids, essential oils, alkaloids, lecithin and polypeptides are also show antimicrobial effect and serve as plant defense mechanisms against pathogenic microorganisms. There are several species of fungi that infect or contaminate at three different stages such as prior to harvest while the nuts are on the tree, after harvesting if the groundnuts remain wet; and in storage, especially when groundnuts are stored under adverse conditions. These are *Aspergillus*, *Fusarium*, *Penicillium* and *Alternaria* species which are mainly found associated with cereals and nuts reducing the yield and decreasing the viability of the seed germination capacity.

Thus, the present study is intended to evaluate the in vitro antifungal activities of the extracts of the leaves of two endemic *Aloe* species (*Aloe harlana* Reynolds and *Aloe megalacantha* Bake) which are found in Harla and Dengego valleys and Chiro selected on the basis of their reported ethnobotanical significance against seed-borne fungal pathogens isolated from groundnuts (*Arachis hypogaea* L.).

Figure 1



Figure 2



P SPP 23

Toxicity study of different amounts of ozone on *Sitophilus granarius* (L.), *Tribolium castaneum* (Herbst) and *Rhyzopertha dominica* (F.) under storehouse conditions

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Introduction: Due to economical importance of stored product insect pests and resistance to phosphine and methyl bromide, it is necessary to replace these insect control agents with novel and suitable compounds.

Materials and methods: To this end, the toxicity of ozone (90 and 180 mg/l) was evaluated under storehouse conditions on three important stored product insects *Sitophilus granarius*, *Tribolium castaneum* and *Rhyzopertha dominica*. These experiments were carried out in the storehouse contained foodstuffs, such date, wheat and rice. The toxicity of the gas was determined after 24 h of exposure.

Results and conclusion: Concomitantly with these trials, the lethal impact of ozone on the *S. granarius*, *T. castaneum* and *R. dominica* in different depths (50, 100 and 150 cm) of date, wheat and rice was estimated as well. Calculated R^2 values showed that, 90 and 180 mg/l of ozone caused 99% mortality on *S. granarius* in rice and *T. castaneum* in date, whereas 99% mortality was recorded of *R. dominica* in rice by 90 mg/l of ozone.

P SPP 24

Toxicity and persistence of spinosad on wheat and rice grains against *Rhyzopertha dominica* and *Tribolium castaneum*

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Introduction and objective: Insect pests in stored grains have been reported to develop a considerable level of resistance to commonly used grain protectants worldwide. Moreover, negative effects of conventional grain protectants on public health and environment necessitate the need to explore newer, reduced-risk pesticides in stored-product protection. Spinosad is a relatively new insecticide of low mammalian toxicity and has a broad spectrum of target species, including stored insect pests. In the present study, we were interested to assess the persistence and toxicity of spinosad on wheat and rice grains against two major stored pests *Rhyzopertha dominica* and *Tribolium castaneum*.

Methods: Spinosad solution was applied to 2 kg lots of wheat and rice grains separately at four concentrations viz., 2, 1, 0.5, 0.25 ppm. Treated commodities were kept for six months at 26 ± 2 °C and $60 \pm 5\%$ RH. The mortality bioassays were done by taking samples from each concentration-commodity combination at 30 day interval for six consecutive months.

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Results: The results revealed that *R. dominica* showed complete mortality at 0.5 and 0.25ppm for up to 2 months, 1ppm for up to 4 months, and 2ppm for up to 6 months. In contrast, *T. castaneum* showed complete mortality at only 2ppm concentration for up to 1 month. There was no significant effect of the grain type on mortality of both tested species.

Conclusion: The study concludes that spinosad could provide effective protection of stored wheat and rice against *R. dominica* for up to 4 months. However, it is not suitable to provide long term protection against *T. castaneum*.

P SPP 25

Volatile organic compounds mediating orientation of *Callosobruchus maculatus* (Fabricius, 1775) (Coleoptera: Chrysomeloidae: Bruchinae) towards dried green peas

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Cowpea weevil, *Callosobruchus maculatus* is a stored product insect of Africa and Asia that presently ranges throughout the tropical and the subtropical world. It infests stored legume seeds including peas (*Pisum sativum* L.). The determination of attractive compounds in dried green peas could be of a great interest for the development of an attractive lure for the monitoring or control of *C. maculatus*. The goal of this study is to investigate the perception of volatile organic compounds emanating from dried peas by *C. maculatus* and the behavior induced by these compounds. The volatile organic compounds from the headspace of dried peas were actively sampled by closed-loop-stripping analysis method. They were then analyzed by gas chromatography coupled to two detectors, a mass spectrometry for the determination of volatile compounds and an electroantennograph for the identification of volatile compounds perceived by adult male and female *C. maculatus*. In addition, the behavioral effects of mixtures (binary, tertiary, etc.) of electrophysiologically active compounds in the beetle were evaluated in a Y-tube olfactometer. The results of the present study will be presented and discussed based on literature data.

P SPP 26

Effects of Rosemary Essential Oil on Edible Chickpea and *Callosobruchus maculatus* (F.)

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Legumes are an important source of protein for people and have an important place in vegetable sources. Larvae of Cowpea weevil (*Callosobruchus maculatus* (F.)) causes a significant loss in stored legumes. Rosemary, *Rosmarinus officinalis* L., essential oil has high fumigant toxicity against stored-product insects. Effects of rosemary essential oil vapor on the properties of edible chickpea are unknown. In this study, the fumigant effects of *R. officinalis* essential oil vapor on *C. maculatus* egg and larvae and, also on hydration coefficient, cookability, color and taste of chickpea grain were assessed. Effectiveness studies of rosemary essential oil were carried out at 28±2°C temperature, %55±5 relative humidity (RH) and darkness in growth chamber. The eggs on chickpea and larvae in chickpea of *C. maculatus* were exposed to 10, 20, 30, 40, 50 and 60 µl L⁻¹ air doses of rosemary essential oil. The highest egg mortality rates were obtained as 71% at the 24th hour and 100% at the 48th hour with 60 µl/l air doses. The most effective dose (50 µl L⁻¹ air) of rosemary essential oil were on larvae mortality rates as 87%, 95% and 100% at the 24th, 48th 72nd, respectively. In addition, edible chickpea seeds were subjected to 10, 20, 30, 40, 50 and 60 µl L⁻¹ air doses of rosemary essential oil for the 24 and 48 hours. The highest dose applied of the essential oil and the longest exposed time did not show negative effect on the hydration coefficient, cookability, color of chickpeas. The chickpea seeds, rosemary essential oil vapor exposed, were ventilated for 1 hour, and then they cooked for 90 min. and the seeds were tasted by 10 different persons. According to taste tests, flavor of the chick pea seeds was changed in 40% of the ratio at 10 µl L⁻¹ air. In the further studies, the taste effects of rosemary should be studied to reduce on chickpeas. Rosemary essential oil vapor was detected first time as potential bio-fumigant in the management of *Callobruchus maculatus* on seed chickpeas and it may use in IPM programs.

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P SPP 27

Development of *Callosobruchus maculatus* (Fab.) on previously infested cowpea seeds

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A laboratory experiment was conducted to evaluate the impact of previous infestation of cowpea seeds on progeny development of *Callosobruchus maculatus* (Fab.). Samples of cowpea seeds containing 0 (check) 20, 40, 60, 80 and 100% previously infested seeds were reinfested with 5 pairs of *C. maculatus* adults for seven days. Number of progeny, days to first progeny emergence, median length of developmental period and weight losses sustained by cowpea seeds were measured. Result showed significant differences in number of progeny and seed weight loss among treatments. Progeny numbers decreased from 86.0±4.8 in the previously uninfested check to 16.4±1.7 in treatment containing 100% previously infested seeds. Days to first adult emergence and median development period ranged from 18.8±5.3 to 24.8±1.0 days and from 27.5±0.5 to 29.1±1.0, respectively with no significant differences among treatments. Seed weight loss decreased with increase in percentage of previously infested seeds. The importance of these findings to cowpea storage are highlighted.

P SPP 28

Studies on the effect of *Azadirachta indica* seed oil on the oviposition and adult emergence of cowpea bruchid (*Callosobruchus maculatus*) (Coleoptera:Bruchidae) on Cowpea seed (*Vigna unguiculata*) conducted at Bayero University, Kano State of Nigeria

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Cowpea is an important legume of worldwide importance. The effect of *Azadirachta indica* seed oil on the oviposition and adult emergence of *Callosobruchus maculatus* from cowpea seeds was conducted at Zoology unit of Bayero University, Kano under ambient condition of temperature and relative humidity. *C. maculatus* was collected from an infested cowpea collected from BUK New site. The insect was identified based on their morphological characteristics. *C. maculatus* was reared on cowpea seeds at the Zoology Laboratory. Cowpea seed used for the bioassay was collected from International Institute of Tropical Agriculture (IITA) Kano and was disinfested in the freezer for 3 weeks. The moisture content of the seed was also determined. The seed of *A. indica* was collected and identified at Afforestation Programme Coordinating Unit (AFCU). Oil was extracted from the seed. Bioassay was conducted in petri dishes. Three (3) different concentrations of oil from the seed of *A. indica* were separately mixed with 20g of cowpea in separate petri dishes. Five (5) pairs of newly emerged *C. maculatus* (24hrs old) were introduced into each petri dish. Control treatment was also set along. The number of eggs laid and adult *C. maculatus* emergence was counted on each treatment. All the seeds coated with the oils significantly ($P < 0.05$) recorded lower egg count and lower adult emergence of *C. maculatus* when compared to the control treatment. *A. indica* oil at 1.0ml and 1.5ml (5%v/w and 7.5%v/w) were the most effective recording the least egg count (0±00) and the least adult emergence (0±00) respectively. The need for further studies to investigate the active compound in the oil responsible for deterring oviposition and adult emergence is therefore of paramount importance.

P SPP 29

Effect of Aceton (C₃H₆O) Steams on *Plodia interpunctella* Hb. in stored rice ecosystems

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Rice is an important staple food throughout much of the world and is damaged by several insect species during storage. Insects cause considerable quantitative and qualitative and viability losses in stored rice every year. The quantitative as mass losses caused by insect are 5-25% per year. The species of insects infesting stored rice, their pest status, sources and mode of infestations, and losses caused by them are discussed with emphasis on rice storage ecosystems in tropical countries. Seventeen species of insects are reported to infest stored rice, with *Plodia interpunctella* Hb Olivier the main pest. Insect pest management in stored rice using holistic ecosystem approaches involves alterations of a biotic components (e.g., grain moisture, temperature inside the storage, condition, type and inner atmosphere of storage structures) in addition to the use of synthetic and botanical grain protectants. For evaluating the efficacy of acetone (C₃H₆O) for control of this pest and determining distribution and

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penetration rate of the gas vapours, experiments were performed in two empty and full containers. Applied concentrations for empty and full containers were 0, 20, 30, 60, 120 and 120, 240, 340, 440 $\mu\text{L/L}$ respectively, with exposure times of 20, 48 and 72 hours. These experiments were done at 15 and 27 $^{\circ}\text{C}$ with 4 replications. Mortality rose with increasing exposure time in all experiments. Thus mortality at 27 $^{\circ}\text{C}$ was higher than at 15 $^{\circ}\text{C}$. In all experiments mortality increased at higher concentrations. The eggs were the most sensitive developmental stage and the pupa was the least susceptible.

P SPP 30

Insecticidal effects of *Thuja occidentalis* (Cupressaceae) essential oil on *Stegobium paniceum* L. (Col.: Anobiidae)

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In order to find recyclable, environment friendly and easy accessible insecticides, the essential oils of *Thuja occidentalis* L. (Cupressaceae), was used against the adult stages of *Stegobium paniceum* L. Analysis of *Thuja occidentalis* L. essential oil used for insect fumigation by phase gas chromatography revealed the presence of 22 compounds including α -thujone (49.64%), fenchone (14.06%), and β -thujone (8.98%). The experiment was conducted in 6 replications using a completely randomized design of factorial experiment. The essential oil was prepared by water distillation method. Experiment was carried out at 30 ± 2 $^{\circ}\text{C}$ and $60 \pm 5\%$ R. H. under dark condition. Concentrations are included 0.00375, 0.00493, 0.00650, 0.00855, 0.01125 $\mu\text{l/l}$ air and a control (untreatment), after 24 h, respectively. At the concentration of 0.01125 $\mu\text{l/l}$ air essential oil of *Lasioderma serricorne* (F.) caused 100% mortalities of adults, respectively. A value of 50% lethal concentration on adults was 0.00650 $\mu\text{l/l}$ air for , *Stegobium paniceum* L. respectively.

P SPP 31

Detection of resistance level in *Tribolium castaneum* (H.) against commonly used insecticides

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Insecticide resistance to pyrethroids (deltamethrin 2.5% SC, deltamethrin 1.5% EC, alpha cypermethrin 10% SC, permethrin 0.5% WP) and organophosphate (DDVP 50% EC) insecticide was evaluated in red flour beetle, *Tribolium castaneum* (Herbst). Insecticide bioassays were carried out by exposing adult insects to different doses of insecticides on insecticide impregnated filter paper and as a residual film in Petri plate. Mortality was recorded after 48 hours of treatment. Resistant insects were identified on the basis of their LC₅₀ values. Highest resistance was observed against DDVP 50% EC followed by Permethrin 0.5% WP, Deltamethrin 1.5% EC, Alpha cypermethrin 10% SC and Deltamethrin 2.5% SC. MATERIALS AND METHODS: Rearing of insects Bioassays Filter paper method: Filter paper (Whatman No. 1) was cut according to the size of Petri plate and placed inside it. One ml of each concentration was dropped on filter paper with the help of glass pipette to soak the filter paper uniformly. Eight concentrations were made for each insecticide and each treatment was performed in triplicates. For comparing the results with control, only acetone was applied to control Petri plates. The Petri plates were then kept open for 5-10 minutes so that the acetone was fully evaporated. Thirty adult beetles were introduced in each Petri plate and Petri plates were covered to prevent the escape of any insect. After 48 hours of exposure, mortality was recorded. The experiment was carried out under laboratory conditions at $30 \pm 1^{\circ}\text{C}$. Residual film method: Glass Petri plates were used for the residual treatment of insecticides on glass surface (Busvine, 1971). Eight doses were prepared for each insecticide and three replicates were taken for each treatment. Concentrations were applied at the rate of 1ml/plate on the centre of Petri plates that were rotated manually for equal spreading of insecticides. Only acetone was used for control plates. After the evaporation of acetone, thirty adult beetles of each test insect were separated and added in each Petri plate in the absence of food. Treated Petri plates were kept at $30 \pm 1^{\circ}\text{C}$ in incubator. Mortality was checked out after 48 hours of insecticide exposure. RESULTS: Percentage mortality of *T. castaneum* (H.) was calculated after treatment with eight different concentrations of tested insecticides by filter paper method and residual film method (Table 1). It was observed that mortalities were increased with increasing concentrations of insecticides. 0.00% mortality was found in control experiment. Analysis of variance revealed that there was significant difference between different concentrations of tested insecticides. LC₅₀ values of deltamethrin 2.5% SC, deltamethrin 1.5% EC, alpha cypermethrin 10% SC, permethrin 0.5% WP and DDVP 50% EC were 16.033, 27.630, 25.720, 46.226 and 111.355 $\mu\text{g/ml}$ for filter paper method while for residual film method, the LC₅₀ values were 32.992, 49.256, 41.780, 56.041 and 463.718 $\mu\text{g/ml}$ respectively (Table 2; Table 3). Insecticides were arranged in ascending order of toxicity on the basis of LC₅₀ values. CONCLUSION The toxicity order was Deltamethrin 2.5% SC > Alpha cypermethrin 10% SC > Deltamethrin 1.5% EC > Permethrin 0.5% WP > DDVP 50% EC.

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P SPP 32

Effects of microwave energy on stored raisins pest

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Introduction: The control of stored pests is an important stage of dried fruits post harvesting procedure. Microwave heating is a friendly environmental treatment technique for controlling the stored pests.

Materials and methods: In this study, microwave heating was used to control of two species of major stored pest of Iranian raisin. The samples of raisins (70 gr each) were infested with adults of *Oryzaephilus surinamensis* and larvae of *Oryzaephilus surinamensis* and were subjected to microwave oven operating at 2450 MHz, at 450,720 and 900 W power for 20, 30, 40 and 50 seconds.

Results: The results showed, for all experiments the mortality was increased with increase of exposure time. Also a direct positive relationship between the mortality rates and microwaves irradiation power levels was obtained. Complete mortality was achieved for pests when the power was 900 W and the exposure time was 50 seconds. Therefore this method can be replaced with common chemical pest control.

P SPP 33

Phototactic response of the main grain storage pests of China to light-emitting diodes

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To develop more efficient methods to monitor main grain storage pests, experiments were conducted to determine whether light functioned as an attractant for them. Light-emitting diodes (LEDs) of various wavelengths (310nm, 395nm, 450nm, 530nm, 580nm, 630nm) were examined as light sources because they produce bright, narrow light spectra. Comparisons of responses to light spectra across the electromagnetic spectrum as follow: 1. *Cryptolestes pusillus* (Seh.) adults preferred 5 wavelength, except UV LED, blue (450nm) was the wavelength most attractive to *Cryptolestes pusillus*, followed by 530nm, 395nm, 630nm and 580nm. 2. *Cryptolestes turccus* (Grouvelle) has positive phototaxis only to 530nm, 395nm, 630nm and the most attractant light was 530nm; 3. *Sitophilus oryzae* (Linnaeus, 1763) showed repellent effects to specific light wavelengths from UV to 580nm, only attracted by red light(630nm). 4. *Tribolium castaneum* (Herbst) was attracted by UV(310nm) and blue(450nm) and repelled by 530nm, 395nm, 630nm and 580nm light wavelengths; 5. To *Tribolium confusum*, Phototaxis from strong to weak as follow: 630nm > 530nm > 580nm > 310nm > 395nm. New traps can be designed to take full advantage of the positive phototaxis of those grain storage pests.

P SPP 34

Insecticidal potential of natural zeolite formulations against stored-grain beetle pests: the effect of particle size

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Question: In recent years there has been an increased interest for the use of inert dusts, especially diatomaceous earth, for the protection of stored-grains against postharvest insects (Subramanyam and Roesli, 2000). Zeolites are inert dusts that, like diatomaceous earth, also contain natural silica and are extensively used in agriculture, mainly for the enhancement of soil properties and as animal feed additive. However, information on the efficacy of natural zeolites against stored-product insects is limited. In the present study the insecticidal effect of natural zeolite (clinoptilolite) formulations was investigated against three major stored-product beetles. Moreover, the effect of particle size on the insecticidal efficacy of the tested zeolites was also examined.

Methods: The insecticidal efficacy of natural zeolite formulations was assessed against adults of *Sitophilus oryzae* (L.) (Coleoptera: Curculionidae), *Tribolium confusum* Jacquelin du Val (Coleoptera: Tenebrionidae) and *Oryzaephilus surinamensis* (L.) (Coleoptera: Silvanidae). Zeolite formulations of different particle size were applied to wheat at rates of 250, 500 and 1000

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ppm and treated wheat was shaken manually to achieve equal distribution of the zeolite particles inside the grain mass. Untreated wheat served as control. Insect mortality was assessed after 2, 7 and 14 d of exposure of insects on treated and untreated wheat. Progeny production was also assessed after an additional period of 65 days.

Results: Complete control (100%) was not achieved in any of the tested insect-zeolite combinations. *Tribolium confusum* was the most tolerant to zeolite among the species tested. In several cases, zeolite formulations with small particles had stronger insecticidal effect compared with ones with larger particles.

Conclusions: The present study demonstrated the insecticidal potential of natural zeolites against the stored-product insects tested. Moreover, it was shown that particle size is a physical property that can affect the insecticidal effect of zeolites and should always be taken into consideration when choosing zeolite formulations for stored-grain protection. Similar results demonstrating the effect of particle size on the insecticidal action have been previously shown for diatomaceous earth (Vayias et al., 2009).

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P VIRUS 1

Role of BION and Allopurinol in Inducing Systemic Acquired Resistance against *Potato Virus Y* in Potato Plants

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Two chemical inducers, BION and Allopurinol, were used in three concentrations (0.2, 0.5 and 1 mM) to induce systemic resistance in potato plants against *potato virus Y* (PVY), and to limit and suppress the incidence and development of virus infection. The results showed that BION and Allopurinol had significant effect on development of virus infection, they reduced the disease severity and percentage of infection. The progress of PVY infection was rapid in control plants (untreated plants), most of those plants developed severe mosaic symptoms, and some leaves showed leaf deformation, percentage of infection reached to (100 %) and disease severity ranged between (15.8 and 41.6) at 25 and 35 days post inoculation (dpi) respectively. In contrast, mild mosaic and mosaic was observed on few BION and Allopurinol-treated plants with delay in time of symptoms appearance, and percentage of infection ranged between (30 and 46 %) , while disease severity ranged between (3 and 12.3) according to concentration. In addition to, BION and Allopurinol reduced stunting rate of treated plants comparison with untreated ones, stunting rate of treated plant ranged between(13 and 33.2 %) while, reached to(46.5 %) in untreated plants at 45 dpi. Yield of treated plants was reduced and ranged between (15.6 and 39.9 %), this reduction was less than in untreated plants (73.4). Treatments led to reduce virus concentration in BION and Allopurinol-treated plant comparing to untreated ones. BION had more efficacy in controlling PVY infection than Allopurinol, and the concentration 1mM had clear significant effect at probability level 5 %.

P VIRUS 2

Proteomic analysis to identify resistant strategies adopted by host plants upon begomovirus infection

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Introduction: Compatible pathogen incidence on plants changes spatial and temporal expression of genome of host plants. Among biotic stress, viruses threaten and contribute majorly to crop loss. Such losses due to viral diseases results in high yield penalty, qualitatively and quantitatively. The molecular events happening during such defense response is utmost importance of study to develop resistant cultivar or variety. Begomoviruses have emerged as major constraint for vegetable production. Studies of interactome of infection will provide valuable insights in understanding combating strategies of host plants.

Objectives:

- 1 Molecular characterization of begomovirus infection on French bean (*Phaseolus vulgaris* L.) from India.
- 2 Proteomic analysis of host pathogen interaction upon viral infection.

Materials and methods: French bean is one of the most important vegetable crops of India which is consumed widely. We have cloned and sequenced begomoviral genomic components associated with bean dwarf mosaic disease (BDMD) manifested leaf samples from Varanasi, India. Proteomic analyses of leaf samples agroinoculated with MYMIV were conducted for studying modulation of host proteome.

Results: Sequence analyses confirmed bipartite nature of begomovirus and MYMIV is causal agent of BDMD. Agro-inoculation studies on *N. benthamiana* and French bean plants further confirm bipartite nature of virus. Accumulation, symptom expression and systemic spread of disease require cognate DNA B molecule (Fig. 1). Proteomics data showed that relative expression levels of most of identified proteins were upregulated. These proteins were mainly involved in defense (25%) signal transduction (15%), energy (15%) and metabolic regulation (20%) (Fig. 2). In our studies, molecular characterization in conjugation with proteomics approach was implemented to get information for molecular events occurring during plant virus interaction. Moreover, our analysis revealed that mock plants had no significant protein profile alteration as compared to virus inoculated plants demonstrating that virus infection initiates modulation of proteomic profile.

Conclusion: In bipartite begomoviruses, DNA A component of virus can autonomously replicate but absence of movement protein restricts their transfer to neighboring cell, which in turn, restricts disease spread and hence the level of defense response. In our studies, we found that, most of the proteins involved directly or indirectly to defense, shoots up at higher level when both DNA A and DNA B components inoculated than DNA A components alone. This suggests an activation of SAR in inoculated plants, which restricts virus copy number accumulation. More in depth insights into host protein modulation will clear defense pathways taken up by host plants.

In summary, all of the above proteins showed changed abundances induced by MYMIV infection on French bean leaves. SAR and induced resistance might be a combinatorial effect of altered interactome of these proteins.

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Figure 1

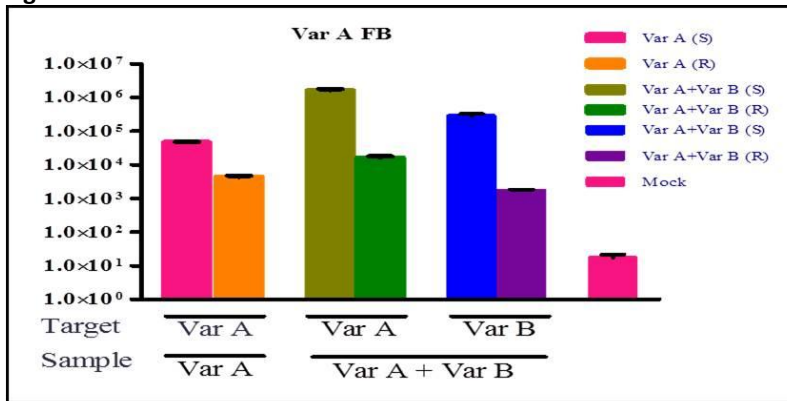


Fig. 1: Representative viral DNA accumulation was detected by RT-PCR. The values on y axis represent Log_{10} values of mean of three independent experiments of viral copy number accumulation. Bars (in black colour) represent Log_{10} values of standard error of mean of the three independent experiments.

Figure 2

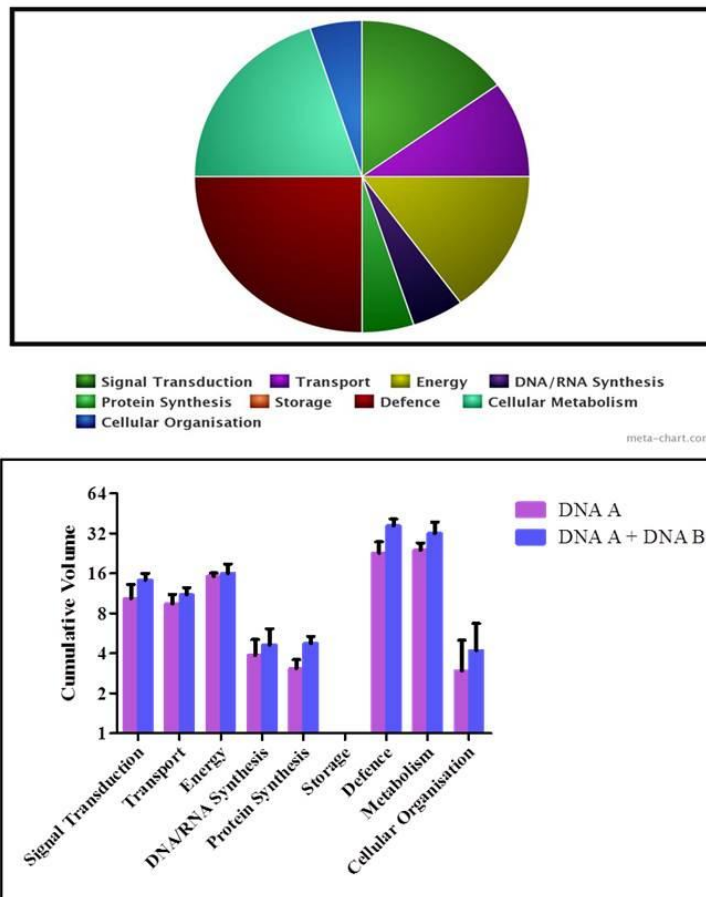


Fig. 2: Pie chart and cumulative volume chart of protein profile of host plant upon virus infection

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P VIRUS 3

Provision of virus indexing services and disease - Free farmer preferred tissue culture banana germplasm for regional food security

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Introduction: Viruses are a major constraint in banana production, germplasm multiplication and exchange as well as to genetic improvement through traditional breeding. Banana virus diseases can cause yield losses of up to 100% and indexing of material from primary tissue culture distributors is essential to stop or at least slow down disease spread. Crossfrontier International Ltd has been established to provide virus indexing services as well as affordable quality farmer preferred tissue culture banana germplasm in the East and Central Africa region.

Methods: Indexing was done using molecular techniques and tissue culture by meristem culture.

Results: Twenty-four percent of bananas which are a source of tissue culture mother plants sampled were positive for BSV virus.

Conclusion: CFI will participate in production of food for all through virus indexing by ensuring disease free tissue culture production and distribution in the East and Central African region.

P VIRUS 4

Complete genome sequence of a carrot virus S isolate from rock samphire from Spain

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In 2008, the 3'-terminal sequence (2.2 kb) of a hitherto unrecognized *Carlavirus* species from a carrot (*Daucus carota*, fam. *Apiaceae*) sample collected in a commercial carrot field near Bingenheim/Germany was determined and the name carrot virus S was coined. Unfortunately, the virus could not be maintained and the rest of the genome remained undetermined. All attempts to rediscover this virus in the following years in hundreds of carrot samples and samples of other *Apiaceae* originating from different carrot growing regions in Germany were not successful. In November 2012, a severely chlorotic rock samphire sample (*Crithmum maritimum*, fam. *Apiaceae*) was collected in the north of Mallorca/Spain. Of that sample, a virus could be mechanically transmitted to *Nicotiana glauca* and subsequently the complete genomic sequence could be determined (8.6 kb). Regarding its putative genome organization it could be assigned to the genus *Carlavirus*. Comparisons with sequences stored at GenBank showed that it shared the highest sequence identities with the previously reported carrot virus S isolate from Germany. Even if the nucleotide and amino acid sequence identities for the coat protein gene are close to or just below the demarcation thresholds (74% nt and 79% aa identity; thresholds being at 72% and 80%, respectively), the isolate should be regarded as deviant isolate of carrot virus S. The Spanish carrot virus S isolate could also be mechanically transmitted to different *Apiaceae*, including carrot, coriander, fennel and celery, which remained symptomless under greenhouse conditions. This first report outside Germany is the second documented discovery at all, but probably indicating that it is a genetically variable virus which is wide spread in Europe even if it does not occur frequently. The Spanish isolate is available at the DSMZ Plant Virus Collection under the accession number PV-1090.

P VIRUS 5

Characterization of a new tobamovirus infecting pepper in Morocco

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In 2010, a sweet pepper (*Capsicum annuum*) sample showing mosaic symptoms was collected in Morocco and kindly provided by A. Remah (IAVCHA, Agadir). By electron microscopy, tobamovirus-like rod-shaped particles could be observed. Following mechanical inoculation, the virus systemically infected different *Nicotiana* species (*N. benthamiana*, *N. tabacum* 'Samsun nn', *N. t.* 'Xanthi nc', *N. glutinosa* '24A'), *Capsicum annuum* and *Solanum lycopersicum* which displayed mottling or mosaic symptoms on systemic leaves, except *N. glutinosa* '24A' which reacted with crinkling and top necrosis. Plants of *Nicotiana rustica* 'NRT 63' and *Chenopodium quinoa* showed only weak chlorosis on inoculated leaves and the virus seemed not to move systemically. Based on dsRNA isolated from *N. benthamiana*, the complete genomic sequence (6,412 nt) was determined. Sequence analysis revealed the typical genome organization of a tobamovirus. The highest nucleotide sequence identity to any other sequence stored at GenBank was 75% to an obuda pepper virus isolate, followed by isolates of paprika mild mottle virus (73%) and yellow

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tailflower mild mottle virus (69%). Based on the molecular ICTV species demarcation criterion (less than 90% overall nucleotide sequence identity) and the predicted genome organisation, this virus can be regarded as an isolate of a new species within the genus *Tobamovirus*. The virus was named capsicum mild mottle virus (CapMMV) and is available at the DSMZ Plant Virus Collection under accession no. PV-1013.

P VIRUS 7

Influence of vector gender of western flower thrips (*Frankliniella occidentalis*) in the transmission of tomato spotted wilt virus (Tospovirus)

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The transmission of Tomato Spotted Wilt Virus in nature is influenced by a range of factors which have led to high variability in its transmission efficiency within a vector population. Some of these factors include: host plant suitability, virus isolate, **vector gender**, timing, individual thrips genetic constitution, feeding style among others, together with their interactions. The contribution of vector gender to the complex virus-vector-host plant interaction was studied using *Frankliniella occidentalis* (Pergande) (western flower thrips (WFT)) reared on either Tomato Spotted Wilt Virus (TSWV) infected or uninfected *Capsicum annum* L. (Solanaceae) leaflets throughout their larval stages. Later pupae were individually transferred on healthy leaf disks to examine the differences in; survival, development rate, transmission efficiency and feeding behavior between males and females. Comparison of fitness and behavioral aspects between *F. occidentalis* sexes showed that males had significantly higher longevity ($F_{1,403} = 72.54$, $P < 0.001$), lower mortality ($F_{1,47} = 76.01$, $P < 0.001$) and higher development rate ($F_{1,239} = 166.22$, $P < 0.001$) compared to females, while in the comparison of feeding behavior, females were found to feed more intensively than males ($F_{1,239} = 324.81$, $P < 0.001$), regardless whether they were exposed or unexposed to TSWV. Conversely, transmission efficiency of the virus was found to be significantly higher in males compared to females ($F_{1,44} = 7.09$, $P = 0.0109$). These findings are envisaged to contribute in deeper understanding of the high variability of *F. occidentalis* vector competence (the ability of a vector to acquire, multiply and transmit a virus) in the transmission of TSWV within a population, which will further help in the development of a model for a sustainable control strategy for *F. occidentalis* as well as TSWV.

P VIRUS 8

Sweet potato virus detection, characterization, elimination and management in Ethiopia

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Sweet potato (*Ipomoea batatas*) is a root crop with worldwide importance from the family *Convolvulaceae*. In area coverage and production, it is the third most important root and tuber crop in Ethiopia, next to enset (*Ensete ventricosum*) and potato. At least 20 million Ethiopians use sweet potato as a seasonal staple crops when there is a shortage of other foodstuffs (Tofu et al., 2007). Plant viruses remains as an important threat to sweet potato production and quality, in particular in Sub-Saharan Africa including Ethiopia. In Ethiopia, studies have shown that most of the sweet potato collections and farmers' fields are infected with viruses (Abraham, 2010, Tewodros et al., 2011). Because of viral diseases, multiplication and distribution of unclean sweet potato planting materials for the millions of poor farmers is spreading diseases rather than contributing to improved yield. Little is known about the viruses associated with sweet potato in Ethiopia. In addition, production and quality are declining because of no provision of virus free planting materials for farmers. A research project initiative was launched between Hawassa University (Ethiopia) and NMBU (Norway) financed by Norad (Norwegian Development Agency) under the NORHED program. A sub-project of NORHED (agreement no ETH-13/0017) is working to detect and characterize the viruses, eliminate viruses from farmers preferred sweet potato varieties, evaluate the relative tolerance of the cultivars and thereby improve productivity and livelihoods through providing clean planting materials for the sweet potato growing farmers of Ethiopia. The presentation will present our progress so far in cleaning stocks for viruses and the way forward.

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P VIRUS 9

Evolution of *Sugarcane yellow leaf virus* Isolates Based on Sequence Analyses of Coat and Movement proteins

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Sugarcane yellow leaf virus (SCYLV) is an economic threat to the sugarcane industry worldwide. Significant genetic diversity occurs in SCYLV. In order to investigate its genetic evolution, three new complete genomic sequences of isolates collected from Colombia, Cuba and Reunion Island were compared with the 30 Sequences available in GenBank. Phylogenetic analysis showed that all isolates were clustered into two distinguishes super-populations: the first I comprises the majority of genotypes (BRA, CHN3, HAW, PER and REU) and the second II comprises CHN1, IND, CUB genotypes. The II diverged in more than 23% in amino acid sequence from I and may be represent even new recombinant polerovirus species infecting sugarcane. Recombination breakpoints were also identified in SCYLV genome based on complete/ partial sequences, it was shown that the whole SCYLV genome is prone to recombination, except in coat protein (CP) and movement protein (MP). Among the 21 recombinants detected, our isolates were included. The ancestral PER, BRA, CUB & CHN1 genotypes have contributed to the REU genotype, while IND4, HAW73-6110, CHN1 ancestral isolates has contributed to the CUB genotype. Selection pressures exerted on overlapping and non-overlapping regions of CP&MP genes were measured as the mean number of nonsynonymous (dN)-to-synonymous (dS) nucleotide substitutions per site (dN/dS ratio) using PAML and HYPHY packages. Our results revealed that CP is more variable and evolve adaptively more than MP in SCYLV. These results elucidate the molecular evolutionary lineages characteristics and allow the proposal of its significant respond to the needs of adaptation to changing vectors, hosts and geographical locations.

P VIRUS 10

Towards the isolation of resistance genes against soil-borne barley yellow mosaic virus disease (BaYMV, BaYMV-2, BaMMV)

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One of the most important diseases of winter barley in Europe and East Asia is barley yellow mosaic virus disease caused by different strains of *Barley yellow mosaic virus* (BaYMV) and *Barley mild mosaic virus* (BaMMV) leading to yield losses up to 50% in susceptible winter barley crop. Due to the transmission by the soil-borne plasmodiophorid *Polymyxa graminis*, chemical measures to prevent high yield losses are neither effective nor ecological sound. Thus, breeding for resistance is of prime importance in order to ensure winter barley production in the growing area of infested fields. Up to now, nine different loci conferring resistance to the different strains of BaMMV and BaYMV are known. In order to get detailed information on the structure and function of resistance genes effective against yellow mosaic disease, map based cloning was conducted to isolate the resistance genes *rym13* located in the telomeric region of chromosome 4HL and a gene, being only effective against BaYMV and BaYMV-2 located in the centromeric region of chromosome 5H.

For marker saturation of the target intervals all available sequence information in barley and the synteny to rice, sorghum, Brachypodium and sequence information of barley included in the genome zipper was used. Phenotyping of respective segmental RILs derived from high resolution mapping populations comprising 5000 F₂-plants each was conducted by mechanical inoculation (BaMMV) and in field tests (BaMMV/BaYMV/BaYMV-2) followed by DAS-ELISA. Based on marker saturation and phenotyping of 691 RILs the resistance gene located on chromosome 5H was mapped in an interval of 0.27% recombination. By an additional exome capture sequencing approach of the parental lines, 249 morex contigs containing 256 genes were located in this interval. Out of these, two candidate genes were identified of which one is co-segregating with the resistance locus. For *rym13* in addition genotyping-by-sequencing (GBS) of 132 RILs and deep sequencing of two differentiating segmental RILs was conducted, which leads to the identification of candidate genes which are presently re-mapped in the high resolution mapping population. The availability of respective genomic tools in barley facilitates an enhanced isolation of resistance genes transferring breeding for BaMMV/BaYMV to the allele level in the future.

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Study of critical points that lead of spotted wilt (*Tospovirus*) outbreaks in sweet pepper in Uruguay

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Tomato spotted wilt virus causes important damages in greenhouse sweet pepper in Uruguay, even in resistant varieties. Some growers have more losses due to some specific conditions that favor the disease. The effect of insect-proof nets (50 mesh) in seedbed and crop and cultivars with and without *Tsw* gene on spotted wilt incidence and its vector (thrips) was studied during 2011-2014. Five replications were planted in two greenhouses, one of them net-isolated. Treatments were net during seedbed/crop and varieties. Number and kind of thrips (inside and outside greenhouses) were periodically evaluated in yellow sticky traps and in weeds and pepper flowers. Percentage of infected (DAS-ELISA) and symptomatic pepper plants was recorded. TSWV infection on weeds was analyzed. In 2011-2012, TSWV was on the property before trial and spread quickly destroying all crops. This was associated with a high number of thrips in traps and flowers and a large number of weeds (some infected). Conversely 2012-2013 and 2013-2014 seasons were had fewer infected plants, less weeds (few infected). 2012-2013 infection started on plants from non covered seedbeds and this of 2013-2014 in the non netted greenhouse. In both disease did not spread highly. The number of thrips/day in the outside of greenhouses was smaller during 2012-2013. All varieties (Troyano, Yatasto, Kaiman, resistants and Margarita susceptible) were not different on frequency and intensity of symptoms. Sanitary measures, as weed eradication and production of seedlings under net, were implemented by the grower after plant removal in 2012. This could account to the subsequent disease reduction. The results remark the importance of preventive disease management by elimination of inoculum sources (weeds and infected seedlings) and vector exclusion by insect-proof nets mainly on seedbeds.

P VIRUS 12

Occurrence of EMARaV and CLRV in *Sorbus aucuparia* and *Betula* spp. in Scandinavia

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Introduction: *Cherry leaf roll virus* (CLRV) is a worldwide occurring RNA virus (*Secoviridae* family, genus *Nepovirus*) infecting naturally several tree species including many birch species (*Betula* spp.) as well as rowans (*Sorbus aucuparia*). It can also cause damage in cultivated crops such as walnut. *European mountain ash ringspot-associated virus* (EMARaV) is distributed in many parts of Europe. It is a RNA containing 4 genome segments. The virus has a restricted host range limited to *Sorbus* species inducing typical symptoms such as chlorotic ringspots and mottling on leaves.

Objective: The objective of this study was the assessment of symptoms indicative of a virus infection occurring in the important Scandinavian deciduous tree species *Betula* spp. and rowans and the detection of CLRV and EMARaV to evaluate the occurrence of these two viruses in forests as well as in urban areas.

Materials and methods: A survey assessing the presence of virus-like symptoms in rowans and birches was conducted in 3 Scandinavian countries in 2012 and 2014. Leaf samples from symptomatic trees were collected and total nucleic acids were extracted. EMARaV detection was carried out by RT-PCR using primer sets targeting all for genome segments. Nested-RT-PCR was applied for CLRV detection with primers amplifying fragments of the RdRp- (RNA1) and the CP-coding region (RNA2). PCR products were sequenced to confirm the infection with the respective virus.

Results: EMARaV occur frequently in rowans as estimated by observation of mottle and chlorotic ringspots on leaves and detection by RT-PCR. Wide geographic distribution of the virus in Scandinavia was confirmed by sequencing. 37 *B. pubescens* and 13 *B. pendula* trees showed chlorotic or necrotic veinbanding, leaf roll or chlorotic spots characteristic for a CLRV infection. However, the virus was only sporadically detectable in birches and *S. aucuparia* trees with the RNA1 specific primer set, but infection was not confirmed with the primers targeting the viral CP.

Conclusion: Results of this study confirm that EMARaV widely affects rowans in different Northern European countries. The distribution in Scandinavia and host range of CLRV, an important virus of main deciduous tree species needs to be further assessed in order to determine its impact on diseased birch species and rowans.

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P VIRUS 13

Molecular characterization of resistance-breaking isolate of *Tomato spotted wilt virus* and searching for resistance on pepper

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In Hungary resurgence of *Tomato spotted wilt virus* (TSWV) frequently causes heavy crop losses in pepper production since the mid nineties. Management of TSWV control was first directed against the thrips (using different insecticides or plastic traps), and against weeds as host plants of the virus and the thrips. Later on *Tsw* resistant gene was introduced into different types of pepper. In 2010 and 2011 sporadically, but in 2012 more frequently a resistance breaking strain of TSWV on resistant pepper cultivars was observed in the Szentes region (South-East Hungary). The presence of a new resistance breaking strain was demonstrated by virological (test- plant, serological and RT-PCR) methods.

Previously, the non-structural protein (NSs) encoded by small RNA (S RNA) of TSWV was verified as the avirulence factor for *Tsw* resistance, therefore we analyzed the S RNA of the Hungarian RB and wild type (WT) isolates and compared to previously analyzed TSWV strains with RB properties from different geographical origins. Phylogenetic analysis demonstrated that the different RB strains had the closest relationship with the local WT isolates and there is no conserved mutation present in all the NSs genes of RB isolates from different geographical origins. According to these results, we concluded that the RB isolates evolved separately in geographic point of view, and also according to the RB mechanism.

In order to find new genetic sources of resistance in *Capsicum* species 82 items from *Capsicum annuum*, *C. chinense*, *C. frutescens*, *C. chacoense*, *C. baccatum* var. *baccatum*, *C. baccatum* var. *pendulum* and *C. praetermissum* were tested with Hungarian TSWV-RB isolate.

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Population of *Plum pox virus* in European Russia seems to be the most diverse in the world

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Plum pox virus (PPV; genus *Potyvirus*; family *Potyviridae*) is a causal agent of Sharka, economically the most important viral disease of stone fruits affecting their yield and quality. A sound understanding the geographic distribution and genetic diversity of the virus is very important for effective management of the disease. Regular monitoring of stone fruit plantings using ELISA and immunocapture reverse-transcription polymerase chain reaction for PPV detection and identification resulted in the findings of numerous focuses of the infection in European Russia. The virus was found in collections, variety test plots, nurseries, commercial orchards, decorative plantings, private gardens, wild stone fruit trees growing in urban and rural areas from many localities all over European Russia. PPV was detected in naturally infected plum (*Prunus domestica*), peach (*P. persica*), nectarine (*P. persica* var. *nectarine*), myrobalan (*P. cerasifera*), blackthorn (*P. spinosa*), downy cherry (*P. tomentosa*), sour cherry (*P. cerasus*), sweet cherry (*P. avium*), apricot (*P. armeniaca*) and Canadian plum (*P. nigra*). Six of the nine known PPV strains (D, M, Rec, W, C, CR) have been revealed in Russia. Most isolates belong to the strains D (38%), W (25%), CR (23%), M (7%) and C (7%). Two distinct PPV-Rec isolates have been found on myrobalan and plum in southern Russia. Population of PPV in European Russia seems to be the most diverse in the world due to wide spread of isolates belonging to the strains W, C, and CR that were never detected or only sporadically identified outside the former USSR until now. Phylogenetic analysis of their genomes shows that these three strains constitute the supercluster clearly divergent from other PPV strains. This evolutionary branch originated from a common ancestor and apparently developed mainly in Russia. The work was supported in part by the Russian Foundation for Basic Research, project no. 14-04-01786.

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P VIRUS 15

Transmission studies of *European mountain ash ringspot-associated virus* (EMARaV) to putative new host plants

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Introduction: Since more than 50 years European mountain ash trees (rowans, *Sorbus aucuparia*), display chlorotic ringspots and leaf mottling. These symptoms are associated with the *European mountain ash ringspot-associated virus* (EMARaV), a (-)ssRNA virus with 4 RNAs which is the type member of the genus *Emaravirus*. In 2013, EMARaV was detected in whitebeam (*Sorbus aria*) and Swedish whitebeam (*Sorbus intermedia*) for the first time. Other potential host species for the virus remain unknown. EMARaV was so far only transmitted successfully between rowan trees by grafting.

Objective: The known host species of EMARaV belong to the family *Rosaceae*. It was attempted to infect herbaceous *Rosaceae* and *Nicotiana* plants with the virus by mechanical inoculation.

Materials and methods: Leaves of EMARaV infected *S. aucuparia* were collected locally. *Nicotiana rustica*, *N. benthamiana*, strawberry plants (*Fragaria sp.*), Lady's mantle (*Alchemilla vulgaris*) and *Potentilla megalantha* were mechanically inoculated with the diseased plant material.

EMARaV infected plant material was homogenized with an abrasive and Norit buffer, 2 % nicotine solution or phosphate inoculation buffer with 2 % nicotine and applied manually onto the leaves of the potential host plants. Alternatively, the infected material was rubbed onto the leaves using an abrasive only. Serial passaging was performed after 7 days. For detection RNA was isolated from the leaf material using the *Invitrap Spin Plant RNA kit* (STRATEC). Reverse transcription was conducted using random hexamer primer. EMARaV detection via PCR was processed according to Mielke et al., 2008.

Results: The majority of plants showed neither virus-specific nor stress symptoms after mechanical inoculation. One *N. rustica* plant displayed leaf mottling after dry inoculation as well as one *N. benthamiana* after inoculation with phosphate inoculation buffer with 2 % nicotine. An infection with EMARaV could not be confirmed in analysis in symptomatic as well as symptomless plants.

Conclusion: It was not possible to transmit EMARaV to a new host plant species testing various inoculation methods and a diverse set of herbaceous biotest plants. This confirms that transmission of the virus from infected rowans by mechanical means, even to related plant species is not possible, which may be due to the narrow host range of the pathogen.

P VIRUS 16

Translation initiation studies of the polyproteins encoded by RNA1 and RNA2 of *Cherry leaf roll virus*

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Introduction: *Cherry leaf roll virus* (CLRV) is a nepovirus of the family *Secoviridae*. The bipartite genome of CLRV consists of positive single stranded RNA serving as mRNA and each encoding one polyprotein P1 and P2, respectively. The pathogen displays various genetic polymorphisms due to its worldwide distribution and wide host range including deciduous and fruit tree species. Sequence variabilities also occur within the 5' terminal regions of the viral RNAs. Some CLRV isolates possess a single startcodon whereas others contain a 2nd in frame ATG in both genome segments which could also serve as translation initiation sites.

Objective: The objective of this study was to ascertain which start codon is utilized to initiate translation of P1 and P2 from the genome segments of CLRV.

Materials and methods: The 5' terminal regions of RNA1 and RNA2 of CLRV isolates originating from 2 differing phylogenetic groups were amplified, cloned into pJet1.2 and sequenced prior to subcloning into the expression vector pET28a(+) containing a T7 promoter region. The pJet1.2 constructs served as templates to insert point mutations by overlap extension PCR disrupting either the first, second or both startcodons in the 5' terminus of RNA1 and RNA2 of the virus strains. Mutated 5' terminal fragments were also cloned into pET28a(+) and sequenced. A coupled transcription/translation system in combination with a non-radioactive detection system (Promega) was used to express peptides from the wild type constructs as well as from the mutated 5' termini of CLRV-RNA1 and RNA2 lacking one or two functional ATG sites. Samples were further analyzed by Western Blot after size separation by SDS PAGE.

Results: Constructs for analyses of translation initiation of CLRV-RNA1 and -RNA2 from different virus isolates were successfully prepared with the described cloning strategy. Sequencing confirmed the correct insertion of mutations without alterations of the reading frame. Results of peptide expression from the CLRV-5' terminal constructs are presented and interpreted in regard to the identification of ATG sites used for expression of P1 and P2.

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Conclusion: Translation initiation studies of various CLRV isolates indicate towards differences in expression strategies of viral strains.

P VIRUS 17

iTRAQ-based quantitative proteomics analysis of rice leaves infected by Rice stripe virus reveals several proteins involved in symptom development

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Rice stripe virus (RSV) can severely damage rice plants; its infection usually leads to chlorosis of newly emerged leaves, plant stunting and eventual death. Proteomic data for rice leaves after infection by RSV and subsequent symptom development can help elucidate mechanisms involved in pathogenesis and symptom development but are still limited; we thus used an iTRAQ approach for a quantitative proteomics comparison of non-infected and infected leaves. As a whole, 681 (65.8% downregulated, 34.2% upregulated) differentially expressed proteins were identified. A bioinformatics analysis indicated that the differentially expressed proteins were mainly located in the chloroplast, and most that changed in response to infection participate in chlorophyll metabolism. Ten of these regulated proteins are involved in chlorophyll biosynthesis, four in defense responses and three in cell death processes. Subsequent qRT-PCR results showed that downregulation of magnesium chelatase was due to reduced expression levels of subunits CHL1 and CHL2, resulting in chlorophyll reduction, which is related to leaf chlorosis. After RSV infection, plant defense was triggered by the expression of Bet v1 allergen family proteins, HSP70, and superoxide dismutase. Three aspartic proteases were implicated in RSV-induced cell death. The findings may yield new insights into mechanisms underlying rice stripe disease symptom development.

P VIRUS 18

The determination of virus diseases for pepper grown into open fields in East Mediterranean region of Turkey

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Main objective of this research are to identify determine of virus on green pepper, bell pepper, sauceboat red pepper and other pepper varieties showing symptoms in open fields in Adana, Mersin, Hatay, Kahramanmaraş and Osmaniye provinces in East Mediterranean region of Turkey.

The samples of fruits and leaves showing symptoms were collected during surveys. All samples were tested with DAS-ELISA using antisera of Alfalfa mosaic virus (AMV), Chilli veinal mottle virus (ChiVMV), Cucumber mosaic virus (CMV), Pepper mild mottle virus (PMMoV), Pepper mottle virus (PepMoV), Pepper veinal mottle virus (PVMV), Potato X virus (PVX), Potato virus Y (PVY), Tobacco etch virus (TEV), Tobacco mosaic virus (TMV), Tomato mosaic virus (ToMV), Tomato spotted wilt virus (TSWV) and Tomato yellow leaf curl virus (TYLCV). Reverse Transcription Polymerase Chain Reaction (RT-PCR) was performed to characterize of positive samples in ELISA.

According to the results of DAS-ELISA and RT-PCR tests, PVY, TEV, CMV, TSWV, PMMoV, PVMV, ToMV, and PepMoV were identified grown in open fields.

P VIRUS 19

Detection and molecular characterization of cotton infecting begomoviruses from Pakistan

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Cotton leaf curl disease (CLCD) is a considerable encumbrance to the cotton production worldwide including Pakistan. The disease is caused by the complex of begomoviruses (Family: *Geminiviridae*) in association with a disease specific satellite known as cotton leaf curl Multan betsatellite. Begomoviral infected cotton plant were collected in Punjab province, Pakistan, during 2011-13. Sixty samples were processed for the presence of begomoviruses virus and betasatellite. Full-length clones were sequenced and analysed. Begomovirus ranged from 2751 to 2759 nucleotides. Complete sequences shown to be an isolates of

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Cotton leaf curl Burewala virus (CLCuBuV) with >96% nucleotide sequence identity-except a clone from Layyah with <93% identity. In common with previous CLCuBuV isolates, the virus from Layyah was recombinant containing sequences derived from two virus species that were predominant in cotton pre-resistance breaking but with distinct recombination break-sites, for which a name was proposed as cotton leaf curl Burewala virus-Layyah. In all viruses, lack of an intact TrAP protein was found as common. Betasatellite were detected and they were ranged from 1349 to 1371 nucleotides. Sequence analysis shown to be an isolate of *Cotton Leaf Curl Multan Betasatellite* (CLCuMB) with >97% identity- containing the recombinant fragment typical of this satellite post-resistance breaking. Agroinoculation of the new strain into *Nicotiana benthamiana* and *N. glauconosa* exhibited symptoms like vein thickening and leaf curling. CLCuBuV-betasatellite complex showed a wide spread and highlights the dominance of resistant breaking CLCuBuV in this region and further emphasis the need for work to combat its damage.

P VIRUS 20

Epidemiology of zucchini viruses and genetic variability of WMV in Flanders between 2007 and 2013: a seven year survey

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During the last 20 years, a more than tenfold increase in zucchini production area as well as turnover was observed in Flanders. This resulted in a gain of importance of zucchini diseases including the three major zucchini viruses *Cucumber mosaic virus* (CMV, *Cucumovirus*), *Zucchini yellow mosaic virus* (ZYMV, *Potyvirus*) and *Watermelon mosaic virus* (WMV, *Potyvirus*). These viruses are transmitted in a non-persistent way by several species of aphids and cause symptoms that are highly similar for all three viruses such as yield reduction and unmarketable fruits due to mottling, mosaic, chlorosis and leaf or fruit distortion. During a seven year survey performed between 2007 and 2013, the virus populations were monitored in the two main zucchini growing areas in Flanders. This revealed a clear shift in virus abundance throughout the years. A gradual decrease of ZYMV was observed while the importance of CMV increased until 2013 when the incidence of CMV dropped dramatically. The incidence of WMV remained more or less constant until it became the most important virus in 2013. In general, it was observed that WMV was the most important virus with regard to the development of symptoms and a consequent reduction of yield and fruit quality. To have a more detailed view on the population structure of WMV, the genetic variability of a selection of WMV isolates from different growing seasons was tested by amplification and sequencing a part of the coat protein (Desbiez *et al.*, 2007). From 2008 until 2010, most isolates that were sequenced belonged to one particular genotype group. However, in 2010 a second clearly distinct genotype was introduced in Flanders and found in both major zucchini producing areas. Both genotypes were also detected in samples of later years indicating that the new genotype was able to establish in Flanders. Further research is required to determine the possible introduction pathways in order to avoid future establishment of more virulent strains in zucchini production.

Desbiez, C., Costa, C., Wipf-Scheibel, C., Girard, M., & Lecoq, H. (2007). Serological and molecular variability of watermelon mosaic virus (genus Potyvirus). *Archives of virology*, 152(4), 775-781.

P VIRUS 21

First report of mixed infection of *Zucchini yellow mosaic virus* (ZYMV) and *Tomato leaf curl New Delhi virus* (ToLCNDV) infecting bittergourd in Punjab, India

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Introduction: Bittergourd *Momordica charantia* L.) of the family *Cucurbitaceae*, is grown as a food and medicine. It has been reported to be a natural host of many viruses which affects its cultivation worldwide. In September 2013, bittergourd plants showing severe yellow mosaic, blistering and curling of leaves were observed. The incidence of the disease was significant (up to 10%).

Objective: Present study was conducted to identify the virus associated with bittergourd linking to complex symptoms, in Punjab, India.

Materials and methods: Tender leaves of bittergourd plants showing pronounced blistering, mosaic and curling kind of symptoms along with healthy leaves were collected from vegetable research farm, Punjab Agricultural University, Ludhiana. Serological detection and identification of virus was carried out by following DAS-ELISA. Total RNA from three of bittergourd samples showing strong positive reaction in ELISA was isolated using the RNA easy Plant Mini Kit and subjected to cDNA synthesis using oligo DT. For ZYMV detection cDNA of BG1, BG2, BG3 along with a healthy sample was used for PCR with set of

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ZY 2 and ZY 3 primer(Thompson et al 1995) and an expected size product of ~1200 bp was amplified in sample BG1 which was further sequenced. The sample BG1 of bittergourd was suspected to be infected with whitefly transmitted begomovirus. To confirm the association of begomovirus total DNA was isolated and subjected to PCR using universal degenerate AV494 /AC1048 primers(Wyat and Brown 1996). An expected size amplicon of ~575 bp was observed from core CP region and cloned in plasmid vector pJET. Overlapping sequences of both clones ZYMV-BG1 and AV/AC-BG1 were assembled and submitted to GenBank with Accession No. KJ614229, KJ744258 respectively.

Results: Results of DAS-ELISA indicated presence of the ZYMV in ten samples which was further confirmed with RT-PCR. Association of Tomato leaf curl New Delhi virus was confirmed with the disease.

Conclusion: This is the first record of mixed infection of ZYMV a potyvirus with ToLCNDV whitefly transmitted begomovirus. Studies through serological and molecular technique would generate important information towards management and check of further spread of the detected viral disease and in addition it will provide an opportunity for studying interaction between RNA-DNA viruses.

P VIRUS 22

Virus complex causing degeneration of cultivated *Allium* species in North-Western India

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Allium sativum L. (garlic) and *A. cepa* L. (onion) are important *Allium* species cultivated in Indian Punjab. Onion is planted both in *kharif* and *rabi* seasons by bulb set or by seed, whereas, garlic in *rabi* by cloves. Among viruses attacking allium, *Onion yellow dwarf virus* (OYDV), *Leek yellow stripe virus* (LYSV), *Garlic common latent virus*, *Shallot latent virus* and allexiviruses are common viruses reported from other parts of India. Present study was focused on serological and molecular detection of viruses associated with both *Allium* species in north-western India. *Allium* growing regions of the state were surveyed and symptoms resembling to be caused by virus (es) were recorded. Major symptoms in onion plants were yellowing, striping and flattening of leaves. Severely infected plants showed curving, twisting, reduction of leaf size, bulb size and stunting of plants. Infected garlic plants were showing stunting, striping and curly wavy leaf margins. To confirm the association of viruses the young leaves samples were collected and subjected to ELISA against *Potato virus Y* (PVY^{o/c}, PVYⁿ), *Potato virus X* (PVX), OYDV, *Zucchini yellow mosaic virus* (ZYMV), *Cucumber mosaic virus* (CMV subgroup I & II) antisera (Agdia, USA) as per manufacturers protocol. ELISA confirmed the presence of PVY^{o/c}, PVYⁿ and OYDV in the samples, whereas, no other virus could be detected. Total RNA was isolated from ELISA positive samples and reverse transcription PCR assay was used for further confirmation using universal primers specific to *Potyviridae*, OYDV, allexivirus and LYSV. First strand of complementary DNA was synthesized using oligo-deoxythymidine for Potyvirus and gene specific RT primers for OYDV, allexivirus, LYSV. A set of primers viz., Nib2F/Nib3R for Potyvirus (Zheng *et al* 2010), OYDVVKB F/OYDVVKB R for OYDV (Arya *et al* 2006), Allex-CP/Allex-NABP for Allexivirus (Chen *et al* 2004), LYSV1/2 for LYSV (Dovas *et al* 2001) were used for PCR. An expected size of amplicon of ~350bp, 1.1 kb, 750 bp and 304 bp of potyvirus, OYDV, Allexivirus and LYSV respectively, were cloned and sequenced from infected samples. Nucleotide sequence analysis confirms the association of OYDV in onion and garlic, allexivirus in garlic and LYSV in onion causing degeneration either singly or in combination recorded first time from Indian Punjab.

Figure 1



P VIRUS 23

Enlightening the association of a plant pathogenic *begomovirus* with the Yellow mosaic disease of an ornamental plant *Catharanthus roseus*

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Begomovirus is one of the largest genus of the family *Geminiviridae*. Ornamentals are considered as a foundation of new viruses and reservoirs in the absence of the main crops. In the present report, we identified a *begomovirus* associated with an ornamental plant *Catharanthus roseus* commonly known as Madagascar periwinkle which belongs to family *Apocynaceae*. This is often cultivated in Indian gardens for decorative purposes and acknowledged for its medicinal values. Twenty symptomatic plant samples were collected from Sikar (Rajasthan) India and the complete viral genome was amplified by rolling circle amplification using TempliPhi DNA amplification kit, digested with EcoRI and Sall, resulted fragment were cloned in suitable pGEMT vector and sequenced (Gene Bank accession no clone C1 KP698312 & C2 KP698313).

BLAST analysis of DNA-A of both clones C1 and C2 showed maximum similarity 97% and 93% respectively with *papaya leaf crumple virus*. The phylogenetic analysis of begomovirus isolates clone C1 2736 bp & clone C2 2736 bp based on DNA-A with corresponding sequences revealed its closest relationship *papaya leaf crumple virus* HM140367 and HM140368 respectively. To our knowledge this is the first report of natural occurrence of papaya leaf crumple virus infecting *Catharanthus roseus* in India. Thus, there is a pressing need for additional information on the diversity and distribution of begomoviruses in ornamental plants.

Figure 1

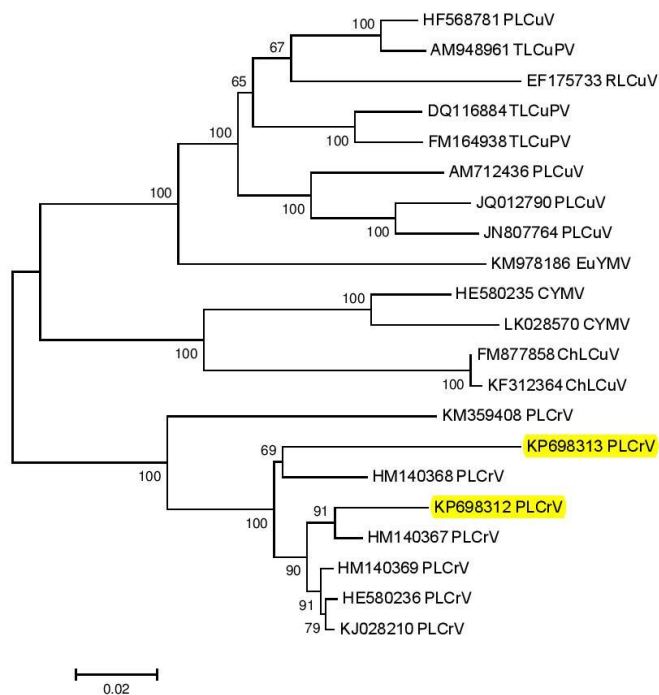


Figure 2



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Serological Identification of Vegetable Viruses Derived from Guizhou Province of China

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Nine hundred and fifty-two symptomatic samples from 8 major vegetable planting counties in Guizhou provinces of China in 2013 and 2014 were detected six viruses by DAS-ELISA methods. The results showed that a kind of CMV, TMV, BBWV, CGMMV, TSMV and TuMV was detected in 273 samples at least. Among 6 viruses, CMV was the most commonly detected, being found in 16.49% of the samples, followed by other 5 viruses, in 2.63%, 1.58%, 0.53%, 1.05% and 2.10%, respectively. It suggested that the major virus types infecting vegetables was CMV in Guizhou Province. According to the infection of a single virus, CMV was detected in all 11 vegetables. TuMV was found in eggplant, Chinese cabbage, radish and cabbage, and TSWV in pepper and tomato. There were 7 complex virus infections types, including CMV+TMV, CMV+TuMV, CMV+TSWV, CMV+BBWV, TMV+BBWV, TuMV+BBWV and CMV+TuMV+BBWV. The positive rate of CMV+TMV was 1.05%, higher than others.

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Localization of EMARaV proteins by *in planta* agrobacterium-mediated transformation

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Introduction: Agrobacteria were used to express and localize the green fluorescent protein (gfp)-fused nucleocapsid and p4 protein of *European mountain ash ringspot-associated virus* (EMARaV) in planta. EMARaV infects European mountain ash (*S. aucuparia* L.) and causes chlorotic ringspots and mottling of leaves. The virus is suspected to influence the decline of branches and even the entire tree.

EMARaV is composed of four ss(-)RNA genome segments and is assigned to the genus *Emaravirus*. Each of the four viral RNAs is coding for one protein (p1-p4). Currently it is unknown, which function the RNA4 encoded p4 protein adopts in the infection process. For many plant viruses the existence of a gene silencing suppressor and a movement protein is essential. These functions could not be associated with the proteins encoded by RNA1-RNA3. It can be assumed that EMARaV RNA4 encodes a gene silencing suppressor and/or a movement protein.

Objective: Inferences about the function of the p4 protein of EMARaV are expected by investigating the localization of the protein *in planta*.

Materials and methods: For localization purposes the nucleocapsid protein (p3) and the p4 protein of EMARaV were C-terminal fused with gfp. Additionally, the movement protein of the *Tomato spotted wilt virus* (TSWV) was cloned as a reference for putative movement protein function of EMARaV p4. Agrobacteria were transformed with these gfp constructs and *Nicotiana benthamiana* leaves were subsequently agroinfiltrated. Confocal laser scanning microscopy was used for localization of viral proteins.

Results: The functionality of the gfp fusion constructs of EMARaV p3 and p4 protein, as well as TSWV NSm protein was proved by use of an anti-gfp antibody in western blot analysis of agroinfiltrated *N. benthamiana* leaf material. The localization of the viral proteins in epidermal cells of *N. benthamiana* leaves was possible. First results of this study will be presented and discussed.

Conclusion: Elucidation of the function of the p4 protein of EMARaV is of great importance for understanding infections of host plants and the virus replication. The localization of viral proteins provides preliminary information on the putative protein function. Further studies using specific cell compartment markers are necessary to prove the hypothesis whether the p4 protein functions as gene silencing suppressor and/or movement protein.

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Detection of *Tomato Chlorosis Virus* infecting Tomato and arable weeds using Multiplex PCR and Dot Blot Hybridization Techniques

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In January 2014, leaf samples from 35 (16 tomato and weeds including nine *Malva parviflora*, three *Chenopodium morale*, and one from each *Emex spinosa*, *pulicaria undulate*, *Brassica tournifotii*, *Conyza bonariensis*, *sonchus oleraceus*, *Brassica tournifotii* and *Rumex vasicarius*) were collected from four greenhouses in peripheral areas of Riyadh region, Saudi Arabia. Symptoms including yellowing, severe stunting, degeneration, upward cupping, distortion and interveinal yellowing observed on the lower leaves referred to possible infection by a whitefly transmitted crinivirus. Objective of this research was to survey tomato plants exhibiting yellowing symptoms and identify the causal agent(s).

Total RNA and DNA were extracted from tomato and weed samples and subjected to Reverse transcriptase-Polymerase Chain Reaction (RT-PCR) and Polymerase Chain Reaction (PCR), respectively. All tomato samples were found to be positive for *Criniviruses* when tested by primers HS-11/HS12 (587bp) and Nested-PCR using specific primer for ToCV while no band was amplified by TICV primers (1). All samples were tested by degenerate primers and specific primer to detect *Begomoviruses* and TYLCV respectively. All tested samples were negative for begomovirus except one sample having mixed infection with ToCV and TYLCV, while all weeds except three *Malva parviflora* were found to be infected with ToCV. To study virus transmission, 40 virus-free *B. tabaci* adults were used to infect the healthy tomato for 48-h inoculation access period (2).

In the transmission tests, ToCV was detected to be present in all tomato and indicator plants which revealed yellowing symptoms 6 weeks post inoculation, whereas no transmission was obtained when non-viruliferous whitefly adults fed on two asymptomatic tomato leaves. All the samples were tested by dot blot hybridization using ToCV cDNA probe for TOCV.

The obtained results revealed that all 14 tomato and 15 weeds samples were found to be ToCV positive while three *M. parviflora* and one *C. morale* sample were found to be negative.

The nucleotides sequence of HSP70 gene amplicons was determined from four selected positive tomato samples and deposited in NCBI (Accession numbers; KJ433488, KJ433489, KJ433490, and KJ433491). Sequences were analyzed with BLAST and revealed 99% identity with the isolate from Japan (AB513442) and Brazil (JQ952601). These isolates of ToCV are the first reports from Saudi Arabia.

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Genetic Diagnosis of L1 and M7 Group Bacteriophages Isolated from *Erwinia amylovora*

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Question: Fire blight of *Rosaceae* plants, one of the economically most important diseases of fruit trees, is caused by the bacterial pathogen *Erwinia amylovora*. Alternatives to the currently available, mostly unspecific fire blight control strategies are highly solicited. Bacteriophages from the virus families *Podoviridae* (L1 group of bacteriophages), *Myoviridae*, and *Siphoviridae* (both M7 group phages) that infect and lyse *E. amylovora* bacteria, are under intensive evaluation as possible highly specific biocontrol agents. M7 and L1 group phages differ considerably in morphology, host range, and efficiency of infection; bacteriophage characterization is mainly done by electron microscopy.

Prior to this study, a set of *E. amylovora* infecting viruses was isolated from *Rosaceae* fruit trees (both from plant material and soil) in the municipality of Chisinau, Republic of Moldova.

The aim of this study was the development of a genetic characterization scheme for *E. amylovora* phages and its use to characterize phage isolates from Moldova.

Methods: Comparative analysis of *E. amylovora* phage genome sequences was used to identify genetic markers for phage characterization. Respective PCR based protocols were developed to evaluate markers using the Moldovan set of phages together with external standards.

Results: The viral terminase large subunit encoding *tls* gene was identified as most suitable among assessed markers for the genetic characterization of *E. amylovora* associated bacteriophages. In particular, a *tls* gene based PCR diagnosis protocol allowed distinguishing positively between L1 and M7 group phages. According to this diagnostic approach, and in congruence

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with electron microscopic data, M7 group phages strongly prevail among *Erwinia amylovora* bacteriophages from the Republic of Moldova.

Conclusions: Genetic characterization of *E. amylovora* associated bacteriophages is a time-, work-, and cost-efficient alternative to electron microscopy. However, the characterization scheme used needs further refinement to improve its reliability and resolution.

As M7 group phages are generally more suitable for biocontrol purposes than L1 phages, the characterization of the *E. amylovora* viruses from Moldova is promising with respect to fire blight control.

Reference: Samoilova AV, Leclerque A (2015) PCR-based identification of *Erwinia amylovora* bacteriophages isolated in the Republic of Moldova. *Journal of Virology and Microbiology*, in press.

P VIRUS 28

The application of high resolution melting real-time PCR for identification of genetic diversity within *Tomato torrado virus* (ToTV) genome

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Introduction: The RNA viruses are thought to be the most excellent models for evolution studies. The high level of genetic variability is generated mainly during the replication process and may be caused by single substitutions, deletions, insertions, reassortments of genomic segments in the multipartite genomes as well RNA recombination that in consequence leads to the emergence of viral evolutionary variants, new strains and virus species.

Tomato torrado virus is single-stranded RNA virus, a member of *Torradovirus* genus. It has been reported worldwide causing burn-like systemic necrosis of leaves, significantly decreasing tomato crops. The virus is transmitted by whiteflies *Trialeurodes vaporariorum*, *T. abutilonea* and *Bemisia tabaci*, by seeds as well by grafting. In Poland we characterized and described three Polish ToTV isolates: Kra, Ros and Wal'03. The sequencing data of Polish ToTV isolates as well as the fact that they are maintained in greenhouse by continuous passages on plant host contributed accumulation of the significant differences within their genomes. The highest genetic variation was indicated within the segment of 3' -UTR of RNA1 but also within remaining part of this strand.

To monitor the changes in viral RNA the HRM real-time PCR protocol for rapid identification of the variability was developed.

Materials and methods: The analysis of three Polish ToTV isolates was performed. The total RNA from ToTV -infected *Nicotiana benthamiana* plants was purified, followed by reverse transcription. Afterwards the variable region was amplified with specific primers and subsequently subjected to HRM curve analysis.

Results: The analyses of melting curves of the Kra, Ros and Wal'03 ToTV amplicons showed a few different temperature melting peaks suggesting the presence of several genetic variants within RNA1 of particular isolates.

Conclusions: HRM real-time PCR assay identified the genetic diversity between and within analyzed isolates of ToTV.

The obtained results suggest the evolution of ToTV virus that may be important in the virus epidemiology and further control strategies.

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Tomato torrado virus requires CP for systemic movement in *Nicotiana benthamiana*

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Question: *Tomato torrado virus* (ToTV) is pathogen that infects susceptible varieties of *Solanaceae*. Infected tomato plants rapidly develop burn-like symptoms (severe systemic necroses) within leaves and stems. The virus is transmitted by whiteflies and can be propagated mechanically. The information on molecular biology of the emerging pathogen is constantly increasing and brings new insights into its pathogenicity. This in turn might benefit with development of new strategies to control both local and global spread of this serious pathogen. Pathogenicity of viruses is connected with their systemic movement in infected plant which might or might not be a coat protein-dependent process.

In the study we asked whether coat protein (CP) of *Tomato torrado virus* (ToTV) is required for its systemic movement in *Nicotiana benthamiana*.

Methods: All performed manipulations of ToTV genome were done using its infectious clones pKra_2014. The full-length RNA2 clone (pKra2) harbouring sequences of Vp26 and Vp23 was modified in such a way to excise them from the viral RNA. This was done by means of inverse PCR with primers del23F1/del23R1 (deletion of Vp23) and del26F1/del26R1 (deletion of Vp26). Then the linear plasmids were re-circularized and transformed into *E.coli* TOP10. Recombinant plasmids: pKraΔVp23 and pKraΔVp26

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were used for transformation of *A. tumefaciens* GV3101:pSoup. Transformed agrobacteria were harvested and suspended in agromix and used for infiltration of *N. benthamiana* in following combinations: pKra1+pKra2 (wild-type virus); pKra1+ pKraΔVp23; pKra1+ pKraΔVp26; pKra1+ pKraΔVp23+ pKraΔVp26 (self-complementation). Plants were grown at greenhouse conditions at 28°C. To verify virus systemic movement total RNA was extracted from upper leaves and taken for RT-PCR with primers RdRP1/RdRP2.

Results: From all tested in this study *N. benthamiana*, ToTV RNA was only detected in plants infiltrated with wild-type virus as well as in Vp-self-complementing variant. On the other hand, plants infiltrated with pKra1+ pKraΔVp23 nor pKra1+ pKraΔVp26 combinations did not give RT-PCR products suggesting lack of ToTV RNA in systemic leaves.

Conclusions: We can therefore conclude that ToTV requires both Vp subunits, Vp23 and Vp26, for long distance transport in host. This might be associated with requirement of spherical structure of the virus for its systemic spread in infected plants.

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Transmission of the Fig Mosaic Disease agents by *Ceroplastes rusci* and *Aceria ficus*

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Fig (*Ficus carica*) was infected by many different viruses (Fig leaf mottle-associated virüs 1, 2 - FLMaV1,2; Fig mosaic virüs - FMV; Fig mosaic associated virüs 1, 2 FMaV-1 ,2; Fig latent virüs 1 FLV-1; Fig mild mottle associated virüs- FMMaV; Fig cryptic virüs- FCV; Fig badnavirüs 1- FBV-1; Arkansas fig closterovirüs 1,2- AFCV-1,2; Fig fleck-associated virüs FFkaV) in different countries . It is known that Fig mosaic virus (FMV) was transmit by *Aceria ficus* but the other viruses unknown. *Ceroplastes rusci* isn't able to transmit FLMaV-1 and FLMaV-2 under experimental conditions. We have little knowledge of the transmission of Fig Mosaic Disease agents by other fig insects.

In this study, after virus acquisition *Ceroplastes rusci* and *Aceria ficus* were transferred on to healthy fig seedlings. Fig leaves were cut under a stereo microscope into small pieces each hosting 10 adult *Aceria ficus* Cotte. and placed directly on the top leaves of healthy fig seedlings. On the other hand *C. rusci* transferred onto healthy fig seedling under experimental conditions. Eriophyid mites and fig wax scale exposed seedlings were checked and observed symptoms. All samples planned to test by RT-PCR using 12 different specific set of primers.

A. ficus infested leaves of fig seedlings showed small leaf deformations, vein bandings yellowish spots after 20 days and 8 week. *C. rusci* infested leaves of fig seedlings showed only leaf deformations symptom whereas no symptoms were observed control plants for six months. Test and control plants were analyzed by RT-PCR using FMV specific set of primers.

A. ficus gave a positive PCR response to FMV whereas no amplification wasn't obtained for FMV from any of the seedlings exposed to *C. rusci*. Our study are continued. We are planing to PCR test for other Fig Mosaic Disease agents.

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A pair of universal primers facilitates the detection of potyviruses occurring in weeds and wild plants in Iran

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Studying on viruses occurring in weeds and wild plants is important epidemiologically, because they may be introduced into agricultural crops and cause new epidemics. The genus *Potyvirus* is one of the largest virus genera; members of this genus occur worldwide and infect many plant families. The aim of this study was to determine potyviruses occurring in weeds and wild plants in Iran. To this end, different geographical regions in Fars, Golestan, Khuzestan, Lorestan, Markazi, Mazadnaran and Tehran provinces were surveyed and symptomatic leaf samples collected. The potyvirus infection of the samples was initially tested by ELISA using the broad-spectrum reacting potyvirus antibodies (Bioreba, Switzerland). Potyvirus infections were confirmed by RT-PCR using universal primers Nib2F (5'-GTITGYGTIGAYGAYTTAAAYAA-3') and Nib3R (5'-TCIACIACIGTIGAIAGGYTGNC-3'). These primers have been previously shown to be more efficient as compared with other reported universal primers for potyviruses. The nucleotide sequences of 350-bp DNA amplicons of fifteen samples were determined and compared with other sequences in

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GenBank using BLASTN analysis. Based on the results, (1) the sequences of two samples from *Narcissus tazetta* showed the highest identities of 78% and 93% to *Narcissus yellow stripe virus* and Indian narcissus virus, respectively, (2) the sequences of four samples from malva plants (*Malva* spp.) matched that of *Malva vein clearing virus* with identity scores of 84-88%, (3) the sequences of two samples from *Arctium lappa* and *Brassica nigra*, and four samples from unknown weeds had the highest identity scores of 77-86% to *Turnip mosaic virus*, (4) the sequence of one sample from an unknown leguminoseous weed matched that of *Bean yellow mosaic virus* with the identity score of 82%, (5) the sequence of one sample from an unknown cucurbit plant showed the highest identity of 99% to *Zucchini yellow mosaic virus*, and (6) the sequence of one sample obtained from aphids on an acacia plant (*Acacia* sp.) matched that of *Wisteria vein mosaic virus* with the identity score of 89%. The results clearly indicate the ability of these primers to amplify a part of genome of some recognized and unrecognized potyviruses occurring in weeds and wild plants, and also seem to show the possible occurrence of new *Potyvirus* species or strains in Iran.

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Natural occurrence of a new *Begomovirus* in cucurbit crops in Iran

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Begomoviruses are one of the most devastating viruses of vegetable crops worldwide. These horticultural crops are extensively grown in commercial greenhouses, plastic tunnels and open farms in many areas of Iran. The aim of this study was to determine begomoviruses infecting vegetable plants in Iran. To this end, different geographical regions in Alborz, Khuzestan and Tehran provinces were surveyed and symptomatic vegetable and alfalfa leaf samples collected. The samples were initially tested by DAS-ELISA using the broad-spectrum reacting *Tomato yellow leaf curl virus* (TYLCV) antibodies (Bioreba, Switzerland). Begomovirus infections were confirmed by PCR using TYLCV-specific primers TYLCV-Sar (5'-CGCCGTCTCGAAGGTTTC-3') and TYLCV-Isr (5'-GCCATATACAATAACAAGGC-3'), and universal primers Begomo-F (5'-ACGCGTGCCGTGCTGCTGCCCCATTGTCC-3') and Begomo-R (5'-ACGCGTATGGGCTGYCGAAGTTSAGAC-3'). The PCR tests using the primer pair TYLCV-Sar/TYLCV-Isr resulted in the amplification of the expected fragment of ca. 0.67-kb in size for ELISA-positive samples tested from alfalfa (*Medicago sativa*), pepper (*Capsicum annum*) and spinach (*Spinacia oleracea*) plants, confirming the presence of TYLCV. The PCR reaction using the primer pair Begomo-F/Begomo-R resulted in the specific amplification fragments of the expected size of ca. 2.8 kb for one melon (*Cucumis melo*) sample from Khuzestan; the sample positively reacted in ELISA with TYLCV antibodies but had no reaction in PCR using TYLCV-specific primers. The nucleotide sequences of the DNA amplicons derived from the isolate, nominated Kz-Me198, were determined and compared with other sequences available in GenBank. BLASTN analysis confirmed the begomovirus infection of the sample and showed 99% identities with *Tomato leaf curl New Delhi virus*, ToLCNDV (KC874506 and AM850115); phylogenetic analysis, performed by the maximum-likelihood algorithm, supported the results of the database searches. This study reports the natural occurrence of TYLCV on different hosts in Iran. Our results also reveal the emergence of a new begomovirus, ToLCNDV, in Iranian cucurbit crops. The virus has a wide host range, including solanaceous and cucurbit plants; so, it seems justifiable to do more studies on the epidemiological and managerial aspects of the virus in the future.

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A survey on distribution of important tomato viruses in Bushehr province of Iran

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Introduction: Bushehr province is one of the major off season tomato growing centers in southern Iran. Wide spread viral infections in tomato fields has been observed in recent years.

Objectives: The present study is aimed to identify viruses infecting tomato plants in Bushehr, in order to help selecting resistant varieties for management strategies.

Materials and methods: During 2013, a survey was conducted on a total of 250 tomato plant samples collected from fields located in Borazjan, Bushehr, Dashty, Dayyer, Esmaeel Khany, Kangan, Khormoj and Ziarat Sahely areas of Bushehr province. The plants showed viral symptoms including leaf mosaic, vein clearing, mottling, and stunting. To identify the tomato infecting

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viruses, samples were tested by DAS-ELISA, using polyclonal antibodies against major tomato viruses known in Iran. Besides, polymerase chain reaction, using specific primers was performed to identify four TYLCV strains.

Results: DAS-ELISA tests showed that samples obtained from tomato fields were infected with Tomato yellow leaf curl virus (TYLCV), Zucchini yellow mosaic virus (ZYMV), Eggplant mottled dwarf virus (EMDV), Cucumber mosaic virus (CMV), Alfalfa mosaic virus (AMV), Potato virus X (PVX) and Tomato mosaic virus (ToMV); with a frequency of 94.5%, 72%, 65%, 56.7%, 27%, 5.4% and 5%, respectively. Mixed infections were observed in most of samples. No infections were observed with Squash mosaic virus (SMV), Potato virus Y (PVY), Watermelon mosaic virus (WMV) and Potato leaf roll virus (PLRV). PCR revealed the presence of TYLCV-IL strain in more than 90% of samples serologically proved to be infected with the virus. Less than 5% of these samples showed a mixed infection with TYLCV-ES strain. No infections with TYLCV-Mld and TYLCV-Sic strains were detected.

Conclusion: The results showed that TYLCV, ZYMV, EMDV and CMV were the most dominant viruses in tomato fields of Bushehr province and control measures should be focused mostly on these viruses, with more emphasis on the TYLCV-IL strain.

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Oleander as a reservoir plant for *Cucumber mosaic virus*

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Introduction: Reservoir plants play an important role in survival of viruses and also as a source of primary inoculums. Some ornamental ever-green plants act as a host for economically important viruses and provide viral inoculums for whole growing season. Varamin is a town located east of Tehran (capital of Iran) and famous for production of melons and ornamental plants. As such, there is even a melon cultivar known as "Varamin Melon". The oleander (*Nerium oleander*) is cultivated as an ornamental plant alongside the highway at some 5 Km distance from the nearest melon farms in Varamin. The aim of this study was to figure out if oleander is naturally infected by the viruses which usually found in the melon farms.

Material and methods: a total of 162 collected leaf samples showing different viral symptoms were checked by different versions of enzyme-linked immunosorbent assay (ELISA) such as double antibody sandwich (DAS)-, triple antibody sandwich (TAS)- or double antigen coated (DAC)-ELISA with antibodies which were specific for viruses commonly reported from melons including *Cucumber mosaic virus* (CMV), *Zucchini yellow mosaic virus* (ZYMV), *Watermelon mosaic virus 2* (WMV-2), *Cucurbit yellow stunting disorder virus* (CYSDV) and *Cucurbit aphid-borne yellows virus* (CABYV). Reverse transcription polymerase chain reaction (RT-PCR) with specific primers, for each of the viruses to confirm the ELISA results.

Results and conclusions: Mosaic, mottling, leaf deformations, yellowing, vein yellowing, crinkling, curling and necrosis were recorded in the melon farms (Figure 1). CMV was the most prevalent virus in the collected samples (Table 1). Interestingly, we also observed mosaic and ringspot symptoms on leaves of oleanders. CMV was detected as an agent associated with these symptoms by the use of DAS-ELISA and RT-PCR. Also, populations of the virus vector, *Aphis gossypii* were found on the oleander. This aphid is also a dominant virus vector of the melon farms. Since this aphid can transmit CMV, it can be concluded that the CMV- infected oleander might be a reservoir plant and source of CMV inoculums for melon farms. Based on our knowledge, this is the first report of CMV from oleander which bears implications as to the control of the viral diseases. Further studies are needed to confirm this finding.

Figure 1



Figure 1. Symptoms recorded in the melon farms of Varamin.

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Figure 2

Table 1. Viruses and their incidence found in samples collected in Varamin

Virus	Infection in samples (%)
CMV	44.4
ZYMV	20.98
WMV-2	17.28
CYSDV	16
CABYV	12.34

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Rice stripe *Tenuivirus* nonstructural protein 3 hijacks the 26S proteasome of the small brown planthopper, via direct interaction with regulatory-particle non-ATPase subunit 3

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The ubiquitin/26S proteasome system plays a vital role in regulating host defenses against pathogens. Previous studies have highlighted different roles for the ubiquitin/26S proteasome in defense during virus infection in both mammals and plants. But their role in the vectors that transmit those viruses is still unclear. In this study, we determined that the 26S proteasome present in the small brown planthopper (SBPH, *Laodelphax striatellus*), and has similar components to that in plants and mammals. There was an increase in the accumulation of *Rice stripe virus* (RSV) in the transmitting vector SBPH after disrupting the 26S proteasome, indicating that the SBPH 26S proteasome play a role in defense against RSV infection by regulating RSV accumulation. Yeast two-hybrid analysis determined that a subunit of the 26S proteasome named RPN3 could interact with RSV NS3. Transient over-expression of RPN3 had no effect on the RNA silencing suppressor activity of RSV NS3. However, NS3 could inhibit the ability of SBPH *rpn3* to complement an *rpn3* mutant in yeast. Our findings also indicate that the direct interaction between RPN3 and NS3 was responsible for inhibiting the complementation ability of RPN3. *In vivo*, we found an accumulation of ubiquitinated protein in SBPH tissues where RSV titer was high, and silencing of *rpn3* results in malfunction of the SBPH proteasome-mediated proteolysis. Consequently, viruliferous SBPH in which RPN3 was repressed transmitted the virus more effectively as a result of higher accumulation of RSV. Our results suggest that the RSV NS3 protein is able to hijack the 26S proteasome in SBPH, via a direct interaction with the RPN3 subunit to attenuate the host defense response.

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The Iranian wheat landraces comprise tolerant and resistant sources against viral diseases

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Iran is located in the Fertile Crescent, the co-epicenter of wheat and some pathogens such as viruses. *Wheat streak mosaic virus* (WSMV) and Barley/Cereal yellow dwarf viruses (B/CYDV) are among the most important viral agents, infecting cereal crops and are widespread in the country. Employing natural resistance and/or tolerance sources is highly advantageous comparing to other management practices. The present research was performed to screen a collection of some Iranian wheat landraces to identify the natural resistance genetic sources against the above viral diseases. A number of native wheat pure lines collected from all around Iran were provided by Seed and Plant Improvement Research Institute (SPII), Cereal Research Department. The biologically isolated sources of WSMV and BYDV-PAV were maintained from Marvdasht (Southern Iran) and used for artificial inoculation of 150 and 98 accession numbers, under greenhouse condition, respectively. Mechanical and aphid based (*viruliferous Rhopalosiphum padi*) inoculations were performed for WSMV and BYDV-PAV, respectively. Assessments were achieved throughout two independent preliminary and complementary steps with five replications, based on diseases symptom scores (0-7 for WSMV and 0-9 for BYDV-PAV) along with ELISA values. According to the results, 2, 3.3, 1.33 and 93.37% of the genotypes were grouped into resistance, semi-resistance, semi-susceptible and susceptible clusters to WSMV, respectively.

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Furthermore, 1, 1, 1 and 97% of tested accessions were classified in the similar above groups for BYDV-PAV, correspondingly. Furthermore, a number of genotypes revealed some degrees of tolerance responses. These findings confirm the presence of resistance sources against viral pathogens, and highlight the value of Iranian landraces, particularly for BYDV, against which no source of natural resistance in wheat is reported. Moreover, use of these novel sources in the future improvement programs against these viral agents is further emphasized.

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Health micro propagation of potato (*Solanum tuberosum* L.)

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Introduction: Every year, negative forms have been evaluated in conforming potatoes process, despite the official medical assurance documents obtained tohile purchasing potatoes seeds, various diseases appeared, most of them viral.

So the micro propagation in vitro is able to solve seed's medical problems in Algeria.

Aims: The viruses contained in potatoes are a serious problem to farming this produce.

- -The principal object in this study is putting a secure method to a safe micro propagation
- -Making synthtic safety plantlues with Elysa test
- -The aims of this search :
- Many years we noticed the increase of viruses on potatoes produces
- -In purpose of micro propagation farming potatoes and reduce costs

Materiels and methods: The application of (ELISA test) has been carried out of a rapid diagnostic of potato's virus reveals the presence of:

- Potato virus M coming from the main tubercles of Elvira and Folva variety;
- Potato virus S coming from the main tubercles of Diamant and Elvira variety;

Results: The plantules at juvenile state are obtained by the micropropagation in vitro technique by using an environment prived of growth hormones.

A serologic test is applied on this plantules revels the presence of:

- Potato virus of Diamant and Elvira variety.
- Potato leafroll virus PRLV of Elvira variety.

Conclusion shows differences between the six variety of potatoes. So we obtained recurrence pourcentage, contamination pourcentage, anormal plantules pourcentage.

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Chilli veinal mottle virus was first identified to infect chilli in Hunan and Fujian provinces, China

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Chilli veinal mottle virus (ChiVMV), a member of genus Potyvirus (the largest genus in the family Potyviridae), is economically important for a wide range of crops, especially for chilli (*Capsicum annuum* L.). In 2013, six of chilli plants exhibiting symptoms of chlorotic, mosaic and leaf-roll were observed in field in Hunan and Fujian provinces, China. All of symptomatic plants were tested for virus infecting by sRNA sequencing, and shown one from Hunan province and two from Fujian Province to be infected by ChiVMV. The ChiVMV positive plants were further tested by enzyme-linked immunosorbent assay (ELISA) specific for ChiVMV. ELISA tests confirmed the presence of ChiVMV. Total RNA extracts from the two positive plants (each from Hunan and Fujian Provinces) were analyzed for the presence of ChiVMV using RT-PCR with primers designed specific to ChiVMV. The PCR products of the expected size (~920 bp) were obtained from the both positive plants. The PCR products were cloned and sequenced to confirm the presence of ChiVMV. The amplicon of sampling in Hunan province was the highest identical (91%) to that in Hainan and Sichuan Province, China (ACX 53640 and AGN92430), otherwise, the amplicon of sampling in Fujian Province was the highest identical (95%) to that in South Korea (AJ972878) and India (AJ237843). Although ChiVMV has been reported to infect chilli in Hainan and Sichuan provinces in Southwest of China, this is the first report of it infected chilli in Hunan and Fujian provinces in South east of China, this also hints ChiVMV spreads orienting to east in China. This may have epidemiological consequences for chill in east of China. The economic loss of chill and molecular characterization of ChiVMV are under investigation.

Poster Presentations

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The impact of regional diversity of Cherry Viral Pathogens on effectivity of their detection

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Cherries are among the attractive fruits suitable for direct consumption as well as for industrial processing, however, the profitability of their production may be adversely affected by a number of viral pathogens. The diagnosis of cherry viruses by the conventional methods such as RT-PCR or real-time PCR is target-specific and the optimisation of such methods requires the access to the genome data. Effective detection can be thus adversely affected by the high potential of viral genetic variability.

We have analysed the molecular variability of two cherry viruses - Prune dwarf virus (PDV, genus *Illarvirus*) and Little cherry virus-1 (LChV-1, genus *Ampelovirus*) obtained from different cherry orchards and plantations in Slovakia.

While PDV belongs to a wide spread and common cherry pathogens, the presence of LChV-1 was reported only sporadically from Europe and it was identified for the first time in Slovakia only in 2014.

Initial detection using the available, previously published RT-PCRs, did not provided reliable results. Therefore, for both viruses, new primer pairs were designed (targeting the capsid and the movement protein gene, respectively), reflecting the increased genome data available in the public databases.

Partial genome characterization showed a substantial genetic divergence among Slovak cherry PDV isolates (reaching 8.3% at the nucleotide level) and their assignment into 2 different phylogenetic groups.

Molecular characterisation of LChV-1 revealed that Slovak isolates form a cluster distinct from previously characterized European isolates. These results further confirms a need for a continual assessment of the virus molecular variability (also at the regional level) as a prerequisite to develop polyvalent detection tools.

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The damage and whitefly transmission of Tomato chlorosis virus in China

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Tomato chlorosis virus (ToCV, genus *Crinivirus*, family *Closteroviridae*), which causes a serious yellow leaf disorder syndrome in tomato plants, originally identified in Florida, USA in 1998. Since then, ToCV epidemics have emerged worldwide. In China, ToCV was firstly reported from Tiwan in 2004. In 2013, ToCV was found on tomato plants in Beijing and Shandong Province, and was detected on sweet pepper plants at the same time. Preliminary investigation indicated ToCV had spread in Beijing, Shandong, Hebei, Tianjing and Jiangsu, causing severe damage to local tomato production (50%-100% infection, 40%-60% yield losses). As its fast transmission, ToCV has become one of the most important viruses for China's tomato production.

In nature, ToCV is transmitted from plant to plant by phloem-feeding whiteflies (Hemiptera: *Aleyrodidae*) belonging to two genera, *Bemisia* (*B. tabaci*) and *Trialeurodes* (*T. vaporariorum* and *T. abutiloneus*). In China, *B. tabaci* is one of the most invasive and devastating insect pests. The investigation has been found to be infected with ToCV in a greenhouse heavily infested with the whitefly *B. tabaci*. The resulting significant yield losses could severely limit tomato production in areas where increased prevalence occurs, which in turn may be determined by changes in vector populations. Whitefly-mediated transmission assays were conducted that ToCV was efficiently transmitted by both *B. tabaci* genotypes, at a rate of 100% for Mediterranean (MED, formerly Q biotype) and 85% for Middle East-Asia Minor 1 (MEAM1, formerly B biotype). In the future, the studies on the molecular mechanism of the ToCV transmission by *B. tabaci* and ToCV disease explosion along with the replacement of MED by MEAM1 will be conducted. Tomato-yellowing caused by the crinivirus ToCV is a good example of a whitefly-transmitted disease emerging in many countries worldwide. Due to the increasing prevalence of ToCV throughout the world, a better knowledge of its host range and syndromes caused on cultivated hosts, virus-vector relationships, and the potential impact on production of affected crops, is crucial to implement more effective and durable crop management practices.

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Numerous novel fungal viruses are associated with the Mushroom Virus X disease

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Introduction: Mushroom Virus X (MVX) is a disease syndrome affecting the commercial fungal crop *Agaricus bisporus* (the common white mushroom). This disease is characterised by brown discolouration of the mushroom cap, delayed fruit body development and misshapen fruit bodies and it has been observed to be widespread across Europe for the last 25 years. The symptoms have been found to coincide with an increased number of double stranded (ds)RNA elements which vary in size between ~20kb and ~600bp.

Objectives: Sequence and characterise the dsRNA species associated with MVX disease. Develop RNA extraction from mushroom compost (straw-based substrate containing fungal mycelium). Develop a detection method for the MVX-associated viruses.

Materials and methods: The dsRNA was selectively purified from the fruit bodies of nine diseased samples and one symptomless sample and sequenced by Illumina MiSeq. The reads were assembled into contigs using Velvet and Cap3. Open Reading Frames and protein domains were predicted using Geneious, DELTA-BLAST and HHpred. Primers were designed for the length of each of the non-host contigs in order to confirm the assemblies and also for qPCR detection.

Conclusion: The dsRNA species associated with the MVX disease were sequenced successfully. Surprisingly the different dsRNA species encode a large number of phylogenetically diverse RNA viruses, with up to 15 viruses appearing in the same sample.

RNA extraction from mushroom compost combined with a quantitative RT-qPCR technique were used to develop a test to measure relative levels of each of the novel viral RNAs, to be used by the growers and to aid epidemiological studies of the disease.

We carried out interaction studies by examining correlations between the viral RNA levels present in MVX-4569 which shows that the majority of the 21 putative viral RNAs present can be categorised into 4 interaction groups.

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Citrus viroids in Tunisia: Prevalence and molecular characterization

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The citrus industry in Tunisia is based on the production of many cultivars of sweet orange (*Citrus sinensis*), mandarin (*C. reticulata*), clementin (*C. clementina*) and lemon (*C. limon*). The total citrus growing area is about 20000 ha mainly located in Cap Bon region (75%) and sour orange is presently the major used rootstock. This prevalence of sour orange explains why viroid symptoms are not observed in commercial orchards except Cassar Clementine and Common Mandarin varieties on which the cachexia symptoms have been mainly observed. Considering the actual Tunisian strategy to prevent tristeza spreading, the substitution of sour orange by new rootstocks giving tristeza tolerant rootstock/scion combinations is a good alternative. However, some promising rootstocks like Citrange Carrizo and Citrange troyer are known to be sensitive to viroids. In this regard, we started an extensive survey including 202 symptomless trees, belonging to commercial orchards in Cap bon region and also in INRAT collection of mother plants to identify the prevalence of viroids in our orchards. Biological indexing was performed using Arizona 861-S1 Etrog citron grafted on rough lemon rootstock as an indicator. Sequential polyacrylamide gel electrophoresis analysis and molecular hybridization using viroid specific probes revealed that all plants are infected with at least two viroids. *Citrus exocortis viroid* (CEVd), *Hop stunt viroid* (HSVd) and *Citrus dwarfing viroid* (CDVd) were widespread and accounted for 70,4; 72,2 and 78%. *Citrus bent leaf viroid* (CBLVd) and *Citrus bark cracking viroid* (CBCVd) were only found in 28,5 and 3% of trees. Reverse transcription-PCR, cloning, sequencing and phylogenetic analysis from isolates of the three important viroids (CEVd, HSVd and CDVd) deposited in the GeneBank databases using the Clustal W (Ver.1.83) program (Thompson et al. 1994) and compared with other isolates from the world showed that tunisian variants CDVd-1 (Genebank N°. KJ538557), CDVd-2 (Genebank N°. KJ538558) and CDVd-3 (Genebank N. KJ538559), were clustered in the same group with Japanese, Iranian and USA strain. The closest one is from Japan (AB054632), with high sequence homology reaching 99%. In addition, a high homology rate between Tunisian variants was observed (between 98.65% and 99.66%) pointing a high conservation. The three CEVd variants

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(CEVd-1 (Genebank N°.KJ538554), CEVd-2 (Genebank N°.KJ538555) and CEVd-3 (Genebank N°.KJ538556) compared with 22 others sequences were belonged to Class "B" (mild) and form a sub-cluster into the main cluster with USA, Iran, Greece and China strains. The Tunisian HSVd-1 isolat obtained have 100% sequence identity with CVD-Ilc (AF131250) (Ca905 from USA) and Iranian variant (JX430798).

Furthermore, it is important to mention that the high contamination rate with viroids must be taken in consideration when new rootstocks will be used to control the tristeza disease. In this case, the control of foundation blocks and nurseries through a rigorous certification scheme would be required.

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Detection and molecular characterization of cotton infecting begomoviruses from Pakistan

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Cotton leaf curl disease (CLCD) is a considerable encumbrance to the cotton production worldwide including Pakistan. The disease is caused by the complex of begomoviruses (Family: *Geminiviridae*) in association with a disease specific satellite known as cotton leaf curl Multan betasatellite. Begomovirus-infected cotton plant samples were collected in Punjab- Pakistan, during 2011-13. Sixty samples were processed for the presence of begomoviruses virus and betasatellite. Full-length clones were sequenced. Begomovirus ranged from 2751 to 2759 nucleotides. Complete sequences shown to be isolates of *Cotton leaf curl Burewala virus* (CLCuBuV) with >96% nucleotide sequence identity -except a clone from Layyah with <93% identity. In common with previous CLCuBuV isolates, the virus from Layyah was recombinant containing sequences derived from two virus species that were predominant in cotton pre-resistance breaking but with distinct recombination break-sites, for which a name was proposed as cotton leaf curl Burewala virus-Layyah. In all viruses, lack of an intact TrAP protein was found as common. Betasatellite were detected and they were ranged from 1349 to 1371 nucleotides. Sequence analysis shown to be an isolate of *Cotton Leaf Curl Multan Betasatellite* (CLCuMB) with >97% identity- containing the recombinant fragment typical of this satellite post-resistance breaking. Agroinocuation of the new strain into *Nicotiana benthamiana* and *N. gluconosa* exhibited symptoms like vein thickening and leaf curling. CLCuBuV with associated betasatellite showed a wide spread and highlights the dominance of resistant breaking CLCD in this region and further emphasis the need for work to combat its damage.

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Detection of *Prunus necrotic ringspot virus* in wild plums (*Prunus domestica* subsp. *insititia*) in Iran

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Plums as one of the major temperate zone fruit trees have a widespread growing habit amongst other deciduous fruit trees. Wild plums (mostly, *Prunus domestica* subsp. *insititia*.) are widely distributed in many parts of Iran and studying on their natural virus infections is important for understanding virus populations infecting them and developing disease management strategies. During a survey in 2014, a total of 29 symptomatic wild plum samples showing chlorotic line and ring patterns and leaf deformation were collected from Mazandaran province, North Iran. Samples were tested for *Prunus necrotic ringspot virus* (PNRSV), *Prune dwarf virus* (PDV) and *Plum pox virus* (PPV) by Double antibody sandwich-enzyme linked immunosorbent assay (DAS-ELISA) using commercial antibodies (Bioreba, Switzerland). Eleven samples (38%) showed positive reaction only with PNRSV specific antibody. These samples were selected and their total RNA were extracted and tested for PNRSV infection by reverse transcription-polymerase chain reaction (RT-PCR) using previously described specific primers designed for amplifying coat protein (CP) gene of PNRSV (1). A DNA amplicon of the expected size (approximately 675 bp in length) was obtained in 9 out of 11 samples. No DNA product was amplified from total RNA of asymptomatic trees. In biological host range studies, sap inoculation of ELISA positive samples on indicator plants produced large chlorotic local lesions in cotyledons of *Cucumis sativus* followed by severe stunting, and systemic mottle in *Chenopodium quinoa*. These data confirm PNRSV infection of the studied wild plum samples which is a first record for Iran. *PNRSV* is transmitted via seeds and pollen at variable rates in several natural hosts, including *Prunus* spp (2). These findings must be considered in establishment of mother gardens, scion blocks and nurseries of stone fruit trees in North Iran.

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Coat protein gene sequence analysis of an Iranian *Prunus necrotic ringspot virus* isolate from sweet cherry

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Stone fruits orchards are widely distributed in almost all geographical regions of Iran. *Prunus necrotic ringspot virus* (PNRSV), a member of the genus *Illarvirus* in the family *Bromoviridae*, was first described on peach in 1931 and affects rosaceous plants worldwide (1). During 2013-2014, a total of 19 symptomatic leaf samples showing mosaic, vein yellows, chlorotic spots and shot-holes, and deformation were collected from sweet cherry trees in Markazi province, Central Iran. Of the 19 samples tested, 16 were positive for PNRSV by DAS-ELISA method using specific antibody (Bioreba, Switzerland). Some of the positive samples had typical chlorotic spots and shot holes symptoms. Three isolates were used for biological assays on cucumber (*Cucumis sativus*) after single lesion transfer on cowpea (*Vigna unguiculata*). Inoculated cowpea plants showed chlorotic spotting on the young leaves and cucumber plants reacted with chlorotic spots followed by systemic mottle and stunting. Total RNA was extracted from ELISA positive samples using RNeasy Plant Mini kit (Qiagen) according to the manufacturer's instructions. Specific primers forward (PNF'-TGATGTC(T/G)ATGGTCCGAAGTAGG) and reverse (PNr-ACGCAGGTAAGATTTCCAAGCA) were designed based on highly conserved flanking regions of the coat protein (CP) gene of the PNRSV isolates available in GenBank databases. CP gene of a PNRSV isolate (Mrk5) was amplified by RT-PCR and its nucleotide sequence was determined and shown to be 681 nt long with an open reading frame (ORF) of 226 amino acids. The CP sequences were assessed for evidence of recombination. After all gaps and nucleotides homologous to them had been removed from the aligned sequences, the likely recombination sites were assessed for evidence of recombination using RDP3. Finally, no 'clear' recombination ($p < 1 \times 10^{-6}$) was found in the genome of them and these isolates seemed not to be recombinants. The phylogenetic relationships of the Iranian Mrk5 with 38 other PNRSV isolates using CP were investigated by NJ method and partitioned most of the sequences into the four phylogenetic groups: PE5, PV32, CH30 and PV96, as reported previously (2). CP genome pairwise identity analysis revealed the highest (97.7%) and lowest (86.3%) nucleotide identity with PV96 and PE5 phylogenetic groups, respectively. The Iranian Mrk5-PNRSV isolate fell into the PV96 group in separate branch with the highest identity (98%) to PchHN9 isolate from China. Our results showed for the first time phylogenetic status of an Iranian PNRSV isolate based on CP gene sequence.

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P VIRUS 46

Distribution of Viruses Infecting Cucurbits Crops in northern Cyprus

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Field surveys were conducted from 2010 to 2012 to detect and determine the incidence of viruses in the major cucurbit-growing areas of northern Cyprus. 502 samples of cucumber, zucchini, melon and watermelon were collected from major cucurbit-growing areas in Cyprus. Zucchini yellow mosaic virus (ZYMV), Papaya ringspot virus type W (PRSV-W), Watermelon mosaic virus (WMV), Cucumber mosaic virus (CMV), Cucurbit yellow stunting disorder virus (CYSDV) melon necrotic spot virus (MNSV) and Squash mosaic virus (SqMV) were detected by enzyme-linked immunosorbent assay (ELISA), and, Beet pseudo-yellows virus (BPYV), Cucurbit aphid-borne yellows virus (CABYV), and Cucumber vein yellowing virus (CVYV) by reverse transcription polymerase chain reaction (RT-PCR). ZYMV was the most prevalent virus of cucurbits in Cyprus with an overall incidence of ZYMV (45.5%), WMV-2 (34.2%), CYSDV (24), CVYV (21), CMV (19.9%), CABY (14), PRSV-W (2.1%), and SqMV (1.8%) BPYV and MNSV were not detected in any cucurbitaceous crop during this survey.

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Virus suppression with the *Polerovirus* 5' sequence-specific RNA-binding protein encoded by the 3' subgenomic RNA

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Introduction: *Potato leafroll virus (PLRV)* causes significant yield and quality losses to infected potato (*Solanum tuberosum* L) world-wide. Through small RNA (sRNA) sequencing and mapping of the *PLRV*-derived sRNAs, we discovered a sgRNA ~500 nt from the 3' terminus of *PLRV* genome. A regulatory role for the *PLRV* sgRNA3 is supported by the 5' binding of the *PLRV* gRNA by the sgRNA3 protein.

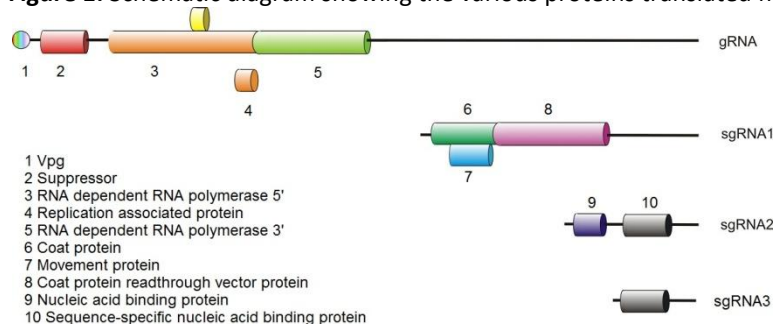
Objectives: To characterize the role of a third sgRNA (sgRNA3) of *PLRV*, through sRNA analyses and *in planta* complementation studies with the *PLRV* 5' sequence-specific RNA-binding protein encoded by the 3' subgenomic.

Materials and methods: *PLRV*-infected and the healthy potato plants were maintained in sterile tissue culture and grown at 20 °C and 16 hrs photoperiod. *PLRV*-derived sRNA sequences were identified using the Genbank BLAST program against the *PLRV* Canadian isolate genomic sequence (accession number D13954.1). The construction of chimeric *PLRV* ORF7 and ORF7-GFP vectors for transient and stable expression experiments were verified with DNA sequencing, transformed into *A. tumefaciens*, and expression examined *in planta*. To test for sequence-specific nucleic acid-binding activity of ORF7 fusion proteins were performed using filter-binding experiments.

Results: The *PLRV*-derived sRNAs covered the viral genome in near saturation with the exception of gaps that aligned to regions of the transcriptional start sites for sgRNA1, sgRNA2, and sgRNA3 and the *PLRV* IRES that allows for ribosomal frameshifting for expression of viral RNA dependent RNA polymerase ORF1/ORF2 and translation of the replication associated Rap1 (Figure 1). The binding activity of pGEX:ORF7 fusion proteins was observed against a specific 5' sequence of *PLRV* close to the IRES. Expression of ORF7 protein in stably transformed plants significantly reduced virus accumulation and disease.

Conclusion: Our results indicate that the *PLRV*-derived sRNAs in of *PLRV*-infected plants covered the viral genome with the exception of four distinctive gaps, which led to the discovery of sgRNA3 for *PLRV*. This is the first sgRNA3 identified in a virus from the genus *Polerovirus* and it exhibits several characteristics that suggest a regulatory role in virus replication. This study advances our characterization and understanding of virus regulation and provides a novel strategy for virus eradication.

Figure 1: Schematic diagram showing the various proteins translated from the *PLRV* genomic and subgenomic RNAs.



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Characterization of a novel Potyvirus of squash (*Cucurbita pepo*) from Florida

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During the 2010-to 2011 growing seasons, leaf samples from various cucurbits were collected in Florida. One of the samples from squash showed unique symptoms, including chlorotic spots, yellowing, mottling, vein clearing and mild mosaic. Total RNA was extracted from symptomatic leaves and was negative by RT-PCR against Papaya ringspot virus (PRSV-W), Squash vein yellowing virus (SqVYV), Watermelon mosaic virus (WMV) and Zucchini yellow mosaic virus (ZYMV). Virus-like particles (VLP) preparation was prepared from symptomatic squash leaves and analyzed by electron microscopy. Typical potyvirus-like particles approximately 700 nm in length and 12-14 nm in width were observed. Total RNA was extracted from VLP and tested by RT-PCR using universal Potyviridae primers to amplify a fragment from the 3' end of the genome (including part of Nib gene, whole coat protein). A band of 1.2 kb was observed when the PCR product was analyzed on 1% agarose gel. PCR product was purified using the QIAquick PCR Purification Kit (QIAGEN, USA), cloned (pGEM-T Easy Vector (Promega, USA) and sequenced in both directions.

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Consensus sequence was obtained from at least 5 clones and submitted to GenBank (KC522958). Sequence analysis and further genome comparison with sequences of other potyviruses will be discussed.

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Wheat streak mosaic virus spreads - first report on the occurrence in Germany and Austria

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Wheat streak mosaic virus (WSMV), the type species of the genus *Tritimovirus* in the family *Potyviridae*, is a serious threat in several wheat producing countries. Incidences of the virus in Europe have so far been reported mainly from countries in Southern Europe, and economically significant crop losses are caused by it particularly in the Ukraine and Southern Russia. In Germany, the virus was observed for the first time in 2013 in volunteer cereals, and in 2014 in a winter wheat field near Hoym (Saxony-Anhalt). In Lower Austria, WSMV was first reported in 2013 in winter wheat variety Lupus. The occurrence of WSMV in Germany and Austria was confirmed by electron microscopy, serology and molecular methods. In electron microscope decoration tests both virus isolates were clearly decorated by WSMV antiserum, and only a weak cross-reaction was observed with an antiserum to the related *Oat necrotic mottle virus*. Ultrathin sections revealed characteristic cytoplasmic inclusions consisting of pinwheels, scrolls, and laminated aggregates. Both isolates were tested with antisera to elongated cereal infecting viruses from the JKI stock collection. In DAS-ELISA and Western blots only WSMV antiserum reacted with the plant samples, whereas in TAS-ELISA a monoclonal antibody specific for two ATCC strains of WSMV (type PV-57 and PV-91), showed no reaction with the German and Austrian isolates and other WSMV isolates originating from different locations in Europe. The complete sequences of isolates Hoym and Austria were obtained and compared to all other known complete WSMV sequences, including a newly collected and sequenced isolate from France. Phylogenetic analyses revealed that European isolates group together with those from the Middle East to form a separate cluster. Further studies are required to make conclusions on the incidence and economic importance of WSMV in Germany and Austria. Recent reports on the occurrence of WSMV in neighboring countries such as Poland, the Czech Republic Slovakia and France, suggest that the virus spreads and could become in the future a threat to the cereal production.

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Investigation of *Potato Y potyvirus* (PVY) Infections and Strain Population in Potatoes (*Solanum tuberosum* L.) in Central Anatolia Region, Turkey

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Potato (*Solanum tuberosum* L.) is one of the most important crop in Turkey, with annual production of nearly 5 million tons from 172.000 ha of arable land. *Potato Y potyvirus* (PVY) has become a serious problem for the seed potato industry, with increased incidence and rejection of seed lots submitted for certification. New PVY strains have emerged in recent years in the world representing recombination events between the common strain (PVY^O) and PVY^N. In the present study, it is aimed to determine the presence of PVY infections and the strains in the potato production areas of Central Anatolia Region in Turkey. For this purpose, surveys were carried out during the year of 2014. A total of randomly collected 50 potato leaf samples were tested by using serological (ELISA) and molecular (Multiplex RT-PCR) methods. Out of 50 samples, 49 were found infected with at least one PVY strain, either single or in mixed infections. Thirty of infected samples were identified as PVY^{N:O} and 2 were found as PVY^{NTN}. In addition, mixed infections were found in 17 samples involving PVY^{NTN} and PVY^{N:O} strains. Only a unique sample reacted negative in multiplex RT-PCR tests. The present results indicates that, the prevailing strain is PVY^{N:O} in potato plants in Central Anatolia (Turkey). Comparing with PCR test, 24 samples were reacted negative when tested by ELISA. This result indicates that, multiplex RT-PCR is much more sensitive and useful than ELISA for both researchers, seed production specialists and also quarantine laboratories in determining PVY infections.

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Electrical penetration graph analysis of *Planococcus citri* (Hemiptera: Pseudococcidae) feeding behaviour on cocoa (*Theobroma cacao* L.)

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Introduction: *Cacao swollen shoot virus* (CSSV) is a mealybug transmitted pathogen that causes severe losses to cocoa (*Theobroma cacao* L.) production in West Africa. At least 16 species of mealybug are thought to act as vectors of CSSV and of these the citrus mealybug, *Planococcus citri* (Risso), plays a particularly important role due to its abundance on the West African crop while its polyphagous status implicates it in the movement of the virus between cocoa and alternative host plants. While adult female mealybugs are wingless and relatively sedentary, wind blown juvenile instars are thought to help spread the virus to uninfected trees. Mealybugs acquire and transmit viruses when they access host plant phloem but to date no detailed information is available on the feeding by *P. citri* on cocoa.

Objective: This work aimed to characterise the feeding behaviour and pattern of individual immature *P. citri* females on leaves of cocoa plants using the DC-electrical penetration graph (EPG) technique which allows for precise monitoring of stylet position and activity within the plant tissue.

Materials and methods: *P. citri* colonies were established from gravid females collected in glasshouses at the Royal Botanic Gardens, Kew. Species identity was established using a combination of scanning electron microscopy and CO1-based DNA barcoding. The colonies were maintained on sprouted potato (*Solanum tuberosum* L.) under controlled conditions (L14:D10; 20±2°C, 50% RH) for several generations. The EPG recording was for a 24 h duration for each of the 16 second-stage instars monitored. Individuals were starved for 24 h before being connected to the EPG apparatus and allowed to feed on cocoa seedlings (variety Amelonado) obtained from the International Cocoa Quarantine Centre, Reading.

Results: Generally, there was a high level of variation in the feeding behaviour observed among *P. citri* individuals. From the start of recording, it took an average of 15 ± 6.8 mins before the mealybugs started probing. Xylem activity (G) and extracellular salivation (E1e) was shown by 14 individuals. The duration spent in G ranged from 0 to 95%. Only 9/16 mealybugs expressed derailed stylet mechanics (F) and this lasted for 3 h ± 36 min. The average time spent in the pathway phase (C) without E1e, F and G, was 41 ± 7 min. Six of the individuals performed phloem and sieve element puncture activities (E1) and 5/16 of these E1 were followed by phloem sap ingestion (E2). The results are discussed in the light of 'mealybug-cocoa' interactions, especially feeding behaviour with implications for the transmission of CSSV.

Conclusion: While some of these waveforms were similar to those reported for *P. citri* on another host, grapevine, this study emphasises the distinct nature of cocoa feeding and the variability of vascular access between individuals. As the search continues for resistance to CSSV in cocoa germplasm these results demonstrate how EPG could be used to characterise the resistance mechanism when such a genotype is identified.

P VIRUS 52

Reliability and inter-annual stability in visual surveys of birch leaf-roll disease symptoms caused by CLRV

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Since the beginning of the 3rd Millennium *Cherry Leaf-Roll Virus* (CLRV) causes a massive outbreak in birch (*Betula* spp.) trees throughout Finland. Number of symptomatic trees has steadily increased in the last 10 to 15 years in whole country. Infected trees seem to be much commoner in constructed environments than in forests. Locally up to over 70% of the trees show symptoms of birch leaf-roll disease (CLRV).

CLRV symptoms are rather clear to excogitate to a positive result; however many factors in the visual surveys weaken the accuracy. Once the symptoms indicate CLRV being involved in the disease, generally, they can be recorded in the coming years, too. Recognition of the symptoms is easiest, when they are strong and low-located. In case of minor symptoms, e.g. one or a few branch tips or short shoots in the canopy only are symptomatic; accuracy of the assessment weakens markedly. If these symptoms are far up in the canopy, also weather conditions affect inspection significantly. In case of currently very minor symptoms the tree may be symptomless next year, possibly related to a cool summer. Further assessments may be biased due

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to other agents with resembling symptoms. Last but not least, human influence on the assessment result is clear. Factors affecting results of visual surveys are introduced and their role discussed.

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Cherry leaf roll virus (CLRV) - a generalist among plant viruses infecting woody hosts

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Cherry leaf roll virus (CLRV) is a worldwide occurring plant virus with an exceptional wide host range, so far comprising 33 plant genera, predominantly deciduous and stone fruit trees and shrubs. The fact that consistently new host species are detected suggests that the host range might be much larger than described so far.

Among natural hosts CLRV is most abundant in birch species (*Betula* sp.), black elderberry (*Sambucus nigra* L.), English walnut (*Juglans regia* L.) and sweet cherry (*Prunus avium* L.). The most recent first descriptions of CLRV host plants being wild potato (*Solanum acaule*) in Peru and nasturtium (*Tropaeolum majus*) from the sub-antarctic Amsterdam Island, apple (*Malus* sp.), Kiwi fruit (*Actinidia deliciosa*) and Hydrangea (*Hydrangea macrophylla*) from New Zealand and Australia, respectively.

The ecologic and economic impact of CLRV is reflected by increasing demands on CLRV monitorings in stone fruit production areas and during sanitary production of propagation material.

Consequently, there is a strong need for intensive studies on the evolution, genetic adaptability of CLRV to specific hosts, and on changes in its virulence. Moreover, the natural modes of transmission have to be elucidated, especially a putative vector transmission.

P VIRUS 54

Occurrence of a new recombinant begomovirus species infecting tomato crops in Al Batinah region of Oman

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Tomato (*Solanum lycopersicum*) is the most important vegetable crop and the second most important agricultural crop, after date palm, in the Sultanate of Oman. Whitefly-transmitted geminiviruses (family Geminiviridae, genus Begomovirus) causing tomato leaf curl diseases (ToLCD) are a major constraint for tomato cultivation worldwide. The aim of this study was to screen the presence of different begomoviruses and their associated satellites on tomato crops grown in commercial farms in Al Batinah region of Oman. Leaf samples were collected from nine tomato plants and total nucleic acid extracts were resuspended in sterile distilled water and stored at 20°C. PCR positive samples were subjected to rolling circle amplification (RCA) to amplify circular DNA using the Illustra TempliPhi 100 Amplification kit (GE Healthcare Bio-Sciences). The resulting linear DNA was digested with restriction endonucleases to obtain DNA fragments of 2.8 kb or 1.4 kb, which were cloned into the pUC19 vector. Clones were sequenced in both orientations using the primer walk strategy (Macrogen Inc.). Phylogenetic relationships were inferred using the neighbour joining method with bootstrapping. Recombination analysis was carried out using SIMPLOT v. 3.2 and the recombination detection program, RDP v. 3. All nine tomato samples were found to be positive for the presence of begomoviruses by PCR-mediated diagnostics and were used to amplify circular DNAs by RCA. The full-length sequences of the five putative begomovirus clones from tomato were determined to be between 2753 and 2757 nt in length. Alignment of the five sequences showed them to share >97% sequence identity. This indicates that the five tomato clones represent a single species based on the present species demarcation threshold for begomoviruses. An initial comparison of the sequences obtained here to all sequences available in the GenBank databases using BLASTN showed the highest levels of sequence identity with isolates of Tomato leaf curl Oman virus (ToLCOMV). Additional pairwise sequence comparisons showed the five sequences to have 85.8-88.6% nucleotide sequence identity with ToLCOMV, followed by 77.4-83.8% identity with Croton yellow vein virus. These results indicate that the five isolates represent a new species in the genus Begomovirus, based on the 89% species demarcation threshold for begomoviruses. The name Tomato leaf curl Barka virus (ToLCBrV) is proposed for this newly identified species. The newly identified virus, ToLCBrV, at this time occurs across a relatively narrow geographical area. The focus of future studies will be monitoring the spread of this virus in Oman and determining whether the virus evolves further. With the diversity of begomoviruses now known to occur in Oman, further recombination is a distinct possibility.

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P VIRUS 55

Assessing the ability of optical methods to detect early *Citrus tristeza virus* infection

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Citrus plants are affected by *Citrus tristeza virus* (CTV), the causal agent of Tristeza. This virus that belongs to the genus *Closterovirus*, is transmitted when infected material is used for grafting and by some aphid species and has already caused devastating epidemics. The outcome of the disease is linked to the CTV isolate and the variety of the infected citrus scion and rootstock, resulting generally in three distinct syndromes, seedling yellows, quick decline and stem pitting. Plants infected with severe CTV isolates must be eradicated. It is therefore crucial to detect infected plants in early stages of the infection in order to control virus dissemination and to protect regions where severe isolates of CTV are not established. Current detection systems for CTV infection include ELISA and PCR which are time-consuming and expensive techniques. Effective detection of the virus in a production setting needs a rapid and inexpensive diagnosis test. The present study aimed to assess the ability of optical methods to detect early viral infection in 'Valencia late' orange (*Citrus × sinensis* (L.) Osbeck) and 'Fina' clementine (*Citrus reticulata* Blanco) by multivariate analysis of reflectance and transmittance spectra in the visible and near infrared and chlorophyll fluorescence data. Healthy and infected plants with the severe CTV isolate T318A, growing in an insect-proof greenhouse, were assayed for the presence of the virus by IC/RT-PCR, RT-PCR and real-time. Reflectance and transmittance spectra of selected leaves were acquired through portable spectrometers. Fluorescence data was acquired using a portable fluorometer. The measurements were performed monthly over 9 months. In this communication we present the first results conveyed by multivariate analysis of the spectra. Standard unsupervised classification methods such as Principal Component Analysis or clustering show promising results by yielding a correct separation of classes (infected vs. non-infected) for some of the sample times but not for others. In order to improve the classification data fusion of spectral and fluorescence data was performed using previously described procedures [1]. Finally we discuss optimization of the measurements directed at obtaining reliable results under field conditions.

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Weeds

P WEEDS 1

Herbicidal activity of *Asphodelus microcarpus* against selected weed species (*Chenopodium album*) of wheat (*Triticum aestivum*)

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The current worldwide demand for cheaper and more environmentally-friendly weed management technologies have motivated a considerable number of studies on the allelopathic potential of some plant species as a resource for weed control in crops. The main objective of the present study was to evaluate the bio-herbicidal potential of *Asphodelus microcarpus* L. (AM) on *Chenopodium album* L. (CA); a major pest of wheat (*Triticum aestivum* L., TA). This research was conducted to study the allelopathic effects of AM crude powder on some growth parameters (fresh weight, dry weight & length of shoots and roots) and photosynthetic pigments of the selected weed species; CA and the crop species; TA in both mono and mixed cultures. Moreover, some chemical constituents (soluble protein, free proline and soluble amino acids) were determined in TA.

The results showed a significant reduction in plant growth parameters in both CA and TA, with considerably stronger allelopathic effects on the growth of CA as compared with TA in the presence of different concentrations of AM. Photosynthetic pigments in CA were also significantly decreased. The organs length of CA under control was lower in mixed than monoculture. This may be an indication of the allelopathic potential of TA on CA. The results also indicated that, proline and amino acids in TA were accumulated with significant level under the allelopathic effect of AM.

In this respect, TA plants have more tolerance and resistance to the different allelopathic treatment when compared with CA especially in monoculture. The study suggested the suppressive potential of allelopathic plant against selected weed species, and offered promises for their usefulness as a tool for weed management.

P WEEDS 2

Effect of Nitrogen Rates on Canola (*Brassica napus* L.) and Wild mustard (*Sinapis arvensis* L.) Competition

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In order to study the effects of wild mustard density on grain yield and yield components of canola cv. Hyola 401 under different rates of nitrogen fertilizer, a field experiment was conducted in 2011-2012. The layout was split-plot in a completely randomized block design and three replications. Main-plots included four levels of nitrogen (0, 70, 140 and 210 kg ha⁻¹) and sub-plots consisted of different wild mustard densities (0, 6, 12, 18 and 24 plants m⁻²). Results showed at densities of 6, 12, 18 and 24 wild mustard.m⁻², grain yield losses were 15.5%, 36.7%, 47.4% and 58.6%, respectively. Grain yield loss was due to reduction in number of grains per plant and 1000-grain weight. Agronomic N use efficiency (ANUE) of canola decreased as the weed density increased. The highest ANUE was obtained in 70 kgNha⁻¹, without weed interference. The lowest ANUE was in 210 kgNha⁻¹ and 24 wild mustard plant.m⁻² treatment. Generally, application of 140 kgNha⁻¹ lead to higher competitiveness and lower grain yield loss in canola compared with 0 kgNha⁻¹, whereas, in 210 kgNha⁻¹, the negative impact of 12 wild mustard.m⁻² and above on canola grain yield, was higher than other N treatments.

P WEEDS 3

Does Salinity Enhance Allelopathic Effects of *Tribulus terrestris* L. in Watermelon Agroecosystems at Nobarria, Egypt?

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Field observations at year 2014 showed that growth and yield of watermelon at Nobarria district, Egypt was highly affected by some unidentified stressful conditions. Presumably, the problem was confined to a large extent to the two combined factors; soil salinity and suppressive effects of *Tribulus terrestris* L. (a common weed dominant in watermelon fields in the study area). Field and laboratory experiments were carried out in summer 2014 to confirm the preceding hypothesis.

The interaction between the two factors was found to be harmful to the watermelon plant. Field experiment revealed that phytomass and leaf area index (LAI) of the study plant were significantly reduced in the vicinity of *Tribulus terrestris* L. (poor weed management) than in good weed management. Likewise, in laboratory test the interaction between both salinity and allelochemicals watery extracted from *Tribulus* plant severely affected germination efficiency, hypocotyl-radicle length and germination index of watermelon compared to the effect of just one factor.

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In conclusion, *T. terrestris* had a considerably suppressive effect on the growth and yield of watermelon, which was increased under slight or moderate salinity. Importantly, weed management in desert agro-ecosystems is an essential strategy to avoid a wide array of interactions between crop-weed from one side and weed-external climatic and edaphic factors from the other side.

P WEEDS 4

Selective Growth inhibitors in *Salvia syriaca* L.

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Syrian sage, *Salvia syriaca* L. (family Labiatae), is a common perennial weed in wheat fields in Jordan. As part of our research on identifying growth inhibitors in plants, we tested the growth inhibitory effects of the aqueous weed extract on different plant species from different botanical families.

Methods: Syrian sage was collected near Al-Kastal south of Amman, Jordan.

Extraction, isolation and identification- published online in Natural Product Research (2014)

Testing- A volume of 10.0 mL of 5 compounds obtained in enough quantities from the weed, was added to each filter paper in a Petri dish containing 10 surface sterilized seeds of the tested plant species. The compounds were tested for their inhibitory activity on most sensitive plant *Wascana* wheat cultivar (*Triticum aestivum* CV *Wascana*), was considered for bio assaying pure compounds at 200 ppm concentration. Each experiment was carried out for two weeks in a controlled chamber with 12 hrs. day and 12 hrs. night regime, temperature was set at 20+/- 1 C. Seedling development i.e. germination percentage, shoot and root lengths were recorded.

Results: Oleanolic and ursolic acids showed remarkable growth inhibition of wheat seedlings as they reduced the lengths of shoots and roots of wheat seedlings when tested at 0.2 mg. L⁻¹ (200ppm). The compounds; 3β hydroxy-11 α-methoxy-17, 22-seco-17(28), 12-ursadien-22-oic acid (a), 1 β, 3 β dihydroxy-11 α methoxy-17, 22-seco-17(28), 12-ursadien-22-oic acid (b), and 1β, 3β-11 α-trihydroxy-17, 22-seco-17(28), 12-ursadien-22-oic (c), did not show growth inhibitory activity on the test plant.

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P WEEDS 5

Field performance comparisons between different tillage implement equipped with herbicides applicator

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Afield experiment was conducted to compare the performance of different tillage implements equipped with herbicide applicator below the soil surface. In this study triflan herbicide was along with three types of inject implements (made, modified and combined with the plows) included: moldboard plow, chisel plow and sweep plow, and two tillage depths 10and 20cm were used in this experiment for weed control. The properties which studied in this research included: Practical Productivity, field efficiency and the percentage ratio of weed growth inhibiting. The experiment was executed according to a split plot design under randomized completed block design (RCBD) with three replications. Least significant differences (L.S.D) were used to compare the means of treatments at 0.05 levels.

The results were as follows:

The interaction between the inject implement with chisel plow and depth 10 cm lead to a significant increasing in field efficiency of injection implement, 77.000 % . The interaction between the inject implement with chisel plow, and depth 10 cm lead to a

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significant increasing in Practical Productivity 0.676. The interaction between the inject implement with sweep plow, and depth 10 cm lead to a significant increasing in percentage ratio of weed growth inhibiting, 94.667 %.

The success of the use of implementry manufactured in the jungles of herbicide and pesticides injected below the soil surface.: We recommend using the plow with the underlying implement to pump the pesticide under the surface 10 cm soil depth and the speed 7.00 km / h in the inhibition of the bush.

Sources: Bailey , W.A. Herbicide - based weed management systems for potato (Solano tuber sum) and heat (Tritical maestivum) and growth and reproduction doractevistic of smooth digital litrary and ordices.,25002.

Figure 1

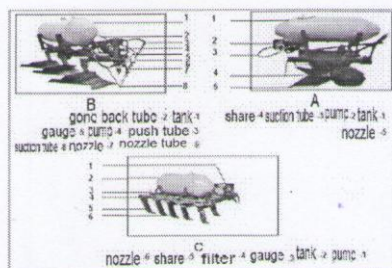


Figure (1) a- Injection implements on sweep plow b- Injection implement on moldboard plow c- Injection implements on chisel plow

Figure 2

Table (3) the Effect of Inject Implements types, and Injection Depth on the Percentage of Weed Growth Inhibition

Type Inject implements on the Plows	Inject Depth cm		Inject Implements Mean
	10	20	
On the Moldboard Plow	0.333	0.310	0.321
On the Chisel Plow	0.676	0.600	0.638
On the Sweep Plow	0.476	0.443	0.460
LS D= 0.05	0.0064		0.0045
Mean of Inject Depth	0.495	0.451	
LS D= 0.05	0.0044		

P WEEDS 6

Herbicidal Activity and Fermentation Culture Condition of PA-2

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(Question)Strain PA-2 was isolated from diseased poplar leaves in Ping An, Qinghai Province,China. Herbicidal activity and fermentation culture condition of PA-2 were studied. (Methods)Efficacy of of PA-2 for the control of different weeds and safety to crops were evaluated by spraying the fermentation broth on 2-4 leaves stage of plants.The optimalculture conditions were tested by liquid and solid fermentation of potential biological agent. The strain was identified by culture characters and 16S rDNA sequence analysis.(Results)PA-2 showed high herbicidal activity to different weeds after inoculation. The fresh weight reductions of PA-2 to the weeds seedlings of *Galium aparine* L. var. *tenerum* Reichb., *Chenopodium album* L., *Malva crispa* L., *Polygonum lapathifolium* L., *Avena fatua* L. were 87.25%, 78.46%, 82.25%, 62.11%, 80.27% respectively. Response of PA-2 to inoculated weeds ranged from light symptoms to 100% mortality. No symptom were observed to *Triticum aestivum* L., *Vicia*

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faba L., *Hordeum vulgare* L., but light spots were exhibited to *Brassica napus* L. and *Pisum sativum* L.. PA-2 could be used as biological control agent of viable alternative to suppress weeds. The optimum carbon of PA-2 was glucose, the optimum nitrogen is soybean meal, the optimum solid substrate is wheat straw. The optimum initial moisture content of PA-2 was 25%, the seed age is 96h, the optimal temperature is 27 °C and the optimum inoculation amount is 20%. According to its culture characters and 16S rDNA sequence analysis, the strain was identified as *Aureobasidium pullulans*.(Conclusions)PA-2 could be a potential microbial herbicide for control of target weeds in the fields of *Triticum aestivum* L., *Vicia faba* L., *Hordeum vulgare* L..

P WEEDS 7

Screening of Lamb's quarters, common groundsel and curly dock population for possible resistance to conventional herbicides in sugarbeet fields of Isfahan

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In order to evaluate the resistance of Lambsquarters(*Chenopodium album*),Curly dock(*Rumex crispus*),Ragwort groundsel(*Senecio spp*) to chloridazon (pyramin), met amitron(Goltix) , phenmedipham (Betanal) ,desmedipham(Betanal AM) herbicides, greenhouse studies were conducted in 1390. Greenhouse experiments were conducted as a randomized design with three replications .In greenhouse screening experiments, biotypes of Lambsquarters, Curlydock, Ragwort groundsel treated during 3-4 leaves stage using the recommended dose of herbicide .visual phytotoxicity rating ,the percentage of survived plants ,and relative dry weight (percentage of control) were used to evaluate seed population response to herbicide treatment. The result of experiments showed that biotypes of lambsquarter ,curly dock are suspected to herbicide resistance.

P WEEDS 8

Effects of seed position along height of mother plant on seed heteroblasty of *Chenopodium album* and *Avena fatua*

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This study was conducted in agriculture college of in 2011. In laboratory and greenhouse, germination and seedling growth studies were made on seed harvested from different seed positions and height of mother plants of London rocket by complete randomized design with four replications. Treatments consisted of three heights of mother plant (40-60, 60-80, 80-100 cm) and three seed positions along the inflorescence.

The results showed that seed germination rate in different parts of two weeds , *Chenopodium album* and *Avena fatua* (initial , middle and end), there was a significant difference and the rate of germination in *Chenopodium album* is end and in *Avena* is more in initial and end of it. Also with compare treatment means in two weeds , there are significant difference at 5% .

P WEEDS 9

Interference of allelopathic wheat with different weeds

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In spite of increasing knowledge of allelopathic wheat as an efficient component involved in weed management, relatively little is known about its impact on a broad spectrum weeds either independently or synergistically with belowground interactions. Accordingly, we selected 38 weeds occurred in wheat fields to examine their performance mixed-culture with allelopathic wheat, and to quantify allelochemical 2,4-dihydroxy-7- methoxy-1,4-benzoxazin- 3-one (DIMBOA) of wheat in response to coexisting weeds in relation to weed-suppressive effect. Furthermore, the weed-suppressive effect and the DIMBOA production in the absence and presence of root-root interactions by means of an experimental design with or without root segregation were evaluated. Subsequently, there were substantial differences in weed biomass and DIMBOA concentration in wheat-weed coexisting systems. Among 38 weeds, 9 weeds were inhibited significantly by allelopathic wheat but other 29 weeds were not. DIMBOA levels in wheat varied greatly with weed species. There was not a significant relationship between DIMBOA levels and weed-suppressive effects. Root segregation led to great changes in weed inhibition and DIMBOA level. Compared with root contact, the inhibition of 8 weeds was lowered significantly while significant increased inhibition occurred in 11 weeds with an increased DIMBOA concentration under root segregation. Furthermore, the production of DIMBOA in wheat was induced by the root exudates from the weeds. The results suggest that the interference of allelopathic wheat with weeds not only is defined as

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the specificity of weeds but also depends on root-root interactions. In particular, allelopathic wheat may detect certain weeds through the root exudates and respond by increased the allelochemical, resulting in weed identity recognition. The occurrence of chemical-identity recognition in interference of allelopathic wheat with weeds, as well as a further understanding of its potential mechanisms and implications in cropping systems, can assist in developing new environmentally safe weed control strategies for sustainable agriculture.

P WEEDS 10

Customized herbicide use in winter wheat in a crop rotation experiment

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Targeted decisions in herbicide use play a significant role in concepts for integrated weed management (IWM). Targeted here means to react on the actual weed infestation with a herbicide decision which reaches the targeted efficacy but avoid “overkill” of weeds as much as possible. To enable quick and repeatable decisions a heuristic which counts for the weed infestation level in terms of species and abundances and a targeted control efficacy was developed. This heuristic was applied to weed control decisions in winter wheat in different crop rotations with energy crops. We report about the dimension of the resulting herbicide use in these crop rotation experiments.

The field experiments are conducted on two sites in Germany: One site is in the North-East with a coastal climate and a sandy soil (Rostock, set-up 2008). The second site is in the center of Germany with a continental climate and a fertile soil (Göttingen, set-up 2009). Three crops, winter oilseed rape (OSR), winter wheat (WW) and maize, are combined in four crop rotations in a way that results in different risks for crop health. Winter wheat is cropped in three rotations: every second year with OSR, every third year with maize and OSR and twice in four years with maize and OSR in between. The factors crop rotation and herbicide intensity (HI) are varied in a split-plot-design with 4 replicates on the treatment level. Each crop of all rotations is cultivated each year. Herbicides are used in the three intensities: a moderate herbicide use in one treatment (HI) is increased (HI+) and in another reduced (HI-). The heuristic enables to underpin these described levels with reproducible decisions. While the weed part is represented by the counted weeds in the wheat crop, the control part is determined by minimum targeted efficacies of 85% in HI, 100% in HI+ and 60 % in HI-. The weed infestations are expected to vary due to crop rotation. In the heuristic weed infestations and targeted efficacies are bridged by ranking the occurring weed species for their damage potential. Thus, reduced and augmented control intensity is realized via controlling species and leaving other uncontrolled.

The resulting herbicide intensities of two cropping years (2013 and 2014) are measured with two indicators for pesticides use: the Treatment Index (TI) and the Active Ingredient Frequency (AIF).

P WEEDS 11

Control and Cross-Resistance of Barnyardgrass to ALS- and ACCase-Inhibitors in Rice Field, Korea

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Barnyardgrass (*Echinochloa crus-galli* var. *crus-galli*) is the most difficult-to-control weed that infests rice fields in Korea. The objectives of this research were to confirm ALS(acetolactate synthase)- and ACCase(Acetyl-CoA carboxylase)-inhibiting herbicide-resistant barnyardgrass in Korea and to determine sensitivity and efficacy of rice herbicides applied for control of resistant and susceptible barnyardgrass biotypes. The putative seeds of ALS- and ACCase-resistant barnyardgrass biotype were collected from rice fields in fall 2010. The response of barnyardgrass biotypes to 10 rates (0 to 10×) of ACCase inhibitors, cyhalofop-butyl and metamifop, and ALS inhibitors, priminobac-methyl, penoxsulam and flucetosulfuron, was evaluated in a dose-response bioassay in a greenhouse. On the basis of the values at at GR₅₀(concentration of respective herbicides required for 50% inhibition of dry weight),the analysis showed about 19 to 42-fold resistance depending upon the type of ALS- and ACCase-inhibiting herbicides being investigated and susceptible biotype used for comparison. The resistant biotype had a reduced sensitivity to ALS- and ACCase-inhibiting herbicides. These results suggested a cross-resistance between ALS- and ACCase-inhibiting herbicides that resulted ineffectiveness for control of barnyardgrass. Barnyardgrass biotypes were effectively controlled(≥ 90%) with mefenacet, and fentrazamide by 2 leaf stage, whereas oxadiazon, thiobencarb and butachlor provided over 90% control by 1 leaf stage of the resistant biotype

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High dominance among ground cover plants and the high weed suppression ability of birdsfoot trefoil (*Lotus corniculatus* L. var. *corniculatus*)

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Introduction: For unused agricultural or industrial land, it is very important to prevent the germinating and growing of weeds to keep the ground looking good. Many kinds of pastures are utilized as ground cover plants to achieve this. However, this causes a big problem as it costs large amount of money to manage and control the weeds that invade the land.

Objectives: We examined 25 species to select the plants with the highest weed control ability, in order to manage the unused land as a green area at as low a cost as possible.

Materials and methods: 14 cold region gramineous grasses, 4 warm region gramineous grasses, 4 leguminous grasses and 3 landscape plants were seeded independently, selected as superior species by their cover degree, plant height and weed suppression rate. We also tested mixed seeding using superior species, and investigated their competitive relationship and weed suppression rate. Dominant species in mixed seeding tests were analyzed with the sandwich method to see whether that dominance was due to an allelopathic factor.

Results: Creeping bentgrass, Kentucky bluegrass, chewing fescue, creeping red fescue, rat-tail fescue and tall fescue as cold region gramineous grasses, centipede grass, carpet grass and bermuda grass as warm region gramineous grasses, birdsfoot trefoil and white clover as leguminous grasses and dichondra as a landscape plant were selected as the superior species.

In mixed seeding tests, birdsfoot trefoil showed perfect dominance against almost all cool region grasses, white clover and dichondra. It divided the cover degree with creeping bentgrass, centipede grass and carpet grass. In both cases, test plots including birdsfoot trefoil showed very high weed suppression rates. Radicle extension rate of lettuce seedlings by the sandwich method among superior species was nearly 0% for birdsfoot trefoil, notwithstanding centipede grass which, having revealed an allelopathic efficiency, showed around 50%.

Conclusion: The invasion into a birdsfoot trefoil field is thought to be easy for weeds because birdsfoot trefoil's germination is slow and its leaves fall in winter. So its high weed suppression ability is thought to be due to its strong allelopathy. We have to think about the possibility that the ground cover plant itself becomes an invader before the use of a too strong plant.

P WEEDS 13

Influence of crop rotation, nitrogen fertilization and herbicide use on weed occurrence in a long term field trial in Germany since 1998

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Introduction: Changes in weed community takes long time, this is true for the number of emerging weeds and also the species. Crop rotation, the nitrogen amount and last but not least the use of herbicides influences the number of weeds and also the species.

Objectives: Long-term fields gave the opportunity to find out what will happen to the weed community not only in some years but in a longer run.

Materials and methods: Since 1998 a long term field trial regarding different crop rotations, application of nitrogen fertilizers and herbicide use are conducted at the Julius Kühn experimental field in Dahnsdorf. The field is located in the state of Brandenburg (Germany) about 50 km South-West from Berlin. The field trial contains two crop rotations (1) peas - winter barley - winter rye - white clover - winter barley - winter rye and (2) continuous cropping of winter rye. In both crop rotations the experimental treatments are: (a) no nitrogen fertilizer and no herbicide, (b) no nitrogen fertilizer but herbicides (c) nitrogen fertilizer but no herbicide and (d) nitrogen fertilizer and herbicide.

Weeds are counted before treatment in autumn and after the herbicide application. *Apera spica-venti* panicles are counted short before harvest.

Results: The influence of the crop rotation regarding the weed infestation was strongest for treatment (a) and declined with fertilizer and herbicide use. Continuous cropping of winter rye (crop rotation 2) promoted *Vicia hirsuta* and *Cirsium arvense* while crop rotation (1) caused more *Matricaria* spp. *Apera spica-venti* is promoted through crop rotation (2), especially in treatment (c).

The difference in weed infestation between crop rotation (1) and (2) decreased with nitrogen and herbicide use. In the treatment (d) only a small difference was found between the two crop rotations (1) and (2) for winter rye. The impact of crop

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rotation to weed infestation and highest if no fertilizer and herbicide were used (treatment a). Regarding winter rye continuous cropping (2) is has nearly the same number of weeds before treatment as the crop rotation (1) in treatment (d).

Conclusion: These rotational impacts weakened if nitrogen fertilization and herbicide are used.

P WEEDS 14

Allelopathic Potential of Saffron (*Crocus sativus*)

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Introduction: Although saffron (*C. sativus*) has been shown to possess many therapeutic properties, its effect on other plants is not yet fully understood. As bioherbicides, allelopathic plants might be used as sources of new natural products with herbicidal activities.

Objectives: The aim of this study is to identify the volatile allelochemicals of saffron and to obtain their allelopathic activity in order to facilitate plant protection strategies.

Materials and methods: Sandwich method (SW) and dish pack method (DP) were used as bioassay methods to evaluate allelopathic activity of style and stigma of saffron flower (1). To evaluate the effective concentration (EC_{50}), 10 ml of 0.75% agar solution was added in the glass vial (20 ml). After solidification, 5 seeds of *L. sativa* were accurately placed into each vial. Double-tipped cotton swab (7.6 cm) was cut in half and then was vertically inserted into the agar on the periphery. Authentic amounts of safranal were dissolved in hexan and appropriate amount was added on cotton swab using glass microcapillary syringe. Glass vial was immediately closed by pressure cup. The percent of inhibition was then calculated based on the actual concentration in the vial air using head space GCMS. In all methods, plants were incubated at 25°C and dark condition then hypocotyl and radicle of tests plant were measured after 3 days.

Results: Results showed that stigma of Saffron flowers could strongly inhibit the radicle growth of Lettuce seedlings in both bioassay methods (100% and 59% respectively). Safranal and isophorone were identified as the main compounds in head space GC-MS analysis of saffron (stigma). Also, pure safranal strongly suppressed the growth of the germinated seeds of lettuce. Moreover, the (EC_{50}) of safranal was 1.2 µg/L when used in gas phase. The EC_{50} was calculated based on actual concentration of safranal. Furthermore, result showed that EC_{50} for methanol extract of stigma of Saffron was 1.3 mg/ml.

Conclusion: To sum it up, this is the first report which revealed that safranal has a strong inhibitory effect on plant growth. It seems that safranal is the main volatile allelochemical of saffron stigma with strong inhibitory activity and could be a candidate for development of bio-herbicides and to facilitate plant protection strategies.

References: Fujii Y, Matsuyama M, Hiradate S, Nakatani K. (2000) Developments of new bioassay and analysis method for volatile allelochemicals. *Journal of Weed Science and Technology*, 45:80-81.

P WEEDS 15

Field evaluation of sulfonylurea herbicides against the broad leaf weed, *Potamogeton distinctus* A.Bennett, in transplanted rice (*Oryza sativa*) in Bhutan

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Introduction: A broad leaf weed, *Potamogeton distinctus*, is the most serious weed of rice in Bhutan. With no chemical weed control strategies available, farmers rely on intensive hand weeding to manage this weed which adds up high labour costs. Up till now, no studies have tested or documented the effectiveness of herbicides against this weed in Bhutan.

Objectives: The main objective of this trial was to evaluate the efficacy of three sulfonylurea herbicides namely, Cormix (Metsulfuron Methyl 10% + Chlorimurion Ethyl 10% WP), Sunrise (Ethoxysulfuron 15 WDG) and Kellion (Orthosulfamuron 50 WG), against *P. distinctus* in transplanted rice and to provide a cost effective control strategy against this weed.

Methods and materials: Field evaluation was conducted from June 2014 to November 2014 in three locations across Thimphu. The fields were treated with the herbicides at 3-5 DAT (days after transplanting). Weed sampling was carried out (after 25 and 40 DAT) to determine the dry matter weight of *P. distinctus*. At harvest, yield difference between the treated and the control plots was recorded. Weed control efficiency (WCE) was determined for all the treatments.

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Results: A *t*-test comparison showed a significant difference in the mean dry matter weight between the treated and control plots for all the treatments. All the herbicide treated plots contributed to higher yield performance compared to control. In location 1 (Kabesa), the WCE for Kellion and Sunrise was 94.87% and 93.04% respectively. In location 2 (Genekha), the WCE for Cormix and Kellion was 75.63%, 78.08% respectively. In location 3 (Tendrelthang), the WEC for Cormix, Kellion and Sunrise was 73.95%, 88.99% and 93.17% respectively.

Conclusion: All three herbicides tested were equally effective in controlling *P. distinctus*. The sulfonylurea herbicides are characterized by broad-spectrum weed control efficacy at low application rates, good crop selectivity, and very low acute and chronic animal toxicity. Therefore, these sulfonylurea herbicides hold promise for use against *P. distinctus*.

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Herbicidal potential of essential oil of *Artemisia scoparia* against some weeds

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Artemisia scoparia (redstem wormwood; Asteraceae) is a faintly scented annual herb widely distributed through out the world. The plants are known for their essential oils that exhibit a wide spectrum of biological activities including antimicrobial, antibacterial, anthelmintic and fungicidal. It forms monospecific strands and does not allow other plants to grow in the vicinity, possibly due to release of certain inhibitors in the environment. We conducted a series of experiments to investigate the herbicidal potential of volatile terpenes emanated from *A. scoparia*. The volatile oil extracted from *A. scoparia* inhibited the emergence, growth and development of weeds namely, *Cassia occidentalis*, *Amaranthus viridis*, *Parthenium hysterophorus* and *Phalaris minor*. *P. minor* was the most sensitive whereas *C. occidentalis* was the least. The post-emergent application of *Artemisia* oil (2%, 4%, and 6%, v/v) on 6-week-old weed plants caused visible injury such as chlorosis, necrosis, wilting of plants and even death. Spray treatment of *Artemisia* oil resulted in a loss of chlorophyll content and cellular respiration thereby suggesting impairment of oil with photosynthetic machinery and energy metabolism. Further, oil treated seedlings of weeds, particularly *A. conyzoides* exhibited a rapid electrolyte leakage, higher contents of conjugated dienes and higher activity of lipoxygenase enzyme indicating membrane disruption and loss of integrity. The study thus concludes that *Artemisia* oil offers a good option for control of weeds and could be exploited as a viable component of integrated weed management under sustainable agricultural practices.

P WEEDS 17

Eucalypt Volatile Oil as an Alternative Tool to Manage Weeds

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The increasing demand of safe green products for sustainable agriculture has resulted in growing interest in many new ecofriendly products to control the weeds. Most of these environmentally safe weed control products are based on volatile / essential oils and their constituents. Eucalypt oils have long been known for their insecticidal and pesticidal activities; however, not much is known to evaluate their herbicidal activity. We, therefore, screened volatile oil from *Eucalyptus citriodora* (lemon-scented eucalypt) and *E. tereticornis* (red gum tree) for their possible herbicidal activity against three crop weeds such as *Echinochloa crus-galli*, *Phalaris minor* and *Ageratum conyzoides*. The volatile oils caused a strong inhibition of emergence and growth of weeds under laboratory and natural conditions. Pre-emergence application of oil significantly reduced the growth and development of the weeds. Post emergence application of eucalypt oil caused wilting, chlorosis, necrosis in weeds. At highest concentration, the plants were even killed due to loss of membrane integrity and severe ion leakage. In addition, the photosynthetic activity and chlorophyll content, energy metabolism of target weeds were severely affected and the effect was comparable to those of sprayed with conventional herbicide glyphosate. Further, the oil showed no residual activity in the soil. Based on these observations, it is concluded that eucalypt oil possess potential to suppress weeds thus can be used as bioherbicide or can be used in the plant based formulations for managing weed.

P WEEDS 19

Studied on the Safeners for Nicosulfuron in Maize

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Nicosulfuron is one of most popular used herbicides on corn in China. It might cause serious phytotoxicity in high temperature and dry weather. To identify the suitable safener, the 33mg/L of fenchlorazole ethyl, cloquintocet-mexyl, mefenpyr-diethyl, isoxadifen-ethyl or cyprosulfamide were used with nicosulfuron of 200mg/L, 400mg/L, 800mg/L. The fenchlorazole ethyl and mefenpyr-diethyl didn't reduce the phytotoxicity and the cloquintocet-mexyl showed relief effect only on low dosage of nicosulfuron. When the isoxadifen-ethyl was used, the inhibition of nicosulfuron on maize's weight were reduced about 36.53%, 12.67%, 17.26%, respectively. Compared to the nicosulfuron alone, the cyprosulfamide could increase the growth of maize about 55.57%, 38.84%, 39.98%. To confirm the result, the mixture of isoxadifen-ethyl or cyprosulfamide and nicosulfuron were sprayed during 3-leaf and 7-leaf stage of corn in the field. The mixture caused lower phytotoxicity degree or yields loss to corn than nicosulfuron alone, which showed the isoxadifen-ethyl or cyprosulfamide could enhance the safety and prolong the use stage of nicosulfuron on corn.

Figure 1

Table 1 The Influence of Safeners in Combination with Nicosulfuron on Fresh Weight of Maize^a

Treatment ($\mu\text{g/L}$)	Concentration ($\mu\text{g/L}$)	Weight ^b (g)	Inhibition (%)
Nicosulfuron	200	6.06 \pm 0.11 cE	85.69
Nicosulfuron	400	6.70 \pm 2.19 eE	84.16
Nicosulfuron	800	5.60 \pm 1.13 eE	86.76
Cyprosulfamide+Nicosulfuron	33+200	29.56 \pm 7.50 bB	30.12
Cyprosulfamide+Nicosulfuron	33+400	23.13 \pm 0.40 bcBC	45.32
Cyprosulfamide+Nicosulfuron	33+800	22.51 \pm 3.50 bcBC	46.78
Isoxadifen-Ethyl+Nicosulfuron	33+200	21.51 \pm 5.52 bcdBCD	49.16
Isoxadifen-Ethyl+Nicosulfuron	33+400	12.06 \pm 2.10 deCDE	71.49
Isoxadifen-Ethyl+Nicosulfuron	33+800	12.90 \pm 7.03 cdeCDE	69.50
Mefenpyr-Diethyl+Nicosulfuron	33+200	9.51 \pm 3.17 eCDE	77.51
Mefenpyr-Diethyl+Nicosulfuron	33+400	5.60 \pm 0.23 cE	86.75
Mefenpyr-Diethyl+Nicosulfuron	33+800	3.74 \pm 0.44 eE	91.17
Fenchlorazole Ethyl+Nicosulfuron	33+200	5.10 \pm 1.72 cE	87.95
Fenchlorazole Ethyl+Nicosulfuron	33+400	5.43 \pm 0.66 eE	87.16
Fenchlorazole Ethyl+Nicosulfuron	33+800	2.59 \pm 0.50 eE	93.88
Cloquintocet-Mexyl+Nicosulfuron	33+200	22.22 \pm 10.82 bcdBC	47.47
Cloquintocet-Mexyl+Nicosulfuron	33+400	7.91 \pm 3.33 eDE	81.30
Cloquintocet-Mexyl+Nicosulfuron	33+800	6.05 \pm 1.52 eE	85.70
CK		42.30 \pm 16.69 aA	

^a The 0.3mL mixture of safeners and nicosulfuron were dropped in the center of tender leaf at 3-leaf stage.

^b The fresh weight of maize acrial part were determined after 15d.

Figure 2



P WEEDS 20

The impact of Tillage Systems and crop sequence on Weed Incidence

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Conventional soil tillage is expensive and power-consuming. Attempts have been made to minimize tillage by replacing deep ploughing with shallow tillage. Reduced tillage has been found to improve agricultural sustainability; however, this technology usually causes increase in crop weed incidence. The objective of the present trial was to investigate the impact of soil tillage systems on weed incidence in different crop rotations. Field experiments were established at the Study and Research Farm "Peterlauki" of the Latvia University of Agriculture in 2008. The paper presents the results of studies carried out during the period 2010-2014. Two tillage systems (conventional tillage - plough tillage (0.22-0.23 m) with mouldboard plough, and reduced tillage - shallow (0.10-0.12 m) tillage with disc harrow) were compared in three different crop rotations: 1) winter wheat - spring oilseed rape - winter wheat - winter oilseed rape - spring barley; 2) winter wheat - winter wheat - winter wheat - winter wheat - spring wheat; and 3) winter oilseed rape - winter wheat - winter wheat - winter oilseed rape - spring wheat. Weeds were counted before the harvest of crops. Annual broadleaf weeds were found to be the most important group of weeds in all treatments. Comparison of the reduced and conventional tillage systems during the five investigation years showed that the average number of weeds in each of the three different crop rotations was respectively: 1) 33 and 16, 2) 47 and 29, 3) 33 and 18 plants m^{-2} . The reduced soil tillage significantly increased the incidence of weeds ($P < 0.05$), but the influence of crop rotation was not essential. On average, the reduced soil tillage increased the number of weeds for 33%. The method of soil tillage and the crop rotation slightly influenced the spectrum of weeds; however, further investigations are necessary to clarify these tendencies.

P WEEDS 21

Post emergence herbicides affect chlorophyll fluorescence in artichoke leaves (*Cynara cardunculus* L.)

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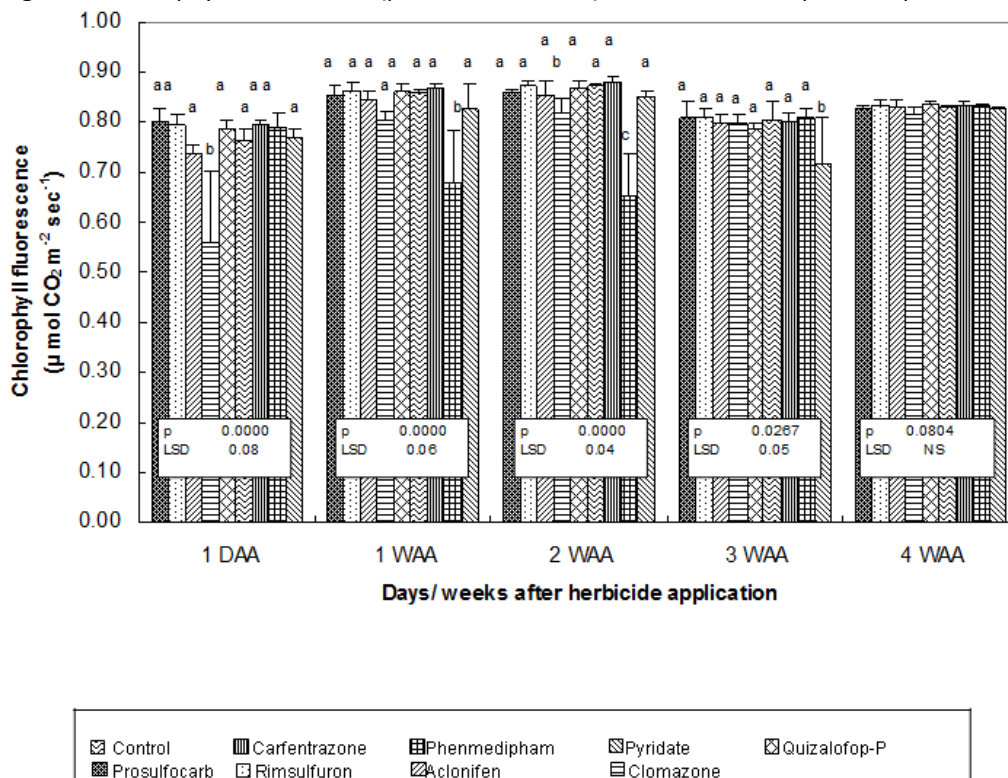
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Artichoke leaves are rich in polyphenols that are accredited to the nutritional and pharmacological belongings. Caffeoylquinic acids, flavonoids and sesquiterpene lactones are some of the important polyphenols in artichoke leaves.

Research project to study the impact of post emergence herbicides on chlorophyll fluorescence of artichoke leaves was (RCBD) with four replications. Gobbo di Nizza cultivar was sown manually in 25 cm P-P and 75 cm R-R distance. Eight herbicides (Carfentrazone, Phenmedipham, Pyridate, Quizalofop, Prosulfocarb, Rimsulfuron, Aclonifen & Clomazone) were used against a manually weeded control. Chlorophyll fluorescence data were recorded by using a portable Mini PAM.

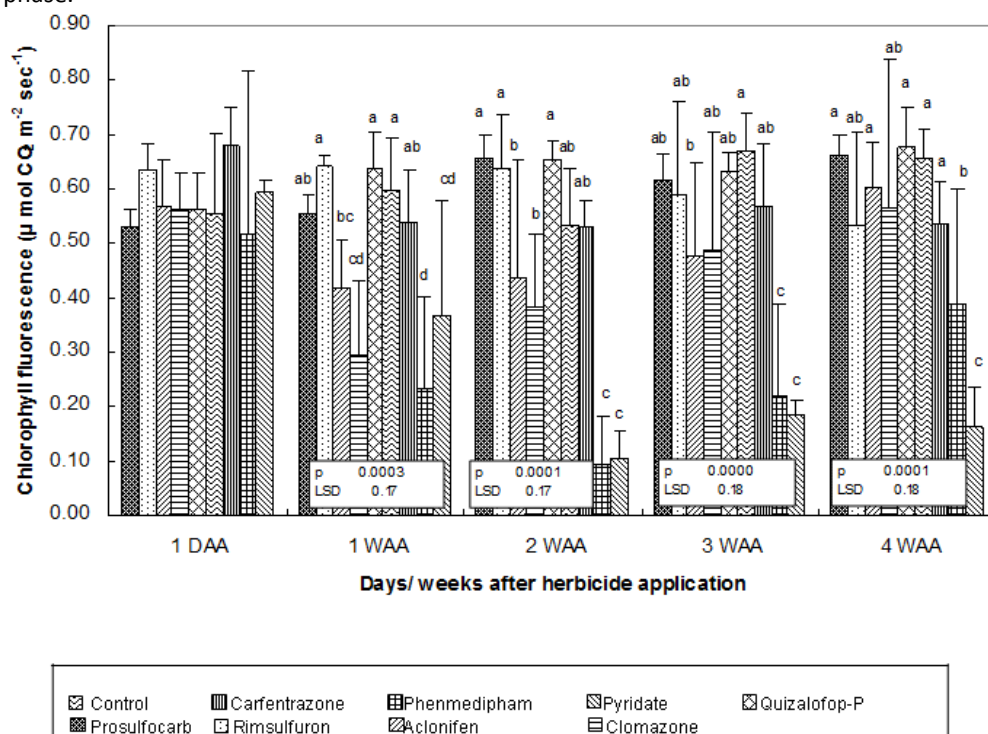
Chlorophyll fluorescence ($\mu\text{ mol CO}_2\text{ m}^{-2}\text{ sec}^{-1}$) in response to applied herbicides during first growth phase was significantly affected by the herbicides. Pyridate affected chlorophyll fluorescence adversely at 1 DAA, which was statistically lower than that of all other treatments. At 1 WAA Aclonifen affected the crop most adversely followed by Clomazone and Pyridate. Artichoke crop recovered against the adverse effects of Pyridate and Clomazone as visible by the chlorophyll fluorescence at 2 WAA. Chlorophyll fluorescence values at 4 WAA exhibit the recovery of the crop against adverse effects of herbicides.

Figure 1: Chlorophyll fluorescence ($\mu\text{ mol CO}_2\text{ m}^{-2}\text{ sec}^{-1}$) of artichoke in response to post emergence herbicides, 1st growth phase.



Minimum chlorophyll fluorescence at 1 DAA during second growth phase 2008 was obtained by the application of Aclonifen. At 3 WAA minimum chlorophyll fluorescence was observed for Aclonifen with a slightly higher one for Clomazone. At 4 WAA chlorophyll fluorescence started increasing for the herbicides showing adverse effect and kept getting closer to that of control depicting that the crop was not able to recover against the toxic effect imposed by a few herbicides particularly Clomazone and Aclonifen.

Figure 2: Chlorophyll fluorescence ($\mu \text{ mol CO}_2 \text{ m}^{-2} \text{ sec}^{-1}$) of artichoke in response to post emergence herbicides, 2nd growth phase.



Experimental study concludes that chlorophyll fluorescence can be used to detect the herbicide stress in artichoke and artichoke can recover this stress with the development stages depending on the severity of stress and prevailing environmental conditions.

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Molecular Identification, Characterization and Transmission Studies on Parthenium Weed (*Parthenium hysterophorus*.L) associated phyllody Phytoplasma in Pakistan

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Parthenium weed (*Parthenium hysterophorus* L.) is one of the 10 worst invasive, the most prevalent, most feared noxious invasive species widely distributed in both arable and grazing lands worldwide. Weeds are good source of pathogens that induce disorders in plant physiology, morphology and flower sterility. The phytoplasma are phytopathogenic wall less, phloem inhabiting bacteria that are transmitted by phloem sap sucking insect vectors. Phytoplasma infected parthenium weeds are characterized by excessive branching (witches' broom), reduced plant height and leaf size, as well as modification of floral structures into leaf-like structures (phyllody) and virescence that lead to sterility. In some countries, the spreading of *Parthenium hysterophorus* is considered to be controlled by using phytoplasma. During a survey conducted in 2012-2014, in Punjab, Pakistan, we observed phytoplasma associated diseases on *Parthenium hysterophorus* L. as well as on other important crops e.g. Sesame, Mung bean, Chick pea, Tomato, Potato, Brassica, Radish etc. The molecular confirmation of phytoplasma presence in infected parthenium weed samples was confirmed by Nested Polymerase Chain Reaction (PCR). Nested PCR product of 1.25 kb were amplified using the universal primers pair (P1/P7) followed by R16F2n. Then, amplified DNA fragment (1.25 Kb) was digested with endonucleases (Kpn I, Hpa II, Taq I, EcoR I) by following the Restriction fragment length polymorphism (RFLP) and confirmed the presence of phytoplasma group (16SrII) in infected samples. The study on transmission of parthenium associated phytoplasma through insect vector (*Orosius albicinctus*), dodder (*Cuscuta campestris*) and grafting showed that parthenium weed not only harbors phytoplasma in itself but is also the source of infection to other important agricultural crops. This is the first identification and transmission of phytoplasma associated with parthenium weed in Pakistan. Our results of transmission studies also suggest that Parthenium weed (*Parthenium hysterophorus* L.) should be managed by other strategies but not be controlled by using phytoplasma because it could be the source of infection to other important major crops.

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Allelopathic Effect of Some Plant Extracts on the Germination of Different Broad Leaved Weed Seeds

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The use of allelopathic components is one of the alternative weed management criteria. Redroot pigweed (*Amaranthus retroflexus* L.), common lamb's quarters (*Chenopodium album* L.) and camelthron (*Alhagi pseudalhagi* (Biev) Desv.) are common weeds that cause problems in agricultural and non-agricultural areas in Turkey. In this study, the effects of sugar beet root extracts, and leaves of wheat, walnut, above-ground organs of sugar beet and mugwort (*Artemisia vulgaris* L.) on the germination of *A. retroflexus*, *A. pseudalhagi* and *C. album* seeds were examined. The germination of *A. retroflexus* seeds was almost completely inhibited by the leaf extracts of wheat ($\geq 10\%$) and sugar beet (in all doses). With 5% and 10% doses of leaf extracts of walnut and the above-ground organs of *A. vulgaris* and the sugar beet root extracts partly was prevented the seed germination and completely with the other overdoses. Sugar beet leaves and root extracts of 5% dose completely inhibited the germination of *A. pseudalhagi* seeds. The above-ground organs of *A. vulgaris* and the leaf extracts of wheat was partly prevented the germination by 5% and 10% doses. On the other hand, 5% and 10% doses of above-ground organs of *A. vulgaris*, sugar beet and wheat leaf, also the root extract of sugar beet, inhibited the germination of test plant *C. album*. There was no germination at 20% and overdoses. By using the leaf extract of walnut, germination decreased depending on the doses and disappeared at 30% dose. According to results, the use of plant extracts on the germination of weed seeds was completely inhibited with some doses of the extracts. Possibility of use as biyo-herbicide studied extracts emerged. Also, effectiveness ratio on *A. pseudalhagi* control should be demonstrated by investigating shooting properties in different climatic area and soil types. This study was summarized from MS thesis of Canan Yurttas Kilinc

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P WEEDS 25

Effects of Millet (*Pennisetum glaucum* [L.] R. Br.) Root Exudates and Extracts on Early Developmental Stages of Sudan *Striga hermonthica* (Del.) Benth.

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Two types of laboratory experiments (*in vitro* and *in vivo*) were undertaken at the Faculty of Agricultural Sciences, University of Gezira, Sudan, to investigate the effects of millet (*Pennisetum glaucum* [L.] R. Br.) root exudates, root extracts on early developmental stages of *Striga hermonthica* (Del.) Benth. collected from different locations in Sudan. Collection of seeds was made in *S. hermonthica* endemic areas in Gadarif, Gazira and Kordofan. A total of fifteen *Striga* populations were collected. Twelve *S. hermonthica* populations, one each, were collected from under sorghum and three *S. hermonthica* populations, one each, were collected from under millet. The fifteen *S. hermonthica* populations were laid in completely randomized design (CRD) with three replications. *Striga* seeds and/or germilings were examined under a binocular for germination, haustorium initiation, attachment and penetration 24, 72, 144, 192 hour after initial incubation. Data were collected and subjected to analysis of variance (ANOVA). Means were separated for significant using Duncan's Multiple Range Test (at $p \leq 0.5$). The results revealed that, root exudates and extracts of all plants induced seed germination and haustorium initiation in *S. hermonthica*. However, the highest germination, haustorium initiation attachment and penetration attained by each of the *S. hermonthica* population, were on their respective hosts. These findings suggest the existence of both inter- and intra-crop specialization. Moreover, the results confirmed the existence of two strains of *S. hermonthica*, one specific to sorghum and the other, to millet.

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Enhancing cereal yield by using plant products as bioherbicides and non-host crop genotypes for suicidal germination to *Striga hermonthica*

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Striga hermonthica is a major biotic constraint to cereal productions in sub-Saharan Africa. The genotypic influence in stimulating *Striga* germination from twenty-seven non-host genotypes was evaluated as trap crops in bio-assay using the root-cut technique. These genotypes belonging to seven non-host crops were compared to *Striga*-susceptible sorghum S29 (control)

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to identify those endowed with a potential capacity to induce suicidal germination of *Striga* seeds. The 10% aqueous extracts of height local plants were also screened in bio-assay to evaluate their allelopathic properties on *Striga* seed germination elicited by GR 24. The genotypes of the non-host trap crops exhibited significant differences for their ability to stimulate the germination of *Striga*. *Striga* seed germination rates of at least 75% were recorded on nine cotton genotypes. *Striga* germination rates induced by both cowpea and groundnut genotypes were lower as compared to sorghum S29 (80%). Sesame genotypes induced *Striga* germination rates ranging from 16.1% to 27.3%, and the variety S42 led to the greatest rate. Among soybean genotypes, G.196 and G.197 significantly induced *Striga* germination with 11.9% and 24.4%, respectively, while Bambara nut genotypes KVS-075 and KVS-143 gave the highest rates of *Striga* germination. The rice bean genotype led to *Striga* germination rate of 6.6%. The 10% aqueous extract from *Eucalyptus camaldulensis* (roots) and *Lipia multiflora* (leaves) significantly reduced *Striga* seed germination with 86.3% and 46.5% inhibition rates, respectively. The 10% aqueous extracts of all plant species stimulated *Striga* seed germination. The most effective in stimulating *Striga* seeds were the 10% aqueous extracts from *E. camaldulensis* (leaves) and *Faidherbia albida* (bark) leading to *Striga* germination rates more than 50%. The 10% aqueous extracts from the four other ones significantly stimulated *Striga* germination and the rates varied between 25.2% and 48.1%. The prolonged use of non-host plants that produced stimulants or inhibitors of *Striga* germination may reduce or inactivate its seed-bank in the soil, respectively. Non-host crop genotypes that induced *Striga* seed germination rates of at least 10% may be recommended for use in cropping systems, particularly in an integrated management approach against *S. hermonthica*.

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Revision of the *Urophora xanthippe* species group (Diptera: Tephritidae)

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The genus *Urophora* Robineau-Desvoidy, 1830, with about 60 species, is one of the largest genera of the family Tephritidae in the Palaearctic Region. All species of known biology are associated with asteraceous plants and induce galls in their host plants (White & Korneyev, 1989).

Some *Urophora* species are potential agents to biological control of astraceous weeds; some species have been successfully introduced into the Nearctic Region for biocontrol of weeds (Turner, 1996 a&b). The *Urophora xanthippe* group of species can be recognize with other *Urophora* species with having yellow notopleura. This group including *U. iani* Korneyev & Merz, *U. impicta* Hering, *U. hermonis* freidberg 1974, *U. kasachstanica* Rikhter, *U. stalker* Korneyev and *U. xanthippe* (Munro). During studies on tephritid flies fauna in Iran, two previously undescribe species of *Urophora xanthippe* species group have been discovered. *Urophora* sp1 is similar with *U. iani* and *U. xanthippe* in having small body and wings (WL<4mm), strongly reduced wing pattern, yellow femora, differing only in aculeus tip with two pairs of distinct preapical primary steps and different host plant (in *U. xanthippe* with one pair of distinct steps and in *U. iani* with one pair of distinct primary and indistinct secondary steps). *Urophora* sp2 is resembling *U. impicta* in having large body and long wings, strongly reduced wing pattern and yellow femora and antennae, differing only in aculeus tip with two pairs of distinct preapical primary steps and different host plant. In addition the following synonymy is established: *Urophora impicta* (Hering, 1942) = *U. hermonis* Freidberg, 1974 new synonym.

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P WEEDS 28

Researches on the Dormancy Conditions of some Common Weeds Seeds in Turkey

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One of the most important factors affecting yield in agricultural production is weeds. As is known, weeds continually compete with crop plants in terms of water, nutrient, place and light. Maturation and germination are the sequential events in a plant's life. The interval between these sequential events can range from a few hours to long years. A seed may occasionally go

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dormant periods from maturity to germination in the negative environmental conditions. Dormancy is very important for the continuity of weed population and their adaptation to the environment.

With this research the germination-dormancy conditions of some common weeds that are widespread in the agricultural and non-agricultural areas in Turkey were examined. In the experiments as the materials, *Amaranthus retroflexus* L. (Redroot pigweed), *Chenopodium album* L. (Common lamb's quarters), *Alhagi camelorum* Fisch. (Camelthron), *Taraxacum officinale* Weber. (Dandelion), *Galium aparine* L. (Catchweed bedstraw), *Peganum harmala* L. (African rue) and *Heliotropium europaeum* L. (Common heliotrope) seeds were used.

To break the seed dormancy; Pre-soaking, Pre-washing, Pre-chilling, Pre-heating, Manual scarification and Removing fruit (just for *A. camelorum*) processes for the weed seeds were used. The process of manual scarification for *A. retroflexus*, *C. album* and *A. camelorum* (without fruit) seeds, pre-washing for *T. officinale* seeds and pre-soaking for *Galium aparine* seeds both for 24 hours, was increased the germination ratio according to the control application of the experiments. Statistically, this method was found different than the others although no statistical difference was found between the methods for the seeds of *P. harmala* and *H. europaeum*.

According to the results, it is necessary to weeds identify, seed biology and to determine of life forms and much more radical solutions for weed control in the cultivated and non-cultivated areas. Investigation of these characteristics has great importance in the basis of weed control programs.

This study was summarized from MS thesis of Huseyin Solak

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Survey and identification of common weeds associated with rice and vegetable production in DMMMSU-Institute of Agriculture, Nagtagaan Campus, Rosario, La Union

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Survey, collection and identification of weeds in the rice and vegetable production of the Don Mariano Marcos Memorial State University Institute of Agriculture- Nagtagaan Campus, Rosario, La Union, were done. Using quadrats measuring 1m x 1m and randomly placed weed- infested areas of each of the plantations, all the weed species therein were uprooted, cleaned, and separately placed in plastic bags. There were three control measures used, in the experiment, namely: manual method, mulching, and chemical method. Total area used was 160 sq.m. Measurement per treatment area was 10 sq. m.

Dominant weeds during the vegetable and rice production during wet season in Nagtagaan Campus, Rosario were *Digitaria sp.*, *Fimbristylis littoralis*, *Ageratum conyzoides*, *Cyperus rotundus*, *Digitaria sp.*, *Chloris sp.*, *Centrosema pubescens* and *Dactyloctenium aegyptium* were the dominant weed species.

On a per hectare basis, results showed mulching was found to be the most economical and beneficial weed control method. Total cost of weed control was P5,080.00 as against herbicide application with P7,224.00 and hand-weeding at P7,620.00. Weeds associated with rice and vegetable production areas in DMMMSU-IA Nagtagaan Campus economically reduced the income per unit area.

P WEEDS 30

Effect of water extracts of *Imperata cylindrica* on germination of some summer annuals

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Question and methods: There have been needs to explore new methods to control weeds. Allelopathy gives new opportunities to control weeds. The question is if *Imperata cylindrica* (IMPCY), which is an important weed species, can be used to control five summer annual weeds, *Amaranthus retroflexus* (AMARE), *Echinochloa colona* (ECHCO), *Physalis angulata* (PHYAN), *Portulaca oleracea* (POROL), and *Solanum nigrum* (SOLNI). Plant parts (below and upper ground) of IMPCY were collected in two different growth stages, vegetative and generative stages, dried, grounded and water extracts were prepared. Seeds of five species were placed in petri dishes and 10 ml of 2, 5 or 10 % of extract were applied. Petri dishes have kept in an incubator, which are adjusted 12 hours dark at 28°C and 12 hours light at 32°C. Percent germination against no extract for each species were calculated, transformed and subjected to ANOVA (P 0.05).

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Results: The effect of IMPCY extracts on weeds was different although all weeds were affected. Effect on POROL and AMARE was significant for rates, parts and growth stage without any interaction among them but for PHYAN and ECHCO effect was depended on three way interactions among rates, parts and stages. Extracts from upper ground parts collected at generative stage at 10 ml gave the highest inhibition for AMARE and POROL. Collecting at generative stage inhibited PHYAN and ECHCO seed germination more than collecting at vegetative stage. In addition the highest rate of extract gave the highest germination inhibition. Root extract inhibited more PHYAN seeds but stem extract inhibited more ECHCO seeds. The effect of rates on SOLNI was dependent to growth stages or plant parts. Highest inhibitions were held from 10% rate of extracts collected at vegetative stages and 10% rate of stem extracts.

Conclusion: Water extracts of IMPCY give promising results. However, further studies are required to show effects in vitro and in vivo as well as to find out chemical(s) is causing this effect.

P WEEDS 31

Estimation of the critical period of weed competition and yield loss due to wild oat competition in wheat.

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The critical period of weed control (CPWC) is a key component of an integrated weed management (IWM). The CPWC is the time period in the crop growth cycle during which weeds must be controlled to prevent un-acceptable yield loss. The CPWC is determined by calculating the time interval between two components of weed interference. These are (i) the critical weed interference period, and (ii) the critical weed-free period or the minimum length of time required for the crop to be maintained weed-free before yield loss caused

Two field experiments were conducted in, Agriculture Research Centre, Alexandria, Egypt, each during two seasons. The first experiment, included fourteen treatments, seven treatments where the wheat plants were kept weed free for 4, 6, 8, 10, 12, 14 weeks after sowing (WAS) as well as for the whole season; in the other seven treatments, the weeds were left present only for 4, 6, 8, 10, 12, 14 WAS as well as the whole season. This first investigation aimed to determine the critical period of weed competition (CPWC) in wheat and resulted on wheat yield losses.

The second experiment, consisted of five wild oats densities in wheat plots namely 0, 25, 50, 100, and 200 plant/ m² to predict the wheat yield losses due to increasing wild oats population densities. The main finding revealed, for the first trail, that the relationship between wheat grain yield and weed infestation periods was nonlinear regression sigmoid models, i.e., MMF and Weibull models, and this were used to estimate (CPWC) based on 5% acceptable yield loss.

As for the second experiment, grain yield was decreased significantly by increasing wild oat density up to 200 plants/ m². The relationship between the grain yield and wild oats density was described by polynomial regression which used to predict wheat grain yield losses due to wild oats densities.

P WEEDS 32

Weed management in direct seeded wet sown rice

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Direct seeding of rice in puddled soils commonly known as direct seeded wet sown rice, involves major change in the production practices for attaining optimal plant density and higher water productivity in the water deficit areas of Karnataka. It also reduces the labour requirement by eliminating transplanting operation, which is laborious, time consuming and high labour oriented. However, weeds pose serious threat to sustainability and viability of direct seeded rice system due to simultaneous germination of rice and weed seeds. So, a field experiment was conducted to identify appropriate, effective and economic methods of managing weeds in direct seeded wet sown rice in Cauvery Command Area of Karnataka. The treatments consisted of two pre-emergence herbicides (bensulfuron methyl 0.6% + pretilachlor 6% GR @ 10 kg ha⁻¹ and pendimethalin @ 1 kg a.i. ha⁻¹) followed by passing of conoweeder and post-emergence application of Bispyribac sodium @ 25 g a.i. ha⁻¹; early post emergence application of bispyribac sodium @ 25 g a.i. ha⁻¹ alone and also another post-emergence application of bispyribac sodium @ 25 g a.i. ha⁻¹. These treatments were compared with weed free, weedy and hand weeding checks. The major weeds associated with direct seeded wet sown rice were *Cynodon dactylon*, *Dinebra retroflexa*, *Echinochloa colonum* and *Digiteria sanguinalis* among grasses, *Ageratum conyzoides*, *Corchorus aestuans*, *Cyanotis cristata*, *Commelina benghalensis* and *Trianthema portulacastrum* among broad leaved weeds and *Cyperus rotundu*, *Cyperus iria* and *Scirpus spp.* among sedges. Application of bispyribac sodium @ 25 g a.i. ha⁻¹ (EARLY POST) at 10-15 DAS + bispyribac sodium @ 25 g a.i. ha⁻¹ (EARLY POST) at 25-30 DAS recorded on par yield

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(4813 kg/ha) as that of weed free check (5262 kg/ha) and hand weeding thrice at 20, 40 and 60 DAS (5193 kg/ha). This was followed by application of pre-emergence herbicide bensulfuron methyl 0.6% + pretilachlor 6% GR fb post-emergence application of bispyribac sodium @ 25 g *a.i.* ha⁻¹ at 15-20 DAS. This increased yield was mainly attributed to effective control of all the species of weeds as indicated by reduced weed count and weed dry weight. Among the herbicides tested, pre-emergence application of pendimethalin @ 1.0 kg *a.i.* ha⁻¹ exhibited slight toxicity to rice seedlings. Among the treatments, application of bispyribac sodium @ 25 g *a.i.* ha⁻¹ (EARLY POST) at 10-15 DAS + bispyribac sodium @ 25 g *a.i.* ha⁻¹ (EARLY POST) at 25-30 DAS found most effective in control of all the three species of weeds (Grasses except *Dinebra retroflexa*, sedges and broad leaved weeds) and recorded 50.8% higher yield over weedy check with higher net returns and benefit cost ratio. Unchecked weed growth throughout the crop growth period resulted in 54.9% reduction in the yield of rice.

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P EGI 1

The status of species diversity in the family Loranthaceae (mistletoes) in Nigeria

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An investigation was carried out to determine the status of species diversity in the family Loranthaceae in Nigeria because of its parasitic nature on economic plants and their very high medicinal value which may lead to overexploitation as a result of destruction and indiscriminate collection of the species respectively. Base on field and herbarium studies, a total number of fifteen species were found to occur in Nigeria out of the twenty-two species documented in literature to occur in the country and they are also found to be locality specific. Eight species out of the fifteen occur abundantly on their respective locality where they are found. *Tapinanthus cordifolius* is endemic to Plateau State and its environs. Out of the fifteen species, the occurrence of *Helixanthera mannii*, *Helixanthera spathulata*, *Phragmanthera kamerunensis* and *Phragmanthera talbotiorum* in Nigeria are only traced to a single herbarium specimen each signifying that they might be threatened in the country. Seven species out of the twenty two species documented in literature were neither found documented in any of the herbaria visited or collected during the field trips. Biodiversity management plans should be developed or modify to take full account of the value of Africa's forest biodiversity especially the parasitic plants.

P EGI 2

Genotypic variation in soybean genotypes in response to NaCl stress

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To investigate the ability of sensitive and tolerant genotype of *Glycine max* to adapt to a saline environment in a field, we examined the growth performance, water relation and activities of antioxidant enzymes in relation to photosynthetic rate, chlorophyll a fluorescence, photosynthetic pigment concentration, protein and proline in plants exposed to salt stress. Ten soybean genotypes were selected and grown hydroponically. Seeds of After 3 days of proper germination, the seedlings were transferred to Hoagland's solution Ten-day-old seedlings were given seven levels of salt in the form of NaCl viz., T1 =0 mM NaCl, T2=25 mM NaCl, T3=50 mM NaCl, T4=75 mM NaCl, T5=100 mM NaCl, T6=125 mM NaCl, T7=150 mM NaCl. The investigation showed that genotype Pusa-24, PK-416 and Pusa-20 appeared to be the most salt-sensitive genotypes as inferred from their significantly reduced length, fresh weight and dry weight in response to the NaCl exposure. Pusa-37 appeared to be the most tolerant genotype since no significant effect of NaCl treatment on growth was found. Chlorophyll fluorescence measurements revealed that non-photochemical quenching increased in genotype Pusa-37 and decreased in Pusa-24. The improved performance of genotype Pusa-37 under high salinity was accompanied by higher leaf water potential and relative water content compared to genotype Pusa-24. Proline and protein contents were also higher in genotype Pusa-37 as compared to their lower accumulation in genotype Pusa-24. The activities of antioxidant enzymes was higher in salt tolerant genotype Pusa-37 than in Pusa-24 at various levels of salt treatments. These results suggest that genotype Pusa-37 is potentially more tolerant to salt damage and is associated with better adaptive responses found in genotype Pusa-24 keeping an active photosynthetic system and strong antioxidant defence system.

Numerous primers were verified on ten soybean genotypes obtained from Operon technologies among which 30 RAPD primers shown high polymorphism and genetic variation. The closer varieties in the cluster behaved similar in their response to salinity tolerance. Intra-clustering within the two clusters precisely grouped the 10 genotypes in sub-cluster as expected from their physiological findings. In conclusion photosynthetic, biochemical and molecular study showed that there is variability in salt tolerance behaviour in soybean genotypes. Pusa-24 is the salt-sensitive and Pusa-37 is the salt-tolerant genotype.

P EGI 3

Effect of seed size on seed germination rate of *Adansonia digitata* from five natural populations in Malawi

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Recently there has been concern over sustainability of natural indigenous fruit trees due to depletion of natural resources. This has been attributed to growing human demands. In Malawi, deforestation has largely eroded much of the indigenous fruit tree

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germplasm. One such indigenous fruit trees that have experienced serious genetic erosion are *Adansonia digitata* L. (baobab), a wild indigenous fruit tree that holds many useful values. Therefore, there is need to comprehend the variation that occurs between and within populations of *A. digitata* if sustainable use is to be achieved in future.

A. digitata is among the top 10 priority indigenous fruit tree species in Malawi. A study was carried out to assess the effect of seed size on seed germination rate of *Adansonia. digitata* from five natural populations in Malawi, namely Mwanza, Salima, Karonga, Chikwawa and Likoma Island. A total of 2500 seeds were collected from five populations and seed traits (seed weight, seed width and seed length) were measured using Vernier calliper and digital balance TR-2101, while germination parameters were analysed in the green house at Forestry department, Mzuzu University where the seeds were grown.

There were significant variations ($P < 0.001$) in mean seed weight, mean seed width, mean seed length between the five populations. Differences were found in mean seed weight 0.37g to 0.58g, mean seed width from 8.87mm to 10.10mm and seed length from 10.87mm to 12.48mm. Differences were also found in seed weight between families. In Chikwawa families ranged from 0.4777g to 0.7439g, Mwanza from 0.3958g to 0.6112g, Likoma from 0.2995g to 0.4674g, Salima from 0.3799g to 0.5875g and Karonga from 0.4151g to 0.6115g. There were also significant variation ($P < 0.001$) in the interaction between pre-treatment and provenances but no significant variation for interaction between pre-treatments ($P = 0.599$).

From these results, it is concluded that there is substantial variation in seed weight, seed width and seed length in *A. digitata* from five populations. Large variation in seed and growth parameters depicts the need for selection if tree improvement is envisaged. From this study, it can be concluded that variations observed are strongly genetically controlled. Future research should strive to partition genetic variation and environmental variation. Parents with superior should be captured with superior traits for domestication and wider cultivation.

Figure 1

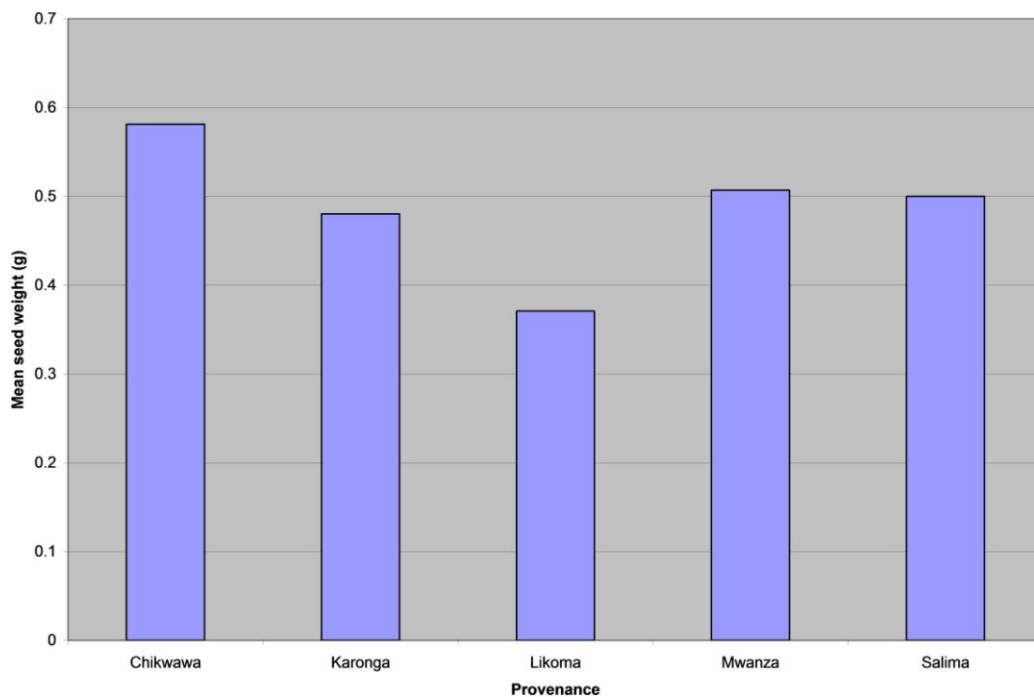
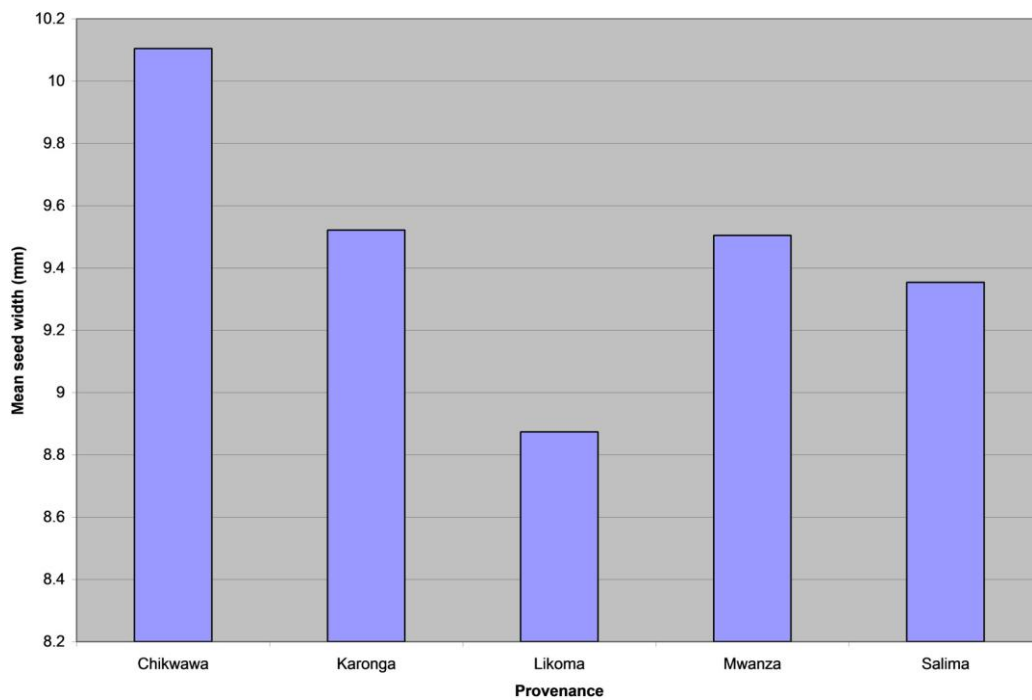


Figure 2



P EGI 4

Mutagenic Effects of Sodium Azide on M₁ Generation of Lagos Spinach (*Celosia argentea* L.)

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Aim: Despite the tremendous benefits of Lagos spinach (*Celosia argentea*) as food and in medicine, its diversity and uses are under threat in Nigeria, due to genetic erosion and low yield. Therefore, this study was aimed at inducing mutation in the seeds of the plant for the improvement of its morphological and yield parameters using sodium azide.

Methods: Two hundred and fifty (250) seeds each were randomly selected and treated with five levels of sodium azide (Control, 2.00mM, 4.00mM, 6.00mM, and 8.00mM) as a chemical mutagen. The seeds were raised both in laboratory for germination study and on the field. On the field, the seeds were planted in experimental pots arranged in a completely randomized block design each with five replicates for morphological and yield parameters study.

Results: With the exception of treatment 6mM, increased concentration of sodium azide resulted in reduction in seed germination and survival. Reduction in Biological injury (Lethality) was observed as an apparent reduced germination, and shoot length increased with an increasing concentration. The dichotomous branching mutant observed in the treated seeds resulted in increase number of leaves in 4mM and 6mM. The highest percentage branching corresponding to the highest number of leaf was recorded in treatment 6mM. The LD₅₀ for mortality were obtained at 4.74mM.

Conclusions: Therefore, treatment 6mM sodium azide was most effective in inducing desirable mutations at the highest frequency in *C. argentea*. Further study would be carried out on this plant to see if this useful character will be transferred to the next generation.

Figure 1: Effect of different concentrations of sodium azide on percentage germination.

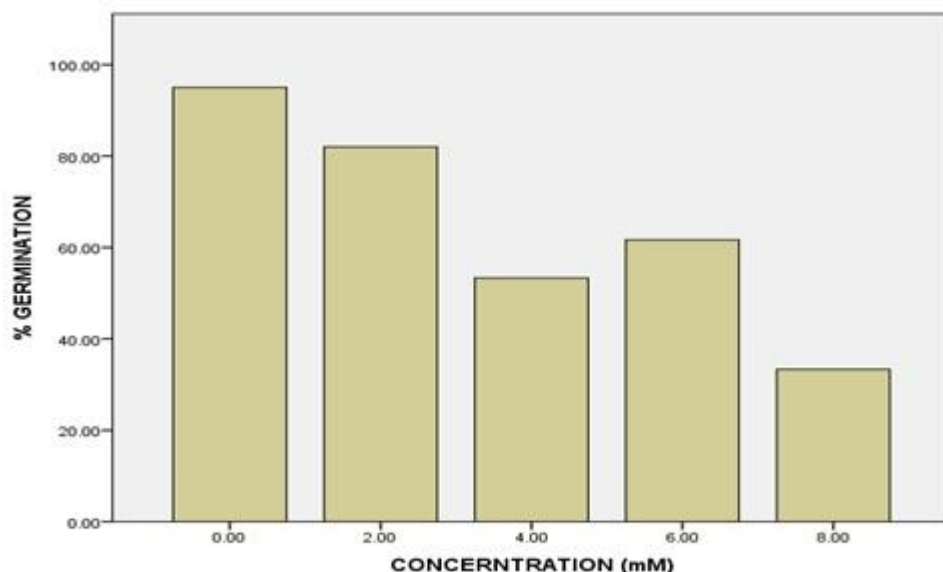
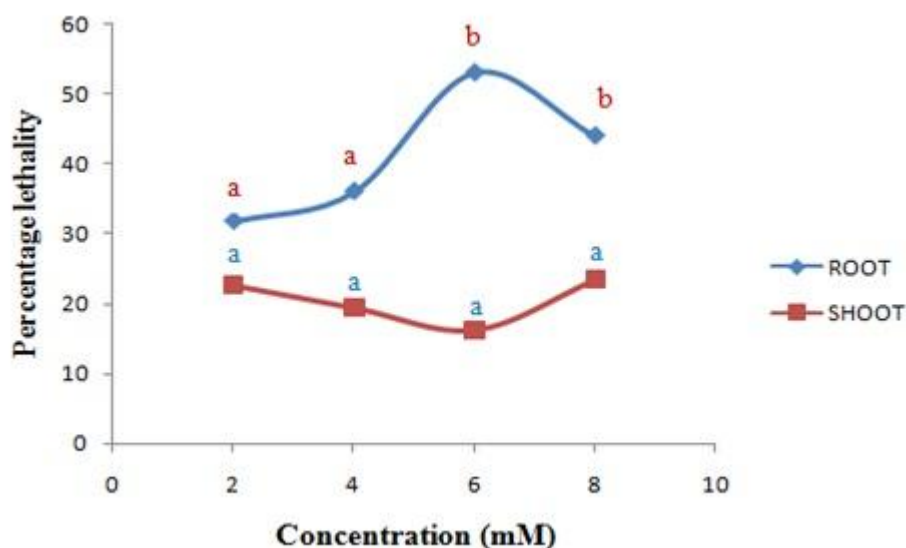


Figure 2: Effect of different concentrations of sodium azide on percentage lethality. Figure 3: Probit regression of *Celosia argentea* after treatment with different concentration of sodium azide



P EGI 5

Molecular study of pear psylla *Cacopsylla* ssp. In middle and southern regions of Syria

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Molecular study of pear psylla *Cacopsylla* spp. (Hemiptera: Psyllidae) males and females were carried out in the Biotechnology laboratory, Faculty of Agriculture, Damascus University during the growing season 2013- 2014. The samples were collected from four provinces located in middle and southern Syria: Homs (Mokhtaria Research Station and Al- Rastan), Hama (Tezeen), Damascus countryside (Al-Zabadany) and Al-Sweida (Al-Sweida Research Center).

The results showed that genetic differences of pear psylla by using ISSR with 19 primers, only 12 primers succeeded in amplifying the DNA of males. and total of bands number were 70 bands, only 64 bands were polymorphism, the percentage of polymorphism between males were (93.06%), the males of Damascus countryside and Al-Sweida were the most closer (86.12%), the clusters analysis separated the males of Hama in an independent group, and the second group was separated into sub cluster, the first was the males of Mokhtaria Research Station and Al-Rastan, and the second was Damascus countryside and Al-

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Sweida. And only 15 primers succeeded in amplifying the DNA of females pear psylla. The total of bands number were 70 bands, only 61 bands were polymorphism, The percentage of polymorphism between females were (74.49%), and the females of Al-Rastan and Hama were the most closer (77.22%), the clusters analysis separated the females of Damascus countryside in an independent group, and the second group was separated into sub cluster, the first was the females of Al-Sweida only, the second was the females of other regions. So males and females of pear psylla separated according geographical distribution.

P EGI 6

Field assessment of sensitivity of some cotton varieties to injury with aphids and cotton whitefly and its impact on productivity

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The field experiments were conducted to study the susceptibility of eight cotton varieties(Aleppo 33 ;McNair 308 ; Tashkent 3 ; McNair 307 ; McNair 235 ; TamCot CAMD ; McNair 220 As imported var. and Moubarak 93 as local var.) to infestation with cotton aphids and cotton white fly. The results indicate that aphid population had three main peaks (1st peak in July, 2nd peak in the 2nd week of August and the minor peak (3rd) occurred at the last week of August).Aphid population showed high reduction with low average of infestation (17.9-24.0 %) at May and June with Temp. (22.9-25 °c), R. H. (48- 56%) Wind speed (1.8 - 2.0m/sec.) and sun shine duration (13.6-14.1 hr and mid of September of cultural season.

The most attractive varieties of cotton and comparatively susceptible to *A. gossypii* were Var. Aleppo 33 , var. McNair235 and var. TashKent 3 followed by var. McNair 307 ,whereas the least attractive varieties of cotton and resistant against *A.gossypii* were var. Tamcot CAMD and var. McNair 220 , comparing with the control (var. Mubarak 93) which was the most tolerant to infestation with cotton aphids.There were high significant difference between infestation of cotton varieties with *A.gossypii* .

The population of cotton whitefly, *B. tabaci* had three main peaks in growing season (July, August and September) with Temp.(26.2-26.6 °c), R. H. (68.3-69.0 %),wind speed (0.77-0.90 m/sec.) and sunshine duration (12.4-13.9 hr) , however ,the lowest population of whitefly was recorded in May with Temp. 23.0 °c), R.h. (51.7 %), W. speed (1.7 m/sec.) and sunshine duration (13.6 hr) . Both *A. gossypii* and *B. tabaci* reached peak population in the third and fourth week of August (2008).The local variety (Moubarak 93) was the most tolerant against infestation with *A. gossypii* and *B. tabaci* . It was found that the rates of cotton yield were decreased with increasing infestation rates of aphid or white fly. The results of seasonal dynamics are detected several weeks differences in peak and main activity period on the host plants.

P EGI 7

Factors influencing culture and enhancement of yam *Dioscorea schimperiana*

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Background: Recent work on the species *Dioscorea schimperiana* has shown that its culture and transformation are endangered in Cameroon. This study aimed to make a diagnosis of the sector to identify the problems faced by actors of the spinneret.

Methods: In this context, 65 transformers *D. schimperiana* were recruited in the Hauts-plateaux, Ndé, Menoua and Bamboutos Department in Cameroon. They were then subjected to an interview through a questionnaire. The collected data were analyzed using descriptive statistics like average percentage, distribution frequency and multiple correspondence analyses.

Results: The results showed that 100% of actors in the sector were women. The majority (69.2%) of respondents were aged 65 and over. About 85 % of respondents were not schooled. The survey results also show that only 67, 3 % of respondents do not cultivate this yam. Among the reasons given to justify this observation, the maintenance difficulty of the case because of its invasiveness ranks was the first with nearly 23.1%. Production and marketing of this yam chips are limited by the availability of fresh tuber. The multiple correspondence analysis shows two types of developments for the four localities surveyed. In Bamboutos and Menoua *D. schimperiana* still positioned in subsistence agriculture. By cons, in Hauts-plateaux and Nde, the chips of the tuber are already an important source of income with the development of several unit of measure.

Conclusion: The spinneret *D. schimperiana* present in several points problems that must be solved if we do not want to witness the disappearance of the plant in Cameroon.

P EGI 8

A Mechanism for Biological Control -Tempo-spatial synchrony of natural enemies to insect pests by cover cropping in apple orchards

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To evaluate the field dynamics of the woolly apple aphid (*Eriosoma lanigerum* Hausm, WAA) and its natural enemies in cover cropping apple orchards, a consecutive two-year field trials were conducted in Yunnan province, China. The investigation revealed that the peak of natural enemies density curve was well synchronized with that of the WAA density on trees in the cover cropped orchard. In contrast, in the clean cultivated orchard, the peak of natural enemy density curve did not synchronize with that of WAA density on trees. In addition, the frequency of both natural enemies and WAA in same sample in cover cropping orchard was obviously higher than that in clean orchard. The density of WAA in cover cropping orchard was much less than that in clean orchard, and the ratio of natural enemy to WAA was higher. Therefore, the temporal-spatial synchrony of natural enemy and WAA populations might be one of the mechanisms for WAA control in cover cropped orchards.

P EGI 9

An innovative approach to enhance biodiversity on farmland: the credit point system

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Farmland biodiversity has often been assessed, but seldom at the farm scale, although it is ultimately the farm level at which decisions are taken. Therefore, a credit point system (CPS) was developed based on 32 options known to enhance farmland biodiversity. It was verified whether the resulting CPS score and farm-scale biodiversity are correlated considering four indicator groups (plants, grasshoppers, butterflies and birds) on 133 farms in the Swiss lowland. We further compared the suitability of the CPS score in reflecting farm-scale biodiversity to three alternative habitat measures, i.e. the amount of ecological compensation areas (ECAs, i.e. agri-environment scheme options), ECAs with a high ecological quality and valuable semi-natural elements (SNEs). Species richness and density of plants, grasshoppers, butterflies and birds were analysed, for 'all species', stenotopic farmland species and 'red-listed' species within each group, resulting in 19 biodiversity measures (dependent variables). Basic models were built, first without, then by including a range of environmental variables and compared to models expanded by the CPS score or one of the three habitat measures (ECAs, high-quality ECAs or SNEs). For each of the 19 biodiversity measures, the CPS score and the three habitat measures were ranked by how much their inclusion improved the basic model, to determine which measure best captured biodiversity at the farm scale. We demonstrate that the CPS score reflects farm-scale biodiversity. For 13 out of 19 biodiversity measures, models including the CPS score performed better than those without. The CPS score was found to be the most suitable predictor for a fast and efficient assessment of farm-scale biodiversity, which makes it suitable for use in large scale agri-environment schemes.

P EGI 10

Genetic diversity and implications for conservation of *Dipteronia* Olive

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The study reported here aims to: 1) to assess the level and distribution of genetic diversity in *Dipteronia* species using a combination of chloroplast microsatellite (cpSSR) and nuclear RAPD and AFLP markers. And 2) to elucidate the cause of endangered state, and provide information for devising scientific strategy for efficiently conservation of this species.

The genus *Dipteronia* Oliv. endemic to central and southern China consists of two species, *D. sinensis* Oliv. and *D. dyeriana* Henry, both of them are rare and endangered. 17 *D. sinensis* populations were sampled across 8 provinces in China, including Shaanxi, Henan, Gansu, Sichuan, Chongqing, Hubei, Hunan, Guizhou, and 4 *D. dyeriana* populations were sampled from Yunnan province (Figure 1). The sampling strategy was designed to cover its whole distribution range as widely as possible (the samples were taken at least 10m apart), and the number of individuals per populations used for study varied from 5 to 15. Young leaves were collected, dried in a plastic bag with silica gel, transported to the laboratory and kept in a -80°C freezer. The DNA was

extracted from dry leaves using a modified CTAB method. In this study, a combination of 18 random amplified polymorphic DNA (RAPD) markers, 8 pair of amplified-fragment length polymorphism (AFLP) primers and 10 polymorphic chloroplast microsatellite loci (cpSSR) were used to access the genetic diversity and population structure of species *D. sinensis* and *D. dyeriana*.

At the species level, the genetic diversity estimated by the two nuclear markers RAPD (*D. sinensis*: $H=0.3864$, $H_{sh}=0.5563$ and *D. dyeriana*: $H=0.3047$, $H_{sh}=0.4450$) and AFLP (*D. sinensis*: $H=0.3319$, $H_{sh}=0.4880$ and *D. dyeriana*: $H=0.3047$, $H_{sh}=0.4450$) were comparable, while that of cpSSR ($H_e = 0.6032$ of *D. sinensis* and $H_e = 0.6711$ of *D. dyeriana*) were much higher than both AFLP and RAPD, And the gene differentiation coefficient (G_{ST}) displayed a similar trend. The resulting values indicated that the three marker types estimated a relative high genetic diversity and genetic differentiation within both two species.

For the implication of conservation, we believe that the habitat destruction by human activities is among the key factors responsible for both species' endangered status. Therefore, in the long term, the strategy for conservation of the two *Dipteronia* species is the protection of its habitat. In addition, increasing small population size to maintain existing levels of genetic variation should be taken into consideration.

Figure 1



P EGI 11

Biodiversity in fruit crops in Balkan

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Introduction: Although Albania and Bosnie Herzegovina is little known to outsiders, there are enormous opportunities to preserve a great many species of fruit crops wild relatives. The Mediterranean basin is regarded as a primary centre of genetic origin and diversification of several temperate and subtropical fruit and nut trees. This paper reports results of a study since 2003 to make an inventory and mapping of fruit crop wild relatives of Albania and Bosnie Herzegovina, as a first step to effective conservation as well as a valuable tool for exploration, surveys and collection.

Material and methods: The study started by compiling geographically filtered list of all genera and species found in Albania and Bosnie Herzegovina. Both native and nonnative (introduced) (IFC) taxa were included in this inventory, although listed separately. In compiling the checklist and matching it with the bibliographic sources, it was necessary to make a taxonomic harmonization, crosschecking and confirming taxon names and recognising synonyms. For the purposes of proper and detailed mapping tens of expeditions were conducted covering all the territory. During the expeditions, a meticulous use of descriptors and descriptions was made. Besides identifying new records and locations for the first time, it was possible to verify the locations found in the references.

Results and discussions: In the natural ecosystems of Albania and Bosnia, we have found spontaneous and wild forms of many fruit trees and shrubs, including apples (*Malus*), pears (*Pyrus*), cherries, almonds, plums, sloes (*Prunus*), pomegranates (*Punica*),

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figs (*Ficus*), grapevines (*Vitis*), olives (*Olea*), cornels (*Cornus*), rowanberries (*Sorbus*), chestnuts (*Castanea*), walnuts (*Juglans*), hazelnuts (*Corylus*), pistachio (*Pistacia*), hackberry (*Celtis*), bearberry (*Arctostaphylos*), blackberries, raspberries (*Rubus*), strawberries (*Fragaria*) and vacciniums. The estimation of the share of taxa found in Albania with those at EU + Mediterranean level show a relatively high share, where almost all genera are found. Beside for mapping purposes, the expeditions carried out served also to assess the level of threatening of certain taxa due to small number of individuals, pest and disease pressure as well as anthropogenic factors.

P EGI 12

Study on population fluctuations of sugarcane mite, *Oligonychus sacchari* Mc Gregor (Acari; Tetranychidae) and its effect on growth of sugarcane in south of Khuzestan province/Iran

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Sugarcane mite, *Oligonychus sacchari* Mc Gregor is an important pest of sugarcane in Khuzestan province. The pest is capable to dry sugarcane leaves in a short period. Study of outbreak time and population fluctuations and damage of the sugarcane mite is important for integrated pest management programme. Sampling was done weekly from June and continued till August. The fifth and sixth leaves were selected as sample unit and the numbers of mites per leaf were counted. To evaluate the damage, experiment was conducted on six fields (each field 25 ha) of CP57-614 cultivar in Amirkabir agro-industrial Co. With starting of mite activity in the sugarcane field in the middle of July, three experimental fields were sprayed with Hexythiazox (Nissorun®) acaricide. Number of infested leaves before spraying operation and number of dried leaves in the late of July were sampled in experimental fields. Also weekly growth of cane was measured in North and South of the fields from the late of June until mid October. The results indicated that mite activity in South of Khuzestan initiate from early June and their peaks occurs in June and gradually reduce in the mid July. In experiment of damage, there was not any dried leaves in treatment but 81% leaves were dried in control and also data analysis showed that aggregative growth of cane between treatment and control had significant different ($P < 0.05$) and it was reduced about 12.37% in control in compare with treatment. Also activity of mite in North and South of the field had significantly different and damage of mite in South was more than North of the field.

P EGI 13

Genome diversity of *Tobacco rattle virus* (TRV) - basic knowledge for virus resistance evaluation

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Tobacco rattle virus is the type species of the genus *tobravirus*. It is transmitted by trichodorid nematodes. In potato tubers, arcs of corky tissue form at some distance around the initial infection sites. These spraing or corky ringspot symptoms disfigure the tubers and can greatly reduce their quality. The most effective measure to keep the damage caused by this virus low is the use of disease resistance of potato cultivars in potato production strategies.

The aim of research co-operations between potato breeders, plant protection services and nematologists/virologists is to develop methods for the evaluation of resistance of potato cultivars against TRV. Prerequisite for that is to know the TRV genome in its diversity.

Tobraviral genomes consist of two RNA species. RNA1 encodes two replication-associated proteins, a movement protein and a silencing suppressor. RNA2s consist of a 5' RNA2-specific and a 3' RNA1-related part. The RNA2-specific part contains the coat protein gene and often two (*2b* and *2c*) or more additional genes. The *2b* gene is required for nematode transmission.

TRV isolates from various parts of Germany were found to contain various combinations of different RNA1 and RNA2 species. Two isolates were obtained from roots of *Nicotiana benthamiana* plants, which had been grown for ca. eight weeks in soil from a corky ringspot-affected potato field, and from roots of field-grown potato plants in a neighbouring field, respectively. The coat protein genes of these isolates were almost identical to those of some previously described Dutch and Polish isolates which had been propagated in tobacco leaves. However, whereas the RNA2s of the previously described isolates consist of only c. 2000 nucleotides (nts), the ones of our isolates contained c. 4000 nts. This difference in size is due to the fact that the RNA2s of our isolates contain two additional genes (*2b* and *2c*). After mechanical transmission of one of our isolates to tobacco leaves most of the genome area containing these additional genes was lost. In addition, the original RNA1-related 3' end of its RNA2 which had resembled that of the RNA1 of a spinach isolate was replaced by that of the clearly distinct one of the supporting RNA1 in our isolates. Deletions and recombinations obviously govern the adaptation of tobnaviruses to various hosts and growing conditions.

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P EGI 14

The diversity of bacterial endosymbiont from cotton leaf hopper in Pakistan

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Introduction: *Wolbachia* and *Arsenophonus* is a maternally transmitted endosymbiont, which is found in the reproductive and steroidogenic tissues of arthropods and nematodes. The cotton leafhopper, *Amrasca devastans* (Distant) (Cicadellidae: Homoptera) is one of the major sap feeding pests of malvaceous and solanaceous crops in Pakistan and is broadly distributed throughout the cotton growing provinces, Sindh and Punjab.

Objectives: The identification, diversity and molecular of secondary endosymbiont isolated from *cotton leafhopper from Pakistan*
Materials and methods

Live field specimens of *A. devastans* were collected from 8 different cotton field locations from Punjab province Pakistan and preserved in 70% ethanol for genetic studies. The presence of *Wolbachia* and *Arsenophonus* in leafhopper was detected by partial 16S rDNA gene amplification and sequencing.

Results: Both *Arsenophonus* and *wolbachia* endosymbiont was detected in different leafhopper. Out of four sequences (SA7, SW1, SW2 and SW3) obtained from this study SA7 and SW2 were found to be most similar (99.5% sequence identity) to the *Wolbachia* strain of *Bemisia tabaci* reported from India. While SW1 showed maximum homology (79% sequence identity) with *Wolbachia* strain of *Guignotus pusillus* and SW3 showed maximum homology with *Wolbachia* strain of *B. tabaci* (99% sequence identity) reported from China. The *Arsenophonus* was also detected in more than 10 samples using *arsenophonus* specific 23S ribosomal RNA primers.

Conclusion: This study is first evidence of *Arsenophonus* and *wolbachia* endosymbiont incidence in cotton leafhopper.

P EGI 15

Crop species richness controls arthropod food web: evidence from an experimental model system

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Effects of plant species richness on the function and stability of ecosystems have been of focus in past decades. In particular, the pest-natural enemy interaction can be sensitive to changes of crop species richness. Elucidating how crop species richness affects pest-natural enemy interactions is the basis for sustainable pest control and important for ensuring food security, yet still largely unknown to date. To explore the mechanism and drivers behind the relationship between crop richness and arthropod community structure, we set up a 4-year experimental model system (EMS) in a micro-landscape that includes 5 levels of crop richness and 10 plots for each richness level from 2007 to 2010. Arthropod richness was found positively correlated with crop richness. High crop richness can enhance the temporal stability of the arthropod community but with the decline of population stability for some species. Quantitative measures of link density, vulnerability, and generality in the arthropod food web significantly increased with high crop richness. The richness and biomass of neutral arthropods, such as honeybees, ants, and mosquitoes, were found positively correlated with those of the natural enemy. As such, neutral arthropods could sustain food web robustness by serving as alternative prey/host for natural enemies. The mediation of neutral arthropods to the interaction between crops and pests plant species can be important for successful biocontrol practices using natural enemies. Planting diverse crop species with a certain level of spatial turnover could benefit grain yield and safeguard multiple ecosystem services.

Figure 1

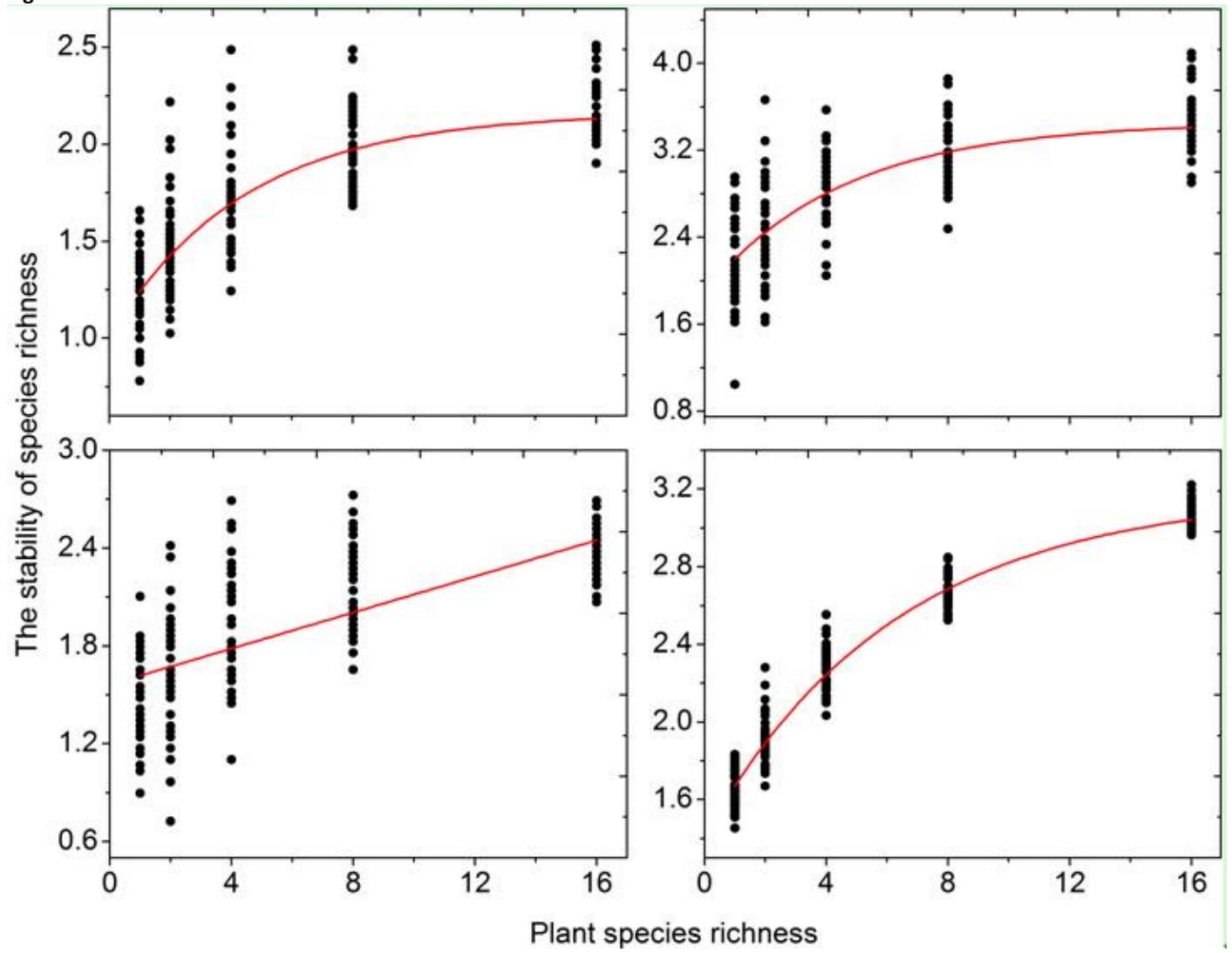
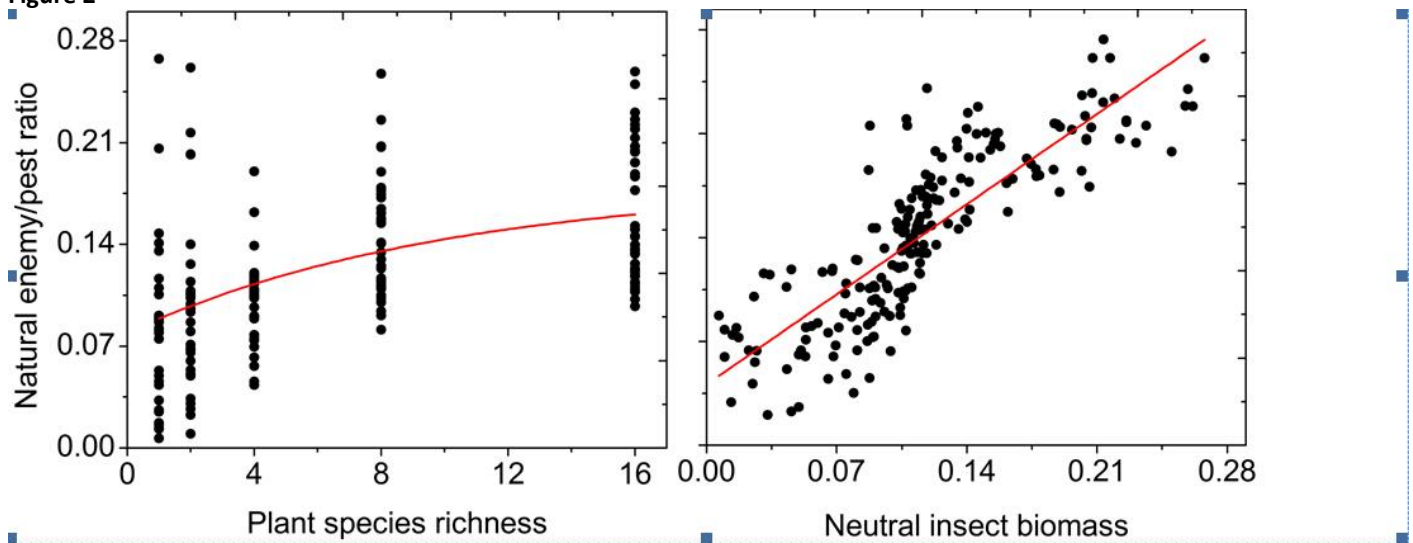


Figure 2



P EGI 16

Pest, Parasitoid and Predator Species Determined in Persimmon Orchards in Southern Turkey

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The pest and beneficial species in persimmon orchards in Adana, Mersin, Hatay, Osmaniye and Kahramanmaraş provinces were determined in 2010-2011. The survey was conducted according to 0.01% of tree number rules in the five provinces. Infected plant materials were brought to the laboratory to obtain the adult pests, parasitoids and predators. Twentyfive pest and 31 beneficial species associated with these pests were found. Among the pest species, Mediterranean fruit fly [*Ceratitis capitata* Wied. (Diptera: Tephritidae)], Citrus Mealybug [*Planococcus citri* Risso (Hemiptera: Pseudococcidae)] and Honeydew moth [*Cryptoblabes gnidiella* Mill. (Lepidoptera: Pyralidae)] were found as important and considered to be the key pests. Among the natural enemies, species of Coccinellidae family were found widespread.

P EGI 17

Spectral phenotyping of *Cercospora beticola* resistance in sugar beet genotypes

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Cercospora leaf spot (CLS) disease caused by *Cercospora beticola* is the most destructive leaf disease of sugar beet (*Beta vulgaris*) causing high losses in yield and quality. Cultivation and breeding of disease resistant cultivars is an important strategy to control this economically relevant plant disease. The breeding progress is limited by time- and cost-intensive phenotyping of the plant and visual assessment of disease resistance may be affected by subjective factors. A non-invasive, objective procedure for phenotyping/evaluating the resistance of breeding lines may contribute to optimize the selection process.

Hyperspectral imaging is an advanced, sensor-based method for precise and objective sensing of plant diseases. The technology was used to identify and to analyze various CLS symptoms on sugar beet genotypes varying in disease susceptibility. Measurements on tissue level were conducted with a hyperspectral microscope. Image analysis and supervised classification, e.g. spectral angle mapper were used.

Different CLS phenotypes were identified which vary in size and spatial composition. Symptoms could be differentiated into subareas based on their optical properties. Genotypes with lower susceptibility to *C. beticola* preferentially had symptoms with smaller center compared to highly susceptible genotypes. Accordingly, the number of conidia per diseased leaf area on resistant plants was lower.

Therefore, the assessment of sporulation per unit of leaf area and phenotypes of disease symptoms may be appropriate methods to identify subtle differences in disease resistance, however, only hyperspectral imaging is suitable for automation.

P EGI 18

Physiology meets Ecology: Carbon allocation patterns as new traits for ecological research questions

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Depending on environmental conditions higher plants develop defenses and undergo acclimatization processes which impact growth, development and reproduction. The underlying physiological key processes alter the carbon allocation patterns in plants, which is mirrored by the macromolecular composition of tissues (e.g. proteins, lipids and carbohydrates). Thereby the carbon pools can be reorganized by e.g. synthesizing more proteins for housekeeping processes or by synthesizing more structural carbohydrates for growing and building up biomass. Therefore the carbon partitioning among these different pools could be used as a physiological trait for a better understanding of community structure and species interaction with regard to biodiversity analysis.

To measure the carbon allocation patterns in higher plants, we want to evaluate infrared (IR) spectroscopy as a high-throughput method as an alternative to biochemical analysis. To date IR analysis is basically used for qualitative analysis, however, for quantitative application the method needs to be calibrated. Accordingly, the method would provide a new tool for physiological fingerprinting in higher plants to bring together physiological and ecological research.

Standard biochemical methods were used to analyze the main macromolecular composition of plant tissues (e.g. carbohydrates, proteins, lipids, lignin and other phenolic substances). The biochemical measurements were then used to calibrate the

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spectroscopic data from the same plant material. To proof the applicability of the calibration, several species were cultivated under different abiotic conditions to induce shifts in their physiological states.

It is shown that the analyzed macromolecules account for 80-90 percent of the dry weight. However, macromolecular changes were species-specific and dependent on cultivation conditions. Furthermore, these changes were clearly reflected in the IR spectra of the plant samples, thus, a calibration of the spectroscopic method based on biochemical data could be completed. Since the IR data can be calibrated because of the obvious shift in carbon pools, we obtain the possibility to provide physiological data for ecological studies.

P EGI 19

Genetic diversity of phytoplasmas causing sesame phyllody and transmission studies with natural vector *Orosius orientalis* in South-western Turkey

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Sesame phyllody is an economically important disease of sesame in Turkey. Phytoplasmas were detected with nested PCR using universal primer pairs P1/P7 and R16F2n/R16R2 from sesame plants and natural vector *Orosius orientalis* (Cicadellidae). Insects collected by mouth aspirator from the experimental field and sesame growing areas in Antalya. To characterize transmission of phytoplasmas with *Orosius orientalis* an insect culture was generated and ten *Orosius orientalis* adults collected from phyllody infected sesame plants were fed with healthy sesame plants for six days. Symptoms of phyllody disease were observed within three weeks insect-fed sesame plants. Characterization of phytoplasmas was done by sequencing of cloned fragment of 16S ribosomal DNA F2R2n region. Using iPhyClassifier, BLAST search and PCR-RFLP assays phytoplasma subgroups and groups were determined. Three different phytoplasmas belong to 16Sr II-D, VI-A and IX-C were detected in sesame phyllody infected plants collected during 2011-2013 growing periods. So far only 16Sr II-D group was detected from *Orosius orientalis* and also this group was in majority in infected sesame plants. This study shows that i) phytoplasmas causing sesame phyllody is highly diverse although in majority 16Sr II-D phytoplasma group detected, ii) *Orosius orientalis* is a natural vector of sesame phyllody in Antalya as its reported by earlier studies. Key words: Antalya, Cicadellidae, *Orosius orientalis*, phyllody, phytoplasmas, sesame, Turkey

P EGI 20

Seasonal abundance of house dust mites in Ordu (Turkey)

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House dust contains various organic and inorganic materials. The most important parts of the house dust are dust mites causing allergic diseases. Many factors may influence mite growth. Temperature and *humidity* are the major *factors* that *influence* the distribution and *abundance* of dust *mites*. In this study, seasonal changes in house dust mites were studied in four different houses in Ordu province (Black Sea coast, Turkey). Dust samples were obtained once a month from August 2013 till July 2014 to assess seasonal variations in mite counts. House dust samples were taken from mattress of bed, couches, carpets and floor of bedrooms. The samples were collected with a portable vacuum cleaner. At each sampling site, 2 m² surface area was vacuumed for 2 minutes. Mites were isolated from dust samples by sieving method. Mite density was reported as mites per gram of dust. During the sampling period, room relative humidity and temperature in each house were measured and recorded. A total of 48 dust samples were examined. The result showed that most abundant and frequent mites were *Dermatophagoides pteronyssinus* (Trouessart, 1897) and *Dermatophagoides farinae* Hughes 1961 (Astigmata: Pyroglyphidae). The mite abundance *reached its peak* in each *house during* August. It may also be concluded that Pyroglyphid mites can survive all year round and they are widely spread in all types of houses in Ordu, Turkey.

P EGI 21

Field evaluation of reaction of determinate sesame lines to sesame phyllody disease in South-western Turkey

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Sesame phyllody is an economically important disease of sesame in Turkey. Reaction of determinate (det) sesame lines which has a determinate growth type for machine harvest was evaluated to sesame phyllody disease. A three year of field experiments was conducted in Akdeniz University campus in Antalya during 2011-2013 sesame growing periods. Three det (3xMug, 36xMug, dt-1) and 4 indeterminate (indet) (Muganlı-57, Muganlıx36, Birkan, Sel-T6) lines were tested for disease incidence, yield and yield components. Disease pressure for selection of lines was high only in 2011 growing period. One det line (dt-1) showed the least susceptible disease reaction in 2011 among all lines. Due to low disease pressure in growing periods of 2012-2013, no difference in susceptibility was determined. Yield was significantly higher in indeterminate lines. Phytoplasmas were detected with nested PCR using universal primer pairs P1/P7 and R16F2n/R16R2 from plants and natural vector *Orosius orientalis* (Cicadellidae). Insects collected by mouth aspirator from the experimental field. Highest number of phytoplasma positive insect samples was found in 2011, none detected in 2012 and only one positive insect was detected in 2013.

This study shows that unless high disease pressure conditions; i) det and indet sesame lines show no difference in disease reaction to sesame phyllody, ii) number of phytoplasma positive insect vector *Orosius orientalis* likely to have a major role in the level of disease pressure.

P EGI 22

Review of the species of *Laelaspis* Berlese (Acari: Laelapidae) occurring in the Western Palaearctic Region

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The Laelapidae is one of the largest families of free-living Mesostigmata, but it has not yet achieved a stable classification (Tenorio 1982, Joharchi *et al.*, 2012a, 2012b). The mite family Laelapidae is ecologically diverse, including obligate and facultative parasites of vertebrates, insect paraphages, and free-living predators that inhabit soil-litter habitats and the nests of vertebrates and arthropods (Evans & Till, 1966; Strong & Halliday, 1994; Lindquist *et al.*, 2009). Species of *Laelaspis* have been collected in many parts of the world, almost are associated with ants or their nests. Joharchi *et al.* (2011) treated *Laelaspis* as a separate genus, and gave a diagnosis and comparison of diagnostic characters for the closely related genera *Gymnolaelaps* and *Pseudoparasitus*. That concept of *Laelaspis* is followed here. The cosmopolitan genus includes 22 identified species have previously been reported from Western Palaearctic Region. The purpose of this research is to review those records and summarize the available information about the species of *Laelaspis* that occur in the Western Palaearctic Region.

P EGI 23

Discovery of single nucleotide polymorphisms (SNPs) in *Sw-5b* resistance gene alleles for *Tomato spotted wilt virus* in tomato and its application

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Tomato spotted wilt virus (TSWV) causes one of the most destructive viral diseases that threaten tomato (*Solanum lycopersicum*) worldwide. So far, eight TSWV resistance genes, *Sw1a*, *Sw1b*, *sw2*, *sw3*, *sw4*, *Sw-5b*, *Sw-6*, and *Sw-7* have been reported. Among them, *Sw-5b* is the TSWV resistance gene that has been widely utilized in tomato breeding because of its durable and reliable resistance to multiple *Tospoviruses*. The objective of this research was to develop single nucleotide polymorphism (SNP) markers to distinguish tomato cultivars resistant to TSWV from susceptible cultivars for marker-assisted breeding in tomato breeding. DNA sequences of *Sw-5* alleles in both resistant and susceptible varieties were analyzed, and the single SNP was found to distinguish tomato cultivars resistant to TSWV from susceptible cultivars. Based on the SNP confirmed, the SNP primer pair was designed. To determine efficiency of this SNP primer pair, resistance of collected 27 commercial tomato varieties was first, determined using one of known *Sw-5b* gene-based markers by PCR. Then, using this new SNP marker and

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high-resolution melting technique, the same tomato varieties were screened. The results were 100% correlated with those from screening with the *Sw-5b* gene-based marker. These results indicate that the SNP maker developed in this study could be useful for tracking resistance to TSWV in tomato breeding.

P EGI 24

Effects of proteinaceous extracts of three native varieties of gramineae family on the digestive α -amylase enzyme activity of *Anagasta kuehniella* Zeller (Lepidoptera: Pyralidae)

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Introduction: *Anagasta kuehniella* Zeller (Lepidoptera: Pyralidae) causes decrease in the quality of the bread during the feeding of flour. Disruption in the insect's digestive system through transgenic plants containing the digestive enzyme inhibitors is one of the control methods.

Objectives: Having enough valide information about the properties of insect's digestive system and introducing natural inhibitor sources are important requirements for developing this method.

Material and methods: The effects of proteinaceous extracts was evaluated on α -amylase activity of fourth (L4) and fifth (L5) instar larvae by spectrometry and gel electrophoresis assay. Ammonium sulfate (70%) was used to extract proteins from seeds. To determine whether the enzyme activity could be inhibited by proteinaceous extracts, the α -amylase activity was assayed by the dinitrosalicylic acid procedure, using 1% soluble starch as substrate.

Results: At the highest dose of proteinaceous extracts of Sivand and Sardari (13 μ g pr) and Triticale (16 μ g pr), 73.08%, 71.07% and 65.88% of the L5 and 58.16%, 42.14% and 50.25% of the L4 enzyme activity was reduced, respectively. Whilst the lowest dose of Sivand and Sardari (0.812 μ g pr) and Triticale (1 μ g pr) inhibited, 19.93%, 17.85% and 25.26% of the L5 and 11.78%, 12.81% and 10.09% of the L4 enzyme activity, respectively. In gel electrophoresis at the highest dose of extracts, in the L5, amylase band disappeared and in the L4, waned band clearly. By reducing protein dose, bonds resolution was increased.

Conclusion: The results showed that inhibitory effect is depending to inhibitor dose. Inhibition of α -amylase in L5 was higher than L4. It is concluded that proteinaceous extract of varieties Sivand, Sardari and Triticale have a potential to be used in this pest management.

P EGI 25

Management practices and environmental conditions influence the impact of plant protection chemicals on soil biodiversity

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Chemical biocides are used to control pests in order to protect crops and produce greater yields. Although the use of plant protection products is becoming more environmentally friendly their effects on soil biodiversity can still be detrimental. Beneficial non-target organisms such as mites, collembolans and oligocheats are affected depending on dose and spraying frequencies as well as a complexity of soil and management factors.

The objective of this study was to measure the impact of biocide usage on the feeding activity of soil organisms representing a variety of species. Conventional and organic practices were compared.

We used the bait lamina technique to asses the feeding activity in differently managed field plots as well as in microosms with soils from the plots in a vineyard in the Western Cape, South Africa. We compared conventionally and organically managed plots. Winter weeds in a vineyard were killed with Glyphosate and pre-emergence weed-control was done with Simazine. A conventionally managed treatment included Mangozeb, copper oxychloride and Penconazole which was applied in September. Bait laminas were inserted every month and removed 13 to 17 days later during the months of September, November, December and April. Soil moisture conditions were also monitored.

The feeding activity of the soil fauna, as indicated by the percentage bait holes eaten, did not differ in the plots prior to the application of the treatments in September. A significantly lower feeding activity was evident in November, December and April in all treated plots compared to the activity before spraying but there were indications of recovery which coincided with an increase in soil moisture conditions. In April the indications were that feeding activity was substantially higher in plots that were organically treated. The bait lamina tests in the microcosms showed a significant difference in activity between soils treated conventionally and organically with the latter showing a higher feeding activity.

Poster Presentations

Ecological/Genetical Issues

We conclude from both the field and microcosm studies that the impact of plant protection products on the feeding activity of soil organisms as an indicator of biological diversity is not only dependent on dose and frequency of application but also on a complexity of soil conditions and management practices.

P EGI 26

Screening of twelve new citrus rootstocks to *Phytophthora citrophthora* by application of a fast test

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To better cope with the problems that threaten actually the Mediterranean citriculture, breeding strategies of citrus rootstocks should take in consideration the tolerance to biotic stresses which prevale in this region. Among these problems, *Phytophthora* diseases are the most important. In this study, twelve citrus rootstock hybrids were obtained by controlled crossing using Cleopatra mandarin (*Citrus reticulata* Blanco), Sunki mandarin (*Citrus sunki* Hort. Ex Tanaka) and pummelo (*Citrus grandis* Osbeck) as male parents and Citrumelo Winter Haven (*Citrus paradisi* Macf. x *Poncirus trifoliata* (L.) Raf.) as female parent. The new hybrids were grown under greenhouse conditions for 18 months and subjected to a screening test for resistance to *Phytophthora citrophthora*, as well as their parents. Rough lemon (*Citrus jambhiri* Lush.) and sour orange (*Citrus aurantium* L.) were introduced in the experiment as controls. The stem of each genotype was divided into many nodal segments and each segment was artificially injured and inoculated with the fungus then kept in a moist chamber at a temperature of 25°C. Most of genotypes developed symptoms of trunk gummosis within a period of six days. By contrast, no symptoms were observed in sour orange, Sunki mandarin and four hybrids of the latter : H28, H56, H80, H87. The estimation of the surface of necrosis revealed also that the severity of *Phytophthora* attack varied significantly between the other rootstocks investigated.

P EGI 27

Global diversity of *Maruca* populations infesting food legumes

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Introduction and objectives: Indo-Malaysian region is considered to be the most probable center of origin for the genus *Maruca*. Although three species have been described in *Maruca*, *M. vitrata* is believed to be the only *Maruca* species causing economic damage on food legumes worldwide. It can feed on at least 45 different host plant species, mostly on legumes in tropical Asia and sub-Saharan Africa. However, variations have been observed in the responses of *M. vitrata* male moths to the same synthetic sex pheromone lures over various geographical locations in sub-Saharan Africa and tropical Asia in recent years. Hence, the objective of the study was to assess the genetic diversity of *Maruca* spp. in the tropics.

Materials and methods: In this study, the partial sequences of the mitochondrial gene, *cytochrome c oxidase I (coxI)* from *Maruca* spp in tropical Asia, Africa, Oceania and Latin America were analyzed. In addition, this study was compared with two earlier studies by Margam et al. (2011)¹ and Agunbiade et al. (2014)² to understand the relationships among the *Maruca* populations from different regions.

Results and conclusion: The results from our study as well as Agunbiade et al. (2014) confirmed that there were no host plant races in *M. vitrata*. *M. vitrata* is composed of morphologically indistinguishable species-complex in Asia and sub-Saharan Africa. In addition, two putative species of *Maruca*, including *M. vitrata* occur in Oceania. However, the *coxI* sequences of the second species of *Maruca* in Oceania did not match with the *coxI* sequences of *M. fuscalis* collected from Oceania (BOLD:AAD9057) and deposited in *International Barcode of Life* project (iBOL). Finally, the *Maruca* species occurring in Latin America is different from the two putative species occurring in Oceania. Thus, this study confirmed the presence of four putative *Maruca* species, including two unknown *Maruca* spp., one each in Latin America and Oceania (including Indonesia), *Maruca* prob. *fuscalis* and *M. vitrata* in Asia and Africa as well as Oceania. Hence, the genetic diversity in *Maruca* spp. should be considered for designing appropriate integrated pest management strategies in different regions.

¹Margam et al. (2011), Mol Biol Rep 38(2): 893-903

²Agunbiade et al. (2014), PLoS ONE 9(3): e92072

P EGI 28

***Fusarium* wilt of date palm: a potential danger to the date palm biodiversity in the south of Algeria**

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The date palm is a tree of interest ecological, economic and social major for many countries of the arid and semi-arid regions. Indeed, by creating in the middle of the desert a microclimate favorable to the development of subjacent cultures, the date palm constitutes the pivot of Saharan agriculture and represents the food and financial main resource for oasis populations.

However, the date palm is subject to several biotic and abiotic stresses, including aging palm trees, the reduction of water resources, the extension of the urban fabric and monoculture.

Among the biotic constraints, the Bayoud disease is the most formidable plug in the south of Algeria. This scourge is caused by *Fusarium oxysporum f.sp.albedinis*, which caused the decline of at least 03 million Palms in Algeria and unfortunately, it did not cease to ravage our palm groves. This devastating scourge causes total decline of the palm groves causing an ecological disorder that the socio-economic consequences could negatively affect the people of the South of Algeria

This disease remains incontestably the main cause of the degradation of the genetic resources of the date palm in the oases of the western south and center of the Algerian Sahara in particular on the level of Adrar department where a very worrying degree of attack was recorded. Some cultivars underwent severe attacks whereas others having a good commercial value are in process of extinction.

Several methods of fight against this disease have been proposed, however, the use of resistant cultivars is the most recommended.

In this context, we have carried out a series of prospection and exhaustive studies on the palm plantations of the area of Adrar, which has more than 03 million trees of date palm, where we determined the degree of morbidity by area since 02 decades. Similarly, we have established a recent epidemiological map of the distribution of the disease Bayoud at the level of Touat, Gourara and Tidikelt regions. Our investigations also revealed a real regression of genetic resources of date palm contributing to the imbalance of biodiversity in the oasis environment. This significant deterioration of the heritage of date palm and plant genetic biodiversity is mainly due to the spread of the disease Bayoud whose expansion has become a source of real concern for the socio-economic interests of farmers and the rural exodus from their palm groves.

P EGI 29

Evaluation of resistance of medium maturing potato clones to *Fusarium* Dry Rot

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Fusarium dry rot is one of the most important diseases of potato affecting tubers in the storage and seed pieces after planting which cause malformation in the field. The disease caused by several species including *Fusarium* species including *F. sambucinum*, *F. solani* var. *coeruleum*, *F. oxysporum* and *F. avenaceum* [1]. In Iran first two species are more abundant and destructive in the storage and field [2]. Evaluation of resistance of potato clones to dry rot disease is among the mandatory assessments during breeding of potato. In the present research, resistance of 29 medium maturing potato clones compared with cultivars Agria, Marfona and Sante and Lady Rosetta was evaluated to dry rot disease. Tubers of the potato clones and cultivars were artificially inoculated with aggressive isolates of *F. sambucinum* and *F. solani* then maintained in a growth chamber at 16°C with 95% relative humidity for four weeks. The experiment was done in a factorial design with two factors including *Fusarium* species and potato clones. After four weeks, data of disease index and tuber sprouting were analyzed with Kruskal-Wallis test and the mean comparison was done by using of nonparametric tukey method. The results showed that potato clones 397009, 397097-13, 397074-3, 397097-2, 397082-10 and cultivar Agria with averages between 168.49 to 233 had the lowest and clone 396124-79*396151-15 and cultivar Lady Rosetta with average of 465.88 and 385.35 had the highest disease index. *Fusarium* species had negative effect on tuber sprouting so that susceptible potato clones and cultivars had the least tuber sprouting rate. All potato clones with the least disease index in this study except clone 397097-13, had a medium to high rate of sprout growth with averages between 296.41 to 466.4. Among resistant potato clones to *Fusarium* dry rot in this study, clone 397097-2 due to other good characters including high yield, dry matter and resistance to some potato viruses was considered as superior one to other potato clones and cultivars.

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Poster Presentations

Ecological/Genetical Issues

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P EGI 30

Entomofaune of *Lavandula multifida* L. (Lamiaceae) : Diversity and Approach Bioecological in the area Maghnia (Tlemcen-Northwestern Algerian)

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The area of Maghnia is located at the extreme North-West of Algeria in Wilaya de Tlemcen. It is characterized by the Mediterranean climate. *Lavandula multifida* is a known aromatic plant for its double gastronomical and pharmaceutical role. It belongs to the family of Lamiaceae. The goal of this study is to carry out a faunistic inventory in various stations of the zone of Maghnia. For that, three stations are prospected. Samplings are carried out from January to June 2012 divided into 12 taking away. We are interested in the entomofaune where various methods of capture are used. The diversified entomofaune account 71 species distributed between 15 orders of which 3 are Apterygota. Pterygota are represented by 12 orders of which most important is that of the Coleoptera with 25 species. Hemiptera, Dermaptera, Mantoptera, Neuroptera, Trichoptera are most slightly represented with only one species each one. Station 3 is richest in entomofaunistic in cash. In spring season, the Coleoptera are most numerous with a wealth equal to 10 in the 3 stations. In May, station 2 counts the largest number of individuals of Hymenoptera. 4 species are constant, 3 accessories species and 64 accidental species whose 52 very accidental is listed. The equitability is higher than 0.5 what implies that manpower of the Coleoptera in the stations with *Lavandula multifida* tend to be in balance between them. About fifty entomofaunistic species are found on the level of the surface of the ground.

P EGI 31

Finding a place to begin integrating research on plant biotic stress

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Crop plants are attacked by large numbers of phylogenetically diverse parasites, including viruses, bacteria, fungi, oomycetes, nematodes, insects, and mites. Methods for protecting plants against parasites are developed by plant pathologists and entomologists, two groups that rarely share conversations about plant protection. A difference in research emphasis during the past 60 years has contributed to this lack of conversation, with plant pathologists focusing more on genetics and entomologists on ecology. In order to develop sustainable and ecologically-sound methods of plant protection, plant pathologists and entomologists need to start thinking about commonalities, including the common strategies that parasites use to attack plants and the common strategies that plants use to protect themselves against diverse parasites. An entry point is needed to begin this conversation, which should also include plant scientists. A suitable entry point is a crop plant that: 1) shows evidence of commonalities across parasite attack strategies and plant protection strategies and 2) is not grown as a genetically modified organism. We propose wheat (*Triticum* spp.) as this crop plant. Wheat will be critical for feeding the world's expanding populations. A common strategy used by wheat's parasites is the production and application of 'effector' molecules in order to suppress plant defense mechanisms and enhance the plant's nutritional status. A common strategy that wheat has to defend against its parasites is 'effector'-triggered plant resistance, which typically is mediated by a *Resistance* (*R*) gene. Wheat has 479 documented *R* genes that protect against plant pathogens, nematodes, insects, and mites. Classical breeding methods have been used to deploy *Resistance* genes in agricultural crops for over 50 years, the result being pesticide-free protection. A problem for *R* gene-mediated plant resistance is parasite adaptation, which reduces the durability of the crop protection that is provided by deploying a single *R* gene. Fifty years of basic research has enriched our understanding of mechanisms of parasite adaptation and plant resistance. This, in turn, has created a hope that, in the near future, more intelligent strategies of *R* gene deployment will provide durable pesticide-free plant protection.

P EGI 32

Dust career impacts on *Pinus halepensis* growth

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Pinus halepensis Mill., is a most common tree in the Mediterranean basin. In Tunisia, specifically in Kroumirie, it is an excellence species. However, for several years, we assist a continual deterioration of this ecosystem type. Several factors are the origin for this degradation: insects and fungi attack, fire, aging populations, low regeneration and hardening climate. This degradation is further accentuated by installing careers around the pine forest. Our objective in this study was to identify the career dust influence on growth and productivity of Aleppo pine; through dendrochronological approach (tree rings study) and dendrometric approach (measurement of diameter, height and survival rate). Study is accomplished on two populations: a reference site 'Charchara' located away from mining and 'Wed el maaden site' near a gravel extraction career. Results showed significant differences of parameters studied between stations both in dendrometric and dendrochronological parameters over time.

Figure 1

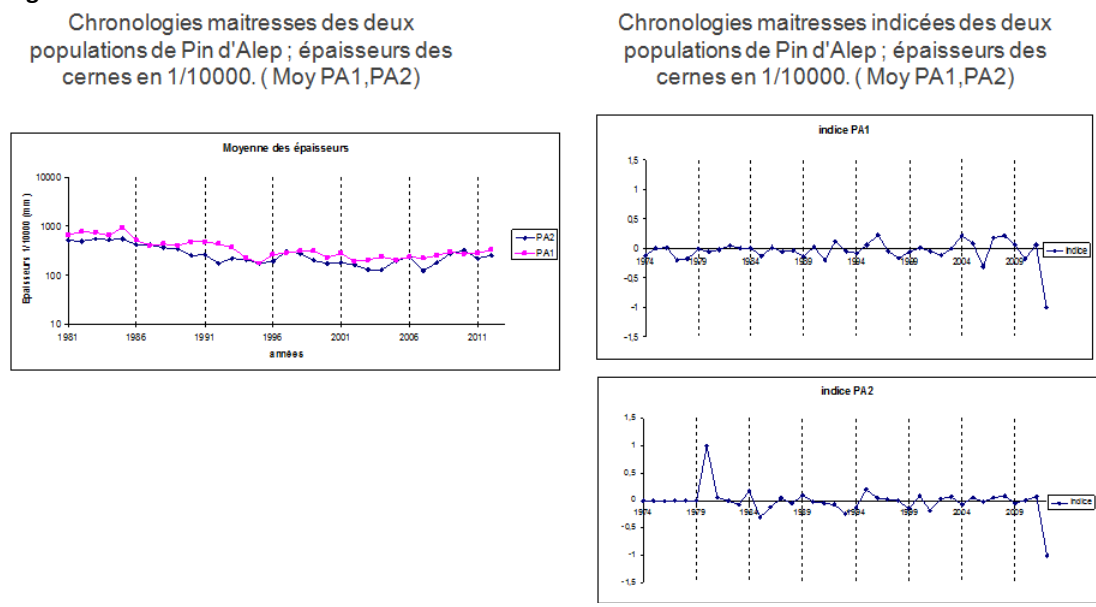
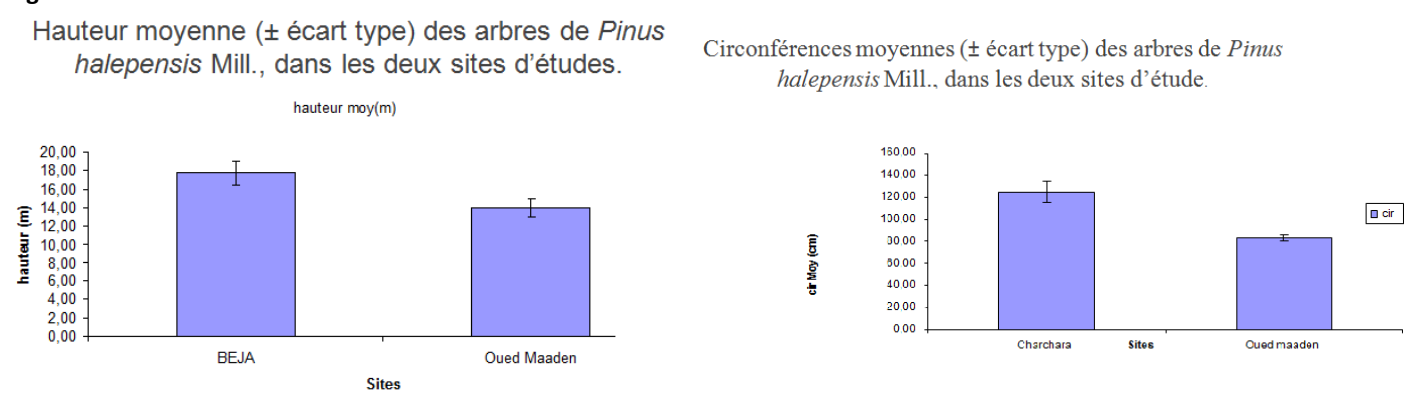


Figure 2



P EGI 33

Monitoring Zygotera(Odonata) in their environnement, Tiaret-Algeria

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Introduction: Freshwater ecosystems include many types of life, of which Zygotera (sub order of Odonata) calls Damselflies. These insects are known by their dependence to wet habitats.

Objective: Our objectives were detecting areas of development of zygotera and improve our information around this group of insects.

Materials and methods: This study deals for the first time the odonatofaune (only Zygotera) in some wetlands in the drainage system of Tiaret-Algeria, this work was carried out by a systematic monitoring in 2013 at 09 stations, of which the objective principal is to explore the areas localization and reproduction Odonata and know the state of diversity in localized stations.

Odonata adults are sampled by a strategy based on direct observation of individuals in areas located along the banks of water bodies, according to the method presented by (Oertli et al., 2000) and applied (Gordeau, et al., 1999) and (Oertli, 1994).

Results: The study focuses exclusively on adult stages (mature and immature) Odonata. The comprehensive inventory of Odonata has established a preliminary list of 07 species: *Sympcma fusca*, *Platycnemis subdilata*, *Calopteryx haemorrhoidalis*, *Coenagrion mercuriale*, *Coenagrion caeruleum*, *Ischnura graellsii*, *Ischnura pomilio* include in 04 families : Lestidae, Platcnimydae, Calopterigidae and Coenagrionidae.

Conclusion: Freshwater ecosystems need to be studied, to protect life in there, Zygotera or Damselflies play an important role in maintaining balance in aquatic ecosystems, and their reduction disappearance will have negative impacts on all living beings in the same medium.

P EGI 34

The culture collection of Phytopathogenic Microorganisms: An important source of information to common bean research

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The culture collection of common bean pathogenic microorganism of Embrapa Rice and Beans was created in 1981 to enrich the Brazilian genetic heritage and the financial support made possible the cataloging, maintenance and monitoring more than 4.000 isolates of bean pathogens, being the most relevant species: *Colletotrichum lindemuthianum* (Figure 1), *Pseudocercospora griseola*, *Fusarium oxysporum* f. sp. *phaseoli*, *Sclerotinia sclerotiorum*, *Macrophomina phaseolina*, *Curtobacterium flaccumfaciens* pv. *flaccumfaciens* and *Xanthomonas axonopodis* pv. *phaseoli*. The challenge was the implementation of the computerized database and a search system, with the most relevant information, including data obtained with the use of molecular markers. Thus, in addition to facilitating access to information contained in the collection to the public internal company also will facilitate access to information by other external research groups, contributing more strongly to the development of research related to pathosystem. The objective of this study was to group the various data relating to the collection of cultures of pathogenic beans of microorganisms, aiming to emphasize its complexity and importance to the development of disease-resistant plants. Samples from several bean producing regions of Brazil were received over 33 years. The data collection of each material was carefully recorded, containing georeferenced cultivar information. After morphological, pathogenicity by using a differential cultivar series, biochemical and molecular characterization, the identified isolates were subjected to long-term preservation in three different methods: cryopreservation, Castellani (in water) and filter paper. A high degree of variability among the isolates of the same species and the predominance of certain pathotypes according to their origin regions, shows the need for sampling and continuous isolation over the years. The isolates are used mainly for the selection of disease resistant genotypes, pathogen-host interaction studies and on the characterization of physiological races.

Figure 2:



P EGI 35

Combined resistance to Bacterial Wilt and Fusarium Wilt in common bean Genotypes derived from a segregating population

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Bacterial wilt (*C. flaccumfaciens* pv. *flaccumfaciens* - *Cff*) and Fusarium wilt (*F. oxysporum* f. sp. *phaseoli* - *Fop*) present similar symptoms derived from the obstruction of xylem vessels. A mapping population obtained by crossing Ouro Branco (resistant) x CNFP 10132 (susceptible), contrasting for bacterial wilt was evaluated for both diseases. The 12 more resistant genotypes of the generation F5:7 were inoculated in Embrapa Rice and Beans greenhouse by injecting 20 uL of bacterial suspension (10^8 UFC.mL⁻¹) of isolates Cff33 and Cff25, ten days after planting. The inoculation of suspension of 10^6 conidia.mL⁻¹ of the isolates Fop101 and Fop102 was made by dipping cut roots during 5 min with subsequent transplant. The control was also inoculated with water. Evaluations were made at 15, 18 and 20 days after inoculation with *Cff*, and 21 days after inoculation with *Fop*, using severity scales of 1 = no symptoms to 9 = dead plants. Data were submitted to analysis of variance and means compared by Skott-Knott test at 5% probability. Plants were considered resistant when the mean rate was at 1 to 3. The more resistant genotypes for both diseases were identified as OBxCNFP 10132.42, OBxCNFP 10132.27 (clear grains) and OBxCNFP 10132.66, OBxCNFP 10132.162 (black grains) as well presented the morphological characteristics and commercial grain favorable to consumers.

P EGI 36

Host Plant Determination of *Brachytrupes megacephalus* Lefebvre, 1827 (Orthoptera, Grillinae) Using Faeces Analysis in the Region of Oued Righ (Algerian Sahara)

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In the southeast of the Algerian Sahara (Touggourt), the host plant determination of *Brachytrupes megacephalus* was studied by faeces analysis (microscopic observations). The experimentation was conducted in mid August until the end of September 2014. The main results of faeces analysis showed that the plants consumed by *B. megacephalus* belong to 8 families: Arecaceae (*Phoenix dactylifera*), Apocynaceae (*Nerium oleander*), Asteraceae (*Chrysanthemum*), Myoporaceae (*Myoporum sandwicense*), Plumbaginaceae (*Armeria maritima*), Rosaceae (*Prunus armeniaca*), Solanaceae (*Solanum lycopersicum*) and Triochylaceae (*Cherry fantasia and White Kristina*). *Phoenix dactylifera* is the most preferred host species by the adults of *Brachytrupes megacephalus*, with a consumption rate 85,87%.

P EGI 37

Varietal differences of barley in susceptibility to feeding by the migratory locust *Locusta migratoria* (Orthoptera: Acrididae) and investigation of the feeding deterrents contained in the barley leaves

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Introduction: The migratory locust *Locusta migratoria* exhibits density-dependent phase polyphenism and is potentially one of the most destructive agricultural pests worldwide. The locust feeds on various Poaceae but does not eat barley. Identification of the feeding inhibitors in the barley leaves is expected to be useful for developing resistant crops.

Objectives: We aimed to investigate the feeding deterrents contained in barley leaves against the migratory locust.

Materials and methods: A laboratory strain of *L. migratoria*, eight barley (*Hordeum vulgare*) cultivars and two strains of *H. spontaneum* were used for the experiments.

Gregarious phase locust hatchlings that were held individually in petri dishes were supplied with fresh leaves of each test plant every day. The percentages of locusts that fed on barley leaves and their survival rate during the first instar were recorded.

Fresh leaves of the barley cultivar Nihon-ichi were freeze-dried and 3.0g of dried leaves were soaked in 300 ml of hexane, dichloromethane or methanol overnight. The extract solutions were concentrated to approximately 10 ml with a rotary evaporator under reduced pressure. 75 µl of each extract and 75µl of 3% sucrose in 50% ethanol were applied to pieces of filter paper (10 x 40 mm), which were then dried and presented to *L. migratoria* adults. The amount of filter paper consumed was determined for each test plant extract and compared with the controls that were treated with wheat extracts and solvent alone.

Results: The survival rate was significantly reduced for the first instars of *L. migratoria* that were fed any of the tested barley cultivars and *H. spontaneum* strains compared with the locusts that were fed the wheat (control). The percentages of locusts that consumed Nihon-ichi and one *H. spontaneum* strain were significantly lower than the value from the wheat or solvent controls.

Locusts consumed a significantly smaller amount of filter paper when it was applied with barley leaf extracts versus wheat extracts, but this difference was obtained only from the methanol extracts.

Conclusion: Our study revealed that the Nihon-ichi and some other barley cultivars and strains contained higher amounts of feeding deterrents against *L. migratoria* than the other tested barley cultivars and wheat. These compounds are considered to be methanol-extractable.

P EGI 38

Biodiversity and Relative Abundance of Arthropods in Citrus Cultivars Using Various Collection Techniques

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Citrus is the major genus of the family Rutaceae and is the most merchandized agricultural produce in the world. The average production of citrus in Pakistan is far below than other citrus growing countries. Many insect pests have been reported in citrus. The objectives of this study were to characterize and document the arthropod community associated with citrus. Sticky traps, Pitfall traps, Berles funnels, aspirator and aerial nets were used to sample the associated arthropods in fourteen of citrus cultivars. The members of insect order Hemiptera were found most in all cultivars followed by Coleoptera and Hymenoptera. The insect pests belonging to order Diptera and Lepidoptera were also found in sufficient numbers. The members of family pseudococcidae and coccinellidae were found in all cultivars. Mars early cultivar of citrus was found with high numbers of termites. The *Olenda Valencia* and Mars early cultivar were found to have more diversity of arthropods while Valencia late the least. This study provides useful information about the seasonal abundance of arthropods in citrus plantation in order to devise a suitable management technique for harmful insects.

P EGI 39

Analysis of the number of sensilla on the labrum and the diet of grasshoppers belonging to the family Pamphagidae (Orthoptera)

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We studied the diet of 10 species of grasshopper belonging to the family Pamphagidae over a period of 3 years at 6 localities in North Eastern Algeria. The species of plants consumed by the grasshoppers was determined by comparing slide mounted specimens of the pieces of plant epidermis in their faeces with those in a reference collection of identified plants collected from the same localities. The percentages of occurrence of the different species of plants in the faeces of the grasshoppers were not related to the abundance of the plants at the sites studied. All the grasshoppers were polyphagous but differed in the percentage of Poaceae in their diets. The diet of *Tmethis* and *Ocneridia* contained a higher percentage of Poaceae than the other species and are considered to be ambivores. The three species in the *Pamphagus* gr. *djelfensis* complex differ in their diets but all tend to avoid consuming Poaceae and are categorized as forbivores. We also compared the frequency of occurrence of Fabaceae in the faeces and in the field and *O. volxemii* is the only species that avoided consuming this plant family.

The number of sensilla on the labrum was also studied in both sexes of each species. Once one corrects for differences in the size of the labrum, the forbivores have higher numbers of sensilla in groups A1, A2 and A3 (but not A10) than the ambivores. The numbers of sensilla in the A10 group on the labrum of species of Pamphagidae is greater than on that of species of Acrididae, which are mainly graminivores and adapted to semi-arid conditions.

P EGI 40

Inheritance of resistance to *Cucurbit yellow stunting disorder virus* (CYSDV) in melon (*Cucumis melo* L.) accessions PI 482431 (TGR 1937) and PI 614479

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CYSDV and whitefly (MEAM1 cryptic species of *Bemisia tabaci*; SPWF) together have devastated fall melon production in the lower deserts of California and Arizona since 2006. Host plant resistance of melon to CYSDV and SPWF are high priorities for sustained summer and fall melon production in this area. Melon accessions PI 482420 (TGR 1551), PI 482431 (TGR 1937) and PI 313970 are partially resistant to CYSDV, but inheritance of resistance has been reported only for PI 482420 and PI 313970. Higher levels of resistance to CYSDV are, however, desired, and toward this end ≈500 melon accessions of India origin were evaluated at the Univ. Calif., Desert Research and Education Center (DREC) in Imperial Valley for reaction to natural infection by CYSDV in open field tests from 2007 to 2013. Six of the accessions were heterogeneous for apparent resistance to CYSDV: PI 122847, PI 123496, PI 124550, PI 145594, PI 614479, PI 614486. Here we report on the inheritance of resistance to CYSDV in PI 482431, which was reported to exhibit moderate resistance in response to controlled inoculations in Spain, and PI 614479, one of the six putative resistant accessions identified in Imperial Valley. A randomly selected plant of PI 482431 was crossed via hand pollination with CYSDV-susceptible 'Top Mark' in a greenhouse to produce the F₁. Likewise, a randomly selected plant from selfed progeny of a putative resistant selection of PI 614479 was crossed with 'Impac'. The respective F₂ and testcrosses were similarly produced in a greenhouse. Inheritance of resistance was studied in naturally infected field tests at DREC in the fall of 2013. The two tests (PI 482431 and PI 614479) were sown 13 to 15 Aug. on standard raised (ca. 20 cm) melon beds on 2.0 m centers. Six seeds were sown by hand ca. 1.2 m apart in each plot (ca. 7.6 m long). The four reps in each test consisted of 12 plots: one each of the parents and F₁, five of the F₂, and two of each testcross. The initial irrigation was applied 16 Aug. via buried drip lines. CYSDV symptoms were evaluated 10 weeks post-planting (WPP; 22 to 29 Oct.) using a 1 to 10 visual scale, where 1 ≤ 10%, 2 ≈20%, 3 ≈30%, ...10 ≈100% symptomatic foliage. SPWF feeding pressure was high based on data collected 3 WPP from 'Top Mark', 'Impac' and PI 482431 in an adjacent field test: 346, 483 and 361 adult SPWF per leaf, respectively. There were significant differences in symptom severity 10 WPP between the resistant parents and their susceptible mates. The F₁ generations did not significantly differ from their respective susceptible parents, which indicated recessive control of resistance to CYSDV in PI 482431 and PI 614479. The F₂ and respective testcross generations confirmed recessive control of CYSDV resistance. CYSDV titer reflected differences in symptom expression; ELISA absorbance (405 nm) means of 'Top Mark', PI 482431 and PI 614479 were 2.405, 0.384 and 0.984, respectively, 10 WPP.

P EGI 41

Isolation and identification of fungal strains producing antifungal substances from the soil of the burned forest of the region of Mila (eastern Algeria)

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The present study was initiated to (i) determine burned forest-inhabiting fungi in Zouagha ,Terri Beinène, Mila and study the antagonistic activity of *Trichoderma* sp against *Fusarium* sp, *Penicillium* sp, *Rhizoctonia* sp, *Alternaria* sp.

18 fungal strains were isolated from Soil samples taken from the forest Zouagha(Burned) in the region Mila representing 6 genera: *Trichoderma* sp et *Fusarium* sp, *Penicillium* sp, *Rhizoctonia* sp, *Alternaria* sp, *Rhizopus* sp.

The tests of dual culture methode on culture medium (PDA) against *Trichoderma* sp et *Fusarium* sp, *Penicillium* sp, *Rhizoctonia* sp, *Alternaria* sp revealed that : *Trichoderma* sp could reduce mycelium growth of *Fusarium* sp 23.13% , *Penicillium* sp 33.13% , *Rhizoctonia* sp 33.75 % and *Alternaria* sp 38.31% in comparaison with the witness after 6 days at room temperature (25°C).

The strains of *Fusarium* sp , *Penicillium* sp , *Rhizoctonia* sp et *Alternaria* sp showed differences sensibility to the antagoniste.

P EGI 42

Biodiversity in Ethnomedicinal practices and Hygiene among Tribals of Wayanad, India

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The present study was conducted to document the biodiversity in ethno medicinal practices followed for oral health and diseases by tribal people of Wayanad district, Kerala, India. Extensive ethno medicinal survey was undertaken to collect information from traditional healers, elders and heads of the tribal community and botanists on the use of medicinal plants and traditional oral care practices of this region. These people belonging to primitive or aboriginal culture possess a good deal of information about medicinal utility of biodiversity. Systematic recording of data was done using a specific questionnaire subsequent to personal interview. This study identified 24 herbs and various traditional methods used by people of this region to maintain oral health and hygiene and as a remedy for dental diseases. Information on 32 plants that were traditionally being used for oral health and hygiene by the tribal people of Wayanad was documented. Many of these plants are still being used. Out of the 24 species, 3 species are used for routine oral hygiene practices, which are used to clean the teeth and massage the gums. The leaves are rolled and one end of the roll is chewed to make it soft and fibrous and used for cleaning. Alternatively whole leaves are chewed to make an infusion of the leaf extract and saliva. This mixture along with fibrous leaf material is rubbed against teeth and gum using finger for cleansing. Similarly 8 species are used for tooth decay, 13 for tooth ache and 6 for stomatitis or oral ulcers. The various parts of plants used. In addition to the herbs some people of Wayanad are also using charcoal, half burnt rice bran mixed with salt, soot formed on the vessels or chimneys after heating with wood for regular cleaning of teeth. Since the use of plant materials with medicinal potential represent a valid alternative for treatment of different diseases, the documentation of the valuable ethno medicinal practices become essential. This would contribute to the body of knowledge and help in exploration of new and novel bioactive compounds to fight against oral diseases.

Figure 1

TABLE I – HERBS USED FOR ROUTINE CLEANING THE TEETH

No	Taxon	Family	Vernacular Name	Part used	Method of use
1	<i>Mangifera indica</i> , Linn	Anacardiaceae	Mavu	Leaf	Folded leaf rub on the surface of teeth with salt
2	<i>Oryza sativa</i>	Poaceae	Nellu	Husk	Burnt husk mixed with salt and rub on the surface of teeth with fingers
3	<i>Piper nigrum</i> Linn	Piperaceae	Kurumulaku	Fruit	Grind pepper fruit along with salt and rub on teeth with finger

Figure 2

TABLE II - USED AS REMEDY FOR DENTAL CARIES/ TOOTH DECAY

No	Taxon	Family	Vernacular Name	Part used	Method of use
1	<i>Curcuma longa</i>	Fabaceae	Manjal	Rhizome	Placed in to the carious cavity of tooth in the form of paste
2	<i>Lobelia nicotianaefolia</i> Roth	Lobeliaceae	Kattupukayila	Leaf	Chewed and kept in the carious cavity of tooth
3	<i>Nicotiana tabaeum</i> Linn	Lobeliaceae	Pukayila	Leaf	
4	<i>Vitex negundo</i> L.	Verbenaceae	Nochi	Leaf	
5	<i>Solanum anguivi</i> Lam	Solanaceae	Puthirichunda	Fruit	Chewed and left in the mouth for some time
6	<i>Piper nigrum</i> Linn	Piperaceae	Kurumulaku	Root	
7	<i>Allium sativum</i> Linn	Liliaceae	Velluli	Bulb	
8	<i>Tabernaemontana divaricata</i> Linn	Apocynaceae	Nandyarvattam	Latex	Milky juice with the help of cotton pellet is inserted into the carious tooth

P EGI 43

Safety of Desert Locust novel management tactics to the faunal biodiversity in the fragile ecosystem in Africa

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This study was conducted to evaluate the effect of phenyl aceto nitrile (PAN) alone or combined with *Metarhizium anisopliae* (Metsch.) on non-target arthropods compared with their effect on desert locust *Schistocerca gregaria* (Forsk., 1775.) The tests were conducted in the Red Sea Coast, at the International Center of Insect Physiology and Ecology (ICIPE) field station in Port Sudan and Sallom. The area is considered as an important breeding area for the desert locust. Different doses of PAN & *Metarhizium* were used including the recommended dose of both. The observed responses include mortality of caged, *S.gregaria* nymphs, field relative abundance of some indicator species such as *Vieta punctipennis* (Tenebrionidae) *Rhyssalus aphodiinae* (Coptypus) (Scarabaeidae), *Cataglyphis bicolor* (Fabricius, 1793) (Formicidae) , *Monomorium spp* (Formicidae), an undefined sp of spider (Araneae) and unidentified species of abundant crustaceans. Environmental impact assessment; before-after -control-impact (BACI), was used. *S.gregaria* nymphs were significantly affected in the second season. Whereas there was no negative effect on other non-target arthropods abundant in the vicinity of desert locust due to all treatments applied. So the tactics used for control of desert locust proved its safety to the faunal biodiversity in the vicinity of desert locust fragile system.

P EGI 44

Two new records of the genus *Holopyga* (Hymenoptera: Chrysididae) from Iran

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Holopyga Dahlbom, 1845 (Hymenoptera: Chrysididae), is a large genus in the tribe Elampini and comprises 91 species in all zoogeographical regions, with highest diversity in the Palaearctic region. According to previous studies, 16 species of this genus had been reported for the fauna of Iran. The aim of this study was to improve our knowledge of the genus *Holopyga* in Iran. The specimens were collected using Malaise traps and sweeping net at Northern provinces (Guilan, Mazandaran, Alborz and Qazvin) and Southern provinces (Hormozgan and Fars) of Iran during 2010-2013. Seven species were collected and identified, of which two species are new records for the fauna of Iran: *Holopyga fasscialis* Linsenmaier, 1959 and *H. jurinei* Chevrier, 1862. *Holopyga*

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fascialis was collected from Hormozgan province and *H. jurinei* from Alborz province. With two new records, the number of *Holopyga* species in Iran has increased from 16 to 18.

P EGI 45

Cold Storage of Eggs and Adults of Phytoseiid Mites, *Phytoseiulus persimilis* Athias-Henriot, 1957 and *Typhlodromus pyri* Scheuten, 1857 (Acarina: Phytoseiidae).

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The research aimed to determine the effect cold storage on the viability of *Phytoseiulus persimilis* eggs and adults, and *Typhlodromus pyri* (adults) stored at 5 °C and 10 °C, as well as its effect on hatchability and incubation period, after 1,2,3 and 4 weeks of cold storage. Results cleared that the incubation period of *P. persimilis* eggs, stored for one week under 10 °C was 3.1 ± 0.32 days and the hatchability percentage was 97.4 %, while the incubation period of eggs stored for two weeks at 10 °C were shortened to 2.5 ± 0.27 days, as well as the hatchability percentage was decreased to 85.3 %. Regarding to the treatment of three weeks storage at 10 °C, the incubation period was sharply decreased to 1.4 ± 0.66 days with hatchability percentage of only 58.6 %. Eggs stored for four weeks at 10 °C didn't hatch. As for the survival rate of the predatory mite *P. persimilis*, results indicated that it gradually reduced by increasing cold storage period, where the survival rate after one week of storage under 5 °C was 91.6 %, decreased to 45.3 % after two weeks, while only 12.5 % of predators were survived after three weeks, while all mites were died after four weeks of storage. As for the results of survival rate of *T. pyri* adult stages stored under cold degrees, results indicated the survival rate was 66.6 and 83.3 % for males and females after two weeks of storage at 10 °C decreased to 50 % and 75 % at 3 weeks periods, for males and females. The survival rate of *T. pyri* was 0 % after four weeks of storage at 10 °C. Finally, it could be concluded that the maximum storage period of the eggs of the predatory mite, *P. persimilis* should not be excess than three weeks under 10 °C. While, *T. pyri* adult females can be successively stored for two weeks under 10 °C. With high rates of viability.

P EGI 46

Resistance of *Hordeum vulgare* against *Pyrenophora teres f. teres*

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Pyrenophora teres f. teres is the causal agent of the net type of net blotch in barley. This fungal pathogen is widely spread and highly damaging, rendering it one of the most serious foliar diseases in barley production. The most cost effective and environment friendly way to prevent and control infection is by growing resistant cultivars. In a previous project between the Institute for Resistance Research and Stress Tolerance of the Julius Kuehn-Institute and the All-Russian Research Institute of Plant Protection more than 10,000 barley accessions, including landraces and commercial cultivars were screened for resistance to *P. teres f. teres* under laboratory, greenhouse and field conditions. In the frame of these studies 449 barley accessions from 50 different countries expressing a different level of resistance were identified. In order to get more detailed information on the resistance these 449 barley accessions are inoculated with two different but very aggressive isolates of *P. teres f. teres*, one from Russia and one from Germany.

To achieve this plants were grown in a completely randomized block design with four replications in a greenhouse at 18°C day/16°C night with 7 hours additional light. Three week old plantlets were inoculated with a hand sprayer until the leaves were completely covered with a spore suspension adjusted to 5000 spores per mL. Fourteen days after inoculation the infection response type was assessed following the scale of Tekauz (1985) from 1 (highly resistant) to 10 (highly susceptible). Out of the 449 tested genotypes 245 (approximately 54%) showed high to moderate resistance to *P. teres f. teres* isolate No 13 from Saint Petersburg, Russia. The resistant genotypes originated from 35 different countries. To confirm the phenotypic data and identify quantitative trait loci associated with resistance against net blotch, genome wide association studies based on genotypic data derived from the Illumina 9k iSelect Chip will be conducted.

P EGI 47

Effect of heat stress on the physiology of the cork oak

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Our study shall focus on the ability of trees cork oak that showed vis-à-vis sensitivity to climate change, including late spring frosts. The combination of these factors resulted in damage alarmed therefore forest ecosystems weakened trees that can affect their ability to support other abiotic and biotic stresses,

For this we tested its tolerance to thermal variations and cold weather conditions by estimating some stress markers (quantification of proteins, RNA, soluble sugars) that are quantified to evaluate the cold tolerance of seedlings.

Sowing of cork oak (*Quercus suber* L.) is grown in controlled conditions at 25 ° C ± 2 ° C in long days 16h. These seedlings are transferred at low temperatures between 5 ° C and -6 ° C for a period of 3 hours. Biochemical analyzes were performed in the various organs of the cork oak seedlings. Cool temperatures induced a significant accumulation of proline in different organs of seedlings and the optimum concentrations were observed in the roots with very high concentrations (4 times larger than those of the control). The accumulation of soluble sugars is significantly in stems and roots at 0 ° C. Protein concentrations are very high in leaves of both growth and high waves in rod at -4 ° C to -2 ° C. Tolerance cork oak seems to be at the thermal limit of -2 ° C. The concentration of these metabolites in the various organs showed the ability oak cork hardening during the winter.

P EGI 48

New records of the family Stratiomyidae (Dip; Brachycera) from Iran

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Introduction: The family Stratiomyidae (soldier fly) belongs to the suborder Brachycera in Diptera. This family includes more than 2650 species in 375 genera composed of 12 subfamilies worldwide of which 426 species in 55 genera in 7 subfamilies occur in the Palearctic region. In the recent years some studies have been done in the northern west forests of Iran that added more than 15 species to the fauna of this family in Iran.

Material and methods: In order to study of the family Stratiomyidae (Dip; Stratiomyoidea) a survey was conducted during the years 2009-2012. Species were collected by sweep net in the Qaradag Forests were identified in northwestern Iran.

Results: A total of 20 species were identified of which eight species [*Nemotelus rudifraci* (Berezovsky & Nartshuk. 1993); *N. nigrinus* Fallén, 1817; *N. notatus* Zetterstedt, 1842; *N. pantherinus* (Linnaeus, 1758); *Oxycera fallenii* Staeger, 1844; *O. meigenii* Staeger, 1844; *O. notata* Loew, 1873; *O. trilineata* (Linnaeus, 1767)] are recorded for the first time from Iran. An adopted key for the studied species along with the supplementary figures for the new recorded species are provided.

Conclusion: The results show that the fauna of the soldier fly in Arasbaran Forests is surprisingly rich and more studies in these forests and other forests in northern Iran are necessary to complete our knowledge of the insect fauna of this region.

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Figure 1: Location of sampling points on satellite image (SPOT) of Qaradag forests.

Figure 2: a-b) *Nemotelus nigrinus*, female, a) frontal view of head, b) lateral view of body; c-e) *Nemotelus notatus*, c) head of female, d) lateral view of female, e) dorsal view of male, f) male genitalia.

Figure 3: a-c) *Nemotelus pantherinus*, male a) lateral view of head, b) lateral view of body, c) male genitalia; d-f) *Nemotelus rudifraci*, d) lateral view of female, e) male genitalia, f) lateral view of male;

Figure 4: a) *Oxycera fallenii*, female; b-d) *Oxycera meigenii*, b) lateral view of female, c) dorsal view of female, d) male genitalia.

Figure 5: a-e) *Oxycera notata*, male, a) lateral view of body, b) dorsal view of body, c) male genitalia; female, d) dorsal view of body, e) lateral view of body; f) *Oxycera trilineata*, dorsal view of female.

No attachments submitted

P EGI 49

Genetic variation among gene sequences of *Phyllosticta citricarpa* isolates obtained in a seven years interval.

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Citrus Black Spot (CBS), caused by the fungus *Phyllosticta citricarpa*, is spread over continents in the world. The disease has been affecting the citrus production for many years causing fruit damage and premature fall of fruits. Thence, CBS is considered A1 quarantine disease for European Union countries. Control of the disease is based on fungicide application, which has shown less effectiveness over the years. Thus, the aim of this work was to check the genetic variability among *P. citricarpa* isolates from citrus fruits collected in the same orchard in a seven years interval.

Methods: Orange fruits with CBS symptoms were collected in an orchard localized in the state of São Paulo, Brazil, in 2005 and afterwards in 2012. A total of 80 and 115 *P. citricarpa* isolates that composed the 2005 and 2012 collections, respectively, were submitted to a partial sequencing of ITS, GPDH and TEF1 genes by applying BigDye Terminator v3.1 Cycle Sequencing Kit (Life Technologies) in a ABI3100 sequencer.

Results: The outcomes revealed higher variability among ITS sequences than GPDH and TEF1. The collection of 2012 has more haplotypes, individuals with different sequences, than the 2005 collection, owing to the accumulation of more variable sites in isolates obtained in 2012. In relation to ITS sequences, there was an increment of 33.8% in the number of haplotypes in the 2012 collection with respect to the 2005. Secondly, analysis of GPDH sequences indicated an increase of 19% in the haplotypes number in the 2012 isolates collection. In turn, TEF1 sequences of isolates from 2005 and 2012 collections did not present significant variation.

Conclusions: *P. citricarpa* species may suffer large accumulation of genic variations in a short period of time.

P EGI 50

Genetic Diversity of *Phyllosticta capitalensis* in guava plants from different environments

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P. capitalensis corresponds to an important species of fungi associated with plant kingdom. Although *P. capitalensis* has endophytic behavior to a wide host range, studies indicated that this fungus species may cause rot on guava fruits, whose symptoms were previously attributed to other species of *Phyllosticta*. Thus, in order to understand better the interaction of *P. capitalensis* and guava, this work aimed to analyze the genetic profile of this isolates species.

Methods: Leaves were collected in guava germplasm bank and in areas of commercial production, while the fruits were only collected in areas of commercial production. The isolates were submitted to a partial sequencing of ITS, actin (ACT) and glyceraldehyde 3-phosphate dehydrogenase (GPDH) genes for previously species identification and afterwards their genetic variability was analyzed by applying AFLP markers. Both analysis were carried out in an ABI3100 sequencer.

Results: 33 isolates derived from asymptomatic leaves and 9 from symptomatic guava fruits were identified as *P. capitalensis*. Although the isolates have been distributed randomly in the AFLP dendrogram, it was observed a subgroup compounded only by isolates of symptomatic guava fruits from commercial orchards.

Conclusions: The genetic difference between a subgroup of isolates from a commercial area that receives chemical treatments and the others from germplasm bank denotes the possibility of *P. capitalensis* speciation between endophytic and pathogenic individuals.

P EGI 51

Population density and spatial distribution of immature stages of *Callosobruchus maculatus* (Col.: Bruchidae) on cowpea in Tehran region

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Population density, sampling program and spatial distribution pattern of egg and larvae of *Callosobruchus maculatus* on cowpea (var. Parastoo) were determined in Tehran region during 2005. Bean pod was selected as a sample unit and the reliable sample size with maximum relative variation of 20% was obtained 50. Taylor's power law and Iwao's patchiness regression methods were used for determining the spatial distribution pattern of egg and larvae of the pest. The relationship between length and diameter of pods with number of laid eggs was determined using linear regression. There was significant positive relationship

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between number of eggs and the length of pods. The relationship between number of eggs and diameter of pods was also significantly positive. There was no significant relationship between number of larvae and length/diameter of bean pods. The spatial distribution pattern of egg using Taylor's method was aggregated and using Iwao's method was random. The spatial distribution pattern of larvae using two ways was aggregated and total of egg and larvae using Taylor's method was aggregated and using Iwao's method was random. Occurrence of the pest in field was observed early summer and irregular population fluctuation was recorded bean growing season.

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Poster Presentations

Endophytes

P ENDO 1

The presence of endophytes and conspecifics determine olfaction-guided host foraging of the New Zealand grass grub.

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Introduction: Grass endophytes of the fungal genus *Epichloë* (asexual: *Neotyphodium*) are widely used in New Zealand to control pest insects of pastures. The primary mechanism for resistance in *Festuca pratensis* x *Lolium perenne* is the shoot-to-root translocation of insect-toxic loline alkaloids produced by the endophyte *E. uncinatum*. However, endophytes can also have an impact on their host plant's metabolism which can subsequently affect soil pests. For instance, below-ground insects rely on root volatiles for long-range host location. Whether endophytic fungi affect root volatile emission of undamaged or herbivore-damaged plants (constitutive and induced volatiles, respectively) and whether this affects plant resistance was the question addressed in this study.

Objectives: To establish whether shoot endophyte colonisation affects constitutive and herbivore-induced root volatile emission and subsequently the behaviour of a belowground insect.

Materials and Methods: Behavioural studies were performed with a 4-arm-belowground olfactometer. Larvae of *Costelytra zealandica* (Col., Scarabeidae) were allowed to choose between odours of (a) undamaged non-endophyte grass (E-) vs. endophyte grass (E+), (b) grass roots (E-) with 0, 5, 10, or 20 *C. zealandica* (c) same as previous but with E+, (d) same as previous but without plants. Proton-transfer-reaction mass spectrometry was used to characterize the volatile metabolome of undamaged E- and E+.

Results: In undamaged plants, grubs preferred volatiles of E- over E+. This correlated with attenuated emission of volatiles in E+. Furthermore, grubs found treatments '20 grubs on E-' and '20 grubs on E+' more attractive than either plant without grubs, but volatiles of 20 grubs without plants were also attractive.

Conclusions: Presence of the endophyte *E. uncinatum* had a positive effect for grass roots as fewer herbivores were attracted, possibly due to reduced root volatile emission. Attraction to the root-herbivore-complex was density-dependent but the pattern was similar for E- and E+ plants. The relative contribution of herbivore-induced versus insect-derived volatiles needs further clarification however.

P ENDO 2

Endophytes in maize and pine in New Zealand

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Introduction: As part of investigations into endophytes in the agricultural and forestry crops in New Zealand, we have examined the naturally occurring fungi colonising maize (*Zea mays*) and pines (*Pinus radiata*). Our interest is not just in occurrence of endophytic fungi, but also in any beneficial effects. Therefore the effect of a common endophyte of pines, *Beauveria bassiana*, was used to effect of above ground and below ground mortality of two species of insects and to determine effect on plant health.

Objectives:

- 1 Identify naturally occurring endophytes in maize and pines.
- 2 Determine any effects of selected endophytes on plant defence against insects.

Methods and materials: Putative fungal endophytes were recovered from maize and pines over several seasons. Fungi were recovered after surface sterilisation on PDA medium, and each isolate identified using morphological and molecular techniques. Selected isolates were inoculated into plants by root dipping and test the effect against *Helicoverpa armigera* and grass grubs (*Costelytra zealandica*) both on insect and plant growth.

Results: To date the study has identified 60 isolates of fungi colonising maize and a further 26 colonising pines. Using pines as the first test system, *C. zealandica* larvae showed reduced weight when endophytic fungus, *B. bassiana*, was present. There was no effect on *H. armigera* which may be due to localised expression of the *B. bassiana* in the roots.

Conclusions: Naturally occurring maize and pine endophytes are numerous but as yet it is not known if any will be useful as a biological control agents. One fungus, *B. bassiana*, has shown promise in pines but requires information on the transmission, colonisation and the persistence within the pine.

P ENDO 3

The endophytic fungus *Piriformospora indica* promotes growth and salt stress tolerance of rice plants

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Piriformospora indica (*P. indica*), an endophyte fungus, can be cultured in artificial medium without plant hosts. Effects of endophytically colonized *P. indica* on promoting plant growth and increasing environmental stress tolerance have been observed in several plant species. However, the effect of *P. indica* on growing of semi-aquatic plant such as rice plants is still need to be further addressed. Rice is one of the most important food crop, and nearly half of the world's population depends on rice as their staple food source. In this study, we focused to reveal the effect of *P. indica* on rice (*Oryza sativa* L. cv. Taichung Native 1, TCN1) growth and the abiotic stress tolerance. The result showed that the biomass of *P. indica*-colonized hydroponic rice seedlings was significantly increased. Using ImageJ analysis to observe the root system, the result showed that architecture of root colonized by *P. indica* was modified. The total number and length of adventitious roots were obviously increased, and the length of lateral roots was also promoted. To reveal whether auxin was involved to the regulatory pathway of *P. indica*-induced root growth, the p-chlorophenoxyisobutyric acid (PCIB), an inhibitor of auxin action, was applied to the seedling culture solutions. The data showed that PCIB can repress the *P. indica*-mediated root phenotype change. Moreover, the *P. indica*-induced expressions of *OsRAA1*, an indicator gene of auxin levels, were also reduced. In addition, cell wall extract of *P. indica* would provide the similar effect with that of endophytic *P. indica* on seedling growth. Rice is sensitive to salt stress especially at seedling stage. The salt tolerance of 3-leaf-stage seedlings inoculated with *P. indica* has been investigated in our study. Malondialdehyde (MDA) level was used as a biomarker to monitor the influence of oxidative stress in rice tissues under high salinity conditions. The increase of MDA level induced by salt stress was significantly reduced in *P. indica*-inoculated rice seedlings. In addition, the activities of some antioxidant enzymes were up-regulated by *P. indica*. In conclusion, reducing oxidant stress would play an important role in the *P. indica*-induced salt tolerance of rice plants at stress conditions and recovery stages.

P ENDO 4

Diversity of Phylloplane and Rhizosphere Bacteria and their potential as Antagonist for blast of rice and bacterial blight of Pomegranate

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Bacteria were isolated from phylloplane of pomegranate and also from rhizosphere of rice plants grown under submerged conditions, following serial dilution method. *Pseudomonas fluorescens* (RPf1) and *Bacillus subtilis* (RBS1) were used as reference culture along with four bacterial endophytes in neem procured from National Center for Biological Sciences, Bangalore. The obtained bacterial isolates were pre-evaluated for inhibitory activity by dual culture method against *Magnaporthe oryzae* and modified well method for *Xanthomonas axonopodis* pv. *punicae* pathogens. The isolates were identified and characterized based on morphological, biochemical and 16S rRNA gene sequencing. Identification of the bacterial strains was done for isolates that showed high inhibition against *M. oryzae* and *X. a. pv. punicae* using bacterial species specific 16S rRNA primers.

A total of 58 bacterial strains from pomegranate phylloplane and 60 bacterial strains from the rice rhizosphere were isolated and along with six reference strains were tested for their biocontrol activity against the devastating *M. oryzae* by *in vitro* dual culture assay and *X. a. pv. punicae* by modified well method on Luria Bertani agar medium. Among these isolates ten paddy rhizospheric isolates, 15 phylloplane bacterial strains from pomegranate and four neem bacterial endophytes effectively inhibited the mycelial growth of *M. oryzae*. The results of *in vitro* dual culture assay revealed that the maximum per cent inhibition against *M. oryzae* was observed in *Enterobacter* spp. (81.71%), followed by *Bacillus cereus* (76.83%), *Alcaligenes* spp. (76.83%), *Serratia marcescens* (76.83%), *Myroides odoratus* (76.83%), *Bacillus subtilis* (75.00%), *Bacillus pumilus* (75.61%), *Bacillus cereus* (74.39%), *Brevibacterium* spp. (74.39%), *Pantoea anthophila* (74.39%), *Myroides marinus* (76.83%), *Pseudomonas fluorescens* (63.41%), *Proteus mirabilis* (63.41%) and *Alcaligenes faecalis* (52.44%) compared to negative control and positive control reference strains *Bacillus subtilis* and *Pseudomonas fluorescens* (71.95% and 56.10% inhibition respectively) (table 1 and plate 1). Among neem endophytes *Pantoea ananatis* recorded 65.30 per cent compared to both the controls. Similarly, maximum zone of inhibition of *X. a. pv. punicae* was recorded with *Bacillus cereus* (3.83cm) followed by *Bacillus pumilus* (3.40cm) and *Myroides odoratus* (2.80cm) compared to both the controls. These bacterial strains were found superior to *Bacillus subtilis* and *Pseudomonas fluorescens* reference strains.

From these studies it was observed that the diversity of bacterial population in three ecological niches of plant and their ability to suppress the blast of rice and bacterial blight of pomegranate along with enhancing the growth of rice seedlings on seed bioprimering. The efficient antagonistic strain and its effective delivery method help not only to suppress the diseases but also enhance the seed indices and seedling growth.

Figure 1

Table 1. The effects of bacterial isolates on *M. oryzae* and *X. a. pv. paniceae* growth under *in vitro*

Bacterial strains	Identification by 16S rRNA sequencing	<i>M. oryzae</i> growth inhibition (%)	<i>X. a. pv. paniceae</i> growth inhibition (cm)
UASR ₁	<i>Alcaligenes faecalis</i>	52.44	1.03
UASR ₂	<i>Pseudomonas fluorescens</i>	63.41	1.00
UASR ₃	<i>Bacillus cereus</i>	76.83	3.83
UASR ₄	<i>Serratia marcescens</i>	76.83	2.70
UASR ₅	<i>Bacillus subtilis</i>	75.00	2.23
UASR ₆	<i>Bacillus cereus</i>	74.39	
UASR ₇	<i>Proteus mirabilis</i>	63.41	-
UASP ₈	Cellulomonadaceae	71.95	2.20
UASP ₉	<i>Alcaligenes sp.</i>	76.83	1.87
UASP ₁₀	<i>Myroides marinus</i>	65.85	2.15
UASP ₁₁	<i>Pantoea anthonomophila</i>	74.39	1.77
UASP ₁₂	<i>Bacillus pumilus</i>	75.61	3.40
UASP ₁₃	<i>Myroides rubicatus</i>	76.83	2.80
UASP ₁₄	<i>Brevibacterium sp.</i>	74.39	1.07
UASP ₁₅	<i>Enterobacter sp.</i>	81.71	1.03
<i>Bacillus subtilis</i>	Standard reference culture 1	71.95	4.27
<i>Pseudomonas fluorescens</i>	Standard reference culture 2	86.10	1.93
A5-I	Reference culture 1	42.87	-
A5-II	Reference culture 2	28.57	
C5-I	Reference culture 3	44.72	-
C5-II	Reference culture 4	65.30	-

Figure 2

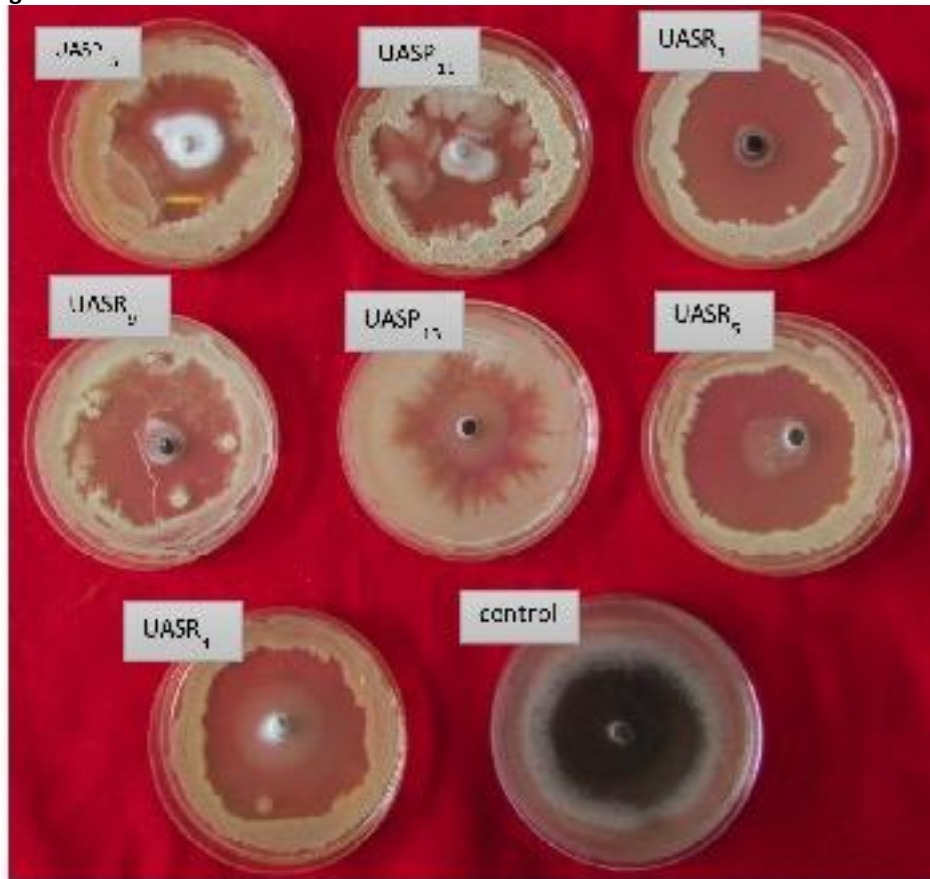


Plate 1. Dual culture for bioantagonistic assay in inhibition of blast fungus by selected bacterial strains. Fungal cultures are in the centre of the plate; bacterial inoculants around the perimeter (UASP₁₅ -*Enterobacter* spp., UASP₁ -*Pantoea anthophila*, UASR₃ -*Bacillus cereus*, UASP₆ -*Alcaligenes* spp., UASP₁₇ -*Mycrodes odoratus*, UASR₅ -*Bacillus subtilis*, UASR₄ -*Serratia marcescens*, Control -*Magnaporthe oryzae*).

P ENDO 5

Endophytes in Oilseed rape: Potential for biocontrol

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Phoma stem canker disease (anamorph *Phoma lingam*, teleomorph *Leptosphaeria maculans*) and Sclerotinia stem rot (*Sclerotinia sclerotiorum*) are two major diseases of oilseed rape (OSR). Endophytic fungi and bacteria may offer a promising alternative to chemical control. Growing sheltered inside the plant, they may have the potential to provide systemic protection over a long period of time. Disease may also influence endophyte communities. We investigated endophyte communities in healthy OSR and in diseased OSR showing symptoms of Phoma stem canker. Root and stem endophytes were isolated from surface-sterilised OSR tissues using nutrient rich (2% MEA) and nutrient poor (SNA) media. The fungal isolates were grouped into morphotypes based on their colony morphology and growth characteristics. Identity of the isolates was confirmed by molecular fingerprinting (internal transcribed spacer sequences ITS).

Cladosporium-like Ascomycetes were only isolated from the roots and shoots of healthy plants and represented the majority of fungal isolates in healthy roots. In roots of diseased plants, fungi related to *Monographella* prevailed. Putative *Plectosphaeria* isolates were isolated from healthy as well as diseased plants. In stems, the differences between healthy and diseased plants

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were less pronounced. Interestingly, *Leptosphaeria*-related fungi formed the largest group of isolates there, irrespective of visible symptoms of Phoma stem canker. *Botrytis*-like isolates were only isolated from symptomless shoots, whereas *Alternaria* was detected only in symptomatic stems. Cryptococcus-like *Basidiomycetes* were present in both healthy and diseased shoots. Endophytic bacteria were more abundant and diverse in the roots (87 isolates) than in the shoots (24 isolates). In both healthy and diseased plants, Pseudomonads were the dominant group of root endophytes, but their species composition differed. Among other bacterial groups, *Enterobacter amnogenes* was more abundant in roots of healthy plants whereas *Serratia* were mainly isolated from roots of diseased plants. *Bacillus* and *Serratia* were the dominant bacterial geni in stems. *Bacillus* isolates prevailed in healthy shoots. *Pseudomonas* species, while frequent in roots, were only found in diseased stems. Selected endophytic fungi and bacteria will be tested in greenhouse and field experiments for efficacy against Phoma stem canker disease and *Sclerotinia* stem rot. In first experiments, *Pseudomonas antarctica* 50 ŘKK 1A, isolated from healthy roots, reduced symptom development of Phoma stem canker significantly when directly applied to leaves, but showed no systemic effect after application as seed and soil treatment. Screening of effective isolates continues.

Acknowledgements: We thank Mr. Dušan Kunc for skillfull technical assistance. The financial support by Technological Agency of the Czech Republic, project Improvement of phytoremediation capacity and production potential of energy crops grown in contaminated and poor soils by means of endophytic and mycorrhizal symbionts contract No. TA03011184, is greatly appreciated.

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P N-CCO 1

Eco-friendly approach to control phytopathogenic fungi Surender Kumar Bhardwaj

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There are concerns about the widespread use of xenobiotic chemicals in crop production in developing countries because of their possible adverse effects on human health. According to World Health Organization survey more than 50,000 people in developing countries are poisoned annually and 5,000 die as a result of the effects of toxic agrichemicals. In India 35,000 - 40,000 tons of hazardous chemicals are sprayed on crops every year and this is considered to increase the risk of cancer, sterility and death. So there is an urgent need to develop sustainable methods for these horrible diseases. The remedy lies in the use of more natural products which do not damage the ecosystems such as biofertilisers, bioinsecticides and biofungicides. The eco-friendly management of crop's diseases is the only safe substitute to be explored to control these phytopathogens and to maintain sustainable agriculture and environment. Plants are known to possess antimicrobial secondary metabolites that can inhibit the growth of plant pathogens and it is possible that these compounds could be used to combat plant diseases. In the present study, experiments were carried out to evaluate the antimicrobial properties of 100 plant parts samples of 100 plants spanning over 45 families against plant pathogenic fungi by the food poisoning method. The various plants tested for their antimicrobial activity have shown varied response. The results are promising and some of the plants have shown inhibitory activity against one or two fungi, whereas others have shown a broader spectrum of activity, some plants showing good activity against all the test fungi. Plants samples of some families such as Apocynaceae, Caesalpinaceae, Combretaceae, Compositae, Ebenaceae, Liliaceae, Lythraceae, Meliaceae, Mimosaceae, Rosaceae, Salvadoraceae, Sapindaceae, Theaceae and Zingibraceae were found to be comparatively more effective against the test fungi and bacteria. In view of the above facts, the present study has elaborated our knowledge by accessing the antifungal properties among the available natural flora which can subsequently be explored for the possibilities towards the identification of the key bioactive agents, through implying modern microbiology and biochemical techniques.

The research work indicates that the use of plant extracts as antimicrobial agent to control plant pathogens is feasible and is cost effective as the material used is inexpensive. The major benefit is its being eco-friendly.

P N-CCO 2

The Effect of Insect Host species on Some Bio-characteristics of *Trissolcus semistriatus* Ness, a Parasitoid of Sunn Pest, *Eurygaster integriceps* Put., in Syria

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Introduction: *Trissolcus semistriatus* Ness is the most abundant species of egg parasitoids of sunn pest (*Eurygaster integriceps* Put.) in Syria (1), which is the most common pest on wheat in Al-Hasaka region in this country (2). There are reports that *T. semistriatus* can be reared in the laboratory on eggs of both *E. integriceps* and *Eurydema ornatum* L. (Het., Pentatomidae) (3), which is a pest of cultivated and wild Crucifera (4).

Objectives: Study the effect of insect host (*E. integriceps* and *Eurydema ornatum*) on some bio-characteristics of *T. semistriatus*, and test cabbage bug if it is possible to be reared as alternative host in the laboratory.

Materials and methods: This study has been conducted during 2009 under laboratory conditions. Some bio-characteristics of parasitoid's female (*T. semistriatus*) was tested when it parasitize on the eggs of both insect hosts (*E. integriceps* and *Eurydema ornatum*). Comparison between eggs of both species and its capability to be parasitized by parasitoid's female was through the tested bio-characteristics.

Results: Results showed that the sunn pest eggs exceeded significantly on cabbage bug eggs for fecundity of parasitoid's female (16.80 and 10.00 eggs), the number of emerged parasitoids (15.90 and 5.60 parasitoids), the hatch percentage (94.92 and 57.67%) and the number of emerged females (10.30 and 5.10 females), but no significant difference for female longevity (6.50 and 4.40 days) and parasitism (22.35 and 24.34%).

Conclusion: This study indicates that it is possible to use alternative insect hosts of sunn pest in laboratory breeding of *Trissolcus* parasitoids, such as cabbage bug (*Eurydema ornatum* L.), which is available naturally in Al-Hasaka region within the integrated management program of sunn pest.

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P N-CCO 3

Development of an efficient approach for genetic transformation of some apple (*Malus domestica* Borkh.) cultivars and rootstocks for improving their fungal diseases resistance using *g2ps1* gene from *Gerbera hybrid* (Asteraceae)

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g2ps1 gene from *Gerbera hybrida* coding for 2-pyrone synthase which contribute for fungal and insect resistance was used. The aim was to establish an efficient and practical reproducible approach of genetic transformation for apple cvs. 'Golden Delicious', 'Royal Gala' and 'MM111', 'M26' rootstocks for improving their fungal resistance using genetic engineering techniques. Putative transgenic shoots could be obtained on MS media with B5 Vitamins, 5.0 mg l⁻¹ BAP, or 2.0 mg l⁻¹ TDZ with 0.2 mg l⁻¹ NAA in the presence of the selection agent "PPT" at 3.0-5.0 mg l⁻¹. Shoot multiplication of transgenic shoots was achieved on: MS + B5 vitamins + 1.0 mg l⁻¹ BAP + 0.3 mg l⁻¹ IBA, 0.2 mg l⁻¹ GA3+1.0 g/l MES+ 30 g/l sucrose + 7.0 g/l Agar, with the selection agent PPT at 5.0 mg l⁻¹. Transgenic clones of the apples studied have been obtained and confirmed by selection on the media containing the selection agent "PPT" and by PCR analysis using the suitable primers in all clones obtained for the presence of the selection bar gene (447 bp) and the gene-of-interest "*g2PS1*" (1244 bp), with transformation efficiency of 0.4%, 0.6%, 0.1% and 0.3% respectively. Results of DNA sequence analysis of the transgenic plants also proved the successful transformation and had 95 to 99% sequence homology with the *g2ps1* gene (accession no. Z38097.2). These transgenic clones were multiplied further *in vitro* in the presence of the selection agent 'PPT' and rooted *in vitro*. Rooted transgenic plantlets were successfully acclimatized and are being kept under-containment conditions according to the biosafety by-law in Syria to evaluate their performance for fungal resistance.

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Figure 1: Physical map of the binary vectors used for apple transformation. (d35S-P: double 35S promoter, 35S-T: terminator, P: promoter, NOS: *Agrobacterium* nopaline synthase gene, Bar: herbicide resistance selectable marker from *Streptomyces hygroscopicus*, RB: right border, LB: left border).

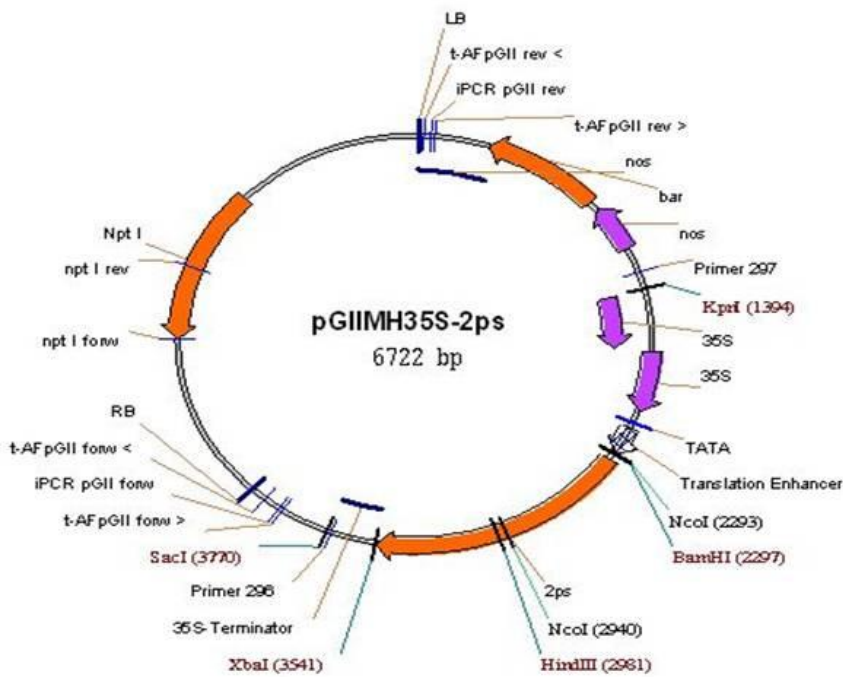


Figure 2

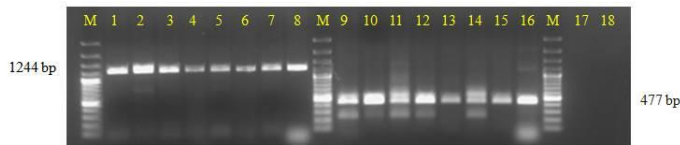


Fig. 5. Molecular Confirmation of Transformation by PCR for the gene of interest g2ps1 and the selection agent bar gene. Lanes: 1,2 GD; 3,4: M26; 5,6: RG; 7: MM111, 8: positive control.

Lanes: 9,10: GD; 11,12: M26; 13,14: RG; 15: MM111, 16: positive control; 17: water; 18: negative control (DNA isolated from non-transformed apple). M: 100 bp marker

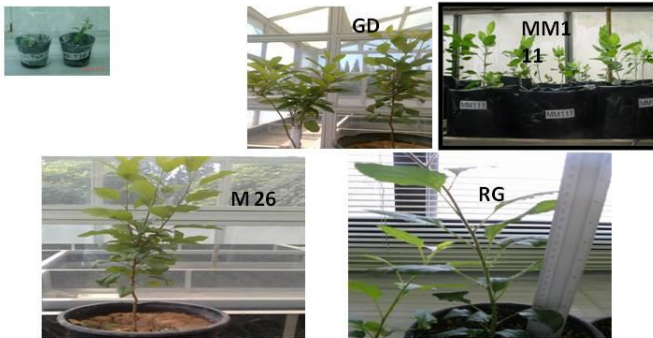


Fig. 6. Acclimatized transgenic apple cvs. and rootstocks studied

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P N-CCO 4

Plant's Strategies for Allelochemical (Benzoxazolinone) Detoxification

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Introduction: Benzoxazinones, phytotoxic secondary compounds of *Secale cereale* L. or *Zea mays*, are used in organic farming for weed control. In soil, benzoxazinones are subject to a cascade of transformation reactions which lead to benzoxazolinones and other compounds. Exposure to the benzoxazolinone BOA affects the transcriptome of receiver seedlings, inhibits germination and growth and can induce death of sensitive species. Differences in the sensitivity are due to species/cultivar-dependent strategies which have been evolved to cope with BOA or other allelochemicals. These strategies include the rapid activation of detoxification reactions and extrusion of detoxified compounds.

Objectives: The intention of the presented and ongoing studies is to elucidate functions of plant associated microorganisms in phytotoxin elimination.

Material and methods: *Zea mays* "Cassilas", *Zea mays* mutant BX-less; *Abutilon theophrasti*, (Herbiseed). Identification of detoxification products, analyses of enzyme assays: HPLC/MS/NMR; proteome analyses: SDS-PAGE; LC-MS/MS.

Results: In *Zea mays* roots, the allelochemical benzoxazolinone is first detoxified by glucosylation of BOA-6-OH and subsequently by glucoside carbamate and malonylglucoside carbamate synthesis. Whereas BOA-6-O-glucosylation is located in the cytosol, detoxification via the glucoside carbamate pathway occurs within the extraplasmic space. Inhibitor studies and proteome analyses revealed enzymes involved in the process. The endophyte *Fusarium verticillioides* participates in the detoxification by providing lactone heterocycle cleavage products. In contrast, the dicot *Abutilon theophrasti* performs BOA detoxification almost exclusively within the extraplasmic space yielding BOA-6-O-glucoside as the major detoxification product. The root associated zygomycete *Actinomyces elegans* supports the plant by stimulating the exudation of absorbed BOA; other microorganisms take over additional detoxification work for the plant.

Conclusion: Our results demonstrate that plant associated microorganisms are important for the tolerance development against phytotoxins.

P N-CCO 5

Biological control of the lesser grain borer *Rhyzopertha dominica* by bioformulated products derived from endophytic fungus *Paecilomyces marquandii* isolated from *Artemisia herba-alba*

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Three bioformulations containing culture filtrates of endophytic fungus *Paecilomyces marquandii* isolated from healthy leaves of *Artemisia herba-alba* (Asteraceae) were tested in laboratory against the lesser grain borer, *Rhyzopertha dominica* (Coleoptera: Bostrichidae). All formulations, namely the stabilized invert emulsion, effervescent powder and seed-coating wettable powder demonstrated insecticidal activities against the targeted pest. Stabilized invert emulsion has a high insecticidal activity which is even more important with the concentration gradient of the filtrates, generating mortality ranging from 64,2% to 100% for culture filtrate concentrations ranging from 20% to 80%, respectively. While insect treatments with effervescent powder caused an average mortality of 53.3%. Lethal concentration 50 (LC₅₀) was 16,26% for the stabilized invert emulsion while the lethal time 50 (LT₅₀) varies between 7,05h, 2,37h, 2,49h and 1,61h depending on the culture filtrate concentrations. Whereas we recorded an LT₅₀ of 3,50h after insect treatment with effervescent powder. Moreover, the average rate of infestation of durum wheat (*Triticum durum*) seeds coated with wettable powder was very low (8,9%) compared to untreated seeds (72%) recording a reduction in the bostrichid attacks of about 88%. It is possible to propose to cereal producers these formulations in order to apply them for protection of durum wheat seeds belonging to the local varieties, as being an alternative to the chemicals.

P N-CCO 6

Toxicological and Biological Effects of Neem and Jojoba Oils on the Black Cutworm *Agrotis ipsilon* (Hüfn).

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The present study aimed to investigate the toxicity and biological effects of neem oil (Neemix 4.5% azadirachtin) and jojoba oil (Nat-1 96%) on 4th instar *A. ipsilon* larvae. The two materials were tested as a bait (with wheat bran) at 0.50, 0.75, 1.00, 1.25, 1.50, 1.75 and 2.00 liters / 25kg. wheat bran under a constant temperature of 26±1°C. The study revealed that the Neemix was

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more toxic than Nat-1. LC₅₀ and LC₉₀ values were 0.84 and 1.85 for Neemix and 0.97 and 2.04 for Nat-1, respectively. In the same time the Neemix was more effective, decreased each of pupation percentage, pupal weight, adult emergence rate, fecundity and fertility. In general the two plant oils caused an increase in larval and pupal durations but decreased the pupal weight. Malformations in pupae and adults were increased with increasing the concentrations of the two oils.

P N-CCO 7

Isolation and identification of the fungus causing leaves spot of Eucalyptus trees species *Eucalyptus Stricklandii* in Sirt region of Libya

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Laboratory results showed insulation and field to cause disease spotted eucalyptus trees verity *E.strickklandii* leaves and seeds , which were imported from Australia and the cultivation nurse Alkardabia productive , which is located in the city of Sirte, Libya during the agricultural season 2014 to the presence of fungus *Alternaria .spp* tested in all the papers . As explained field survey of the disease to the results of a high sensitivity of the disease , where the severity of the injury recorded a rate of 12.2% at the upper limbs of trees and 17.5 % at the lower extremities , while the prevalence of the disease record 75% of the trees tested in the study area .

P N-CCO 8

Entomopathogenic fungi, *Beauveria bassiana* (Bals.) and *Metarhizium anisopliae* (Metsch.) As A biological Control Agents on Some Stored Product Insects.

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Egyptian isolates of Entomopathogenic fungi, *Beauveria bassiana* and *Metarhizium anisopliae* were isolated and tested against three Stored Product insects, *Rhyzopertha dominica* (F.), *Sitophilus oryzae* (L.) and *Oryzaephilus surinmensis* (L.). The result revealed that *M. anisopliae* was more effective against tested insects than *B. bassiana*. The *R. dominica* was the most effective insects to both fungi, followed by *O. surinmensis*, while *S. oryzae* was the least. Different concentration of Entomopathogenic fungus, *B. bassiana* (**0.12 x 10⁶ conidia /g, 0.22 x 10⁶ conidia /g, 0.32 x 10⁶ conidia / g, and 0.42 x 10⁶ conidia /g**) were tested against *R. dominica*, *S. oryzae* and *O. surinmensis*. LC₅₀ were **1.2 x 10⁵ conidia / g, 1.6 x 10⁵ conidia / g and 1.4 x 10⁵ conidia / g**, respectively. The same concentrations of Entomopathogenic fungus, *M. anisopliae* were tested against *R. dominica*, *S. oryzae* and *O. surinmensis*. LC₅₀ were **2.7 x 10⁵ conidia / g, 1.3 x 10⁵ conidia / g, and 3.5 x 10⁵ conidia / g**, respectively.

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Laboratory Evaluation of the efficacy of biopesticides on the management of American cockroach (*Periplaneta americana* L.)

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Laboratory experiment was carried out to evaluate the efficacy of *Allium sativum*, *Citrus sinensis*, *Lycopersicon lycopersicum*, *Ocimum gratissimum* O. santum, *Piper guineensis* and *Zingiber officinale* on the management of American cockroach (*Periplaneta americana* L.). Five concentrations (0.05, 0.10, 0.15 and 0.20 g/ml) of the plant extracts were sprayed on cages that contained ten adult P. americana each. An untreated treatment was included to serve as control. Mortality was recorded at 24 h, 48 h, and 72 h exposure period. At 24 h, only P. guineensis at 0.05g/ml gave 23% mortality which was significantly (p = 0.05) different from the control. However, 0.20 g/ml, significant (p= 0.05) differences were observed among other plant extracts except P. guineensis, A.sativum, O. gratissimum. But at 48 h exposure, 0.20g/ml of Piper guineensis gave 100%, which was significantly (p=0.05) different from O. gratissimum (43%), A. sativum (37 %), L. lycopersicum (37 %), O. sanctum (30 %), C. sinensis (27%) and Z. officinale (20%). Percent mortality at 72 h was: P. guineensis (100 %), O. gratissimum (50 %). L. lycopersicum (43 %), A. sativum (40 %) and O. sanctum (30 %), C. sinensis (27 %), Z. officinale (23 %). No mortality was recorded in the control throughout the experiment. In conclusion, P. guineensis resulted in significantly (p=0.05) higher percent mortality than all other plant extracts except O. gratissimum hence can be used in the control of P. americana. However, this result should be confirmed with trials outside the laboratory.

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Abundance comparison and sex index of ladybird (*Coccinella septempunctata* L.) (Col.: Coccinellidae) in wheat fields and apple orchards in Urmia region

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Abundance of ladybird (*Coccinella septempunctata* L.) evaluated in two conditions contain wheat fields and apple orchards between 2010-2011 in Urmia region. Ladybird population monitoring was began in April and continued in field to late of June and in orchard to late of September. Abundance of population in field was higher than orchards and it is considerable because the length of sampling time in wheat field was shorter than apple orchards. Regular application of insecticide against codling moth, decrease pest population in orchards furthermore high biodiversity and abundance of aphid population as prey for ladybirds in fields, justificate high density of ladybird in field conditions. Average of ladybird sex index during sampling was 0.744 and 0.753 in fields and whereas it calculated 0.676 and 0.73 in orchards in 2010 and 2011, respectively.

P N-CCO 11

Species diversity of aphids (Homoptera: Aphididae) and coccinellids in apple orchards of Urmia

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Abundance and diversity of aphid and coccinellid species were monitored in apple orchards in Urmia during 2010 and 2011. In this study, eight aphid species were collected and identified in 2010 and nine were found in 2011, while; only two coccinellid species were found in both years. In 2010, *Aphis pomi* (De. Geer) was the most abundant aphid (55.5%) followed by *Dysaphis plantaginea* (Passerini) (34%). However, in 2011, *Eriosoma lanigerum* (Hausmann) had the largest population (57%) in apple orchards followed by *D. plantaginea* (21.5%) and *A. pomi* (18%). Generally, more aphids were collected in June and July of 2010. However, aphids population in 2011 was high from mid-May until early October. 1 Shannon and Simpson diversity indices were measured. The Shannon and Simpson indices of aphids and coccinellids in 2011 were more than those in 2010. Simpson index of aphids in 2010 and 2011 were 0.573 and 0.594, respectively, indicating that the possibility of selecting two different species of aphids was 57% in 2010 and 59% for 2011.

P N-CCO 12

Insect pheromones as biodegradable, renewable and sustainable biopesticides in future IPM suitable for mechanized application

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Experience in plant protection with conventional insecticides accumulated for more than a century. For environmental reasons, poisons indiscriminately affecting target and non-target organisms alike and contaminating ecosystems should, with advancing knowledge, now be replaced by softer, non polluting, biodegradable, renewable, and sustainably synthesized biopesticides with high target specificity, high effectivity, low (eco)toxicity and with negligible selection for resistance.

Insect pheromones, a few natural products of the class of pyrethrins, and neem are well known examples for such selective approaches. Their current market share in organic agriculture is by no means trivial. Pheromones can be partly or totally synthesized by advanced methods of organic chemistry starting from renewable and sustainable resources. They are modifiers of insect behaviour. With properly adjusted dosages in time and space, they had reached a competitive status in *Pectinophora gossypiella* control in cotton by the late 1970s. Typically, they are dispensed with rates of 100 g/ha/season for mating disruption of a number of economically important pest insects. For monitoring timing and abundance of insect flight, a small fraction of 1% of this dose is sufficient.

Three examples studied extensively by this lab, the beetle *Diabrotica v. virgifera* (Chrysomelidae), and the two moths *P. gossypiella* (Gelechiidae) and the grape vine moth *Lobesia botrana* (Tortricidae) are cited. This research also included novel developments in high tech areas like patented mesofibers for behavioural mating disruption in vineyards. Electrospun

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mesofibers, specifically Ecoflex®, are biodegradable dispensers suitable for the *L. botrana* pheromone. One application is sufficient for 7 weeks, without leaving any residues or showing side effects on other vineyard organisms. Considerable costs for the disruptant (*E,Z*)-7,9-dodecadienyl acetate can be defrayed by the cheap polyester Ecoflex® and by mechanized deployment of the pheromone-Ecoflex® mesofiber combination with existing vineyard cultivators, thus realizing savings in both labour and treatment costs. Removal of spent dispensers is now obsolete.

Currently, extension of this technique to commercial fruit and nut growing, to cotton and to vegetable pest management is envisioned.

P N-CCO 13

Neem ingredients - a precious natural resource of *Homo agronomicus* for meeting the needs of organic plant protection

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Azadirachta indica (Rutales: Meliaceae)(neem) and closely related plant species of this genus are natural resources still holding many surprises. Their botanical diversity and applications of their natural products for plant protection are by no means exhausted.

Neem research was boosted by the structure elucidation of its main ingredient, azadirachtin (C₃₅H₄₄O₁₆), 25 years ago. Research on biosynthesis, total chemical synthesis, explanations of its multiple modes of action, and dozens of applications in medicine, agriculture and IPM suddenly gained a solid scientific basis. Likewise, marrangin (C₃₅H₄₄O₁₅) from the Malaysian/Philippine neem tree *A. excelsa* (marrango) shares with azadirachtin most structural features. In some insects, antifeedant and development modifying properties of marrangin are superior to those of azadirachtin.

At the neuroendocrine level, azadirachtin, marrangin and congeners are interacting with RNA synthesis. Subsequent biochemical pathways induce morphological and behavioural disorders in insects without killing them as conventional insecticides do. General defects, exploitable for IPM, are reductions in physiological and reproductive fitness.

Even crude neem extracts, i.e. neem oil, can reduce and inactivate notorious pest populations in *Zea mays* such as *Diabrotica* spp. in Illinois, without noticeable impacts on ecological cycles, simply by interrupting feeding of immature stages and slowing down orientation responses of adults. Typical concentrations between 10-100 ppm of azadirachtin on foliage are sufficient. Also, sex pheromone communication is reduced, presumably by interference of neem with PBAN dependent female sex pheromone synthesis and pheromone perception by male antennae.

Not only agriculture, but also human and veterinary medicine profit from recent advances. Neem products are non toxic and are compatible with beneficial insects, pollinators, and bees. They are environmentally benign, renewable, and are affordable to developing countries. Neem is an eldorado for natural product chemists, entomologists, physicians and practitioners of organic agriculture. In contrast to frequent cases of resistance in conventional pesticides, neem is the medium of choice where and when no other active, registered preparations remain available.

P N-CCO 14

The Effect Of Four Local Plant Extract On The Control Of Rice Weevil, *Sitophilus oryzae* L.

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Four local species (*Allium sativum*, *Capsicum annum*, *Anethum graveolens* and *Ocimum basilicum*) were evaluated in the laboratory of Biolog Department, College of Education, for their ability to protect stored rice from the infection by weevil *Sitophilus oryzae*. Aqueous extracts of the plant species were applied as direct admixture of three concentrations levels of 1%, 2.5% and 5% (W/V) to assess for mortality, adult emergence, and repellency and weight losses. The results showed that *Allium sativum* extracts was the most effective as it gave the highest mortality (90%) at 5% concentration followed by *Capsicum annum* (80%) on the 4th day post treatment, the result showed that the plant extract of different concentrations exhibited different level of reduction in adult emergence and different repellency of adults of *Sitophilus oryzae*. *Allium sativum* recorded the lowest mean number of adult emergence (8) followed by *Capsicum annum* (10) at 5% concentration, while *Capsicum annum* was found to be revealed complete repellent agent (100%) repellency on the 6th hours against *Sitophilus oryzae* followed by *Allium sativum* and *Anethum graveolens* (81.8%). There was a significant (P>0.05) reduction in the weight lost by the weevils with less damaged recorded on grain treated with *Allium sativum* and *Capsicum annum* (1.6%) and (2.3%) respectively.

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Effect of mycorrhizal fungi on seed germination and seedling establishment in *Cucumis sativus* L under drought condition

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Green cucumber is a vegetable that is used in the world but fruiting and growing of it requires a lot of water. Due to the recent crisis "huge water shortage" cultivation of this plant is a problem. The aim of present study was conducted on the effect of mycorrhizal fungi on seeds germination and seedling establishments of this plant against drought stress. This research conducted on factorial experiment based on randomized complete block design with 5 replicate and 4 treatments. For mycorrhizal inoculation *Glomus mosseae*, and for drought treatment PEG (Poly ethylene glycol) with -0.2 MPa were used. Results showed that drought stress reduced seed germination rate, germination index, and seedling growth significantly but in seeds that inoculated with mycorrhizal fungi seed germination beginning with delay. In interaction of mycorrhizal fungi and drought stress seed germination beginning with delay but germination rate and index and seedling growth significantly increased in comparison with drought stress. Finally from this experiment concluded that inoculation with *Glomus mosseae* increased seed protection against drought stress.

P N-CCO 16

Recent Developments in Bio-pesticide Industry in India

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Introduction: Over the last few years, there has been significant growth in the use of bio-pesticides worldwide. This paper will examine the status of Bio-pesticide industry in India.

Objectives: India is one of the largest manufacturers of chemical pesticides in India. During 2013-14, India exported more than 2 Billion USD of chemical pesticides. In view of the fast growth in global bio-pesticides segment, we decided to undertake a study on the status of bio-pesticide industry in India.

Materials and methods: The Central Insecticides Board (CIB) under Government of India is the authority which gives permission to manufacture, import and market all types of pesticides including bio-pesticides. Information on the permission given to Indian companies for manufacture or import during 2014 was collected from the data published in CIB website. The information on growth of bio-pesticide industry in India was obtained from different industry sources.

Results: Analysis of CIB data showed that a very large number of Indian companies got approval in 2014 for diverse types of bio-pesticides. From market data, it was observed that the growth rate of bio-pesticides in India was much higher than the chemical pesticides. Detailed analysis will be presented.

Conclusions: Like many other countries, the growth rate of bio-pesticides in India has been significantly higher than the conventional chemical pesticides. As per our analysis, some of the factors contributing to this are: (a) Growth in organic farming (b) Environmental concern associated with use of chemical pesticides (c) Awareness amongst farmers about bio-pesticides (d) Degradation of soil quality (e) Safety considerations (f) Inclusion of bio-pesticides in Integrated Pest Management (g) Current low base.

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P N-CCO 17

Induced proteome of *Botrytis cinerea* by L-amino acid oxidase from *Trichoderma*

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Introduction: *Botrytis cinerea* is a major fungal pathogen to over 200 host plants worldwide including fruits, vegetables, and ornamental crops. The disease frequently occurs near the time of harvest, following rainfall or long periods of high humidity, and develops into the characteristic appearance of gray mold. Gray mold outbreaks lead to major financial losses for growers, reducing both yield and quality. Consequently, control of *B. cinerea* infections in plants, mainly by a combination of chemical and

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cultural control methods, remains a challenge. To compensate the deficits, biocontrol of *B. cinerea* infections by *Trichoderma* spp presents a promising alternative.

Objectives: Previous studies in our laboratory identified several extracellular enzymes secreted by *Trichoderma* when deactivated *B. cinerea* was served as foster antagonist. An L-amino acid oxidase (Th-LAAO) was one of the enzymes shown to inhibit the growth of *B. cinerea* through the mitochondria-mediated apoptosis. The induced proteome of *B. cinerea* by Th-LAAO was investigated here to further understand the antagonistic mechanism of *Trichoderma* against fungal pathogens.

Materials and methods: Th-LAAO was purified from extracellular protein extracts through two-step chromatography including ConA-Sepharose and Superdex HR 75 employing ÄKTA prime plus 100 FPLC. Two dimensional electrophoresis analysis and in-gel digestion were performed prior to MALDI-TOF analysis. The MS/MS data were subjected to search algorithms against Swiss-Prot sequence database using Mascot software.

Results: As the results, proteins related to the translational machinery and the biochemical energy production, chaperons, and F₀F₁-type ATP synthase were induced and identified in the extracts of Th-LAAO treated *B. cinerea*.

Conclusion: The proteome data here showed in addition to the antagonistic role played by Th-LAAO, Th-LAAO treated *B. cinerea* was also trying hard to survive through rebooting the energy production and translational machinery though in the end turnout not so successful. This is the first ever proteome of Th-LAAO treated *B. cinerea* to be reported.

P N-CCO 18

Efficacy of *Beauveria bassiana* isolates Against *Sitophilus oryzae*

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This study was carried out to evaluate the efficacy of entomopathogenic fungal isolates, obtained from soil samples of Ordu Province, on *Sitophilus oryzae*. Fourteen *Beauveria bassiana* isolates were tested against *Sitophilus oryzae* adults at 1x10⁷ spore/ml concentration *in vitro* conditions. All isolates were inoculated by immersing the insects in 10 ml of a fungal suspension for 10 s. 0,2% tween 80 solution was used as control. The experiments was conducted with 3 replications and 10 adults were used for each replication. Mortality was evaluated after 24 and 72 h incubation periods. The results indicated that increase in incubation period increased the mortality rates of the insect. Percentage mortalities ranged from 0% to 33% were recorded at the end of 24 h incubation period. At the end of 72 h incubation period, percentage mortality ranged from 14% to 65.5% and three isolates (O5, O55, and O66) were found to be relatively more virulent. The highest mortality was obtained with isolate O66 (65.5%) at the end of 72 h incubation period. The preliminary results foresee the potential of *B. bassiana* isolates in managing *S. oryzae*.

P N-CCO 19

Survey of Entomopathogenic Fungi From Field Soils In Tokat Province, Turkey

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Present study was aimed at isolation and identification of entomopathogenic fungi from field soils in Tokat province, Turkey. Total of 176 different soil samples were collected from different field crop production areas of Tokat in 2014. Bait insect method was used for isolation of entomopathogenic fungi. Bait insects, *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) larvae, five larvae were placed into each soil sample in 90 mm glass petri dishes and kept at 25±2 °C in darkness were monitored for 15 days. Entomopathogenic fungi were found in 15,34% (27 entomopathogen fungal isolates) of the soil samples. Twenty isolates were identified as *Beauveria bassiana* (Bals.-Criv.) Vuill., 4 isolates as *Fusarium* spp., 2 isolates as *Lecanicillium lecanii* (Zimm.) Zare & W. Gams and one isolate as *Paecilomyces* sp. The results of the study will be evaluated on the account of the use of entomopathogenic fungi for control of different insect pests

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Encapsulation of *Metarhizium brunneum* as basis for an attract and kill strategy

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Several soil-borne herbivorous insect pests such as wireworms and western corn rootworm larvae cause tremendous losses in different crops like potato and maize. The INBIOSOIL project aims at developing innovative beads containing entomopathogenic fungi (EPFs) as well as novel synergistic co-formulations of EPFs with efficacy enhancing agents to control soil-borne insect pests. These innovative formulations should display properties such as high mechanical stability, high entrapment efficiency, high shelf life and optimal nutrient additives for growth and sporulation of EPFs (C/N ratio, pH). For application in the field these formulations were dried with a focus on high cell survival, low a_w values and good re-swelling properties. For scale-up, high throughput encapsulation technologies like jet cutting and several drying processes e.g. fluidized bed dryer, drum dryer or the Innojet dryer were investigated, respectively.

Here we report on the encapsulation and drying of novel formulations on lab and technical scale. Furthermore data will be presented on investigations on the influence of nutrients on sporulation, re-swelling of dried capsules as well as the influence of fillers on a_w value and survival of dried encapsulated aerospores of the *M. brunneum* isolate BIPESCO 5 and virulence against *Tenebrio molitor* larvae.

Dry beads with additives such as starch or other nutrients showed survival up to 80 % for *Metarhizium brunneum* spores and a_w values down to 0.1 depending on the formulation and drying method. Depending on the composition, dried beads rehydrated in water to 70-90 % of their initial bead diameter. Furthermore we encapsulated *M. brunneum* aerospores together with CO₂-releasing *Saccharomyces cerevisiae* in a co-formulation to implement an "attract and kill" - strategy to control wireworms and other soil-borne insect pests in agricultural fields. Selected field results, using *M. brunneum* (isolate ART 2825) formulated beads to control wireworm larvae will be presented by the collaborating group of S. Vidal, demonstrating that these novel formulations are capable to significantly reduce the wireworm populations in the field.

P N-CCO 21

Biological control of crown gall on grapevine by nonpathogenic *Agrobacterium vitis* strain ARK-1

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Graft unions of nursery stock of grapevine collected in Japan yielded nonpathogenic strains of *Agrobacterium*. On the basis of classic diagnostic tests, a sequence analysis, and a previously reported multiplex PCR method, the nonpathogenic strains were identified as *Agrobacterium* (= *Rhizobium*) *vitis*. Stems of grapevine seedlings were inoculated with both a cell suspension of *A. vitis* (Ti) as a pathogen and one of a new nonpathogenic strain as competitors to assay the suppression of tumor formation caused by the pathogen. In a test with a 1:1 cell ratio of pathogen:nonpathogen, all new strains of nonpathogenic *A. vitis* reduced the tumor incidence. In particular, one of the new strains named "ARK-1" was most effective in inhibiting tumor formation on grapevine and appears to be a promising new agent to control grapevine crown gall. When roots of grapevine were soaked in a cell suspension of strain ARK-1 before planting in the field, the number of plants with tumors was reduced in treated plants. Results from 7 field trials in 2009 to 2012 were synthesized in a meta-analysis. The integrated relative risk of treatment with ARK-1 was 0.15 (95% confidence interval: 0.07-0.29, $p < 0.001$), indicating that disease incidence was significantly reduced by ARK-1. The ultimate goal in conducting this study is to evaluate strain ARK-1 as bio-pesticide.

P N-CCO 22

Impact of Biocontrol Agent *Streptomyces fumanus* on Bacterial Communities in the Rhizosphere of Wheat and Soybean in Newly Cultivated Soil

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Question: The balance between the rhizosphere microflora and plant pathogens and soil microflora is important in host-pathogenic relationships.

Streptomyces fumanus isolated from the rhizosphere was intended for seed and soil application as a biofertilizer to increase plant growth and to protect against pathogens in our study.

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We aim to investigate the role of *Streptomyces fumanus* gn-2 in the regulation of the functional diversity of rhizosphere bacteria of wheat and soybean in the experimental field, where soils have not previously been used as farmland.

Methods: The quantity and diversity of a rhizosphere functional microbial community's: ammonifiers, oligotrophs and diazotrophs after processing of wheat (*Triticum aestivum*) and soybean (*Glycine max*) seeds in *Streptomyces fumanus* gn-2 suspension (10⁴ spores/ml) were examined. The rhizosphere microflora of wheat was investigated in shoot, tillering, heading and maturation phases by classical microbiological and molecular biology methods. The soybean rhizosphere microflora in three phases of vegetation, formation of the first trifoliate leaf, flowering and maturation was investigated by the same methods.

Results: A 16S ribosomal RNA analysis revealed a rich biodiversity of bacteria in the rhizosphere of the wheat's maturation phase, which differs from the biodiversity of bacteria in the rhizosphere of soybean in the same phase. Bacteria of *Microbacterium* genus from Actinobacteria phylum dominated in the rhizosphere of wheat. The bacteria species of *Chryseobacterium* genus from Flavobacteriia phylum dominated in the rhizosphere of soybean, which proves the different chemical composition of these organic plant exudates that attracts the preferred species of microorganisms.

Conclusions: The use of a formulation of *Streptomyces* gn-2 has improved the composition of rhizosphere microflora, attracting saprophytic microorganisms: ammonifiers and oligotrophs. The presence of the biocontrol microorganism *Streptomyces fumanus* in the rhizosphere plays an important role in enhancing the growth and development of useful groups, such as nitrogen-fixing bacteria. *Streptomyces fumanus* is an ideal biological agent for use against soil infections, due to its high colonization of the root system of soybeans and significant colonization of wheat. Treatment of seeds by biological fertilizer such as *Streptomyces* gn-2 is an important prerequisite for profitable crop production and ensuring a full and environmentally healthy crop.

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Effects of a new organic residue of *Stellera chamaejasme* on crops seedling growth and its control efficacy against root-knot nematode

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The new organic residue, a waste material of *Stellera chamaejasme* was extracted with ethanol, has good potential as a valuable agricultural resource. The influence of the new organic residue on two different crops (wheat and maize) seedling growth and its control efficacy to *Meloidogyne incognita* were investigated with pot cultivation experiments in greenhouse. The wheats and maizes were cultured in potting soil that mixed with the residue of *Stellera chamaejasme* at four different concentrations (750, 900, 1050 and 1200 kg/ha). The plant length, root length, root weight and upper ground weight were measured and analyzed in seedling stage. Plant growth in the residue treatment was significantly better than blank control. In four different treatments, the growth and development of crops were improved obviously at the concentration 1200 kg/ha, and the plant length, root length, root weight and upper ground weight of wheat compared with blank control increased 2.7%, 20.8%, 7.5% and 5.6%, respectively, while maize increased 15.2%, 22.8%, 11.8% and 10.4%. In control efficacy experiment, the soil was collected from the heavy disease field with *Meloidogyne incognita* and its concentration remain the same. Based on the disease index of roots analyses, control efficacy of the residue at four different concentration to *Meloidogyne incognita* was 32.2%, 46.4%, 52.3% and 60.0%, respectively. These results suggested that the organic residue of *Stellera chamaejasme* is a good agricultural resource as a kind of new biological fertilizer with pesticide.

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Performances study of *Ooencyrtus pityocampae* (Mercet) (Hymenoptera: Encyrtidae) on the new factitious host *Philosamia ricini* (Danovan) (Lepidoptera: Saturniidae) to optimize its rearing

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The Pine Processionary, *Thaumetopoea pityocampa* Denis & Schiffermüller, (Lepidoptera: Thaumetopoeidae) is the most damaging pest of pine trees in the Mediterranean region. *Ooencyrtus pityocampae* (Mercet) is a polyphagous egg parasitoid, known mainly as an egg parasitoid of the Pine Processionary. Studies were conducted to assess the biological parameters of this parasitoid on the new factitious host *Philosamia ricini* (Danovan) (Lepidoptera: Saturniidae) under laboratory conditions. Parasitism of *P. ricini* eggs by *O. pityocampae* is influenced by host egg age, number and age of the female parasitoid. To evaluate the effect of egg age of host and age of female parasitoid, groups of 50, 60, 80, 100 eggs of (1-2) and (3-4) days old were exposed to 1, 3 and 5 days old age, for one or two female parasitoid. Then the parasitized eggs were incubated at 25°C ± 1, R

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H 65%±5 and L:D 16:8 h photoperiod. As results, higher percentage of adult *O. pityocampae* emergence was obtained on (1-2) days old age *P. ricini* eggs. Emergence rate was affected positively by the parasitoid age. Fifty host eggs and five days old age one female parasitoid was more suitable for the economic and effective mass production programme. Developmental period of adult parasitoid was not affected by the host age. Longevity was significantly higher for parasitoid fed with bio-honey. Mass rearing of parasitoids is an essential step for biological control programs. Based on the results of this study, *P. ricini* is an appropriate host species for the mass rearing of *O.pityocampae*.

P N-CCO 25

Screening of antagonistic bacteria against *Pseudoperonospora cubensis* and their control efficacies on cucumber downy mildew

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Introduction: Downy mildew, caused by *Pseudoperonospora cubensis*, is one of the most prevalent diseases of cucumber. Control of this disease in present is greatly depended on chemical fungicides over the world. However, it becomes a common and important problem that many chemicals show the potential toxic effects on humans, wildlife, environment, as well as result in pathogen resistance. Therefore, microbial fungicide with environmental-friendly were focused on and developed.

Methods: The antagonistic activities of tested bacteria against the pathogen of cucumber downy mildew were tested by using leaf-disc floating method. The control effects of the antagonistic bacteria were evaluated against this disease separately by pot test method and plot experiment in greenhouse.

Results: A total of 200 bacterial isolates, obtained from rhizospheresoil samples collected from the field cultivated with different plants, were tested for their antagonistic activities against *P. cubensis* by leaf-disc floating method. Among them, 14 isolates could reduce the disease severity over 40%. Then, eight antagonistic bacterial isolates were evaluated for their abilities to control this disease by pot test method in greenhouse. The results determined that all tested bacterial isolates could significantly decrease the disease severity. 4 isolates including HMB19198, HMB20199, HMB20428 and HMB20729 expressed a significant high control efficacy against the disease, but not significantly different from the chemical fungicide “INFINITO” (Fig. 1). This suggested that the 4 bacterial isolates would be potential agents to control cucumber downy mildew. Furthermore, these four isolates were tested for their ability to control this disease by plot experiment in greenhouse. The results showed that 3 bacterial isolates could significantly reduce the disease index with the control efficacy over 50%, and the control effects of isolate HMB 20729 and HMB 20248 were significantly higher than the chemical “KOCIDE 3000 ” (Fig. 2).

Conclusion: These findings may lead to the development of a new eco-friendly fungicide to control cucumber downy mildew.

Figure 1

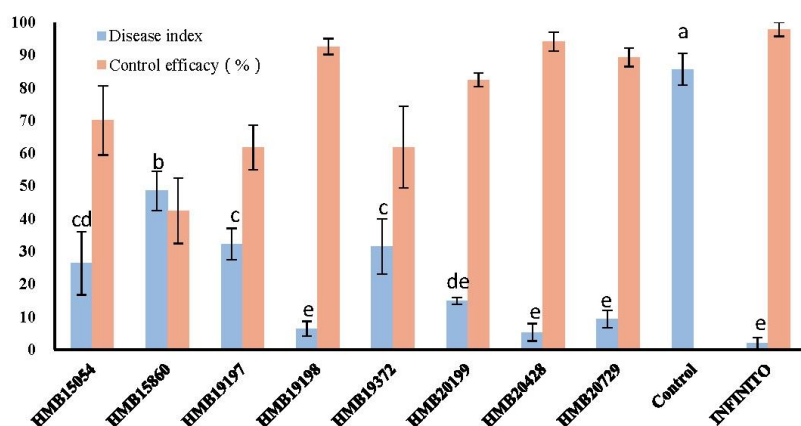


Fig. 1 Eight antagonistic bacteria were tested for their ability to control cucumber downy mildew by pot test method in greenhouse

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Figure 2

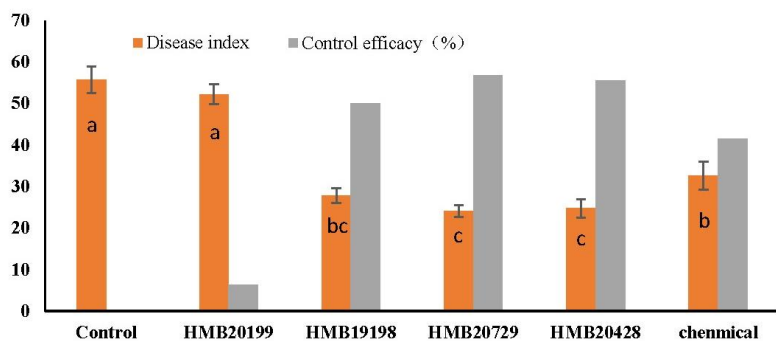


Fig. 2 Four antagonistic bacteria were tested for their ability to control cucumber downy mildew by field plot experiment in greenhouse

P N-CCO 26

Induced Systemic resistance (ISR) in *Cucumis sativa* using Bacterial and Fungal Isolates to Control *Tetranychus urticae* koch

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Tetranychus urticae koch is an important pest on *Cucumis sativa* causing losses up to 95%. Chemical control is not possible against *T.urticae* because it is resistant of many pesticides.

The efficacy of microbial antagonists against *T.urticae* in greenhouse was tested. *Serratia plymuthica* HRO-C48 and *Gliocladium catenulatum* J1446 were able to reduce the mite intensity on plants of *Cucumis sativa* infested with *T.urticae* at rates 44% and 52% respectively. Plants treated with a suspension of the antagonists (2×10^5 cfu/plant) and inoculated with either pycnidiospore suspension (2×10^7 cfu/ml), showed a reduced infestation rate of 53% - 93% in the presence of *S. plymuthica* and 46% - 77% in the presence of *G. catenulatum*. The efficacy of the antagonist depends highly on their concentration inside seeds. Below 105 cfu/seed no significant difference was recorded between control untreated and infested plants.

P N-CCO 27

Control effect of organic agricultural materials on several diseases and pests

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Using chemical to control diseases and pests is an important factor of sustaining the mass production of modern agriculture. However substitutable control method to chemical should be investigated for organic farming. In Korea, the demand of consumers for organic products is increasing and many organic materials are being used to control diseases and pests. In this experiment, we tried to select useful organic materials. Inhibition of organic fungicidal materials to spore germination as essential process for fungal penetration to host was tested and effects of organic pesticidal materials were tested for killing Beet armyworm(*Spodoptera exigua* Hübner) larva. The study was conducted at Organic Agriculture Research Institute, Korea. 128 products of organic fungicidal extracts and 28 products of organic pesticidal extracts were bought. The fungicidal extracts were tested of inhibiting the spore germination of *Colletotrichum*, *Alternaria*, *Botrytis* and *Penicillium* spp. by 96 well microplate in vitro. The extracts were diluted at $\times 50$, $\times 100$, $\times 200$, $\times 400$, $\times 800$ and $\times 1,600$ and mixed with 50 spores in each cell. Spore germination was observed for 5 days by inverted microscope(Olympus CKX41, Japan). The effect of 28 products of pesticidal extracts was tested to 3th and 4th larva of Beet armyworm in vitro. The extracts were diluted at $\times 100$, $\times 200$ and $\times 400$ and 5 larvae of Beet armyworm were dipped in the solutions for 1 sec. We observed the development from a larva to an imago. 9 fungicidal materials, Spil(turpentine extract), Kalkumi(kaolin and copper sulfate), Poex(grapefruit extract), White(castar extract), KG-sukhaeuwhwang(sulfur and calcium hydroxide), Laces330(*Pae. polymyxa* AC-1), Hinjabi(sodium bicarbonate), Tori(*T. harzianum*) and Neobordeaux(Copper sulfate basic 58%), inhibited spore germination of four fungi. Kalkumi was the most effective. Pesticidal effects of 5 organic products, Chungtajin(sophora root extract), Everkill(plant essential oil), Daeudaipos(plant extracts), Neosuncho(natural complex plant extracts) and Nabangtuk(medicinal plant extracts), were higher. Everkill was the most effective. In this study, we selected several effective products of organic materials to control fungal spore germination and the

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larvae of Beet armyworm in vitro and in field(Unpublished). We think that the selected products of organic materials can substitute the chemicals and be used to maintain the production for organic farming.

P N-CCO 28

Plant symbioses: understanding the “double life” of the entomopathogen *Beauveria*

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Introduction: Plant-fungi symbioses represent a natural phenomenon widespread on nature that in most cases offers to the plant more advantages than disadvantages. Fungal endophytes are well known examples of beneficial organisms showing to be involved in several roles such as plant protection against insects. Entomopathogenic fungi such as *Beauveria* have been described as an important control for insects and some of their species have been isolated as endophytes.

Objectives: The role of *Beauveria* in the three-component relationship involving plant, fungus and insect has been poorly studied and several questions arise. What makes to an entomopathogen fungus becomes a plant endophyte (or vice a versa)? How does the penetration into plants occur? Does the presence of the fungus have any effect on both plant and insect? Our research proposes the use of fluorescence microscopy techniques for a better understanding of this triangular system.

Materials and methods: Vector carrying a red fluorescence marker was designed for tracking the growth of three *Beauveria bassiana* isolates, BG11, FRH2 and J18 within three plant systems, *Arabidopsis*, maize and tomato. Transformation of *Beauveria* strains was achieved by a PEG-blastospores method and inoculation of plant tissues was performed by using conidia suspensions. In order to avoid detection of epiphytic hyphae, reliable surface sterilization methods were applied to plant samples.

Results: Preliminary results have shown a close relationship between root plants and *B. bassiana* strains. Fungal hyphae were found attached to roots before any sterilization treatment was applied and within the tissue in the post-treatment. However the mechanisms of penetration remain unclear and more analysis will be needed to determine whether the presence within the plant produce any effect.

Conclusion: Our research is based on the fact that biological control organisms are an essential tool in sustainable agriculture systems in twenty-first century. By understanding the role of *Beauveria* in their association with plants, we will be able to develop compatible and efficient plant-fungus systems that act against plant pathogens.

P N-CCO 29

Management of strawberry anthracnose by application of *Bacillus subtilis* endospore formulations and potential resistant cultivars

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Strawberry anthracnose caused by *Colletotrichum gloeosporioides* severely impacts the strawberry nurseries and greatly reduces yields and quality of strawberry in Taiwan and worldwide. Toward environmental friendly control strategies, we focus on the selection of microbial agents and resistance cultivars for management of the disease. The microbes that antagonize against anthracnose fungi of strawberry were isolated from plant potting mixes and rhizosphere soils in Taiwan. Two *Bacillus subtilis* strains TKS1-1 and SP4-17 showing superior antagonistic activity against anthracnose fungi of strawberry, various pathogenic fungi and bacteria of *Xanthomonas* species were subjected to biopesticide development. *B. subtilis* strain TKS1-1 was previously shown to reduce disease incidence of citrus bacterial canker and bacterial blight of rice. Here, we evaluated the colonization and survival ability of *B. subtilis* strains TKS1-1 and SP4-17 on leaves of strawberry plants. Results indicated that 21 days post-treatment of these strains, 10^5 colony forming units per cm^2 of strains TKS1-1 and SP4-17, which was similar to the numbers of cells inoculated, colonized on leaves of strawberry plants, suggesting the superior colonization and survival ability exhibited by these strains. The disease severity of strawberry anthracnose was greatly reduced when treatment with 100-fold diluted endospore formulations of *B. subtilis* TKS1-1 and SP4-17. Our data also indicated that culture filtrates of endospore formulations of *B. subtilis* TKS1-1 and SP4-17 inhibited spore germination of *C. gloeosporioides* and caused deformation of the spores. Additionally, results from assessment of the susceptibility of strawberry cultivars *Fragaria X ananassa* Duch. cv. Tauyuan No. 1, cv. M-99-20, *Fragaria X ananassa* cv. Tauyuan No. 4, and 12 hybrids of cv. Tauyuan No. 1 (as female parent) and cv. M-99-20 (as male parent) to *C. gloeosporioides* indicated that hybrids no. 4, 10, 13, and 19 were relatively resistant. In conclusion, endospore formulations of *B. subtilis* TKS1-1 and SP4-14 are potential biocontrol agents for strawberry anthracnose which may be

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attributed to their inhibitory effects on spore germination. Four strawberry hybrids exhibiting relatively resistant to *C. gloeosporioides* would be candidates for further study on their growth, yield and quality of strawberry fruits.

P N-CCO 30

***Perofascia lepidii*- the causal agent of downy mildew on garden cress (*Lepidium sativum* L.)**

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Introduction: In Germany, the organic production of garden cress seeds comprises about 50-60 ha of farm land. Since 2006, German farmers are increasingly complaining about yield losses in organic garden cress seed production due to the occurrence of the devastating downy mildew pathogen, *Perofascia lepidii*.

Objectives: By now little is known about the biology and the primary inoculum source of *P. lepidii* in garden cress. It is assumed that like other downy mildew pathogens *P. lepidii* can survive in seeds and soil. Our main goal was to establish methods for detection of the pathogen in seeds and plant tissue based on molecular tools and to improve our knowledge on the biology of *P. lepidii*.

Material and methods: For establishment of a PCR based diagnostic method for *P. lepidii* specific PCR primers were identified by an iterative development method. This approach includes inter alia the comparison of DNA-fingerprints of various oomycetes species followed by candidate gene selection and primer design. The influence of temperature (13 - 25 °C) and leaf wetness periods (1 - 24 hours) on disease incidence and severity was investigated. The importance of contaminated seeds as primary inoculum sources was tested under controlled conditions and in the field. Soil as potential inoculum source was also tested under controlled conditions by sowing pathogen free seeds in soil samples originating from various garden cress production areas. Plants growing out were examined for downy mildew outbreak.

Results: Initially, a DNA diagnostic method (PCR) was successfully established. The contamination of garden cress seeds with the pathogen *P. lepidii* was shown by specific PCR. Temperatures of 15 to 25 °C and leaf wetness durations higher than 4 hours favored the development of downy mildew disease in garden cress. Downy mildew disease outbreak was observed in contaminated soils and seeds sown in field.

Conclusions: The results confirm our assumption that contaminated seeds and soils play a major role as inoculum sources responsible for the dissemination of the downy mildew disease in organic garden cress seed producing fields.

This research was funded by the Federal Office for Agriculture and Food (BLE, Germany).

P N-CCO 31

Population reduction of *Xanthomonas citri* subsp. *citri* by rhizobacterial strains on the leaves of Satsuma mandarin

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Citrus canker caused by *Xanthomonas citri* subsp. *citri* (*Xcc*) was one of the important diseases in citrus cultivating orchards. Chemical fungicide is mainly used for preventing diseases including citrus canker in the field. However, due to the side effect of the chemicals, alternative method of disease control is extremely required. In our previous study, we found that four rhizobacterial strains such as *Burkholderia gladioli* MRL408-3 and TRH423-3 and *Pseudomonas fluorescens* THJ609-3 and TRH415-2 could suppress disease severity on leaves of Satsuma mandarin. To illustrate the mechanism by these selected rhizobacterial strains, the number of *Xcc* on the citrus leaves pre-treated with the selected strains was counted. On the leaves pre-treated with the rhizobacterial strains, the number of *Xcc* was significantly reduced compared to that of non-treated control one, indicating the direct suppression by the selected rhizobacterial strains against *Xcc*. The counted colonies were identified as *Xcc* and the rhizobacteria, respectively, by the gel electrophoresis of polymerase chain reaction (PCR) amplified and analysis of bacterial rDNA sequence using the specific primer sets. Generally, the observations using with a scanning electron microscope showed the total bacterial cells of the treatments reduced more than one of the control. Based on the result, it is suggested that these rhizobacterial strains may express any antibiotic activity on the leaves against *Xcc* resulting in suppression of disease severity.

P N-CCO 32

Comparative Study of Life Table and Predation Rate of *Eocanthecona furcellata* (Hemiptera: Pentatomidae) Fed on *Spodoptera litura* (Lepidoptera: Noctuidae) and *Plutella xylostella* (Lepidoptera: Plutellidae)

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We compared the life table and predation rate of the predator *Eocanthecona furcellata* (Wolff) reared on two major crucifer pests, *Spodoptera litura* (F.) and *Plutella xylostella* L. Newly hatched nymphs were selected from different egg mass of *E. furcellata*. At total of 81 and 82 nymphs of *E. furcellata* were used for the life table study on *P. xylostella* and *S. litura*, respectively. The raw data of the developmental time, survivorship, and female daily fecundity of *E. furcellata* individuals were analyzed based on the age-stage, two-sex life table by using the computer program TWSEX-MSChart. The net reproductive rate, intrinsic rate of increase, finite rate, and net predation rate of *E. furcellata* reared on *P. xylostella* were 292.4 offspring, 0.1389 d^{-1} , 1.1490 d^{-1} , and 644.1 third instars of *P. xylostella*, respectively; they are significantly higher than those reared on *S. litura*, i.e., 272.3 offspring, 0.1220 d^{-1} , 1.1298 d^{-1} , and 863.1 third instars of *S. litura*. For a comprehensive evaluation of the predation potential of *E. furcellata* feed on *P. xylostella* and *S. litura*, we analyzed the data by using the computer CONSUME-MSChart and combined both the growth rate and predation rate to calculate the finite predation rate (ω); our results showed that *E. furcellata* is a devouring predator for both *S. litura* ($\omega = 1.6029$) and *P. xylostella* ($\omega = 1.4277$). Our study demonstrated once again that life table study incorporating predation rate is a more promising and persuasive way for biological control.

Figure 1

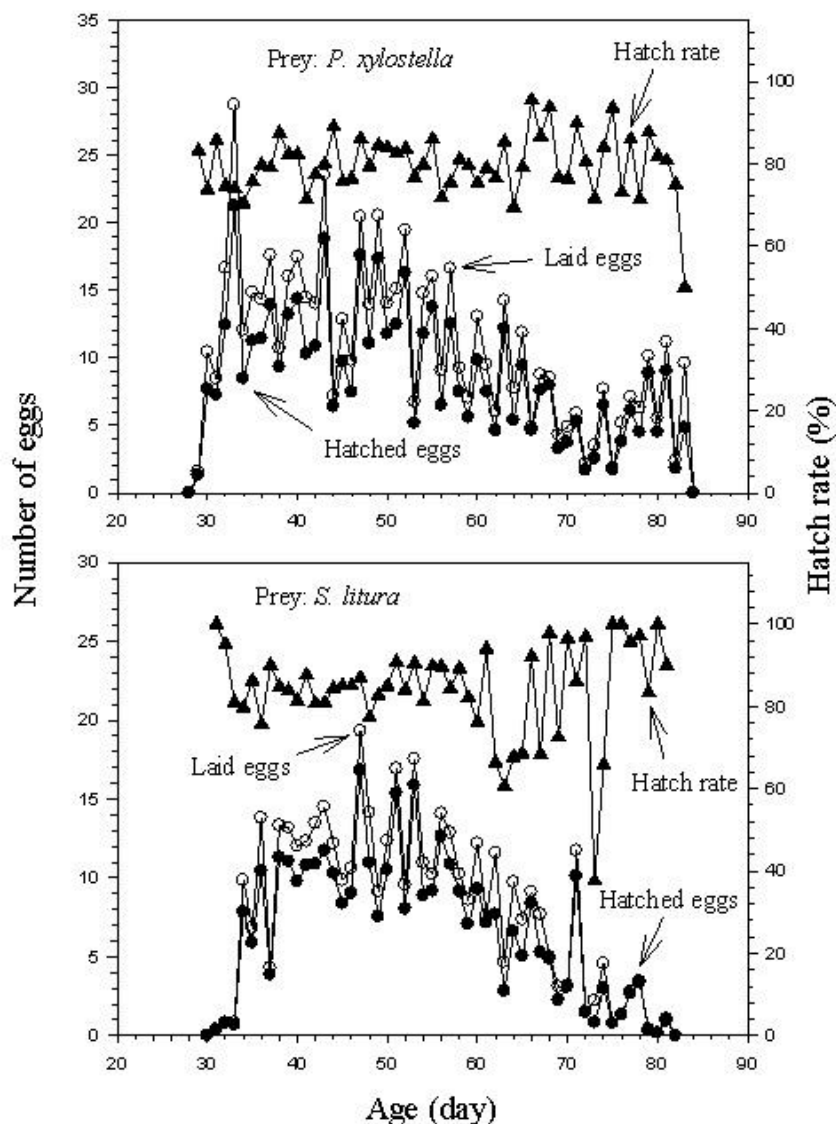
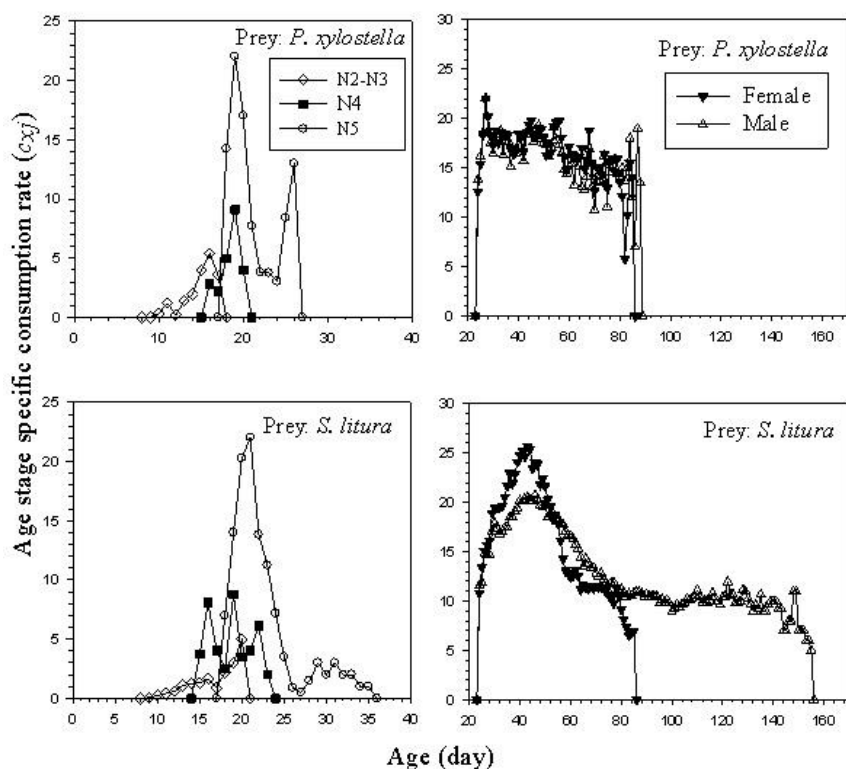


Figure 2



P N-CCO 34

The insecticidal effects of *Laurus nobilis* essential oil against immature stages of the flour moth, *Ephestia kuehniella* Zeller

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Among the plants species, some of them are able to produce secondary metabolites which play an important role in the defense mechanisms of plants against arthropods. Phytochemicals are usually less environmentally harmful than synthetic agrochemicals. Mediterranean flour moth, *Ephestia kuehniella* Zeller (Lep.: Pyralidae) is a worldwide pest and its close association with human foods makes it prime target for control methods other than chemical pesticides. At this study, insecticidal effect of essential oil of *Laurus nobilis* on *E. kuehniella* was evaluated. Experiments were carried out at the dark conditions in germinator (27±2°C, 65±5% RH). Mortality percentages of immature stages were tested at six different concentrations ranging from 0.5 to 3.5 µl/L air, during several times intervals from 6 to 24 hours with six replications. Results showed that with increasing dose and time, percentage of mortality increased significantly. LC₅₀ values after 24 h fumigation with *L. nobilis* essential oil were 32.37, 10.67, 18.18 and 26.08 µl/L air, for egg, 2th instar larvae, 3th instar larvae and 4th instar larvae, respectively.

P N-CCO 35

Laboratory developmental traits and functional response of *Pseudapanteles dignus* (Hymenoptera: Braconidae) attacking *Tuta absoluta* (Lepidoptera: Gelechiidae) in eggplant.

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Laboratory studies were carried out to investigate some developmental traits and functional response of *Pseudapanteles dignus* (Muesebeck), a larval endoparasitoid of *Tuta absoluta* (Meyrick) in eggplant, *Solanum melongena* L., another crop attacked by this pest. Stage specific developmental times were evaluated by exposing 10 2nd and 3rd *T. absoluta* larvae to *P. dignus* wasps. To determine *P. dignus* potential efficiency to control *T. absoluta* in eggplant crops, the parasitoid functional response to varying host densities (3, 7, 10, 15 and 20 2nd and 3rd -instars larvae; 24 h- exposition period, 8 replicates per host density) was assessed.

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Egg-pupal developmental time was similar for males (13.74 ± 0.66 d) and greater for females (13.57 ± 0.4 d) than when reared on *T. absoluta* in tomato. Pupal stage was longer for both sexes (~ 13 d) in eggplant, yielding a longer total preimaginal period for this parasitoid in eggplant than in tomato crops. As in tomato, *P. dignus* showed a density independent type I response in laboratory (only significant $p_0 = -1.39 \pm 0.4$ SE) in *S. melongena*, but attacked a lower number of hosts per day (attack rate (a') = 0.19 ± 0.03 SE, 95 % CI 0.13-0.24). These results suggest that *P. dignus* has an inferior performance when reared on *S. melongena*, which could have implications for use in biological control of *T. absoluta* in eggplant.

P N-CCO 37

Evaluation of the causes of legume yield depression syndrome using an improved diagnostic tool

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Due to their ability to fix atmospheric nitrogen in symbiosis with rhizobacteria, legumes are of outstanding importance in organic agriculture, and are often included in crop rotations. Despite their importance for soil fertility, the use of legumes has been declining in organic agriculture for many years, mainly because productivity is much below expectations based on pedoclimatic conditions. A multitude of factors and their interactions may contribute to such yield depressions, including abiotic (e.g. impaired soil structure, lack of nutrients, toxic compounds) as well as biotic (pathogens) factors.

The aim of the study was to establish a diagnostic tool to narrow down the causes for pea yield depressions. A differential two-level diagnostic test system was established under controlled conditions using peas (*Pisum sativum* L.) as test plants. Soils from 22 organically managed sites with unexplained moderate to high pea yield losses were tested in level 1 diagnostics (g-irradiation to eliminate potentially harmful organisms, nutrient additions to compensate for potential nutrient deficiencies or activated charcoal amendment to bind and thereby to immobilize potentially phytotoxic compounds). Results showed that pathogens were the primary cause of limited germination and growth in most of the sampled soils, whereas a positive effect of nutrient addition was rarely found and toxins were not involved. Level 2 diagnostics (pesticides targeting ascomycetes, oomycetes, *Rhizoctonia* spp., nematodes) further narrowed down the organisms involved in yield depressions. Oomycetes were identified as the primary reason for limited germination rates, and, in some soils, also for limited growth of established seedlings. In other soils, a multitude rather than a single group of pathogens was involved in limited growth. Plant-pathogenic nematodes were never found to be limiting. Harmful effects of pesticides were found in several soils, hinting at an important role of beneficial soil organisms in the suppression of pathogens causing yield depression in legumes. The bioassay used in the present study was robust and could thus serve as a low-cost tool for agricultural advisors and farmers to predict the risk of yield losses in legumes and to narrow down causes, helping them to develop appropriate strategies.

P N-CCO 38

Effect of seed- and soil-borne fusaria on the development of maize seedlings

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Introduction: In maize, fungicidal seed treatments are mainly applied to protect against species of *Pythium* on the one hand and seed- or soil-borne infections by *Fusarium* spp. on the other. However, while *Fusarium* ear and stalk rot of maize are well described and understood, much less is known on the pathogenic effect of fusaria on germinating maize kernels and on seedling development.

Objectives: For the development of seed treatments targeting seed- or soil-borne *Fusarium*, an adequate methodology for determining the efficacy under controlled conditions is required. In order to develop such a methodology, we performed pot tests in the greenhouse involving different fusaria, maize varieties and inoculation techniques.

Materials and methods: Different species of *Fusarium* were isolated from maize kernels and identified. In the tests a seed lot of maize naturally infected with fusaria was used, or healthy maize kernels were inoculated artificially. The fusaria were grown on agar media, and suspensions with different concentrations of conidia were prepared and applied to maize kernels. Alternatively,

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inoculum was prepared in shake cultures and applied to pre-heated potting substrate in seed trays. After sowing, the seed trays were incubated in a growth room under fluorescent lamps at 20 °C and watered regularly according to weight. Fifteen to twenty days after sowing the above-ground plant parts were harvested, and their dry weight was determined.

Results: Of the fusaria from maize kernels, highest pathogenicity was observed for isolates belonging to *F. verticillioides* and *F. semitectum*. Generally, the dry weight was reduced by 30 - 50%. Cultivation of the plants at low water content of the potting substrate increased the pathogenic effect. Differences between maize varieties were observed. The results obtained with the described methodology were well reproducible.

Conclusions: The method of soil inoculation mirrors the natural conditions closely and is suited for testing seed treatments. If naturally infected seed is not available, artificial seed inoculation can be used. In our experiments it resulted in a clear reduction of the biomass formed. However, interpretation of results from seed treatment experiments should consider that inoculum on the seed surface may be easier to control than internal infections.

P N-CCO 39

Effect of essential oil against bacterial knot disease caused by *Pseudomonas savastanoi* pv. *savastanoi* in *in vitro* conditions

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The antibacterial effect of essential oils of thyme (*Thymus vulgaris*), bergamot (*Citrus bergamia*), garlic (*Allium sativum*), fennel lavender (*Lavandula stoechas*) clove (*Caryophyllus aromaticum*), eucalyptus (*Eucalyptus globus*) on *Pseudomonas savastanoi* pv. *savastanoi* were investigated. Thyme, bergamot, garlic, fennel lavender, clove, eucalyptus essential oils have been found effective more than others. It is detected that as the dose of essential oils increased, the antibacterial effect increased. Bacterial population did not change after 24 h growth in different treatment of essential oils at different times, but relative decrease in bacterial population at 0, 1, 3, and 6 h growing were observed. Especially, thyme essential oil showed the highest antibacterial effect in all of the applications in different doses. Thyme essential oil the most effective. Bergamot, lavender and eucalyptus essential oils followed the thyme essential oil, respectively.

P N-CCO 40

Effects of Some Antagonists as Seed Treatments on Biological Control of Watermelon Fruit Blotch

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Watermelon fruit blotch agent, a seed borne bacterium, *Acidovorax citrulli* is one of the destructive pathogen of watermelon growing areas in the world including Turkey. Cotyledon symptoms are water-soaking, brown, sunken, necrotic spots or large necrotic lesions. Control strategies of the disease should focus on using disease-free seeds. In this study, 322 candidate antagonists were isolated from healthy watermelon leaves, blossoms or soils. Of those, 14 antagonists were selected from the highest inhibition zone growth *in vitro* tests. Antagonists were tested for their ability to suppress the pathogen on seeds. Pathogen and antagonists treated seeds were sown in plastic containers, kept in 30 °C and 85% relative humidity. A week after germinations, infected cotyledons were determined with 0 to 7 scale. 100 seeds were used for per treatment. In the experiment, antagonists reduced the pathogen incidence from 6 to 94% and disease severity was ranged from 10 to 93%. Seven antagonists (Antg-12, Antg-57, Antg-79, Antg-147, Antg-197, Antg-198 and Antg-273) were reduced the pathogen incidence and disease severity over 85% and 88%, respectively. This study demonstrated that antagonists are able to reduce disease occurrence and serves as our first attempts on biological control of watermelon fruit blotch for further studies.

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Morphological and molecular characterization of *Fusarium* spp. isolated from Crown rot of organic bananas.

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Crown rot is a postharvest fungal disease with a great negative impact on fruit quality. It is caused by several fungal pathogens, including some *Fusarium* species. We studied *Fusarium* species associated to crown rot of organic bananas grown in Dominican Republic. Bananas were collected in five organic farms and in the corresponding packing stations over two years. A total of 316 hands were collected and more than 2034 fungal colonies were obtained from crown tissues. Out of them 275 representative *Fusarium* colonies were purified, characterized and identified. Combinations of two identification keys were used for morphological identification. In addition to, these isolates were characterized by DNA sequencing of nuclear rDNA internal transcribed spacer (ITS), beta-tubulin gene and microsatellite markers. Finally, 11 representative strains were tested for their pathogenicity. *Fusarium* spp. were isolated from all the analyzed samples from field and packing stations. Moreover they were isolated from different crown layer and also from crown internal tissues. The *Fusarium* population was composed mainly by seven species and five were the most frequent: *F. verticillioides*, *F. chlamydosporum*, *F. solani*, *F. equiseti*, and *F. oxysporum*. *F. verticillioides* was the most virulent specie which caused the highest disease incidence and severity in experimental pathogenicity tests.

P N-CCO 42

Melanosporales: Potential biocontrol agents against fusaria?

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Introduction: Different species in the order Melanosporales (Ascomycota) occur naturally in association with plant pathogenic fungi. Some of them were shown to cause damage to species of *Fusarium in vitro* (1), and there are few reports demonstrating biocontrol activity *in planta* (2,3).

Objectives: The aim of the work described here was to characterize *in vitro* the host spectrum of one isolate each of *Melanospora zamiae* and *Persiciospora moreau* and to determine certain traits such as culturability and formation of perithecia and conidia on different laboratory media.

Materials and methods: The isolates of *M. zamiae* and *P. moreau* used were originally co-isolated with *Fusarium solani* from cucumber and *F. proliferatum* from asparagus, respectively (4). They were cultured singly on malt extract peptone agar (MPA), tryptic soy agar (TSA), strength tryptic soy agar (0.1xTSA), or paired with different *Fusarium* species on specifically nutrient poor agar (SNA).

Results: Both ascomycetes formed perithecia on complex media such as MPA, 0.1xTSA or on wheat kernels. *M. zamiae* generally produced more perithecia than *P. moreau*. On nutrient poor medium (SNA) mycelial growth was very poor, and no perithecia were formed. When paired with fusaria, mycelial growth was much faster, and perithecia were formed. However, this was only seen in pairings of *M. zamiae* with *F. solani* and of *P. moreau* with *F. oxysporum* and *F. proliferatum*. In pairings between *M. zamiae* and *F. solani*, hook-like structures of *M. zamiae* were observed in close contact with hyphae of *F. solani*, indicating a parasitic relationship. However, damage of the hyphae was not observed. On TSA, both fungi produced phialidic anamorphs with conidia approx. 5 x 3 µm in size. In the case of *P. moreau* their germination could not be observed (not attempted in the case of *M. zamiae*).

Conclusions: The observations correspond largely to the literature describing interactions of members of Melanosporales and their *Fusarium* hosts and constitute a base for further studies aimed at evaluating the potential of *P. moreau* and *M. zamiae* for use in biocontrol.

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- (3) Arch Microbiol (2012) 194:707-717
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Poster Presentations

Non-chemical control options

P N-CCO 43

Alternative products from CO-FREE for protection of organic potato crops. Are they effective?

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Introduction: The search for methods and means that can be an alternative to copper, and may be used in organic farming is one of the tasks of the project CO-FREE. Results from field trials in potato done by IPP-NRI (Poland) and JKI (Germany) are shown.

Objectives: In 2012-2014 field experiments were conducted to evaluate the effectiveness of CO-FREE test products (CTPs), single and combined with disease control strategies in potato / late blight (*Phytophthora infestans*).

Materials and methods: Different CTPs (resistance inducers (RI) and agents with direct effects) were tested against *P. infestans* in potato varieties ('Ditta', 'Jelly', 'Allians', 'Sante'). In 2012-2013 CTPs were sprayed with standard flat fan nozzles. In 2014 special flat fan nozzles were used to improve the wetting of the underside of the leaves. CTPs were separately and preventively applied 4 to 6 times at intervals of 7 to 10 days according to prognosis models. Low dose copper was combined with tested products, also. In Germany, additionally, phosphonates were tested.

Results: In Poland in 2012-2013 in 'Ditta', no significant increases in potato production compared to untreated control or 0.5 and 1 kg Cu/ha were noted. However, the highest yield was reached after the treatments with a RI (as stand-alone or together with 0.5 kg Cu/ha). In 2014 two strategies (CTPs in combination with different susceptible varieties and different levels of nitrogen fertilization) were compared. The highest total yield was obtained for plants treated with 03E (direct effects) and 6715B (RI) ('Sante'). Treatments delayed disease symptoms for 3 and 6 days, respectively.

Field trials 2012 in Germany have shown that after copper applications, the late blight infestation was delayed by about 6 or 7 days, while treatment with CO-FREE24 and CO-FREE25 (RI) delayed disease by 3 days and increased yield by ca. 10 % ('Ditta'). In 2013 and 2014 low doses of copper were combined with CTPs. Here CTPs have not yet contributed satisfactorily to yield stability.

Conclusions: The potato cultivar had the most influence on yield. Field trials with 6715B and 03E showed promising results in a moderate susceptible variety 'Sante'.

P N-CCO 44

Change in growth of phytopathogenic fungi by the biostimulant Frutogard®

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Introduction: Plant diseases cause high economic losses. Therefore, the control of plant pathogenic fungi is very important. In order to protect the environment, biological and ecologically acceptable control of plant pathogens has been a considerable topic for research. Regarding the importance of this control method, in this survey we will study the effects of a plant strengthener on the control of fungal plant pathogens both in vitro and on plants under greenhouse conditions. This plant strengthener is a liquid formulation.

Objectives: Comparing the growth behavior of the fungi on the plant strengthener with control medium will help to answer the following questions:

A) Is the fungal growth completely stopped? **B)** Does the fungus show abnormal growth as result of stress? **C)** Or is fungal growth not affected at all? **D)** How does Frutogard® induce tolerance or resistance in plant to pathogenic fungi?

Materials and Methods: Frutogard® is a plant strengthener, which is produced by Tilco biochemical company and is a liquid formation. Its active substances are including 24% brown algae extract (*Ascophyllum nodosum* and *Laminaria species*) and 7% plant amino acids.

In preliminary experiments different concentrations of Frutogard® are tested with a range of fungi. We prepared three different media:

- PDA+1% Frutogard®
- PDA+5% Frutogard®
- PDA as a control

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The fungi were transferred on the medium and then were incubated at different temperatures. Fungal growth was daily measured and the pictures were taken.

Results: In preliminary experiments some fungal species showed growth inhibition to Frutogard® in comparing with control samples and some of fungi grew better in comparing with control samples. Of the 17 tested, 4 were promoted and all others inhibited.

Conclusion: The plant strengthener can be tested in plant-fungus interaction for disease control.

P N-CCO 45

Protective effect of lipopeptide extracts from *Bacillus* sp. isolates on leaves of *Arabidopsis* and sugar beet infected with bacterial pathogen *in planta*

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Introduction: The increasing demand for a healthy food supply requires an efficient control of plant diseases. Antagonism to pathogens is the main mechanism of biocontrol that has been proposed for *Bacillus* species. Lipopeptides, mainly classified into three families (iturins, fengycins and surfactins), are among the antibiotic compounds frequently produced by *Bacillus* species. It has been shown that each of these families can be included in direct antagonism to different plant pathogens.

Objectives: The present study was aimed at investigating direct antagonism *in planta* of lipopeptide extracts of 5 *Bacillus* isolates, obtained with various methods of extraction. Also, potential interactions of the individual extracts in different combinations on antimicrobial activity were examined.

Materials and methods: For the *in planta* assay, mixtures (10 µl) of individual methanolic (2.5 mg/ml) or ethyl acetate extracts (1 mg/ml) and each isolate of *Xanthomonas arboricola* pv. *juglandis* and *Pseudomonas syringae* pv. *aptata* were simultaneously inoculated into wounds of pin-pricked leaves of greenhouse grown *Arabidopsis thaliana* L. and *Beta vulgaris* L. plants at a ratio of 4:1 (v/v). When testing combination 1/2 or 1/3 of final concentrations of individual lipopeptide extracts were used.

Results: After 14 days from inoculation, control *A. thaliana* plants, inoculated with *X. arboricola*, dried almost completely. Plants inoculated with a mixture of pathogen and methanolic extracts of SS-12.6, SS-27.2 and SS-38.4 didn't show any signs of drying. *B. vulgaris* inoculated with *P. syringae* and individual extracts SS-12.6 and SS-38.4 from both method of extraction did not show necrotic lesions in a leaf tissue, unlike the extract SS-10.7 with necrotic zone similar to those of the positive control. When reviewing the results, all showed some antagonistic relations, except for combination of SS-12.6 and SS-38.4, and mixtures of SS-10.07, SS-12.6 and SS-38.4, which successfully suppressed pathogenic infection due to synergistic or additive effect.

Conclusion: The potential of lipopeptide extracts in direct antagonism against bacterial pathogens was confirmed. Perseverance in antagonistic action 7 and 14 days after incubation with the pathogens recommend specific combinations for further analysis in terms of biocontrol agents.

P N-CCO 46

Entropy of *Helicoverpa armigera* infected with entomopathogenic fungus, *Metarhizium anisopliae*

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Question: The cotton bollworm, *Helicoverpa armigera* Hübner (Lepidoptera: Noctuidae) is one of the most important agricultural pests worldwide. Undesirable side effects of synthetic insecticides, including development of resistance, have necessitated a shift to more eco-friendly methods for its control. The entomopathogenic fungus, *Metarhizium anisopliae* (Metsch.) Sorokin (Hypocreales: Clavicipitaceae) is a valuable biocontrol agent attacking larval stages of many lepidopteran pests including *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). In this research, sub-lethal effect of *M. anisopliae* isolate M14 was investigated on entropy values of *H. armigera*.

Methods: Based on mortality data from bioassays, third instar larvae of *H. armigera* were exposed to sub-lethal concentrations (LC₅, LC₁₀, LC₁₅, LC₂₀ and LC₂₅) of *M. anisopliae*. In order to determine the type of survivorship curves, entropy was used as a criterion.

Results: The entropy values were 0.17, 0.20, 0.22, 0.29, 0.33 and 0.40 for control and other treatments respectively. The entropy value less than 0.5 shows the survivorship curves near to type 1 suggesting that the probability of death was higher in late compared with early stages.

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Conclusions: Isolate M14 can be considered as one of the effective biocontrol agents of *H. armigera* because it decreases the total population of this pest by reducing its survival.

P N-CCO 47

Potential biopesticide against GTD pathogens isolated from asymptomatic grapevines

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Grapevine trunk diseases (GTD), caused by different phytopathogenic fungi threaten the vineyards worldwide. Endophytic, mycoparasitic fungi, like *Trichoderma* species have huge potential against grapevine plant pathogens colonizing woody tissues. Testing new isolates with biocontrol potential and ability to grow under different environmental conditions may provide effective biopesticide to control GTD pathogens.

Endophytic *Trichoderma* isolates were obtained from asymptomatic plants in the Tokaj Wine Region Hungary. Their mycelial growth rates were tested on different temperatures. The mycoparasitic potential of the trichodermas against two GTD pathogens (*Diplodia seriata* and *Neofusicoccum parvum*) was also tested.

Eight *Trichoderma harzianum* were identified based on their ITS1,2, *tef1* and Chit42-1a; 2a marker sequences. The mycelial growth was determined from the two average colony diameters on PDA, while the mycoparasitic potential was detected according the method of Szekeres et al. (2006; *Journal of Microbiological Methods*, 619-622). Field test with mixed spore suspension of the three fastest-growing *Trichoderma* strains (10^7 conidia ml⁻¹) on the pruning wounds of canes of GTD symptomatic grapevines were also tested.

All the eight tested *T. harzianum* isolates were able to grow on the tested temperatures (5-37 °C), but they showed different mycelial growth activity. 30 °C were the optimal for all isolates. TR02-03 and TR05 had the highest growth rate at all the tested temperatures on PDA. TR01-05 and TR09 isolates showed the most intensive growth at the lowest temperature (5°C). TR08 and TR10 grew significantly slower at the lowest, while TR07, TR09 and TR10 at the highest tested temperature. The Biocontrol Index of all *T. harzianum* was 100%. Eleven GTD symptomatic plants were treated with *Trichoderma* spore suspension, containing TR04 and TR05 spores collected from seven day old colonies. The diseases-specific symptoms decreased in all but one of the treated trunks. Moreover the *T. harzianum* could be reisolated from their woody tissues.

The tested *T. harzianum* are potential biopesticide, overgrowing GTD pathogens and decreasing symptoms on grape plants with the ability of growing well on broad temperature range.

P N-CCO 48

Influence of entomopathogenic fungus, *Metarhizium anisopliae* on developmental time, survival and fecundity of *Helicoverpa armigera*

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Question: The cotton bollworm, *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) is a polyphagous pest attacking wide range of crops throughout the world. Due to its quick adaptation against synthetic insecticides, control of this pest has necessitated the development of safer methods. The entomopathogenic fungus, *Metarhizium anisopliae* (Metschn.) Sorokin (Hypocreales: Clavicipitaceae) is a valuable biocontrol agent of many insect pests. In this research, sub-lethal effects of *M. anisopliae* isolate M14 were studied on developmental time, survival and fecundity of offspring from treated larvae of *H. armigera*.

Methods: The immersion method was used to bioassay *M. anisopliae* against third instar larvae of *H. armigera*. Based on mortality data from bioassays, third instar larvae of *H. armigera* were exposed to sub-lethal concentrations (LC₅, LC₁₀, LC₁₅, LC₂₀ and LC₂₅) of *M. anisopliae*.

Results: The results revealed that sub-lethal concentrations of fungus could adversely affect next generation of *H. armigera*. There were significant differences in duration of immature stages of *H. armigera* ($F = 158.64$; $df = 5, 158$; $P < 0.0001$). Isolate M14 reduced the age-specific survivorship (l_x), daily and total fecundity (m_x) of *H. armigera*. The highest and lowest total fecundity values were 836.36 and 425.87 (egg) for control and LC₂₅ treatments, respectively.

Conclusions: Our results indicated remarkable changes in *H. armigera* population control with special emphasis on its immature stages and progeny.

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P N-CCO 49

Biocontrol with the Southern Grey Shrike (*Lanius meridionalis*) in Algeria

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A total of 5513 preys are noted in 431 pellets of the Southern Grey Shrike *Lanius meridionalis* (178 in East Mitidja, 599 in Oum El Bouaghi, 487 in Biskra and 249 in Tlemcen). The average of prey per pellets varies ($6.1 < \text{Avg.} < 16.5$). In Ramdhan, *Geotrupes* sp. (11.9 %) dominates in winter, Gryllidae sp. indet. (27.6 %) in spring and *Messor barbara* in summer (18.4 %) and in autumn (79.4 %). In Baraki, *Messor barbara* dominates in spring and autumn successively (13.1 %) and (70.6 %). In El Medfoun, *Geotrupes* sp. dominates in winter (10.5 %), *Anisolabis mauritanicus* in spring (13.8 %), *Acinopus* sp. in summer (28.0 %) and *Messor barbara* in autumn (22.5 %). In Sidi Okba, *Sepidium* sp. dominates in winter, *Bothynoderes* sp. in spring and *Cataglyphis bicolor* in summer and autumn. In Bouhannaq, *Geotrupes* sp. dominates in winter (20.8 %) and *Aethiessa floralis barbara* in summer (7.9 %). The total richness of prey about *L. meridionalis* in all areas is 375 species (97 species in Bouhannaq and 222 species in Baraki). In the menu the class of Insects is the most represented ($83.5 \% < \text{AR} \% < 91.8 \%$). H' varies ($1.2 \text{ bits} < H' < 4.1 \text{ bits}$) in Ramdhan, it's ($1.7 \text{ bits} < H' < 3.7 \text{ bits}$) in Baraki, ($2.9 \text{ bits} < H' < 3.5 \text{ bits}$) in El Medfoun, ($3.4 \text{ bits} < H' < 3.8 \text{ bits}$) in Sidi Okba and ($2.8 \text{ bits} < H' < 3.8 \text{ bits}$) in Bouhannaq. The Values of E varied ($0.3 < E < 1$).

P N-CCO 50

Antimicrobial Activity of *Syzygium aromaticum* and *Zanthoxylum*

xanthoxyloides* Essential Oils Against *Phytophthora megakarya

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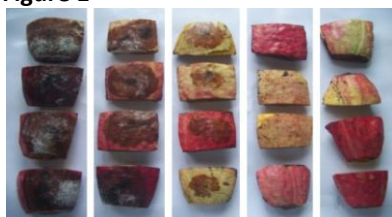
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The aim of this study was to assess the chemical composition and the antimicrobial activity of essential oils of dried fruits and buds of *Zanthoxylum xanthoxyloides* (*Z. xanthoxyloides*) and *Syzygium aromaticum* (*S. aromaticum* or clove), respectively, against *Phytophthora megakarya* (*P. megakarya*). Essential oils were extracted by hydrodistillation, and their composition was determined by gas chromatography and by gas chromatography coupled with Mass Spectrometry. The minimal inhibitory concentration (MIC) and minimal lethal concentration (MLC) of the essential oils against *P. megakarya* were assessed by the Agar dilution method. The *in vivo* efficacy study consisted of spraying the essential oil emulsions on cocoa pod husk pieces (CPHP), followed by the inoculation with *P. megakarya* zoospores. The hydrodistillation yielded 10.54 and 1.89% of essential oils for *S. aromaticum* and *Z. xanthoxyloides*, respectively. Both oils were mainly made up of oxygenated monoterpenes (89.58 and 88.2%, respectively). Eugenol (83.02%) and eugenyl acetate (9.15%) were the main components of clove oil while α -citronelol (25.83%) and trans-geraniol (16.49%) were mostly found in the *Z. xanthoxyloides* oil. Clove oil exhibited stronger antimicrobial activity with a MIC of 250 μl than *Z. xanthoxyloides* with MIC of 350 μl . The symptoms were totally suppressed on pod husk treated with clove oil at 2000 μl . The decrease in the growth rate of the necrosis (GRN) and the sporulation of *P. megakarya* (PS) on cocoa husk after the successful infection was significant after the treatment with essential oils. These results are promising and indicate that the studied essential oils might be further investigated as natural alternatives to synthetic fungicides for the control of cocoa black pod diseases.

Figure 1



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The use of *Trichoderma Asperellum* as a bio-control agent of tomato white mould disease caused by *Sclerotium Rolfsii*

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Existing evidence suggest that pre-treatment of seeds with *Trichoderma* spp could significantly reduce deleterious effect of pathogenic fungi on various economic crops. Studies were carried out to determine the most suitable application method of *Trichoderma asperellum* as an alternative to the use of fungicide that would reduce the incidence and severity of white mold disease of tomato caused by *Sclerotium rolfsii*. *Trichoderma asperellum* was applied using 5ml of 10⁵ conidia/ml to moisten 10 seeds of tomato as seed treatment, secondly as a preventive treatment on 18 day-old tomato seedlings at 24hrs ahead of *S. rolfsii*, and lastly as a curative treatment applied after 24hrs inoculation of *S. rolfsii*. The controls involved an untreated seeds and seeds treated with fungicide (mancozeb) as a chemical control. The data were subjected to ANOVA and the means separated by Tukey test. It was observed that preventive application of *T. asperellum* gave 7% which was significantly the least disease incidence and also recorded the least value for white mould severity of 1.2 which was significant when compared with both untreated and fungicide treated tomato plants. Thus, *Trichoderma asperellum* could be further developed as a bio-fungicide for the control of white mould disease of tomato.

P N-CCO 52

Antimicrobial activity and characterization of the ethanolic extract of the peels of sweet orange fruit (*Citrus sinensis* (L.) Osbeck) in the control of *Lasiodiplodia* sp. IMI 503248.

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Introduction: Due to the great nutraceutical and economic importance of citrus essential oils, numerous investigations have been performed aimed at identifying the chemical composition, antimicrobial activities of the essential oils from peels of different citrus species, but the peels have been less studied.

Materials and methods:

Preparation of Peel Extracts for Antifungal Activity: Extracts were prepared according to the method of Harbourne (1998) but with slight modification.

Test for Antifungal Action of Peel Extract: The antimicrobial activity of the extract was assayed using agar well diffusion method described by Madigan *et al* (2002) but with slight modification.

Fractionation of Peel Extract: The ethanolic extract of the peel was purified using column chromatography technique.

Gas Chromatography- Mass Spectrum Analysis (GC-MS) of the EthanolSoluble Fractions of the Peel Extract: GC-MS analysis of the fractions was performed using PerkinElmer Clarus 500 GC system and gas chromatography.

Results:

Antifungal Activities of the Crude Peel Extract: The zone of inhibition for ethanolic extract against the test isolate on day 3 of incubation was 1.87±0.03mm.

Antifungal Activities of the Ethanol Soluble Bulk Fractions: Fractions 2-5 had 1.96±0.40mm as their zone of inhibition while fractions 6-10 had 1.98±0.65mm as zone of inhibition.

Structural elucidation of the active antifungal extract from the peel of *Citrus sinensis*: Gas Chromatography-Mass Spectrometry of the ethanol soluble fractions that are active against the test pathogen showed the presence of one major bioactive component, having highest peak (m/z) at 402 and retention time of 26 minutes.

Conclusion: The Gas Chromatography- Mass Spectrometry analysis revealed that ethanolic peel extract of *Citrus sinensis* (L.) contains 5,6,7,8,3,4¹ - hexamethoxyflavone which is commonly called nobiletin.

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P N-CCO 53

The influence of temperature on the vertical transmission of a mutualistic tall fescue endophyte

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Introduction: Many Poaceae grasses form a symbiosis with *Epichloë* endophytes. Initially a serious problem in agriculture due to their toxicity to livestock, endophyte strains were identified that were less toxic to livestock whilst also possessing advantageous traits, including insect deterrent alkaloids. Selection and transfer of these endophytes into elite grass cultivars has resulted in pastures with improved persistence with no, or reduced, livestock toxicosis. These novel associations are now successfully marketed in New Zealand, Australia, USA and South America. *Epichloë* species exhibit strong host specificity and the asexual form is exclusively vertically transmitted through maternal lines via the seed embryo. This process is far from perfect with endophyte transmission failures attributed to factors including endophyte and host genotypes, environmental factors and crop management regimes.

Objectives: To determine the influence of temperature on the vertical transmission of a tall fescue endophyte in its original host compared to that in a novel host.

Materials and methods: Two endophyte-grass associations were used in this study. The first consisted of *Epichloë* sp. strain AR501 within its original host background, namely tall fescue and the second with AR501 within an artificial host background, a cultivar of perennial ryegrass. Seedlings of both associations, original and novel, were arranged in a controlled environment at four temperature regimes ranging from 6-25°C. After set amounts of time, endophyte transmission frequencies and endophyte biomass was quantified. Mature plants from both associations were transferred from 12/6°C to 25/16°C environment and their endophyte biomass calculated again.

Results: For perennial ryegrass, the endophyte transmission frequency was significantly higher in plants kept at a cold (day/night 12/6°C) temperature regime compared to the warm (day/night 25/16°C) regime. This was not observed in tall fescue. The endophyte biomass concentrations of both associations increased when plants were transferred from the cold to warm temperature regime.

Conclusion: Temperature is a significant factor in the transmission of certain novel grass-endophyte associations and this should be taken into account during their research and development.

P N-CCO 54

Selection of entomopathogenic fungi based on plant growth promotion and insect pest control for its use in seed coatings

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Introduction: The development of fungal entomopathogens as biocontrol agents requires an understanding of the dynamic interactions between fungi, insect pests and plants. Recently, it has been shown that *Metarhizium* spp. can act as both insect pathogen and plant-growth-promoting agent. This bifunctional lifestyle of *Metarhizium* is illustrated by the differential expression of genes involved in the adhesion to insect (*mad1*) or plant surfaces (*mad2*). In addition, a plant carbon transporter, *Metarhizium* raffinose transporter (*mrt*), was reported as required for successful root colonization. The present work is focused on selection of entomopathogenic fungi to both colonize the plant rhizosphere and have biocontrol activity when delivered through seed coating.

Objectives: Delivery of *Metarhizium* spp. isolates through seed coating to protect crops against insect pests.

Materials and methods: The growth response of *Metarhizium* spp. isolates to maize root exudates was determined using microplate assays. The presence of *mad1*, *mad2* and *mrt* was determined by PCR. Finally, the effects of *Metarhizium* spp. on maize plant growth and plant resistance against larvae of the scarab beetle *Costelytra zealandica* was determined. Fungal isolates were applied to maize seeds by seed coating.

Results: *Metarhizium* isolates showed different growth rates in the presence of maize root exudates, suggesting differing abilities in associating with maize roots. While the *mad1* or *mad2* genes were not detected in all isolates, the *mrt* gene was widely conserved among the *Metarhizium* spp. The strains with the highest growth rates in exudates contained the *mad2* gene. Although some resistance to *C. zealandica* was found, it was unclear whether there was enhanced plant growth in the presence of the fungi.

Conclusion: The selection of root-competent strains is advisable for improve establishment and persistence of entomopathogenic fungi delivered as seed coating.

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Isolation, identification and biological activities of alkaloids from *Anisodus tanguticus*

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Two kinds of alkaloids were isolated by using varieties of column chromatography separation from the aerial parts of *Anisodus tanguticus* and identified their chemical structures by MS and NMR, they were hyoscyamine and scopolamine, respectively. Contact activities of hyoscyamine and scopolamine against nine species of aphids including *Brevicoryne brassicae*, *Myzus persicae* and four species of mites including *Tetranychus cinnabarinus*, *T. urticae* were conducted by micro spot method and slide dip method. The results showed that hyoscyamine had stronger contact effects against *Rhopalosiphum padi*, *Hyalopterus arundinis*, *Aphis craccivora* and *A. citricola*, the LC₅₀ values were 257.863 mg/L, 275.459 mg/L, 344.645 mg/L and 344.717 mg/L, respectively, and scopolamine had stronger contact effects against *A. craccivora* and *R. padi*, the LC₅₀ values were 311.585 mg/L and 392.309 mg/L, respectively. Rotenone (control pesticide) had stronger contact effect against *B. brassicae* and *Aphis gossypii*, the LC₅₀ values were 399.542 mg/L and 436.124 mg/L, respectively. Contact toxicities against nine species of aphids including *B. brassicae*, hyoscyamine was stronger than scopolamine or quite. Hyoscyamine and scopolamine were stronger than rotenone in contact toxicities against *A. craccivora* and *R. padi*, and hyoscyamine and scopolamine were weaker than rotenone in contact toxicities against *B. brassicae*, *M. persicae* and *A. gossypii*. Hyoscyamine and scopolamine had stronger contact effects against *T. truncates*, *T. viennensis*, *T. urticae* and *T. cinnabarinus*. Contact toxicities of two kinds of alkaloids against four species of mites were quite, toxicities against *T. urticae* and *T. cinnabarinus* (201.027~224.172 mg/L) were higher than that of *T. truncates* and *T. viennensis* (257.014~332.698 mg/L). The LC₅₀ values of rotenone against *T. truncates* and *T. viennensis* were 257.863 mg/L and 275.459 mg/L, respectively, its toxicities were higher than that of hyoscyamine and scopolamine.

P N-CCO 56

Biological control of charcoal-rot of sorghum by actinomycetes

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Introduction: Charcoal-rot of sorghum (*Sorghum bicolor*), caused by *Macrophomina phaseolina* (Tassi) Goid., is endemic to tropical and temperate regions of the world. Significant losses of yield (up to 64%) have been observed under conditions favoring the disease development in the post-rainy sorghum. With the ever increasing cost and concern over environmental pollution, efforts are being taken to develop environment-friendly methods of disease control.

Objectives: The objective of this study was to isolate, characterize and evaluate actinomycetes from herbal vermicompost for their antagonistic potential against charcoal-rot of sorghum under both greenhouse and field conditions.

Materials and methods: The actinomycetes were isolated from herbal vermicompost and screened for their antagonistic potential against *M. phaseolina* by dual culture and metabolite production assays. The ten most promising isolates were characterized for the production of siderophore, chitinase, hydrocyanic acid (HCN), Indole acetic acid (IAA), β -1, 3-glucanase, lipase and protease and further evaluated for their antagonistic potential under greenhouse and field conditions.

Results: A total of 10 actinomycetes antagonistic to charcoal-rot were selected based on dual culture and metabolite production assays. The isolates were found to produce siderophore, chitinase, cellulase, lipase, protease, HCN, IAA and β -1,3-glucanase. Under greenhouse conditions, the isolates significantly reduced the extent of charcoal-rot infection. SEM analysis revealed that the actinomycetes colonized the roots of sorghum. qRT-PCR analysis on selected biocontrol and PGP genes of actinomycetes revealed up-regulation of β -1,3-glucanase and chitinase genes. The isolates are further being evaluated against the pathogen under field conditions.

Conclusion: The 10 actinomycetes were demonstrated for their biocontrol potential against charcoal-rot of sorghum under greenhouse conditions and are being further evaluated under field conditions.

P N-CCO 57

Establishment of pre-harvest residual limits of amisulbrom and flubendiamide in cherry tomato and their biological half-life

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Introduction: Pesticides are extensively used to control the pest population in intensive agricultural production. Pesticides can easily reach agricultural products while spraying directly against target organisms and transferring from water and soil. Recent studies show that serious concerns have been raised about risks of residues in various foodstuffs.

Objectives: The present study was performed to establish pre-harvest residual limits (PHRLs) of amisulbrom (fungicide) and flubendiamide (insecticide) in cherry tomato and to evaluate their biological half-life.

Materials and methods: Amisulbrom and flubendiamide was diluted two thousand fold separately and sprayed single time on cherry tomato in the cultivation areas of Chuncheon (field 1 and 2). Cherry tomato (*Lycopersicon esculentum* Mill) samples were randomly collected after 2 hr treatments and then at the end of 1, 2, 3, 5, 7, 9 and 10 days collected for residue analysis. Samples were extracted twice (80 and 70 ml) with dichloromethane and purified through NH₂ SPE cartridge, respectively. Finally, the residual amounts of both pesticides in all samples were analyzed using liquid chromatography-tandem mass spectrometry (LC-MS/MS).

Results: In this study, the method limit of quantification (MLOQ) for both pesticides in cherry tomato was 0.005 mg kg⁻¹. The results of this study shows the recovery levels of both pesticides were found to be 84.7% ~ 101.0%, 86.6% ~ 90.9%, respectively (Fig 1). Further, the calculated biological half-life for amisulbrom and flubendiamide in cherry tomato were 13.3 and 11.2, and 6.5 and 4.9 days in site 1 and 2, respectively (Table 1). Based on the results obtained from this study we recommend the level of PHRLs on cherry tomato is 1.27 mg kg⁻¹ for amisulbrom and 1.27 mg kg⁻¹ for flubendiamide at 10 days before harvesting.

Figure 1: Levels of PHRLs of amisulbrom and flubendiamide for cherry tomato.

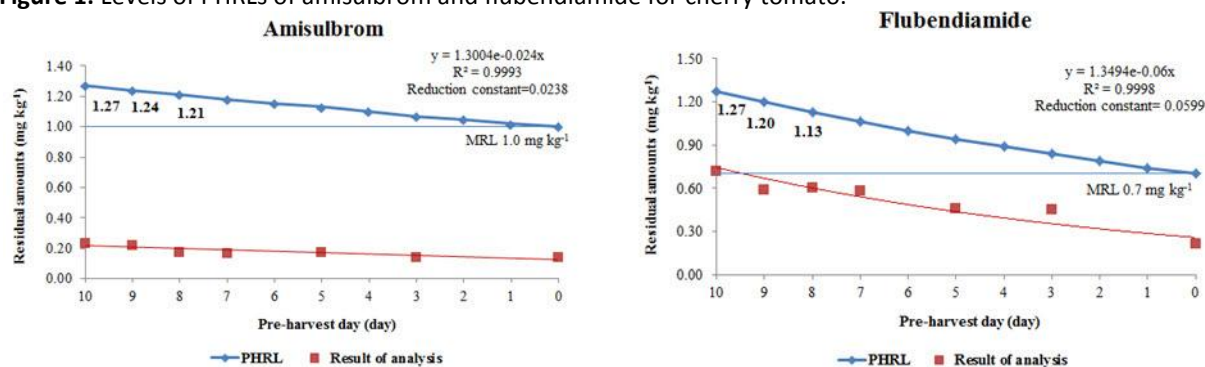


Table 1: Biological half-life of amisulbrom and flubendiamide in field 1 and 2.

	Amisulbrom		Flubendiamide	
Field	1	2	1	2
Half-life	13.3 d	11.2 d	6.5 d	4.9 d

Conclusion: The present study concludes that the residue level (1.27 mg kg⁻¹) of both pesticides in cherry tomato is safe for human consumption at 10 days before harvesting. Further, we suggest that the residue levels of both pesticides found in this study will be lower than the maximum residual limits (MRLs) stated by National Agricultural Products Quality Management Service (NAQS), Government of Korea. Moreover, the study will be very helpful in establishing PHRLs for many other pesticides, and for the safety of agricultural products and the people of Korea.

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Potential of *Amblyseius (Neoseiulus) californicus* (McGregor 1954) to suppress *Polyphagotarsonemus latus* (Banks) (Prostigmata: Tarsonemidae) on tea plant

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Polyphagotarsonemus latus (Banks) (Prostigmata: Tarsonemidae) is a serious pest of tea *Camellia sinensis* (Theales: Theaceae). Several phytoseiid mites have been described to control this mite. In this study, the potential of *Amblyseius (Neoseiulus) californicus* (McGregor 1954) on control of *P. latus* population was assessed on seedlings of Tuglali-10 tea cultivar at 1:10 and 1:20 predator:prey release ratios in Rize province (Black Sea coast, Turkey). Completely randomized plot design with four treatments including the sprayed, unsprayed control and release plots was set up. The mite populations were evaluated by sampling weekly young leaves from the top of the plant from 4 August to 29 September 2014. The lowest *P. latus* population occurred in 1:10 prey: predator ratio, followed by 1:20 prey:predator ratio, sprayed control and unsprayed control plot. Results showed that *N. californicus* is an important mortality factor of *P. latus* and should be considered as a key biological control agent in *integrated pest management* programs targeting *P. latus* on tea in Rize province. Also, the study revealed that population densities of *P. latus* reached its peak levels during the period of August-September, year.

P N-CCO 59

Effect of soft soap, the tobacco leaf and garlic bulb extract on *Polyphagotarsonemus latus* (Banks) (Prostigmata: Tarsonemidae) on tea plant

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The efficacy of the soft soap, the tobacco leaf and garlic bulb extracts in controlling *Polyphagotarsonemus latus* (Banks) (Prostigmata: Tarsonemidae) on tea plant in Rize (Black Sea coast, Turkey). For preparing the tobacco extract, the dried *tobacco leaves* (50gr) were crushed into smaller pieces, taken into a 1 lt capacity glass jar and 500 ml distilled water was added to it. The glass jar was then tightly sealed. The jar was incubated at 70 °C and 60% relative humidity for 24 hours. The obtained product was separated using fine muslin cloth. To obtain the stock solution of garlic extract, peel garlic cloves (60 gr) were crushed and mixed with mineral oil (20 gr) into a glass jar. The jar was tightly sealed and incubated at 25°C ±1 and 60% relative humidity for 24 hours. After incubations, the obtained products were mixed with 500 ml distilled water and separated using fine muslin cloth. It was considered as a stock solution. The solution were diluted with distilled water to a total volume of 1 lt (final volume) to give the desired concentration. For the desired concentration of the soft soap, 150 gr soft soap was mixed with distilled water (1 lt) and shaken until the soap was fully dissolved. Completely randomized plot design with five treatments including the sprayed, unsprayed control. Morecare DF 800 gr (%80 Micronized Sulfur) was used on sprayed control plot. During the sampling period, *temperature* and *relative humidity* were also recorded using the *data logger*. The mite populations were evaluated by sampling weekly young leaves from the top of the plant from 4 August to 29 September 2014. When the motile mite densities (all stages except eggs) of *P. latus* were above an average of 4 mites leaf⁻¹, the spraying was done. While *P. latus* densities at tobacco, soap and sprayed control plots were over 4 motile mites per leaf three times during the experiment, the density at the garlic plot reached above the level four times. The highest population levels observed in the soap, garlic, tobacco, sprayed control and unsprayed control plot were 29.4, 38.4, 43.7, 54.2 and 67.8 mites leaf⁻¹, respectively. When all data were compared, we concluded that the soft soap, garlic bulb extract and tobacco leaf extract were found to be promising for practical application for controlling *P. latus* on tea plant in Rize, Turkey.

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Application of invasion ecology theory to bacterial inoculation of maize crops in southern Brazil

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The use of bacterial inoculants to improve crop productivity reduces the need for fertilizer and other agrochemicals without reduction on grain yield. These inoculants, however, might not be always effective under field conditions. As interaction between the inoculants and the native microbial community plays a key role in effective root colonization and plant growth promotion, classical invasion ecology might be applied for inoculants on crops to predict results and improve microbial management effectiveness. In this work, we apply hypothesis based on invasion ecology to crop fields under bacterial inoculation. We evaluate the associated microbial community composition of inoculated and non-inoculated plants to test if: (I) an inoculant with a higher invasion potential will provide a higher plant growth promotion effect; (II) if lower diversity/resource ratio in the environment facilitates invasion. To do this, we analyzed 3 different maize crops locations in south Brazil, which received the same 4 bacterial inoculants (one *Azospirillum*, one *Achromobacter*, and two *Pseudomonas*). Samples were taken from bulk soil immediately before planting, and from rhizospheric soil of 10 days old plants. Metagenomic DNA was extracted, the V4 region of the 16S rDNA gene was amplified, amplicons were sequenced on the MiSeq platform, and sequences were processed using QIIME. OTU frequency data was used on PCoA and SIMPER tests. All SIMPER results were then plotted on a PCA, showing how dissimilar were the differences in the control-treatment pairs across the locations. This approach was used as a proxy for inoculant invasion ability. 12.1 million valid reads with 300 bp were grouped into 1.3 million OTUs. PCoA showed that samples clustered by the 3 locations, but the PCA based on SIMPER results suggested that 2 different inoculants might induce very different community changes across two locations. These larger differences, however, were not associated to greater crop productivity. The diversity/resource ratios are still to be calculated, so invasion difficulty across environments must yet be estimated. Our preliminary conclusion is that it is not possible to predict the most effective inoculants by looking at shifts on the rhizospheric community 10 days after planting - what would be extremely useful in field trials.

P N-CCO 61

Antifungal activities of some plant extracts against phytopathogenic fungi *Sclerotinia sclerotiorum* Lib. De Bary, *Alternaria solani* (Ell. and G. Martin) *Rhizoctonia solani* Kühn. and *Ascochyta rabiei* (Pass) Labr.

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Nowadays, the emergence of a clearly negative aspects of the pesticides used in agricultural production has led researchers to focus on alternative control methods. These alternative methods of pest and disease control are to use plant extracts. The aim of this study was to determine methanol, acetone, ethyl acetate and n-hexane extracts of different parts (leave, stem and flower) of *Ricinus communis* L., *Vitex agnus-castus* L., *Heracleum platytaenium* Boiss., *Isatis glauca* Aucher ex Boiss., ve *Polygonum cognatum* Meissn. plants against *Sclerotinia sclerotiorum* Lib De Bary, *Alternaria solani* (Ell. and G. Martin) *Rhizoctonia solani* Kühn. and *Ascochyta rabiei* (Pass) Labr. which are important pathogens of cucumber, tomato, potato and chickpea all over the world. To evaluate the effects of these plant extracts, the rate of MGI (mycelia growth inhibition) were compared with thiram 80% (Hektaş group) which is a standard fungicide. 10, 50 and, 100 mg/ml doses of plant extracts were used. At the same time, the antifungal activities of the extracts were also statistically evaluated. It was shown that the extracts of *R. communis*, *V. agnus-castus*, *H. platytaenium*, *I. glauca* and *P. cognatum* caused remarkable inhibition rate against plant pathogens. In addition, the extract of *R. communis* caused a strong antifungal activity against these selected fungi in comparison to other plant extracts. According to this result, natural antifungal agents are cheap and less side effect to environment and human health could have promising results for the control of plant pathogenic fungi.

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Challenges in the development of a microbial fungicide based on a strain of *Lysobacter capsici*

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Introduction: *Lysobacter capsici* AZ78 (AZ78) is a Gram-negative bacterium that effectively controls oomycetes, in particular *Plasmopara viticola*. Non-spore forming bacteria populations quickly decline when exposed to harsh environments as phyllosphere and formulation can play an important role to achieve consistent efficacy in field applications. Limited information is available in literature on formulation of Gram-negative bacteria. Survival and fate in the environment are also important traits to be assessed for the registration as a biopesticide.

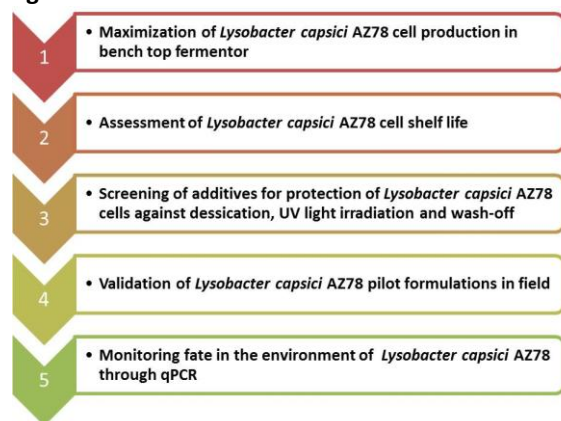
Objectives: The main aim of this work was to set up and apply a stepwise flow diagram to design performing formulations for AZ78 and follow its fate in the environment (Figure 1).

Materials and methods: Cell mass production was maximized in a benchtop fermenter and shelf life of the harvested cells was assessed. We screened compounds capable to protect the bacterial cells against desiccation, UV irradiation and wash-off. We tested the ability of combinations of selected compounds to preserve the efficacy of AZ78 against *P. viticola* and to establish populations on grapevine. A specific primer pair was developed starting from REP-PCR fingerprinting and subsequently used in a qPCR procedure for monitoring the fate of AZ78 in vineyards.

Results: The optimised fermentation protocol gave a harvest of at least 10^{10} AZ78 cell/ml. Viability of cells decreased only one order of magnitude after one year of storage at 4°C. The use of a combination of polyethyleneglycol, corn steep liquor and lignosulfonate in the formulation improved the survival of AZ78 cells in response to environmental stresses and the efficacy against *P. viticola* on grapevine in field conditions. Moreover, the qPCR procedure showed that the AZ78 population reached 10^6 cell/gram of leaf after its application and revealed that the use of additives in the tank mix enhanced the persistence of AZ78 cells in vineyards.

Conclusion: The stepwise flow diagram allowed us to achieve high biocontrol efficacy of AZ78 in field conditions and fulfill some of the requirement for the registration of AZ78. The same approach could be extended to other members of the genus *Lysobacter* for the development of biofungicides.

Figure 1



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Suitability of an electrolytic disinfectant to sanitize irrigation water contaminated with plant pathogens

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Introduction: The transmission of plant pathogens through irrigation water and nutrient solutions is a serious problem in agricultural production. Different physical and chemical techniques such as pasteurization, UV-light, filtration and water treatment by chlorination, ionization and surfactants have been described to decontaminate irrigation water and nutrient solution. Beside cost effectiveness and ecological concerns none of the methods is suitable to inactivate the multitude of relevant viral, bacterial and fungal plant pathogens.

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Objective: Evaluation of the efficacy of a disinfectant produced by an electrolytic disinfector processing a salt solution to inactivate plant pathogens *in vitro*.

Materials and methods: The efficacy of the disinfectant, a low concentrated potassium chlorid solution, to inactivate plant pathogens was tested *in vitro* according to the standard for disinfection in plant protection (OEPP/EPPO, 2008). Eight pathogens were selected: *Fusarium oxysporum*, *F. verticillioides*, *Pythium aphanidermatum*, *Botrytis cinerea*, *Verticillium dahliae*, *Rhizoctonia solani*, *Xanthomonas campestris* pv. *Campestris* and *Pseudomonas syringae* pv. *syringae*. Dose-effect relations were calculated.

Results: Applying the disinfectant with a concentration of 6 mg KClO/l and a contact time of 60 min, achieved total inactivation of all tested pathogens with exception of *Rhizoctonia solani*. This fungal pathogen even presented activity with 10 mg KClO/l and a contact time of 120 min.

Conclusion: The efficacy of Potassium Hypochlorite (KClO) to inactivate plant pathogens is confirmed *in vitro*.

P N-CCO 64

Electron treatment of sprouting seed

An efficient, economical and environmental-friendly process for pathogen reduction

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Providing the world's growing population with nutritious food is an enormous challenge, that solution starts very early in food production. Beside the known chemical seed dressing there is another way for killing pathogens. This environmental friendly, purely physical disinfection of seed, bases on the biocidal effect of accelerated electrons.

Electrons are a versatile tool for numerous applications in all fields of industry. Beside the known and well established processes in medicine and pharma the electron treatment of seed became more and more important. This technology is well established for treatment of cereal seed. Hence the treatment of sprouting seed is a challenging topic as well. Systemic problems with Bacteria, such as *E.Coli* cannot be solved completely with the common technics.

Due to the current demands, after EHEC crises in 2011, FEP investigated the behavior of electron treated sprouting seeds. When treating seeds, the applied dose, which can be determined by regulating the current strength, and the electron energy, which can be adjusted with the acceleration voltage, are important. When electrons penetrate matter, they lose their energy through collision processes. Once the energy is spent, they do not penetrate further into the material. This fact is used to precisely control the sphere of action during electron treatment.

Infected seeds are treated with electrons and there germination force, germination rate and load of pathogens are investigated. More than 90 % of the fenugreek and clover samples and more than 80 % of the mung bean samples are sterile, proved with fluid turbidity tests, after electron treatment. Not to influence the embryo, can be proved by testing germination rate and germination force. Both are kept unchanged.

Tests show that the treatment of sprouting seed (Mung bean, clover and fenugreek) to reduce bacteria load is possible, without influencing the embryo.

P N-CCO 65

Effect of Essential Oil Against Bacterial Cancer Disease Caused by *Clavibacter michiganensis* subsp. *michiganensis* in *in vitro*

Conditions

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Bacterial wilt and bacterial canker on tomato caused by *Clavibacter michiganensis* subsp. *michiganensis* is gram-positive bacteria. In this study, the antibacterial effects of essential oils of sage (*Salvia* spp), anise (*Pimpinella anisum*), juniperus (*Juniperus* sp.), aloe vera (*Aloe vera*), safflower (*Carthamus tinctorius*), bergamot (*Citrus bergamia*), rosemary (*Rosmarinus officinalis*), laurus (*Laurus nabilis*), senien urticae piluliferae (*Urtica dioica*), clove (*Caryophyllus aromaticum*), centaury (*Hypericum perforatum*), priest (*Lavandula stoechas*), thyme (*Tymus vulgaris*), cumin (*Carum carvi*), lavender (*Lavandula officinalis*), melissa (*Melissa officinalis*), myrtle (*Myrtle* sp.), mint (*Mentha piperita*), eucalyptus (*Eucoylytus globus*), fennel (*Foeniculum vulgare*), daisy (*Matricoria chamomilla*) on *Clavibacter michiganensis* subsp. *michiganensis* were investigated. 100 µl of the pathogen bacterial suspension (10^8 cfu/ml) was spread on 9 cm diameter petri dishes containing King medium B. Sterile discs (Watman No.1 diameter 5mm) were put on the medium and 10 µl of each essential oils was dropped on discs and cover petri with parafilm. Sterile distilled water was used for negative control. All plates were incubated at 25°C for three days. The diameter of clear zone around the disc was measured as millimeters. All treatments were three times replicated and for each

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replicate were used three paper disk. As a result of these study sage, juniperus, aloe vera, senien urticae piluliferae, centaury and cumin essential oils have not been found effective on *Clavibacter michiganensis* subsp. *michiganensis*. Antibacterial activity on growth of the pathogen was found with essential oils of anise, melissa, eucalyptus and fennel. The diameters of clear zones around the disc were ranged between 2.3 and 10,0 mm. Inhibition zones were recorded as 7, 5.6, 2.3 and 10 mm, anise, melissa, eucalyptus, fennel, respectively. However, Bergamot, rosemary, laurus, lavender, priest, clove, thyme, mint, melissa, myrtle, essential oils have been found not effective on *Clavibacter michiganensis* subsp. *michiganensis*. This study clearly demonstrated that essential oil of anise, melissa, eucalyptus and fennel are effective bacterial wilt and bacterial canker of tomato for traditional and organic production.

P N-CCO 66

Analysis of pathogen resistance induced by the root endophytic fungus *Piriformospora indica*

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Endophytic fungi of the order *Sebacinales* (*Basidiomycota*) are distributed all over the world and colonize roots of a wide range of host plants. Within this group, *Piriformospora indica* became a root endophyte model system, as it can be axenically cultivated, its genome has been sequenced, and it is colonizing important crop plants and model plants.

Previous studies have shown that *P. indica* improves growth, and enhances systemic pathogen resistance in leaves of host plants, e.g. against powdery mildew in barley and in *Arabidopsis*. To investigate what kind of signaling processes are induced from roots colonized by *P. indica*, we utilized a hydroponic split-root cultivation system. Using quantitative real-time PCR, we show that *P. indica* colonization of *Arabidopsis thaliana* triggers a local, transient response of several defense-related transcripts. This induction is also present in distal, non-colonized roots of the same plant. These systemic effects on distal roots include inhibition of secondary *P. indica* colonization. Faster and stronger induction of defense-related transcripts during secondary inoculation revealed that *P. indica* triggers root-wide priming of defense responses. This priming could be the basis for reduced secondary colonization levels observed in split-root experiments and could also lead to enhanced pathogen resistance of the host plant.

To characterize reprogramming of *P. indica* colonized roots and to identify possible signaling pathways, we are analyzing the levels of several plant hormones and perform metabolite profiling using ultra-high pressure liquid chromatography coupled with tandem mass-spectrometry (UPLC-MS/MS).

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Molecular components required for the FB-MR5 mediated resistance against Fire blight caused by *Erwinia amylovora*

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Fire blight caused by *Erwinia amylovora* is a bacterial disease in the family of Rosaceae, economically very important in apple production. With help of QTL (quantitative trait loci) analysis a fire blight resistance gene (FB-MR5) could be detected in *Malus xrobusta* 5 (Fahrentrapp et al. 2013). Mutation analysis on the pathogen *Erwinia amylovora* suggesting a gene for gene relationship for the R-gen FB-MR5 and the virulence gen AvrRpt2ea (Vogt et al. 2013). Based on Amino acids alignment we assume a similar mechanism than in the interactome of *Pseudomonas syringae* and *Arabidopsis thaliana*. To analyze the function of the interactome FB-MR5 and AvrRpt2ea in detail we made transgenic apple plants expressing the AvrRpt2ea under control of the heat shock promoter (HSP) GmHsp 17.5-E from Soybean.

1) We will present a molecular characterization of the plants.

2) By bioinformatic analysis in the genome of the apple cultivar Golden Delicious we detected a set of candidate genes featured by putative cleavage sites for the AvrRpt2ea gene coding for a cysteine protease.

3) To proof the supposed cleavage candidates we transiently express AvrRpt2ea and the supposed candidates in *Nicotiana benthamina* followed by monitoring of the cleavage fragments.

4) One candidate gene with a putative cleavage site is the cinnamate 4-hydroxylase. Hence we are analyzing components of the phenylpropanoid pathway by HPLC in the AvrRpt2 expressing plants.

5) Furthermore we are searching for binding partners by Y2H analysis or FRET.

Fahrentrapp et al. 2013: A candidate gene for fire blight resistance in *Malus x robusta* 5 is coding for a CC-NBS-LRR. *Tree Genetics & Genomes*, 2012, DOI 10.1007/s11295-012-0550-3

Vogt et al. 2013: Gene-for-gene relationship in the host-pathogen system *Malus x robusta* 5-*Erwinia amylovora*. *Plant Biotechnology Journal* (2014), pp. 1-6

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Effect of some insecticides on the occurrence of *Brevicoryne Brassicae* K. and its hyperparasite *Diaeretiella Rapae* (M'intosh)

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In Lithuania cabbage is an important vegetable, which is attacked by various pests, including the cabbage aphid *Brevicoryne brassicae*. *B. brassicae* causes only some losses of yield in *Brassica* crops, but this aphid may also transmit the viruses. The Braconid wasp *Diaeretiella rapae* is an important primary hyperparasite of *B. brassicae*. A potential consequence within integrated crop management systems is that insecticides can take out susceptible genotypes and reduce the population sufficiently to allow biological control of the resistant individuals by surviving natural enemies. Also the use a lot of insecticides on vegetable crops has caused increasing concern among growers, markets and consumers. The objectives of this research were to determine the effect of insecticides Proteus 1100D (a.i. Tiachlopid 100 g l⁻¹ and Deltamethrin 10 g l⁻¹), Karate Zeon 5SC (a.i. Lambda-cihalotrin 50 g l⁻¹ and Fastac EC (a.i. Alfa-cipermetrin 100 g l⁻¹) to *B. brassicae* and their hyperparasite *D. rapae*. Experiment were carried out in the experimental fields of the Lithuanian Institute of Horticulture (Central Lithuania 55° 60' N 23° 48' E) in 2012. The experiment was designed by randomized blocks at four replications. The number of pests was compared among treatments using a single factor analysis of variance (ANOVA). The cabbage plants were colonized by *B. brassicae* at the same time, but the number of aphids on plants differed, also abundance of aphids was low. Quantity of parasitized aphids by *Diaeretiella rapae* (M'intosh) was highest in plot treated with Fastac and differ from 34.3 till 47.4%. In plot treated with Karate parasitized aphids differ from 10.9 till 44.4%. In untreated plots the number of parasitized aphids was lower (from 7.3 till 15.6%), because the abundance of aphids was higher than in with pyrethroids treated plots. In plots treated with Proteus any aphids was not found. We not found any significant differences between number of parasitized aphids in all treated plots.

This research was funded by a grand ("Horticulture: agro-biological basics and technologies" implemented by Lithuanian Research Centre for Agriculture and Forestry") from the Research Council of Lithuania.

P N-CCO 69

Developing bioformulations of antagonistic strains of rhizobacteria for managing seedling damping-off, root rot, and tuber diseases caused by various soilborne pathogens

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Several novel antagonistic strains of rhizobacteria *Bacillus*, *Paenibacillus*, and *Pseudomonas* were isolated from a potato field and soybean leaves and evaluated for their potential to be developed as biofungicides. These strains were identified based on phenotypic characteristics, biochemical tests, and sequence analysis of 16S rRNA gene. Antagonistic potential of the strains was also characterized for production of antibiotics, metabolites, volatiles, phytohormones, and lytic enzymes. In agar plate assays, most of the strains showed broad-spectrum antagonistic activity against several fungal and oomycete pathogens. In pot assays, irradiated peat formulations of these bacteria provided control of *Pythium* damping-off and root rot and *Phytophthora* blight and root rot of cucumber, *Rhizoctonia* damping-off of radish, and *Fusarium* crown and root rot and *Fusarium* wilt of tomato. Bacterial treatments also resulted in higher fresh weights of plants produced in pathogen-infested substrate. In micro-plot trials, coating of seed potato tubers with irradiated peat formulation of some of these antagonistic bacteria reduced scab severity on daughter tubers and increased tuber weights, when planted in a potato soil with a history of common scab disease. Antagonistic and plant-growth promotion activities of these bacterial strains might be related to the production of several types of antibiotics, lytic enzymes, phytohormones, secondary metabolites, siderophores, and volatile compounds. Tomato root colonization by two *Pseudomonas fluorescens* strains indicated their endophytic potential. The results of these studies indicated that several indigenous antagonistic bacterial strains showed potential to be developed as biofungicides for minimizing crop losses due to seedling damping-off and root rot and tuber diseases caused by various soilborne pathogens.

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P N-CCO 70

Laboratory evaluation of the effectiveness of botanical substances on *Phytophthora Infestans*

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Potato late blight of is one of the most devastating diseases in the world with significant losses in yield and tuber quality declines. The use of pesticides is still the most common way to manage the disease in fields. However, the use of chemical fungicides in conventional agriculture or minerals fungicides in organic farming can cause environmental problems. As part of a prospective approach to the development of biocontrol and especially the use of natural plant substances in plant protection, we study here the efficacy of essential oils on *Phytophthora infestans*. We test the efficacy of 7 essential oils on zoospores in a liquid medium (microplates) in accordance with a range of concentrations. Essential oils were purchased from specialist suppliers: they are pure and chemotyped. Essential oils are compared to fungicides active substances such as chlorothalonil, fluazinam and copper sulfate. The experiments are carried out at least three times in independent way. The fungicidal effectiveness is modeled by the calculation of the IC₅₀. The IC₅₀ values of the tested products are compared by an F-test within a nonlinear regression approach. The results show that the IC₅₀ fungicides are lower than the IC₅₀ essential oils. Among the chemical fungicides, fluazinam is more effective than chlorothalonil. As a fungicide mineral, copper sulfate is less effective than chemical fungicides. If all essential oils have good fungicidal activity *in vitro*, 3 are statistically more effective than others. As natural substances, essential oils are less effective than the chemical fungicides. They are also less effective than the copper sulfate even if some of them approach the effectiveness of the mineral substance. The results of this innovative screening of essential oils in the laboratory is part of a larger research program carried by the Technical Institute of Organic Agriculture and co-funded by the Ministry of Agriculture ("evaluation of the interest of using essential oils in crop protection"; 2013-2015).

P N-CCO 71

Efficiency of wheat associated plant growth promoting rhizobacteria along with biochar under reduced chemical fertilizer

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The objective of this study was to isolate plant growth promoting rhizobacteria possessing nitrogen fixation, phosphate solubilization and growth hormone producing activities from the rhizosphere of wheat grown in three different ecological zones (Mailsi, Multan and Bahawalpur) of Pakistan. Among the total 20 bacterial isolates, 9 were positive for Acetylene reduction assay (ARA), 11 isolates exhibited P solubilisation activity. All the 20 bacterial isolates produced growth hormone indole-3-acetic acid (IAA) in culture medium. From these, three bacterial isolates with maximum potential to fix nitrogen, solubilize phosphorous and IAA production were selected to test their performance as PGPR along with biochar under field conditions at three different locations in 2012 with full and half recommended dose of chemical fertilizer. The experiments were laid out in Randomize Complete Block Design. Results indicated that, combined application of PGPR and Biochar significantly increased the grain yield at all 3 locations (Mailsi, Multan and Bahawalpur) over control and their sole application with half of the recommended fertilizer. The finding of this study suggested that application of biochar and PGPR along with half of the recommended dose of fertilizer has appeared the best dose and significantly increased growth and yield of wheat.

P N-CCO 72

Toxic factors in seeds and leaves from *Morinda citrifolia* Linn

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Morinda citrifolia Linn, popularly known as noni, is a native plant from Southeast Asia and belongs to Rubiaceae family. All parts of the plant are used as medicine by Polynesians for the treatment of numerous diseases. The aims of this study were to evaluate the presence of toxic and/or antinutritional potential factors in the seeds and leaves of *Morinda citrifolia* (Noni), including lectins, trypsin inhibitors, urease and fungicidal and/or insecticidal actions. Aqueous extracts from mature seeds and dried leaves were prepared in 20 mM NaPB pH 7.5 containing 10 mM β-mercaptoethanol. The soluble proteins were quantified using the Bradford method. Both protein extracts were analysed by electrophoretic mobility, urease activity, hemagglutination activity, trypsin inhibitory activity and antimicrobial and insecticidal activities. Leaves and seeds extracts, respectively, has a

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protein concentration of 1,46 µg/µL and 2,24 µg/µL. The protein profile on SDS-PAGE showed a higher occurrence of proteins with apparent molecular mass of 25 kDa in the seed extract, and a greater variety of proteins with molecular mass among 10 to 75 kDa in the leaf extract. It was also possible to detect the presence of urease activity in the seed extract, in the order of 5,44 U/mg.min, indicating that the noni seeds probably have at least one isoform of urease. Hemagglutination activity was positive in both extracts. Using the BAPNA as the substrate, the aqueous leaf extract was able to inhibit about 40% of bovine trypsin activity. The antifungal assays showed a strong negative correlation for the development of *S. cerevisiae* when both extracts, from seed or leaves were used. However, with *C. tropicalis*, a positive correlation was observed for fungal growth using leaf extracts. In the insectidal experiments, we observed that the number of *C. maculatus* subjects was significantly influenced by the development instar as well as between different treatments using both, leaves or seeds extracts. Therefore, the aqueous extracts from noni leaves and seeds, present toxic and/or antinutritional potential factors that can be used for biotechnological applications.

P N-CCO 73

Fungicidal potential of aqueous and organic solvent extracts of allelopathic plants leaves against phyto-pathogenic fungi *Fusarium solani*.

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The use of toxic fungicides results into adverse effects on human health and also treacherous to the environment. It has provided opportunity to formulate alternative control measure plan. For the last many years, the use of allelopathic plant extract against pathogenic fungi is gaining attention of the scientists because of non hazardous and environment friendly. Allelopathic compounds are tested as natural substituent of fungicides. Keeping in view this aspect of plants, the present study was therefore designed to evaluate some allelopathic plants for its antifungal properties against *F. solani*, the causal organism of various wilt and rot diseases. For this purpose the test fungal species was grown in 100 mL ME broth medium in various concentrations (0, 5, 10 & 15% w/v) of aqueous and organic solvent extract for 10 days. Five plants species viz., *Acacia nilotica*, *Bauhinia verigata*, *Cassia fistula*, *Eucalyptus camaldulensis* and *Syzygium cumini* of two families Myrtaceae and Fabaceae were selected. The aqueous and organic solvents extract of leaves of all these plants were used for their fungicidal activity. The results of this study showed that growth of target fungus was checked by all test plants. However, the extent of inhibitory effect of leaves varied with the plant species. The study revealed that all leaves extract whether aqueous or in volatile solvents equally showed the inhibitory effect against *F. solani*. Diverse bio-control based fungicides have been designed and circulated globally. Present study is the effort to elaborate the importance of biological based bio-control agents.

P N-CCO 74

Growth enhancement and Biocontrol potential of two endophytic *Trichoderma spp* from *Terminalia catappa* against *Fusarium solani* responsible for Beans Root Rot in Cameroon

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Root rot disease, caused by the fungus *Fusarium solani* is an important soil-borne disease reducing common bean (*Phaseolus vulgaris* L.) yields, in the world and particularly in Cameroon. The present study aimed to investigate the promotion of beans seeds germination, plant growth and biocontrol potential of two *Trichoderma spp* against beans root rot disease causing fungi *F. solani*. Several types of assays were carried out with *Trichoderma spp*. In dual culture assay it was found that *T. atroviridae* inhibited the growth of *Fusarium solani* (90.65%) more than *Trichoderma sp* (86.99 %). The inhibition percentage on spore germination was 34.3 6% and 27.93% respectively. It is observed that at 50% concentration, culture filtrate from *Trichoderma atroviridae* shows 80% and 100% against mycelia growth and spore germination respectively. The extracted ethyl acetate metabolites of *T. atroviridae* inhibit the germination of *F. solani* with Minimum Inhibitory Concentration of 0.66mg/ml. Among test species, *T. atroviridae* was the most potent and exhibited 100% beans seeds germination at 2×10^5 conidia/ml from day 1 to days 4 and was found to increase the germination percentage and protect beans seeds from deleterious effect caused by *F. solani*. In vitro *T. atroviridae* shown to be best biocontrol agent (PBCI = 0.73529) and less susceptible to resistance development by *F. solani* (R value equal 0.8). In pot experiment, *Trichoderma atroviridae* significantly reduced (p Therefore, can be used for further field studies to confirm the feasibility of using to expresses growth promoting effects in plants, increasement of crop productivity and root rot disease management.

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Understanding endophyte colonization behaviour in oil palm plantlets via Ergosterol, PCR and Plate Assays

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Endophytes have been extensively studied for their role as biocontrol agents and growth-promoters of various crops. Although *in vitro* and in some cases, glasshouse trials yield good results, the biocontrol activity is seldom repeated in the field. Many possible reasons have been proposed, but rarely on the colonization behavior of the endophyte upon introduction into the plants. In our study, we aim to understand the colonization behavior of endophytes introduced into oil palm plantlets (*Elaeis guineensis*). Oil palm is a golden oil crop in Malaysia and Indonesia, which unfortunately succumbs to the Basal Stem Rot (BSR) disease caused by *Ganoderma boninense* (Gb). We assessed the colonization extent of three selected endophytes and one pathogen, Gb in oil palm plantlets by firstly, inoculating the plantlets with $6 \log_{10}$ cfu ml⁻¹ of the inoculum and over 7-days period, these plantlets were sampled. The samples were subject to three assay methods; via plating of surface-sterilized tissues, DNA sequencing of isolates extracted from sampled tissues, and via ergosterol quantification using HPLC to detect presence of isolates. Results revealed that isolates WAA02 (*Diaporthe phaseolorum*), T2 (*Trichoderma asperellum*), BTF08 (*Penicillium citrinum*) and Gb (*Ganoderma boninense*) were able to colonize oil palm plantlets after 7 days. Endophytes were detected from tissue samples (leaf, stem and root) on potato dextrose agar with Rose Bengal. DNA sequencing further validated the presence of endophytes in tissues sampled as same species were identified. Ergosterol was most highly detected from plantlets inoculated with WAA02 followed by T2, BTF08 and Gb. This suggested that endophytes not only colonize but may also grow and proliferate in plant tissues. However, results shown that there was no significant increase in ergosterol concentration for all inoculated plantlets during 49-days incubation, indicating endophytes can colonize but is not able to proliferate inside the plantlets. To conclude, our study using plate, PCR and ergosterol methods gave new insights into endophyte colonization in host plants. Endophytes and pathogen can colonize host plants but may not be able to proliferate, hence leading to the hypothesis that colonization behavior may influence the biocontrol expression of endophytic biocontrol agents.

P N-CCO 76

Integrated Andean blackberry (*Rubus glaucus*) crop management using beneficial microorganisms by small farmers in the Ecuadorian Andes.

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The Ecuadorian Government is promoting Agricultura Limpia (Clean Agriculture) as a strategy to improve environmental health and rural incomes. Andean fruits can be a profitable option for small farmers but productivity is limited by pests and diseases. Andean blackberry (*Rubus glaucus*) is grown by small farmers in the mountain valleys but suffers from root disease causing dieback resulting in a loss of yield and persistence of the crop.

Responding to farmer priorities, a participatory study was carried out with blackberry farmers' in Ecuadorian province Tungurahua to evaluate the effect of *Trichoderma* spp. and for control of dieback and promotion of plant growth. Trials were carried out on farmers' field in three locations comparing two preparations of *Trichoderma*, chemical and untreated controls on both young and established plants with disease incidence and fruit yield monitored.

The results showed that *Trichoderma* reduced disease incidence and produced yield similar to the chemical control on both young and productive plants. Yield increase with *Trichoderma* averaged 21% and plant loss to dieback was reduced. Application of the microbe improved yield components, such as the number of fruits per plant (17%), number of branches (7%), fruit size (23%), compared with the untreated control.

The beneficial effect of *Trichoderma* was influenced by weather, soil and crop management conditions with greatest benefit from the more vigorous plants. The use of *Trichoderma* for prevention of dieback has been incorporated into Integrated Crop Management (ICM) protocols for sustainable production and has been widely adopted by farmers in the affected zone where it has been used in both clean and organic production systems. The technology benefits a large number of small scale farmers, who are able to reduce chemical use and integrate into higher value supply chains for clean produce, improving their income and standard of living.

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***Hypothenemus hampei* as inducer of laminarinase and chitinase of *Beauveria bassiana* in liquid cultures**

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Introduction: In Perú, the coffee berry borer (CBB) *H. hampei* has been reported since 1962. It is the most important insect pest that causes specific damage to coffee producing the falling of cherry fruits; affecting the production between 30% and 50%. The most common methods are the use of synthetic insecticides that penetrate the fruit and are toxic to health and the environment. *B. bassiana* is considered the natural controller of *H. hampei*. It is known that entomopathogenic fungi produce extracellular enzymes that degrade insect wall allowing the hyphal penetration through the cuticle. It is necessary to quantify the catalytic activity of the enzymes produced by *B. bassiana* to relate pathogenicity as one of the parameters to be evaluated within the mechanism of infection of *B. bassiana* and promote the development of controlling agents to replace synthetic chemicals.

Objectives: Find an association between chitinase and laminarinase activities of *B. bassiana* induced with *H. hampei* and other characteristics of their development and growth (conidia amount, biomass and total protein exudate), obtained at different times of evaluation.

Materials and methods: The strain of *B. bassiana* was provided by SENASA. Three different media liquid culture were prepared. Biomass, total proteins, conidias and enzymatic activity specific activities were evaluated. All data was analyzed using SPSS 19.0. Pearson correlation between variables was used.

Results: Significant differences ($p < 0.05$) for biomass ($F = 5.306$), conidia ($F = 190.870$), total proteins ($F = 91.038$), laminarinase activity ($F = 27.605$), specific laminarinase activity ($F = 25.306$) chitinase activity ($F = 32.661$) and specific chitinase activity ($F = 26.908$) were determined between each treatment evaluated. Enzymatic activities determined that induction of enzymes was possible in liquid culture supplemented with powdered extract of *H. hampei*. There is a positive correlation between biomass, total proteins, and laminarinase activity.

Conclusion: The positive correlation between chitinase enzyme activities, laminarinase, biomass and the amount of total protein, and the days of increased expression are fundamental to further study since this contributes to knowledge related to the pathogenicity of *B. bassiana* over *H. hampei*.

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Cabbage resistance against *Botrytis cinerea* involving *Trichoderma* metabolites

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Introduction: It is well known *Trichoderma* spp can contribute crucial benefits to the host plant through varieties of proposed mechanisms such as promote host plant immunity against phytopathogens. How the host plant acquired the immunity by the presence of *Trichoderma* spp remained an interesting issue. *Trichoderma* spp have earned fame in producing cell wall degrading enzymes both in quantity and quality. It is also well known in secondary metabolites production. Many *Trichoderma* metabolites showed antimicrobial activities, but little is known about any beneficial effect of these metabolites to the host plants. Further investigation into the roles played by these metabolites would shed the lights on *Trichoderma*'s biocontrol mechanisms.

Objectives: Chrysophanol and anthraquinone are two major secondary metabolites produced by one *T. harzianum* strain isolated from Taiwan. Using cabbage as a plant host model, this research would elucidate the involvement of these two major secondary metabolites during *Botrytis cinerea* infection.

Materials and methods: Cabbage *Brassica oleracea* var. *capitata* was mainly used as the host plant in this study. The seed germination rates were calculated following chrysophanol/anthraquinone treatment. The whole plant development, as well as susceptibility to *B. cinerea* infections, was assessed following chrysophanol/anthraquinone treatment or *Trichoderma* colonization (hydroponic growth system was employed for the three-way interaction study). Further investigation with q-PCR to learn the variation of pathogenesis related proteins (PR-1), cell wall degrading enzymes (chitinase, β -1,3 glucanase), and reactive oxygen species scavenging enzymes (GST & ascorbate peroxidase), was also conducted.

Results: The *B. oleracea* var. *capitata* leaf infected area causing by the *B. cinerea* can be reduced significantly either by the presence of *Trichoderma* in rhizosphere or chrysophanol/anthraquinone treatment. As the results of q-PCR analysis, PR-1 was significantly suppressed while GST & ascorbate peroxidase were induced with chrysophanol/anthraquinone treatment.

Conclusion: Both compounds indeed promoted the defense mechanism of *B. oleracea* var. *capitata* against *B. cinerea*.

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Prophenoloxidase encoding genes in *Spodoptera exigua*: cloning, expression profiling and transcription in response to the combined effect of nucleopolyhedrovirus infection and parasitism by *Microplitis pallidipes*

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Prophenoloxidase (PPO) is the unactivated form of phenoloxidase (PO). It has been suggested that PO plays a critical role in innate immunity. There are some studies on insect PPO under single biocontrol agent, but up to now information about an insect PPO in the presence of two biocontrol agents is still scarce. Here we cloned a conserved segment of PPO gene from the beet armyworm (designated as SePPO1), which contains 1215 bp, with high consistency to PPO1s of other insects. The results indicated that the mRNA levels of SePPO1 and SePPO2 (known in Genbank) have a positive correlation in different periods and tissues by real-time quantitative PCR. SePPOs were mainly expressed in hemolymph instead of other tissues, and the transcription of SePPO was higher in adults than larvae and pupae. It suggests that the PPO of beet armyworm may be a heterodimer composed by subunit of SePPO1 and SePPO2. When the *S. exigua* were treated with the combination of parasitism by *Microplitis pallidipes* and infection of nucleopolyhedrovirus (NPV), the results revealed that parasitism down-regulated the expression of SePPOs, however the NPV infection up-regulated the expression of SePPOs on hours 48 and 72 after treatment, down-regulated the expression on hours 6, 12, 24 and 96 after treatment. The transcription of SePPOs was lower in the combined treatment than in the NPV-infected treatment. Our results also suggested that the activity of PO was positively correlated with the mRNA level of SePPOs basically. And the combined effect of the two biocontrol agents had a synergistic enhancement to the suppression of host immunity. This is the first report on the insect PPO under the combined effect of two biocontrol agents.

P N-CCO 80

Use of the vacuum system to artificially inoculate watermelon seeds with *Acidovorax citrulli* causing bacterial fruit blotch

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Bacterial fruit blotch caused by the Gram-negative bacterium *Acidovorax citrulli* is a contagious disease in watermelon, melon, and other cucurbits. *A. citrulli* causes the disease symptom in cotyledons, hypocotyles, leaves, or fruits. This bacterial pathogen has been shown to be transmitted by seeds, which makes more difficult to manage this disease. To study seed transmission, infected seeds are needed. However, it seems that only very small portion of seeds even from infected fruits carries bacteria inside seeds. Due to this reason, to secure certain amount of infected seeds is very important for studying seed transmission or for developing technology to specifically select infected seeds from mixed seed pool. In this study, we tried to set up and optimize the artificial seed inoculation method to inoculate *A. citrulli* into watermelon seeds used by vacuum system. Healthy watermelon seeds were soaked in *A. citrulli* suspension (1.2×10^8 CFU/ml) and then vacuumed at the 760 mmHg. To remove bacteria simply attached to the surface of seeds, disinfestation by treatment of a chlorine bleach solution or by simple drying was compared. In the case of the former method, seeds were soaked with a chlorine bleach solution, then washed with distilled water, and dried for two days. Then, the bacterial number in each seed separately was counted. The seeds that were just soaked in the bacterial suspension without vacuum application were used as a control. As time for vacuum increased up to 30 minutes, inoculation efficiency also increased. In contrast, vacuum time did not affect seed viability although 30 min treatment decreased viability very little. These results indicate that vacuum inoculation system could be a good method to generate artificially infected seeds with bacterial pathogens.

P N-CCO 81

Determination of fluthiacet-methyl in Corn, Soybean and Soil using a Modified Easy, Cheap, Effective, Rugged, and Safe method and Liquid Chromatography/Tandem Mass-spectrometry

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A modified quick, easy, cheap, effective, rugged, and safe (QuEChERS) method for determination of fluthiacet-methyl in corn, soybean and soil based on the use of liquid extraction/partition and dispersive solid phase extraction (dispersive-SPE) followed by ultrahigh-performance chromatography coupled with tandem mass spectrometry (UHPLC-MS/MS), was established. Acidified

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acetonitrile (containing 1% (v/v) acetic acid) as the extraction solvent and simultaneous liquid-liquid partitioning formed by adding anhydrous magnesium sulfate (MgSO₄) and anhydrous sodium chloride (NaCl). The extract was then cleaned up by dispersive-SPE using primary secondary amine (PSA) as selective sorbent. Further optimization of sample preparation and determination achieved recoveries of between 82 and 110% for clodinafop-propargyl with RSD values lower than 14% in corn, bean and soil at three levels (10, 100 and 1000 µg/kg). The method showed excellent linearity ($R^2 \geq 0.9991$) for fluthiacet-methyl. The method is demonstrated to be convenient and reliable for the routine monitoring of fluthiacet-methyl in corn, soybean and soil.

P N-CCO 82

High-throughput batch and fed-batch cultivations of endophytes in a microbioreactor

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The potential of endophytes to produce insecticidal or pharmaceutical compounds clarifies their promising potential for industrial usage. The major problem of an in vitro cultivation of microorganisms is the durable production of effective compounds such as secondary metabolites. With regard to this challenge it is essential to investigate, how culture conditions affect the secondary metabolite production. The current practice to understand the interaction between the cultivation of endophytes and the production of interesting compounds is still cost and time intensive. A novel microbioreactor with an automated pipetting and screening system (RoboLector® with an integrated BioLector® Pro from m2p-labs GmbH) allows the on-line measurement of pH, pO₂, biomass and metabolite production of endophytes as well as the development of fed-batch processes in microtiter plates.

Within the BMBF-supported project we will use the microbioreactor to develop new strategies for the in vitro cultivation of endophytes. The system enables 48 parallel batch or 32 fed-batch cultivations in a volume under 1 ml. Furthermore, the potential to define the nutrient availability, the CO₂ content and the light intensity during the cultivation combined with the non-invasive detection of pH, biomass and product concentration accelerate the process to achieve optimal product yields. Afterwards, the knowledge gained from the microfermentations facilitates the scale-up to an in vitro mass production process in a 2 L stirred tank reactor.

We will report first cultivation data of batch and fed-batch microfermentations of endophytes. Besides, we illustrate the advantages of this novel tool compared to commonly used cultivation methods. Finally, we will present results how the RoboLector® with an integrated BioLector® Pro can be used for experiments with submerged plant cell cultures as well as for bioassays based on *Spodoptera frugiperda* (Sf9) cells.

P N-CCO 83

Peroxidase Enzyme Activity of Rhizobacterial Introduced Soybean's Which the Ability to Induce the Resistance of Soybeans toward Bacterial Pustule (*Xanthomonas axonopodis* pv. *glycines*).

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Bacterial pustule caused by *Xanthomonas axonopodis* pv. *glycines* is a major constraint in soybean cultivation. To control this disease we have found six rhizobacterial isolates, which have the ability to induce systemic resistance (ISR) of soybean. One of the mechanisms of ISR on plants are changes of peroxidase enzyme activity. The aim of this experiment was to study the activity of peroxidase enzyme in rhizobacterial introduced soybean which have ISR against *Xanthomonas axonopodis* pv. *glycines*. The experiment has been done in triplicate. Six rhizobacterial isolates were introduced on soybean seed and two weeks old seedling. Peroxidase enzyme activity was assayed on soybean seedlings (0, 2, 4, 8, 10, 12, 14, 16, 18, 20, 22, 24, 30, and 35 days after rhizobacterial introduction). The results showed that rhizobacterial could increase the activity of peroxidase enzyme, and the highest enzyme activity was the isolate SR2RZ2.1 (*Serratia marcescens* strain N.2.9) on the roots compared to control. Activity peroxidase enzyme 0.063 µm.mL⁻¹ and 0.059 µm.mL⁻¹

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Test of culture and production of Fungi Entomogenous *Beauveria Bassiana* on whey, olive pomace and vegetable water

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Entomopathogenic microorganisms prominently among alternative control methods against insect pests. In Algeria the first studies on the entomopathogenic fungus *Beauveria bassiana* as a biological control agent, were made by CHAHBAR in 1996 and HALOUANE in 1997. It can be used against various insect pests. To the extent that use of bio-insecticide, high biomass production is required by simple and inexpensive techniques, using by-products of the food industry. A study is being conducted in the laboratory on *Beauveria bassiana* biomass production on organic substrates, whey, vegetable water and olive pomace. Our study revealed a very good mycelial growth on crude whey, a significant growth in the vegetable and significant sporulation rate on olive cake and a considerable biomass yield on deproteinized whey, rated 5, 56g / liter of substrate.

P N-CCO 85

Contact toxicity of Silica and Silver nanoparticles against *Brevicoryne brassicae* L. (Hemiptera: Aphididae)

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The cabbage aphid, *Brevicoryne brassicae* is one of the most serious pests of rapeseed. *B. brassicae* causes direct damage, resulting from searching for food, which may induce plant deformation and indirect damage caused either by honeydew or by transmission of viruses. The cabbage aphid is a vector of 20 virus diseases in a large range of plants. The use of nanomaterial products in various sectors of science increased during the last decade. Nanotechnology has already shown great potential for application in environmental protection. Nanoparticles help to produce new pesticides, insecticides and insect repellants. In this study, two type of nanoparticles were tested against adults of cabbage aphid by leaf dip method. The experiments were conducted at 25±1°C, 65±5% RH and 16L:8D h photoperiods. The mortality of adults was tested at different concentrations and in two exposure times (24 and 48 h). Results showed that LC₅₀ values of silica nanoparticles against *B. brassicae* were 154.4 and 67.76 mg.mL⁻¹ after 24 and 48 h, respectively. In addition, LC₅₀ values of silver nanoparticles were 337.87 and 121.46 mg.mL⁻¹ after 24 and 48 h, respectively. Results indicated that both silica and silver nanoparticles had contact toxicity on cabbage aphid adults. The results also showed that silica was more toxic than silver nanoparticles, and the mortality increased with increases in concentration and exposure time.

P N-CCO 86

Evaluation of *Acacia nilotica* Pods Water Extract on Growth of Selected *Xanthomonas campestris* Pathovars

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Sunt (*Acacia nilotica* spp. *tomentosa*) pods water extract was tested for its efficacy in inhibiting *Xanthomonas campestris* bacterial growth which causes bacterial leaf spot disease. Seed dressers namely Oxilinic acid (Starner) and Bronopol (Bronotak) were also tested. *Xanthomonas campestris* pathovars isolated from Cotton, Tomato, Pigeon pea, Commelina and Hambouk were treated with the dressers. Bronopol recommended dose 0.5 g/100ml was used. Then, increased to 1.0 g/100ml and decreased to 0.25 g/100ml. Starner was also tested at the recommended dose 0.4 g/100ml. Then, increased to 0.8 g/100ml and decreased to 0.2 g/100ml. 0.5 g/100ml was tested for Sunt pods water extract. Then, increased to 1.0 g/100ml and decreased to 0.25 g/100ml. At the lower concentrations, seed dressers used showed no inhibition to the bacterial growth except *Xanthomonas campestris* pv. *cajani* which inhibited by starner. At the recommended doses *Xanthomonas campestris* pv. *malvacearum* showed less sensitivity to the chemicals, whereas other bacterial pathovars showed variable reactions. Both saturated filter paper disc and mixed media techniques were applied and the former gave results. Sunt pods water extract proved very good bacterial growth inhibition and the effect was increased by increasing the concentration.

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Figure 1: Sunt (*Acacia nilotica* sp.tomentosa) pods .



Table 1: The recommended, higher and lower concentrations

Concentration	Starner g/100ml	Bronopol g/100ml	Sunt g/100ml
Lower	0.2	0.25	0.25
Recommended	0.4	0.5	* 0.5
Higher	0.8	1.0	1.0

Figure 2: Bacterial leaf spot on cotton.



Figure 3: *Xanthomonas campestris* pv. *malvacearum* colonies.
 No attachment submitted

Table 2: Effect of Sunt pods water extract on the growth of *Xanthomonas campestris* pathovars.

Concentrationg/100ml	<i>X.c.pv.malvacearum</i>	<i>X.c.pv.vesicatoria</i>	<i>X.c.pv.cajani</i>	<i>X.c.pv.Commeli</i>	<i>X.c.pv.theophrastii</i>
0.25	+	+	+	+	+
0.5	+	+	+	+	+
1.0	++	++	++	++	++

+ = Good inhibition zone.

++ = Very good inhibition zone.

Table 3: Effect of starner doses on the growth of *Xanthomonas campestris* pathovars.

Concentrationg/100ml	<i>X.c.pv.malvacearum</i>	<i>X.c.pv.vesicatoria</i>	<i>X.c.pv.cajani</i>	<i>X.c.pv.commeli</i>	<i>X.c.pv.theophrastii</i>
0.2	-	-	+	-	-
0.4	+	+	+	+	+
0.8	+	+	+	+	+

- = No clear area or inhibition zone.

+ = Clear area or inhibition zone.

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Antagonistic activity of fungal root endophytes from solanaceous plants against potato late blight (*Phytophthora infestans*)

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Fungal endophytes have been shown to produce secondary metabolites that can protect plants from pathogens. The antifungal activity of 354 root-endophytic fungi isolated from four solanaceous species obtained from Kenya against *Phytophthora infestans* was screened *in vitro*. Accordingly, 60 isolates were selected and further evaluated in dual culture tests. The results revealed that mycelial growth of *P. infestans* was differentially effected by the tested endophytes. *Trichoderma harzianum* along with two endophytes (KB1S2-4 and KA1S1-1) suppressed mycelial growth of the pathogen by 84.5%, 78.2% and 76.5%, respectively. Other endophytes however, were either only moderate (KB1S4-7, NA1S2-10, KT1S1-6) or slight (KT2S2-5, KB2S2-5) inhibitors. The potential of bioactive crude extracts of culture filtrates obtained from 10 endophytes was evaluated against sporangia germination of *P. infestans*. The results revealed that, sporangia germination was repressed by more than 88% in the presence of extracts from isolates KT1S1-2, KB1S1-10 and KB1S1-8 compared to the control. The activity of crude extracts was further assessed using thin layer chromatography and bioautography techniques. Out of 10 crude extracts tested, two showed inhibition zones corresponding to the Rf values of 0.23, 0.43 for isolate KB1S1-8 and 0.40 for the isolate KT1S1-2. Both isolates were identified as *Aspergillus aureofulgens* and *Aspergillus flavipes* using ribosomal gene sequence analysis of the ITS region.

P N-CCO 88

Effect evaluation of the biological antagonists against *Septoria tritici* agent of wheat septoria

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In Morocco, leaf Septoria is a fungal disease found in all regions of Wheat production caused primarily by *Septoria tritici* and *Septoria nodorum*. The losses in yield caused by this disease can reach up to 40%. Resistance in Moroccan germoplasm breaks down easily and many currently grown varieties are susceptible so the research of alternative way to control this disease is important. The "in vitro" study of the effect of some biological antagonists in direct confrontation and remote confrontation between five isolates from different areas of *septoria tritici* and five antagonists has been done.

The tests were conducted on PDA medium at 22°C. Results revealed that all antagonists could inhibit the mycelial growth of these pathogenic isolates with reduced percentages of the diametric growth (PRCD).

PRCD can reach up to 84.21%, 52.43% and 37.36% respectively for antagonists A1, A2, A3. The same results were obtained for remote confrontation tests with an important reduction of percentages of mycelial growth.

P N-CCO 89

Mass production of the endophytic entomopathogenic fungus *Metarhizium brunneum*

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Introduction: Classic biocontrol of insect pests with *Metarhizium* spp. is challenging because of the lower efficacy, difficult handling and limited shelf life compared to synthetic pesticides. However, recent studies have provided evidence that these fungi can grow endophytically in plant tissues, paving the way for novel plant protection measures.

Objectives: The aim of our current investigations is to gain insights into innovative fermentation and formulation approaches of endophytic *M. brunneum* that enhance penetration and colonization of the fungus to systemically protect plants from herbivorous insects.

Materials and methods: *M. brunneum* strains were raised in shake flask cultures. By variation of the carbon and nitrogen source as well as concentration, C:N ratio, water activity, pH, viscosity and shear forces, the selective production of fungal biomass was achieved. The produced biomass was immobilized in beads and film coatings based on a broad variation of biopolymers. Spray formulations were developed by using different adjuvants, like wetters, nutrients, sunscreens, humectants and adhesives.

Results: Here we present data on the submerge mass production of endophytic entomopathogenic *M. brunneum* strains based on agricultural residues. After fermentation, the fungi were formulated in a novel spray formulation applied on tomato leaves against whiteflies. First promising results on increased persistence, germination and growth on leaves, penetration, endophytic colonization as well as efficacy in bioassays will be shown.

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P N-CCO 90

Development of a biotechnological plant protection agent for control of oomycetes

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Question: Biotechnological fungicides based on antagonistic bacteria or yeast with known activity against oomycetes are developed in a project, supported by funds of the Federal Ministry of Food, Agriculture and Consumer Protection (BMELV) based on a decision of the Parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the innovation support programme. *Phytophthora infestans* infections on tomato leaves were used as a test system during the developing process. In addition, tests were done in cucumber to control *Pseudoperonospora cubensis* at Julius Kühn-Institut (JKI) to compare the formulated test preparations for efficacy against both pathogens.

Methods: Six bacterial and two yeast strains with known antagonistic effect were selected for the trials in the project. Besides the efficacy in *in vivo* test systems, toxicity and pathogenicity data from the literature were considered during the selection process. Also data for economical producibility (fermentation, downstream processing) and suitable formulations were elevated. Each step in the production process was investigated for determination of general and/or strain specific factors responsible for improving the efficacy against *P. infestans* on tomato leave disks. In addition, promising test preparations were also tested for their efficacy against downy mildew on grape vine and cucumber on potted plants and late blight on potato in field trials.

Results: In this presentation we will focus on the cucumber trials done in climate rooms at the JKI in Darmstadt. Besides an untreated control, a chemical standard and a copper standard, the antagonists were tested in different formulations and in combination with copper. Based on the preceding experiments, formulations were adapted and improved. Selected formulations controlled *P. cubensis* comparable to the chemical standard and copper - microorganism combinations allowed a copper reduction to a tenth of the recommended dose for copper standalone treatments.

Conclusion: Production procedures and formulations were developed for antagonistic bacterial and yeast strains yielding in biotechnological fungicides with high efficacy against *P. cubensis* on potted cucumber.

P N-CCO 91

Efficacy of Velum Prime[®] SC 400 in cucumber and tomatoes against *Meloidogyne incognita* in Turkey

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In this study nematicidal effect of commercial new generation nematicidal chemical product *Velum Prime[®] SC400* containing 400 gr/L Fluopyram active ingredient was investigated to control *Meloidogyne incognita* in two cucumber and two tomato greenhouses in Antalya, Turkey. Experiments were conducted in a completely randomized block design with four replicates in the four greenhouses. There was heavy infestation with *M. incognita* at all trial sites.

Velum Prime[®] SC400 was applied with drip irrigation system at three different doses (400+400, 500+500, 600+600 ml/ha) at 1-3 days after planting and two weeks later. Iprodione SC 500 and Fosthiazate EC 150 were used as comparison chemical compounds for the experiments. Moreover, untreated control plots were included in the experiment for positive nematode control plots. Approximately 10 weeks later experiments were ceased and 20 plants in each plot were harvested and evaluated by using 0-10 scale of root gall index (Zeck, 1971).

Velum Prime[®] SC400 600+600 ml/ha had the highest biological effect for both cucumber greenhouses. Root gall indices were extremely low level in the roots and ranged from 0.5 to 0.6 in the *Velum Prime[®] SC400* 600+600 ml/ha ($P<0.05$). However, root gall indices were 3.8 in Iprodione SC 500 plots in both cucumber experiments. *Meloidogyne incognita* severely galled on cucumber roots in untreated control plots, root gall indices were 7.9 and 8.3 in cucumber 1 and cucumber 2 experiments respectively ($P<0.05$).

Similarly, *Velum Prime[®] SC400* 600+600 ml/ha doses extremely reduced root gall on tomato roots in both experiments ($P<0.05$), and the highest biological effect was observed on this treatment in the two tomato greenhouses (0.4 root gall indices / $P<0.05$). Biological effect of Fosthiazate EC 150 was found close to *Velum Prime[®] SC400* 600+600 ml/ha doses and there was no significant differences between *Velum Prime[®] SC400* 600+600 ml/ha and Fosthiazate EC 150. Root gall indices were found 3.7 in tomato roots in Iprodione SC 500 plots. However, tomato roots in untreated control plots had 8.2 scale of root gall indices in both tomato experiments ($P<0.05$).

Velum Prime[®] SC400 600+600 ml/ha significantly reduced the galling index in tomato and cucumber, and has provided the best control of *Meloidogyne incognita*.

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Effects of Aqueous Extracts of Seeds of *Peganum harmala* L. (Zygophyllaceae) on 5th Stage *Schistocerca gregaria* (Forsskål, 1775) (Orthoptera: Acrididae)

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The work is a study of the toxic effect of aqueous extracts of *Peganum harmala* (Zygophyllacée) on L5 larvae locust *Schistocerca gregaria* (Forskål, 1775). After treatment with 5 different doses, some individuals exhibit morphological changes, which are characterized mainly by a deformation of the wings. On the other were those who undergoes physiological changes which pigmentation turned red indicating that individuals are quiescent which is a form of resistance encountered in the Desert Locust in cold period. Also the treatment had a significant impact on other physiological parameters in this case the delay moulting which characterizes the majority of individuals. Unlike most plant-based substances whose activity typically occurs from the 11th day the aqueous extract of *Peganum harmala* proves toxic after the first day especially with doses of 2.4 g / l and 4.8g / l which cause high mortality of 80% and 86.7 %.

P N-CCO 93

Application of plant growth-promoting rhizobacteria for control of *Meloidogyne incognita* race 2 on soybean

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Question: The aim of this study was to screen plant growth-promoting rhizobacteria (PGPR) for activity against *Meloidogyne incognita* race 2, and to elucidate the modes of action of effective strains.

Methods: PGPR strains were first screened for compatibility with *Bradyrhizobium japonicum* using an antibiosis test. Compatible strains were screened for control of *M. incognita* using a soybean seedling bioassay and a juvenile mortality assay. In the seedling assay, rhizobacteria suspended in quarter-strength Ringer's solution were pipetted onto soybean cv. LS 5995 R seeds before covering the seed with growth medium. At emergence of the first true leaves, each seedling was inoculated with *M. incognita* second-stage juveniles. After 14-19 days gall formation was assessed. The seedling assay was repeated with strains that reduced the galls per plant by at least 30%. In a greenhouse trial conducted with the promising strains, nematode reproduction was assessed 7 weeks after inoculation with *M. incognita*. Broth culture filtrates from promising strains were tested for reduction of juvenile motility, juvenile mortality and egg hatching. The promising strains were also tested for induction of systemic resistance against *M. incognita* using a split-root assay.

Results: During the seedling bioassay *Lysinibacillus* strain T19, *Paenibacillus* strain T22 and *Pseudomonas* strain N04 reduced the number of galls per plant by 31%, 38% and 32%, respectively, compared to nematode-only controls. When the seedling assay was repeated with a lower amount of nematode inoculum, aforementioned isolates reduced the number of galls per plant by 67%, 32% and 44%, respectively. None of the strains significantly affected juvenile mortality. In the greenhouse trial, strain T19 reduced Oostenbrink's reproduction factor by 48% and the number of eggs and juveniles per gram root by 57%. Culture filtrates from strain T19 and strain N04 significantly reduced juvenile motility. Culture filtrates from all 3 strains reduced egg hatching. Strain T22 and strain N04 reduced the number of galls in the split-root assay, indicating induction of systemic resistance.

Conclusion: Three PGPR strains significantly reduced disease expression on soybean seedlings, through different modes of action. These results warrant further evaluation of these strains.

P N-CCO 94

First report of genus *Tumidiclava* (Hymenoptera: Trichogrammatidae) from Iran

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Green stink bugs, *Brachynema germari* (Heteroptera: Pentatomidae), feeding on different host plants, is one of the most important pests in pistachio orchards in Iran. In order to identify its parasitoid wasps, several egg masses of *Brachynema germari*

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were collected from 106 locations of Kerman province. The egg samples were transferred and kept under laboratory conditions. Among the parasitoids which were identified based on morphological characters including fore wings venation, ratio of width to length of fore wings, number of setae on marginal vein, funicular and clava segmentation, size of funicular segments, shape and length of ovipositor and structures of male genitalia some samples collected from Haft Bagh of Kerman were identified as *Tumidiclava* sp. belonged to family Trichogrammatidae. The genus constitutes an economically important group of hymenopterous parasitoids attacking eggs of various groups of insect pests, mostly Lepidoptera, few Coleoptera and Orthoptera. This is the first report of *Tumidiclava* sp. from Iran, and first record of its parasitization on the eggs of *Brachynema germari*.

Figure 1



Figure 2



P N-CCO 95

Endophytic fungi promotes growth and reprograms the adverse affect of stem rot by regulating systemic acquired resistance in Sunflower

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Disease resistance in crops is highly desired for sustainable agricultural industry. The endophytic fungi having gibberellins (GAs) secreting potential are now widely known for stimulating plant growth, but rarely reported previously for their favorable role in plant disease resistance. We studied the role of *Penicillium citrinum* LWL4 and *Aspergillus terreus* LWL5 in sunflower (*Helianthus annuus* L.) growth and their capacity to regulate hormone signaling networks involved in plant defense against stem rot caused by *Sclerotium rolfsii*. Results showed that plant growth attributes i.e. shoot length, shoot diameter, shoot fresh/dry weight, transpiration, stomatal conductance, photosynthesis and chlorophyll contents were promoted in fungi treated plants as the negative impact of stem rot on growth attributes were greatly recovered in these treatments. The signaling of plant-defense hormones, such as salicylic acid and jasmonic acid fluctuated with the application of fungal endophytes in the diseased plants. The endogenous SA and JA contents were significantly higher in diseased plants as compared to endophytes treated plants. Furthermore, we observed that the association of *Penicillium citrinum* LWL4 with sunflower yielded better results as compared to *Aspergillus terreus* LWL5. It was concluded that the inoculation of fungal endophytes reprogrammed plant growth during disease incidence by regulating the defense-linked responses of host plant. Strategies involving endophytic symbiosis can help achieve sustainable agriculture in an eco-friendly way, thus checking excessive use of fungicides.

P N-CCO 96

Insecticidal activity of two methanols extracts of the plant *Ammi visnaga* on the black citrus aphid *Toxoptera aurantii*

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This study aims to propose alternative solutions based on the use of natural products in order to fight against the black aphid orange *Toxoptera aurantii*.

To respond to this objective, we evaluated the toxicity of topically methanol extracts of the plant *Ammi visnaga* L. tests are carried out in vitro in laboratory conditions following a completely randomized design. Two methanolic extracts of *Ammi visnaga* L., were used, it is extracted from the seeds and the extract of the leaves during flowering. Five concentrations were prepared

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for each type of extracts: 250, 500, 3000, 5000, 10000, 30000 ng / aphids. One microliter of each sample is placed in the adult aphid wingless thorax using a Hamilton type of glass micro-syringe with accuracy and repeatability of $\pm 0.05\%$. Controls are aphids solvent 1 μ l. The experimental unit comprises a Petri dish containing twenty adult aphids released on an orange film. Each treatment was repeated three times. The results show that 88.33% mortality was caused by the higher dose (30,000 ng / aphid) after 24 hours. This mortality reached 100% after 72 hours. For seed extract, the same dose resulted in a mortality of 86.67% after 24 hours and 100% mortality after 96 hours. LD50 values of two methanol extracts of the plant *Ammi visnaga* L. obtained for *Toxoptera aurantii* aphids are small. LD50 values obtained after 24 hours are of the order of 171.65 ng / aphid for the extract of the leaves and in the range of 0.054 ng / aphid for the extract of the seeds.

P N-CCO 97

Comparative study of the tolerance of fifteen citrus rootstocks to salt stress

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Introduction: Citrus are classified among the most sensitive species to salinity. This constraint affects the morpho-physiology of the plant and ultimately leads to a reduction in yields.

Materials and methods: To assess the effect of salinity on some physio-biochemical parameters in plants, an in vivo screening test was performed under controlled saline conditions of fifteen different citrus rootstocks, Citrumelo 57-98-502, Citrumelo swingle F9-22-55 (80-11), Citrumelo 57-98-506, Citrumelo swingle 74-1, Citrumelo Winter Haven B2, Citrange. Carizo 28608, Citrange Troyer C35B6A11, Citrange Troyer B2 31655, Citrumelo 4475 B2G3, Citrumelo 4475 B B6A5, Citrumelo 4475 A B6A4, Citrumelo Sacaton 30057, Gou-Tou SRA 506, C. Volkameriana B2 28613 and Citrange Troyer. Plants were subjected to four salt treatments 0 (control), 2 and 5g NaCl for 90 days in sand culture. Salinity affected all of the parameters under study.

Results and conclusion: The high salt concentrations caused a great reduction in growth parameters such as fresh and dry weights of shoots and roots especially for Citrumelo 57- 98-502, Citrumelo swingle F9-22-55 (80-11), Citrange Carrizo 28608 and Citrumelo 4475 BB6A5. These changes were associated with decrease in chlorophyll contents in leaves in Citrumelo 4475 B B6A5. With the increase in salinity level, the proline and sugar contents were increased especially for Citrumelo 4475 A B6A4 and Citrumelo sacaton 30057, and it was concluded that these osmolytes play a key role in generating tolerance against salt stress.

P N-CCO 98

Effect of gamma ray irradiation on the variability of some quality criteria in Marisol clementine

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Introduction and objective: citrus fruits are a great socio-economic importance nationally. This work is part of a program of improvement and genetic diversification of citrus its purpose is to study the variability of selection criteria related to the quality of the fruit.

Material and methods: 107 clones of clementine from gamma ray irradiation Buds Marisol's clementine. They were grafted onto macrophylla and planted with a spacing of 3x5 m². The evaluation was focused on the organoleptic and pomological criteria.

Results: Statistical analysis showed significant differences between the control and irradiated clones Clementine (Marisol) for all variables studied (sugar content, number of seeds per fruit, average fruit weight and percentage of juice and maturity index) except for the acidity of the fruit.

Conclusion: Four clones of clementine from irradiation were selected because of the good quality (seedless fruits, large caliber) and later that Marisol clementine.

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Application of plant growth-promoting rhizobacteria for control of *Meloidogyne incognita* race 2 on soybean

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PGPR strains were first screened for compatibility with *Bradyrhizobium japonicum* using an antibiosis test. Compatible strains were screened for control of *M. incognita* using a soybean seedling bioassay and a juvenile mortality assay. In the seedling assay, rhizobacteria suspended in quarter-strength Ringer's solution were pipetted onto soybean cv. LS 5995 R seeds before covering the seed with growth medium. At emergence of the first true leaves, each seedling was inoculated with *M. incognita* second-stage juveniles. After 14-19 days gall formation was assessed. The seedling assay was repeated with strains that reduced the galls per plant by at least 30%. In a greenhouse trial conducted with the promising strains, nematode reproduction was assessed 7 weeks after inoculation with *M. incognita*. Broth culture filtrates from promising strains were tested for reduction of juvenile motility, juvenile mortality and egg hatching. The promising strains were also tested for induction of systemic resistance against *M. incognita* using a split-root assay.

During the seedling bioassay *Lysinibacillus* strain T19, *Paenibacillus* strain T22 and *Pseudomonas* strain N04 reduced the number of galls per plant by 31%, 38% and 32%, respectively, compared to nematode-only controls. When the seedling assay was repeated with a lower amount of nematode inoculum, aforementioned isolates reduced the number of galls per plant by 67%, 32% and 44%, respectively. None of the strains significantly affected juvenile mortality. In the greenhouse trial, strain T19 reduced Oostenbrink's reproduction factor by 48% and the number of eggs and juveniles per gram root by 57%. Culture filtrates from strain T19 and strain N04 significantly reduced juvenile motility. Culture filtrates from all 3 strains reduced egg hatching. Strain T22 and strain N04 reduced the number of galls in the split-root assay, indicating induction of systemic resistance.

Three PGPR strains significantly reduced disease expression on soybean seedlings in greenhouse conditions, through different modes of action. These results warrant further evaluation of these strains.

P N-CCO 100

A preliminary study in vitro on the antagonism capability of entomopathogen fungi and *Penicillium* spp. against *Fusarium oxysporum* f.sp. *lycopersici* and *F. oxysporium lycopersici* f.sp. *radicis* in tomato

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Fusarium oxysporum f.sp. *lycopersici* (FOL) and *F. oxysporium lycopersici* f.sp. *radicis* (FORL) are the most important soilborne pathogens of tomatoes causing wilting and tomato root / root rot diseases and they seriously loss yield in tomato. The aim of the study is to clarify the antagonism capability *in vitro* of the *Beauveria bassiana*, *Metharizium anisoplia*, *Paecilomyces lilacinus*, *Pochonia chlamydosporia*, *Fusarium subglutinans* and *Penicillium* spp. against these pathogenic fungi, FOL and FORL. Dual cultures method was used for screening antifungal effect of each fungus. All experiments were repeated in three times and antifungal effect was measured miselial growth in petri dishes.

M. anisoplia, *P. lilacinus* and *Penicillium* spp. effectively inhibited the mycelial growth of FOL and FORL 7 days after incubation on PDA medium. *M. anisoplia* was the most effective as biocontrol agent, and it reduced mycelial growth of FORL and FOL ($P < 0.05$). The mycelial growth rates of FORL and FOL for *M. anisoplia* were 1.8 ± 0.34 cm and 2.16 ± 0.24 cm respectively. There was significant differences compared with untreated control (9 ± 0.1 cm) ($P < 0.05$). *Fusarium subglutinans*, *B. bassiana* and *P. chlamydosporia* caused slight inhibition of the mycelial growth. The highest mycelial growth rates for FOL (5.8 ± 0.24 cm) and FORL (6.33 ± 0.3 cm) were obtained with *P. chlamydosporia* ($P < 0.05$). It has been shown that some fungal traits can control the soilborne fungal pathogens, FORL and FOL.

P N-CCO 101

Evaluation of induced resistance to powdery mildew disease in barley using the endomycorrhizal fungus *Piriformospora indica*

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Introduction: Powdery mildew caused by *Blumeria graminis* f. sp. *hordei* (*Bgh*) is one of the most destructive diseases on barley (*Hordeum vulgare* L.). The endophytic fungus *Piriformospora indica* colonizes barley roots, and helps in protection against biotic and abiotic stresses and eventually in higher yield.

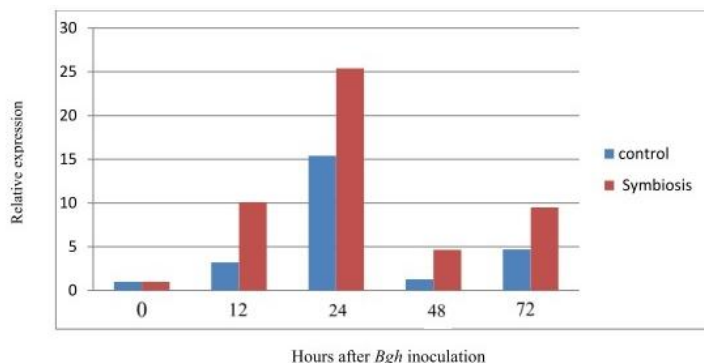
Objectives: The impact of resistance induced by this mycorrhizal fungus on disease progress in barley was studied in this research.

Materials and methods: To evaluate the resistance induction ability of the fungus, the most sensitive barley genotype, L.527/Sawson/Bco/3/jonoob, was selected and the leaves were inoculated with *Bgh* spores by air current dispersion in an inoculation tower, with a density of 50 conidia per cm². Samples were incubated at 22°C under 16 hrs light photo period and at 70% RH. One week later, the number of colonies on 2cm² of leaf segments were counted under dissecting binocular field. Expression of plant defense genes *NPR1*, *PR1b*, *PR5* in barley under symbiotic association with *P. indica* was studied using quantitative PCR.

Results: The expression rate of these genes in symbiont barley genotype reached its highest level 24 hours after inoculation with *Bgh*, which was significantly higher than that of the non-symbiotic plants (Fig 1 and 2).

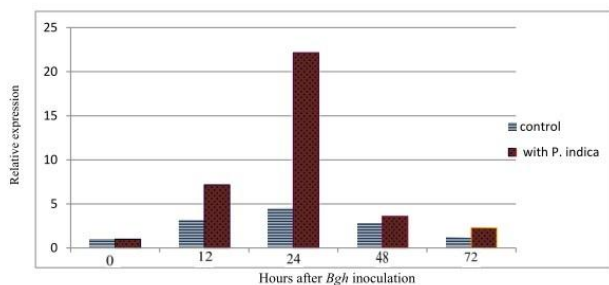
Conclusion: This study shows that *NPR1*, *PR1b*, *PR5* genes are involved in induction of resistance responses in barley against the powdery mildew fungus. On the other hand, results confirm the potential use of *P. indica* in management strategies based on induced resistance.

Figure 1



Expression level monitoring of *PR1b* in the symbiosis and non-symbiosis genotype during challenge with powdery mildew fungus, *Blumeria graminis* f.sp. *hordei* (*Bgh*).

Figure 2



Expression level monitoring of *PR5* in the symbiosis and non-symbiosis plants during challenge with powdery mildew fungus, *Blumeria graminis* f.sp. *hordei* (*Bgh*).

P N-CCO 102

Response of *Tuta absoluta* (Lepidoptera: Gelechiidae) to superparasitism of *Pseudapanteles dignus* (Hymenoptera: Braconidae): Implications for biological control.

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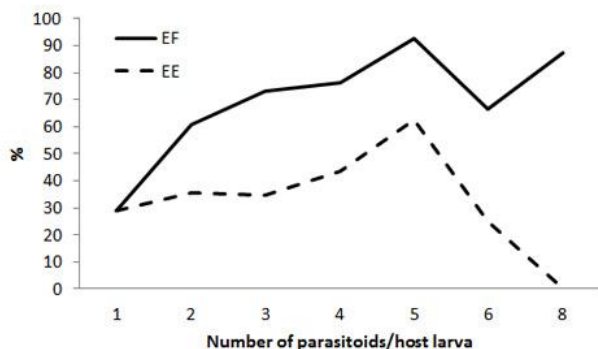
South American tomato pinworm *Tuta absoluta* causes great economic losses in tomatoes and disperses rapidly in Europe, Africa and Asia. *Pseudapanteles dignus* is the main larval endoparasitoid and despite being solitary, superparasitism is detected in field and laboratory. Host larvae in turn, respond to parasitism through encapsulation. Superparasitism of *P. dignus* and response of host larvae were examined in laboratory.

We exposed 20-25 *T. absoluta* larvae to each *P. dignus* female (n=10) daily. Host larvae were removed and reared for 72h until dissection. Eggs and larvae/host and encapsulated individuals were recorded. Monoparasitized vs. superparasitized larvae was compared daily for each female (*t* Test). Frequency (% EF) and efficiency of encapsulation (% EE) were calculated for different number of parasitoids/host larva (1, 2,... n individuals). Percentage of offspring loss was estimated by adding percentage loss due to superparasitism (encapsulation + larval competition) + percentage loss in monoparasitized larvae due to encapsulation, for each number of parasitoids/host.

Of 1,705 dissected larvae, 50% were parasitized; 63% of them were monoparasitized and 37% were superparasitized ($P < 0.05$). In superparasitized larvae, the most frequent number of parasitoids/host was 2 (22%) and 3 (10%), and the maximum number was 9. Superparasitism was observed throughout the lifetime of *P. dignus* female. During the first 3 days, no differences were found between mono and superparasitism ($P > 0.05$), but the latter decreased relative to monoparasitism with female age. EF markedly increased with the number of *P. dignus* offspring/host whereas EE only exhibited a substantial increase at the unusual number of 5 parasitoids/host (Fig. 1). *P. dignus* females presented a loss of 42% of offspring: 19.20% (encapsulation of monoparasitized hosts) and 22.80% (encapsulation and larval competition in superparasitized hosts).

This suggests that due to a significant encapsulation response of *T. absoluta*, superparasitism would reduce the offspring that *P. dignus* will leave to the next generation, decreasing its potential capacity to grow. The implications for parasitoid mass rearing and for the pest biological control are discussed.

Figure 1



P N-CCO 103

Positioning pre-mix formulation Isoprothilane 28%+ Fipronil 5% EC against Stem Borer, Brown Plant Hopper, Green Leaf Hopper, and Whorl Maggot and Blast Disease in rice it's Phytotoxicity and effect on natural enemies in India

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Introduction: Rice is India's pre-eminent crop, and is the staple food of the people of of the country. Chewing and sucking pests and blast disease occurs throughout India and South East Asia during different crop growth stages and occurs in kharif and rabi seasons. Moderate to severe incidence is noticed in nursery, planting to mid-tillering and panicle initiation stages causing 40-60 per cent yield loss. Now to meet the global urge towards IPM Isoprothiolane 28%+ Fipronil 5% EC a suitable insecticide and fungicide pre-mix formulation for rice eco-system. Safe for human being, animal, crops natural enemies etc and also environment.

Objective: To evaluate the efficacy of the Isoprothiolane 28%+ Fipronil 5% EC against pests and blast disease of rice vis-a-vis natural enemies and pollinators and its phytotoxicity, if any, on rice.

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Materials and methods: Experiment was laid out RBD with seven treatments transplanted in 25 sq.m. plots in 20 x 10 cm inter and intra row spacing with variety 'Lal Swarna'. test pesticides were applied as foliar spray with knapsack sprayer fitted with hollow cone nozzle. The requisite quantity of the treatments were mixed with water @ 500 litres/ha (1.25 litre water/25 m²) and imposed coinciding with the ETL of pests and disease at 40 and 80 DAT . The effect on natural enemies was based on parasitisation of stem borer eggs by parasitoids (*Tetrastichus* sp., *Telenomous* sp. and *Trichogramma* sp.) and the larval stage of leaf folder by *Bracon* sp. and *Apanteles* sp.were taken careof.

Results: All the treated plots showed significant reduction of the pest damage /population over untreated control. It is evident that Isoprothiolane 28%+ Fipronil 5% EC @ 1000 &1500 ml/ha resulted in 2.21 & 2.30 % dead heart and white ears, 77.82-78.13% mortality of brown plant hopper , 3.45-3.41 % least damage to leaves by whorl maggot and 64.07-65.31%. GLH mortality .Leaf and neck blast disease control ranged from 90.58-90.58% and 100% respectively over untreated control.Further, this insecticide has least adverse effect on important natural enemies as indicated by high egg parasitisation (66.25 - 63.75 %), high larval parasitisation (23.50 - 24.38 %).Spider population varied between 5.15-4.89/hill at 15 DAT

Figure 1

Table I: Relative Efficacy of different treatment schedules of Isoprothiolane 28% + Fipronil 5% EC against pests and diseases on Rice West Bengal

Serial No.	Treatment	Formulation (ml/ha)	Mean % damage / reduction over untreated control				Percent disease reduction over untreated control	
			White Ear head At pre-harvest	BPH	Whorl Maggot	GLH	Leaf Blast At 15 DALS	Neck Blast At 15 DALS
1	Isoprothiolane 28%+ Fipronil 5% EC	500	5.00 (12.92)	75.62 (60.14)	4.82 (12.62)	34.81 (36.15)	74.11 (59.42)	55.60 (48.22)
2	Isoprothiolane 28%+ Fipronil 5% EC	750	4.00 (11.54)	75.18 (60.12)	4.23 (11.87)	50.49 (45.28)	90.58 (72.12)	60.00 (50.27)
3	Isoprothiolane 28%+ Fipronil 5% EC	1000	2.21 (8.54)	77.82 (61.09)	3.45 (10.70)	64.07 (53.17)	90.58 (72.12)	100.00 (90.00)
4	Isoprothiolane 28%+ Fipronil 5% EC	1500	2.30 (8.72)	78.13 (62.13)	3.41 (10.64)	65.43 (53.98)	90.58 (72.12)	100.00 (90.00)
5	Fipronil 5% SC (Standard)	1000	2.50 (9.09)	77.13 (61.43)	3.46 (10.47)	65.31 (53.91)	7.09 (15.29)	2.00 (8.13)
6	Fipronil 5% SC (Standard)	1500	1.50 (7.04)	77.37 (61.59)	3.19 (10.29)	66.79 (54.81)	4.70 (12.52)	3.90 (10.93)
7	Isoprothiolane 28%+ Fipronil 5% EC	750	9.12 (17.56)	20.36 (26.82)	8.64 (17.09)	42.79 (71.30)	94.11 (75.95)	100.00 (90.00)
CD (p<0.05)			0.13	4.28	5.71	6.25	3.90	4.73

* Data in parentheses are angular transformed values. F.T.C. - Pre Treatment Count
 Data were arc-sin transformed and then analysed by one-way ANOVA. UTC - Un-treated control
 DALS: Days after sowing

Figure 2

Table II: Relative effect of Isoprothiolane 28%+ Fipronil 5% EC against natural enemies present in Rice in West Bengal,India.

Serial No.	Treatment	Formulation (ml/ha)	% parasitisation		Predators/SP	
			% egg parasitisation	% larval parasitisation	Wolf spider (<i>Lycosa</i> sp)	
					FTC	15 days after last spray
1	Isoprothiolane 28%+ Fipronil 5% EC	500	67.75 (55.39)**	26.00 (10.65)**	4.78 (12.62)**	5.00 (12.92)
2	Isoprothiolane 28%+ Fipronil 5% EC	750	67.00 (54.94)	25.00 (30.00)	4.63 (12.42)	5.25 (13.24)
3	Isoprothiolane 28%+ Fipronil 5% EC	1000	66.25 (54.48)	23.50 (28.99)	4.92 (12.81)	5.15 (13.11)
4	Isoprothiolane 28%+ Fipronil 5% EC	1500	63.75 (52.98)	24.38 (29.59)	4.75 (12.58)	4.89 (12.77)
5	Fipronil 5% SC (Standard)	1000	65.25 (53.87)	23.35 (28.89)	4.84 (12.70)	5.00 (12.92)
6	Fipronil 5% SC (Standard)	1500	64.75 (53.57)	23.05 (28.66)	4.85 (12.45)	5.00 (12.92)
7	Isoprothiolane 40% EC (Standard)	750	64.88 (53.65)	23.10 (28.73)	4.38 (12.08)	4.50 (12.24)
8	UTC	-	69.78 (56.65)	27.55 (31.678)	4.65 (12.45)	4.85 (12.72)
CD (p<0.05)			NS	NS	NS	NS

*Data in parentheses are %>0.5 transformed values. analysed by one-way ANOVA.** Data in the parentheses are angular transformed values.
 • UTC - Un-treated control. FTC-Pre-treatment count

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P N-CCO 104

Root-associated endophytes from *Musa* spp. can promote banana plant growth and inhibit both pathogen and beneficial microorganisms

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The use of tissue-culture (TC) planting material is a management practice recommended in banana worldwide to preclude disease spread. However, bacterial and fungal endophytes, known to provide benefits to banana plants, are removed during the TC process. The enrichment of TC-planting material for more effective plant vigor and disease resistance is seen as a promising strategy, but its success requires an understanding of the complex relationships of endophytes and banana plants. In this work, nineteen root-associated endophytic microorganisms from *Musa* spp. were characterized in relation to a) antagonism against *Fusarium oxysporum* f. sp. *cubense* (Foc), b) growth compatibility in culture medium and c) growth promotion of TC-plants in greenhouse. Eleven fungi were previously characterized as *Trichoderma asperellum* (ML023, PS029, TG022, YK024, NM026, ML021, TG025, YK026), *T. spirale* (ML032 and ML001) and *T. koningiopsis* (C019). The eight bacteria corresponded to *Pseudomonas plecoglossicida* (BD001, AM012 and YK001), *P. geniculata* (GN005), *Bacillus aryabhatai* (GN010), *Wautersiella falsenii* (B021), *Enterobacter soli*, (C001) and *Kluyvera ascorbata* (PP032). Most of fungal isolates inhibited Foc growth at certain levels. ML023 and PS029, *T. asperellum* isolates, obtained from *Musa laterita* and Pisan Seribu, respectively, inhibited Foc up to 82%. Among bacteria, only *P. geniculata* (GN005) inhibited Foc (47%). Different interactions were verified in the compatibility analyses between endophytes with some endophytes showing total (i.e PS029) or partial (ML023) incompatibility. *P. geniculata*, was the only bacteria that inhibited fungal endophytes. Most of microorganism promoted TC-plants growth, but bacteria were more efficient than fungi, especially BD001, which significantly increased plant height and root dry weight. NM026 showed no differences or even lower performance than controls. Our preliminary results contribute to a better understanding of relationships among endophytic microorganisms and banana roots, and to an approach to disease crop management based on single or combined formulation of microorganisms to enhance TC-planting material.

P N-CCO 105

Development of compost tea for control of plant diseases and enhancement of plant growth promotion in organic farming

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Compost tea plays new tools in plant growth promotion and decreases disease against various plant pathogens on organic farming. Fourteen compost teas were applied as foliar sprays on cucumber plant with 7 days intervals. All treatments suppressed powdery mildew disease of cucumber. The vermicompost tea was significantly improved cucumber growth compared to the control. A total of 100 bacterial isolates were isolated from aerated compost tea and screened for antagonism against six plant pathogens, *Botrytis cinerea*, *Colletotrichum gloeosporioides*, *Fusarium oxysporum*, *Phytophthora capsici*, *Rhizoctonia solani*, and *Sclerotium sclerotinia*. 83% isolates showed antagonistic activities against six pathogens. All most of them formed siderophore and indole acetic acid, and had nitrogen fixation activity in vitro. Selected bacteria were identified as *Bacillus amyloliquefaciens*, *Bacillus pumilus*, *Paenibacillus terrae*, and *Sphingobacterium composti*. 20 seeds of cucumber and hot pepper treated with 10⁷ cfu/ml of each isolate. All isolates increased the germination rate, the elongation of shoot and root, and improved fresh weight of seven day old cucumber and hot pepper seedlings. Specific primer for zwittermicin A, iturin A, and bacillomycin D were used to amplify biosynthesis genes from these bacteria. The majority of strains harbored Iturin A (50/56) and Bacillomycin D (35/56) biosynthesis genes. Foliar application of the compost tea significantly reduced powdery mildew and downy mildew incidence in organic cucumber farm, and increased with 35% cucumber yield than conventional farm. The results recommended that the compost tea may be useful ways to control of plant disease in organic cultivation field.

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Figure 1: Changes of fungal (A) and bacterial (B) population density according to the compost tea boosted with rice-seed germinator for four days. Fig. 2 Screening of the presence of the biosynthesis genes, Bacillomycin D, Iturin A, and Zwittermicin A in 56 strains isolated from compost tea and showed the antifungal activities for six plant fungal pathogens.

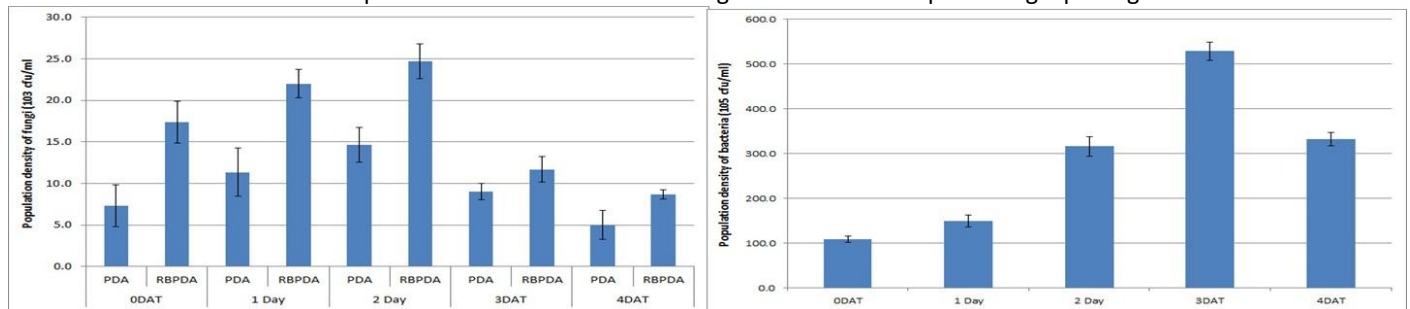
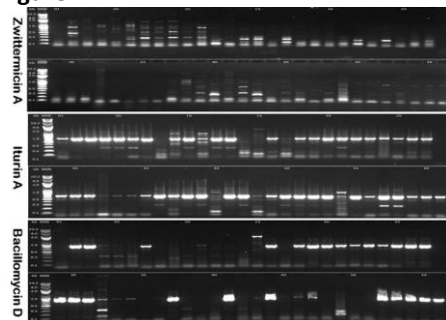


Figure 2



P N-CCO 106

Control effect of velvet bean seed extract against root-knot nematode, *Meloidogyne* sp

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One of the techniques for the management of coffee root-knot nematode is the use of plant extracts that have nematicidal effect. In this study, the anti-nematode activity of water extract of velvet bean (*Mucuna pruriens*) seed has been investigated against coffee root-knot nematode (*Meloidogyne* sp.) in laboratory. Experiments were carried out with extract, concentration and time level using randomized completely design in vitro. For this purpose, the effect of water extract of velvet bean seed with concentration of 0, 0.15, 0.3, 0.6, 1.2, and 2.4% (w/v) on the percentage of immobility of second stage juveniles was evaluated. The results indicated that all of concentrations of water extract of velvet bean had anti-nematode activity. Overall water extract of velvet bean had the most effect on immobility of second stage juvenile of nematode in vitro.

1) Rabie, E. C. and Tustin, H. A. 2009. The effect of different cover crops on nematode populations and yield in 'Queen' pineapple cultivation. In: VI International Pineapple Symposium. ISHS Acta Horticulturae 822.

Figure 1: Effect of velvet bean extracts with water on mortality of coffee root-knot nematode, *Meloidogyne* sp. Fig. 2. Observation of mortality of coffee root-knot nematode, *Meloidogyne* sp. after treatment of velvet bean extracts.

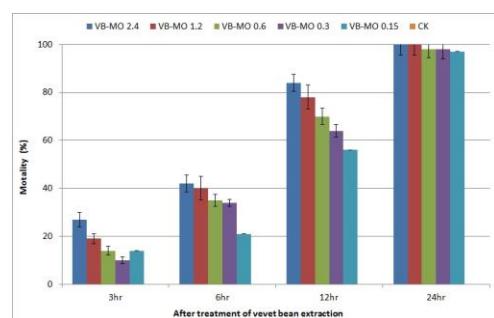
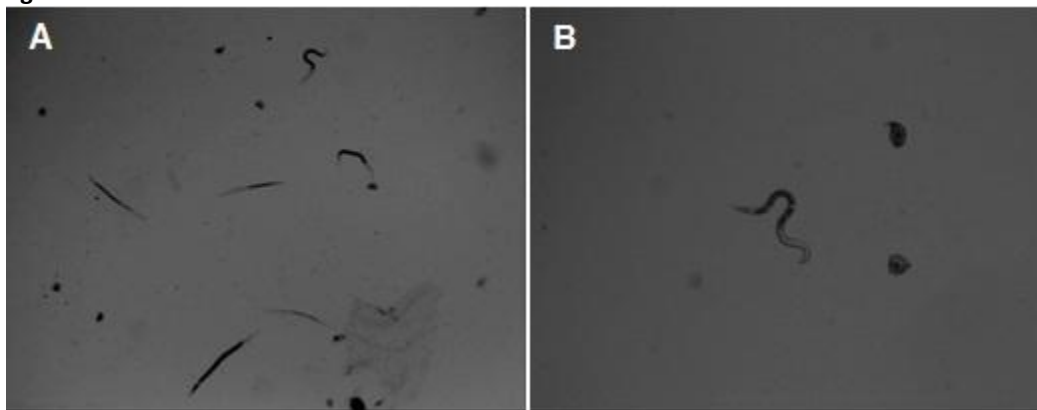


Figure 2



P N-CCO 107

Antibacterial activity of wild mushroom extracts on bacterial wilt pathogen, *Ralstonia solanacearum*

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Alternative methods of disease control are needed to complement conventional therapies faced by the limitations on use and the low efficacy of existing compounds against bacterial diseases e.g. against bacterial wilt disease of potato and other plants caused by the quarantine *Ralstonia solanacearum*.

Our aim was to explore mature and healthy sporocarps of fungi as a source of antibacterial proteins against *R. solanacearum* and to assess their efficacy *in vitro* and *in planta*.

Proteins were extracted from mushrooms collected from forest stands or grasslands by acetone precipitation and tested for antibacterial activity against NIB Z 30 (NCPB 4156) with the adapted MIC method. Selected active extracts were mixed with *R. solanacearum* and stem inoculated into plants of tomato cv. Moneymaker and/or potato cv. Désirée. Percentage of plants with certain symptom severity (1) were recorded at different dpi and compared between the test and the control groups (buffer/bacteria).

Altogether, 150 mushroom extracts (including one sterile mycelium cultivated *in vitro*) from 94 different species of Basidiomycetes and Ascomycetes were tested. Of these, 15 extract inhibited *R. solanacearum in vitro*. The *in-vitro* inhibitory effects of the *Tricholoma saponaceum*, *Suillus variegatus* and *Clitocybe geotropa* extracts were confirmed *in vivo* on tomato and potato plants. Overall, extracts of two edible mushrooms *S. variegatus* and *C. geotropa* had the strongest effect *in vivo* seen as delayed symptom development in tomato and potato plants (Fig. 1) respectively and were identified as the most promising source of effective antibacterials (2, 3).

References:

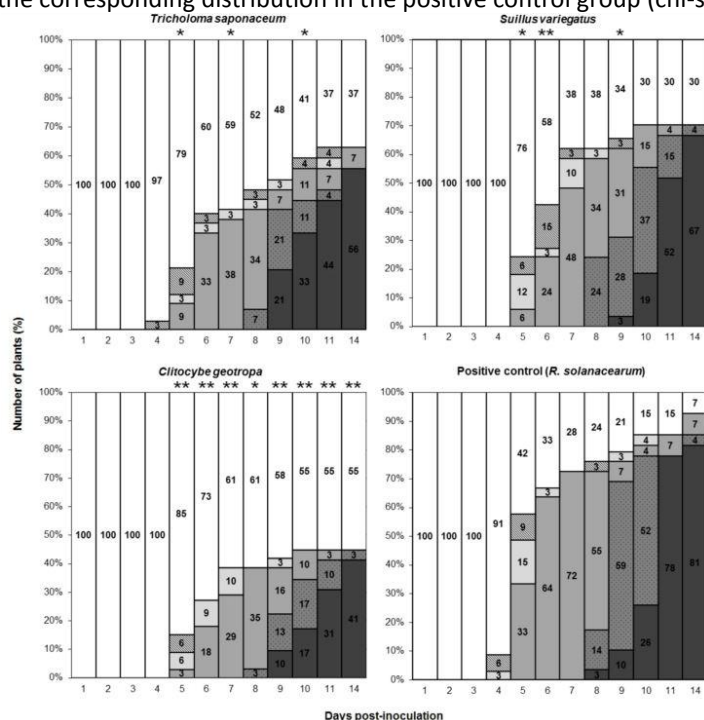
(1) Winstead & Kelman, 1952. Phytopathology, 42:628-634.

(2) Erjavec *et al.*, 2015. Plant Disease, accepted for publication.

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Figure 1: Influence of selected mushroom extracts on the bacterial wilt disease progression in artificially inoculated potato plants of cv. 'Désirée'. Percentage of plants of different symptom severity is shown and colour coded, ranging from no symptoms (white) to dead plant (dark grey). Asterisks indicate time points at which the distribution of symptoms differs from the corresponding distribution in the positive control group (chi-squared test; *, $p < 0.05$; **, $p < 0.01$).



P N-CCO 108

Efficacy of pH regulated Cu-chitosan nanocomposite against pathogenic fungi

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The objective of the current research was to develop chitosan biopolymer based pH regulated Cu-chitosan nanocomposite and evaluated their growth promotory and antifungal efficacy in tomato (*Solanum lycopersicum* Mill). Physico-chemical characterization of the developed Cu-chitosan nanocomposite was carried out by DLS, FTIR, TEM, SEM-EDS and AAS. The study highlighted the stability and porous nature of pH regulated Cu-chitosan nanocomposite. Laboratory synthesized nanocomposite showed substantial growth promotory effect on tomato seed germination, seedling length, fresh and dry weight at 0.08, 0.10 and 0.12% level. At 0.12% concentration these nanoparticles caused 70.5 and 73.5% inhibition of mycelia growth and 61.5 and 83.0% inhibition of spore germination in *A. Solani* and *F. oxysporum*, respectively in an *in-vitro* model. In pot experiments, 0.12% concentration of Cu-chitosan nanocomposite was found most effective in percentage efficacy of disease control (PEDC) in tomato plants with the values of 87.7% in early blight and 61.1% in Fusarium wilt. The overall results confirm the significant growth promotory as well as antifungal capabilities of Cu-chitosan nanocomposite. Our model demonstrated the synthesis of pH regulated Cu-chitosan nanocomposite and open up the possibility to use against fungal disease at field level for other important crops. We also demonstrated that upon infection of fungi Cu ions releases from nanomaterials and act on fungi and increase the antifungal efficacy of developed biocompatible nanomaterials. Further, developed porous nanomaterials could be exploited as smart delivery system for other antifungal compounds to enhance the efficacy of fungicides.

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Seed Health and Film Coating

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Reproductive development and growth by crops is especially important for human welfare because we depend on crop fruits and seeds, directly and indirectly, for most of our food. Seed is the main source of reproduction. Producers want to have high quality seeds for a high gain, especially high vigor, quality, yield and also seedborne diseases-free. There are many seedborne diseases in the World, especially *Clavibacter michiganensis* subsp. *michiganensis*, *Acidovorax citrulli*, Tobacco Mosaic Virus etc. Although seed production has been moved to disease free regions to escape seedborne pathogens, seedborne diseases (bacterial, viral and fungal) continue to be problematic and cause significant economic losses worldwide. Contaminated seeds are responsible for the re-emergence of diseases to date, movement of pathogens across international borders, or the introduction of diseases into new production areas. There are many applications to control seedborne diseases. These are seed treatments, resistant variety, clean seed production, seed health tests and quarantine preventions, but not enough. Pesticides can be applied to protect the seeds. But, they should be homogeneously dispersed on seed surface to effective control of pathogen and minimizing negative effects on seed quality. Seed film coating is effective and protective technique for pest control, seed quality and plant growing. Therefore, this article aimed to explain the advantages and disadvantages of seed film coating in seed health and present research findings. Many chemical and a few biological (organic) film coating studies were investigated. Findings of these researches indicated that seed film coating (chemical or organic) was found to have repellent effect against to pests. Therefore, this technique should be developed and could be used effectively against seedborne pathogens in future.

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Stability of Rhizobacterial isolate in some formulas to control bacterial Pustule Disease (*Xanthomonas Axonopodis* PV. *Glycines*) and to increase growth and yield of Soybean

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Previous research had showed, that two of rhizobacterial endophytes isolates from soybean root effective to control bacterial pustule cause by *Xanthomonas axonopodis* pv. *glycines*. To maintain the effectivity of this rhizobacterial isolate during storage, transportation and application, so need to be formulated. The aim of this research was to get the best carrier for formulation to maintain the effectivity of rhizobacterial isolate on storage to control bacterial pustule on soybean. The experimental was designed in complete randomized design where 16 treatments and three replicates with three plants /treatment for each variety was taken. The treatments were combination of material carrier for formulation of rhizobacterial isolate (peat soil, tapioca flour and coconut water + 1 % palm oil) and time of storage of formula (0, 1, 3, 5 and 7 weeks) and control. Every formula of rhizobacterial endophytes were inoculated on soybean seed as seed treatment. *Xanthomonas axonopodis* pv. *glycines* were inoculated on leaves of three weeks old soybeans. The parameter were observed include: the viability of rhizobacterial isolate in the formula during storage, bacterial pustule development (incubation periode, disease incidence and disease severity of bacterial pustule on leaves and pods), growth and yield of soybean. The results showed that all formulas of rhizobacterial isolate were able to suppress the bacterial pustule on soybean. The best combination of rhizobacterial formula which effective to control bacterial pustule and to increase growth and yield of soybean were five weeks and one week stored tapioca flour compare than control.

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Biological control of *Fusarium oxysporum* f.sp.albedinis using *Trichoderma viride*

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Bayoud of date palm (*Phoenix dactylifera* L.), caused by the fungus *Fusarium oxysporum* f.sp. albedinis (FOA), is the most important disease of this crop. A promising strategy for reducing diseases is based on Biological control involves the use of one or more biological organisms to control pathogens or diseases. The microbial inoculants as biocontrol agents are effective and attractive alternatives to prevent the deficiencies brought about by the exclusive reliance on chemicals (Nakkeeran et al., 2002). The objectives of this study were to evaluate the antagonistic activity of *Trichoderma viride* against different isolate of *Fusarium oxysporum* f.sp.albedinis and the effect of extracellular metabolites of *Trichoderma viride* on the radial growth of pathogen.

Biocontrol efficiency of *Trichoderma viride* was determined using dual culture method against different isolate of *Fusarium oxysporum* f.sp albedinis. The isolate *Trichoderma* showed 47.33%, 53.36%, 59%, 60.25% growth inhibition against S07, S15, S33, S13 respectively. The cell free culture filtrate of *T. viride* showed 92.30%, 85.7%, 87.25%, radial growth inhibition at 10% concentration against, S13, S33, S09, respectively. While, 20% concentration observed 100% mycelial growth inhibition. The current study assures the efficiency of *Trichoderma* as biocontrol agents against fungal soil pathogens and indicates the need of production and development of *Trichoderma* based biocontrol agents to serve as a model for environment friendly biocontrol agent. Thus, the *Trichoderma viride* isolate Tr V could be further exploited for commercial scale up under localized climatic conditions

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Seed Film Coating with Commercial Essential Oils against to *Clavibacter michiganensis* subsp. *michiganensis* and its efficiency on tomato seed and seedling quality in seedling company conditions

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Clavibacter michiganensis subsp. *michiganensis* (Cmm) causes bacterial cancer and wilt on tomato (*Solanum lycopersicum* L.). There is no resistant varieties to this disease and many seed treatments have negative effect seed quality of tomato. This is the first study aimed to control Cmm using film coating with commercial essential oils (EOs) and its effects were investigated on tomato seed and seedling quality in seedling company conditions. Cmm free tomato (cv. Rio Grande) seeds were film coated by different doses (500, 1000 and 5000 ppm) of EOs (*L. stoechas*, *O. onites*, *O. vulgare* ve *R. officinalis*). Then, its effects were investigated on some tomato seed and seedling quality parameters, especially emergency rate (ER), mean emergency time (MET), growing of uniform seedling (GUS), weight of fresh seedling (WFS) and dry seedling (WDS) by three replicated randomized complete block experimental design in factorial arrangement. Controls were sterilized seeds, unsterilized seeds and copper sulfate (nanocopper, 250 cc /100 l water). Effects of EOs x dose interaction, EOs and doses on ER, MET, GUS, WFS and WDS was not statistically different (P=0.809, P=0.4982 ve P=0.7145, respectively). But, EOs were given ER the lower than controls. Its ER were 69 to 72 % and *O. vulgare* was the lowest ER (69 %). EOs film coating was not negative effect on GUS, but some EOs (*O. vulgare* and *L. stoechas*) were created abnormally seedlings (about 1 to 1,5 %). Results of this study showed different data. Although EOs film coating have not statistically negative effect on ER, MET, GUS, WFS and WDS, It was the lower ER than control and created abnormally seedlings. Therefore, data of EOs film coated by control of Cmm were promising on tomato seed and seedling quality. Finally, findings indicated that It should be studied too much on EOs film coating and its effects have to tomato seed and seedling quality.

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Field Development of *Copidosoma conii* in Guangxi Province of China and its susceptibility to insecticides imidacloprid and matrine

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The rice leaffolder, *Cnaphalocrocis medinalis* Guenee, is a migratory insect which damages rice seriously. A large number of pesticides were used to control the pest that have many negative effects. Parasitoids also reduce the quantity of the pest. So, we research the field development and susceptibility of a wasp which can parasitize the pest.

Copidosoma conii, which belongs to Hymenoptera, Encyrtidae, is a polyembryonic parasitic wasp of the rice leaffolder. We investigated the field development of the wasp in Guangxi Province of China. This wasp occurred from June to November, and the average field parasitism rate was up to 18.4% during the late rice growth period. The female of the wasp oviposits into the egg of the rice leaffolder. The larvae of *Copidosoma conii* lived in the body of host larva until the adult wasp emerged. One host larva could breed 200~400 parasitic wasps.

The wasp can be a potential bio-control agent of rice leaffolder. However, as using pesticides is still believed to be the most important measure in pest management, huge application of pesticides may pose damage to this parasitic wasp. Imidacloprid and matrine are the two commonly used pesticides in rice production. We assessed the susceptibility of *C. conii* to the two insecticides in laboratory by drug velum contacting method. The results showed that the parasitoid was highly susceptible to these two insecticides. The 50% lethal concentration (LC50) of matrine was 13.45mg/L after treated 12 hours, and 8.52mg/L after treated 24 hours, respectively. The LC50 of imidacloprid was 6.8mg/L after treated 12 hours and 3.26mg/L after treated 24 hours. The common field application concentration of these two insecticides was 8-16 mg/L and 30-50mg/L, respectively. When we spray any types of insecticides we need avoid contacting the parasitoids such as *C. conii*, or else they might be hurted.

Figure 1



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Efficacy of a plant extract from plum poppy, *Macleaya cordata*, against the plant pathogenic oomycetes *Pseudoperonospora cubensis* and *Phytophthora infestans*

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Question: A plant extract from plum poppy, *Macleaya cordata* (Willd.) R. Br. (Papaveraceae), is known for high activity of its active substances sanguinarine and chelerythrine against economically important phytopathogenic fungi and bacteria under in vitro conditions. The objective of this work was to investigate the efficacy of *M. cordata* plant extract against plant pathogenic oomycetes.

Methods: Trials ad planta were performed using the host-pathogen system cucumber (*Cucumis sativus*) and downy mildew (*P. cubensis*). Bioassays on potted cucumber plants were carried out under climate room conditions. The extract of *M. cordata* was applied prophylactically in concentrations ranging from 0.63 µg/ml to 40 µg/ml to the lower leaf surfaces.

To investigate the effects on mycelial growth under in vitro conditions, the facultatively biotrophic oomycete *P. infestans* was used as model organism. A radial growth test was carried out with concentrations ranging from 1 to 100 µg extract per ml vegetable juice agar.

Results: On potted cucumber plants, the extract of *M. cordata* was very effective at low concentrations. Treatments with an extract concentration of 40 µg/ml resulted in an efficacy of about 95%; whereas at 5 µg/ml the corresponding value was still 66%. The observed protective effect was clearly dose-dependent.

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Under in vitro conditions nearly the entire Petri dish was covered with mycelia of *P. infestans* after 14 days of incubation in the water controls. An extract concentration of 10 µg/ml caused a low inhibitory effect of 10%, whereas mycelial growth was completely inhibited at 100 µg/ml. Across the full range of concentrations assessed, inhibition was positive dose-dependent.

Conclusions: The results demonstrated that *M. cordata* extract has a high potential to control downy mildew on cucumbers. Based on the in vitro activity against another economically important oomycete, *P. infestans*, an effect against oomycetes in general can be expected. However, validation of this potential for plant protection requires further study under both laboratory and field conditions.

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Biosynthesis of silver nanoparticles using Curvularin compound isolated from the endophytic fungus *Epicoccum nigrum*: New approach with great promise

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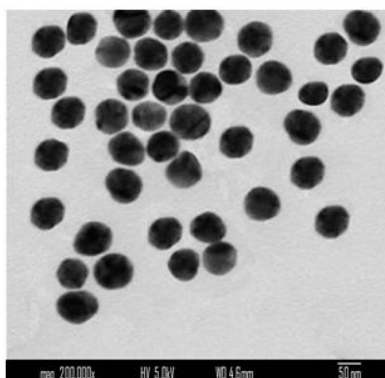
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The field of nanotechnology has a wide spread applications in different areas of science. The usual methods for synthesis of Nobel nanoparticles are more expensive and usually involve hazardous chemicals which results in drawbacks like high energy consumption, application of toxic material, and production of hazardous wastes.[1]Nowadays, synthesis of metal nanoparticles, in particular silver nanoparticles (AgNPs), and using fungi has become a major focus for most researchers.[2]Due to their simplicity of procedures, stability, and their wide potential applications in different mankind lives.[3]*We focused on* synthesis of controlled size and stable AgNPs with potent antimicrobial activity using biomolecules derived fungi acting as reducing and capping agents. *By isolation of* the endophytic fungus *Epicoccum nigrum* from healthy tomato plants **Fig.1..** The anti-oxidant Curvularin compound was isolated from the ethyl acetate (EtOAc) extract of *Epicoccum nigrum* and purified. Curvularin was utilized as reducing/capping agent in the AgNPs synthesis process. The biosynthesized AgNPs were authorized by UV-vis spectrophotometer with surface plasmon resonance at 430 nm. Transmission electron microscopy (TEM), X-ray diffraction (XRD) analyses were used to determine the size of the Ag NPs.. *The obtained results showed* appearance of a strong absorption peak centered at 430 nm at different time intervals of the absorption spectra which is characteristic for *surface plasmon resonance* of silver and hence indicate the formation of Ag-NPs. Also, we proved the formation of spherical, well-dispersed Ag-NPs with an average size of 37 nm **Fig. 2**. The XRD exhibited 2 θ values confirmed the crystalline nature of AgNPs. *Finally*, we proved that biomolecules derived fungi can be utilized as bioreductants and stabilizing agents in the synthesis process silver nanoparticles, by adopting the principles of green chemistry. *Undoubtedly*, further research is needed in this area to explore other possible biomolecule derived fungi acting as reducing/capping agents in the metallic synthesis of nanoparticles without the need to use such expensive and toxic chemicals.[1]Narayanan, K.B. and Sakthivel, N., *Journal of Advances in Colloid and Interface Science*, **2010**, 156, (1-2), 1-13.[2]Lamabam Sophiya and Devi, S.R. Joshi; **2014**.<http://dx.doi.org/10.1016/j.jmau.2014.10.004>.[3]B. Sogra F. and Raj M. Balakrishnan, *Materials Letters*, **2014**,132, 428-431.

Figure 1



Figure 2



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***Aeromonas media* in compost amendments contributing to suppression of *Pythium ultimum* in cress**

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Introduction: Soil-borne diseases such as damping-off caused by *Pythium* sp. are responsible for high yield losses in organic vegetable production and are difficult to control. Compost amendments have been shown to be able to improve survival and growth of plants in soils infested with soil-borne diseases. Yet, not all composts are equally disease suppressive and little is known about the microbial species directly involved in disease suppression.

Objectives: The objective of this study was to compare the microbial community in the rhizosphere of cress grown in substrates amended with composts suppressing *Pythium ultimum* damping-off at different levels.

Materials and methods: Cress was grown in a standard peat substrate amended either with coco fibre (conductive control) or with composts differing in their disease suppressive abilities. Bacteria were isolated from the rhizosphere and the most abundant species determined by Maldi-ToF MS. In a second experiment the most abundant bacterial species isolated of protected plants was added to all treatments to evaluate its role in disease suppression.

Results: The bacterial composition was essentially different with *Aeromonas media* being the main species present in the highly suppressive compost whereas *Enterobacter cloacae* was the dominating species in the less suppressive one. Addition of *Aeromonas media* improved suppressiveness against *P. ultimum* of less suppressive compost to the level of the highly suppressive compost.

Conclusion: We can therefore conclude that presence of *Aeromonas media* in composts is indeed contributing to disease suppression at least in this particular test system.

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Identification of endophytic microorganisms from *Olea europea* L. for the biological control of *Colletotrichum acutatum* and *Verticillium dahliae*

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The olive tree is one of the most important crops in the Mediterranean basin countries including Portugal. Anthracnose and verticillium wilt are two of the major olive diseases due to their high incidence and related losses. In Portugal and Spain, anthracnose is mainly caused by the fungus *Colletotrichum acutatum*, leading to losses in production up to 100%. Verticillium wilt is caused by the fungus *Verticillium dahliae*, causing yield losses up to 89%. The main control strategy is based on the use of fungicides, which is not completely effective and is associated with environmental risks and toxicity problems. For this reason, the biological control is a sustainable alternative for this problem. The endophytic fungi are a group of microorganisms with great potential to be explored as biologic control agents. In this work we evaluate the endophytic microorganisms community inhabiting roots, leaves and twigs of three olive cultivars (Picual, Galega and Cobrançosa) with different susceptibilities to the abovementioned diseases through sequencing (Illumina) of the amplicons ITS (fungi) and 16S (bacteria). The correlation between the presence/absence of each endophyte in different olive cultivars is discussed.

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Entomophages in the modern industrial greenhouses: survival strategies and tactics

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At the present time, the main and the most effective method of biological control of pests in hothouses is preventive colonization of entomophages.

When preventive introducing, the key requirements to entomophages are:

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1 longterm preservation in the greenhouse in the absence of the pest (those species of entomophages, which are resistant to food stress, primarily survive);

2 high biotechnological potential that ensures costeffective mass reproduction of speciesproducer under the conditions of technocenosis (when using natural feed substitute, in case of increased density, that is under the stress conditions).

The study objective is to optimize the existing complex of entomophages in the light of needs of the modern glasshouse cropping and its technological characteristics. As a model group, the lady beetles were chosen, because they show significant diversity of morphoecological adaptations to insect feeding. Good deal of bioresources of Coccinellidaepolyphages remains undeveloped. In the world practice Coccinellidaeoligophages, specialized in one particular group of pests, are primarily used. So, it is likely to find universal species that can be used for broadspectrum pest control among lady beetles.

In our work laboratory populations of entomophages from the collection of VIZR, including 8 species of the lady beetles from three dimensional classes, were used:

1 small (average weight of imago is up to 15 mg): Propylea japonica (population origin is Ussuriysk, 2012), Propylea 14-punctata (SaintPetersburg, 2014), Propylea dissecta (Nepal, Chitwan, 2013), Cheilomenes sexmaculata (Nepal, Chitwan, 2013), Cycloneda sanguinea limbifer (Cuba, 1972),

2 middle (1530 mg): Harmonia axyridis (Serbia, 2013; Sochi, 2012; AlmaAta, 2014; Irkutsk, 2012), Harmonia 4-punctata (Serbia, 2013).

3 large (more then 30 mg) Harmonia dimidiata (China, Guangzhou, 1990; Nepal, Pokhara, 2013).

The morphoecological criteria for the evaluation of biotechnological potential of the lady beetles were revealed. It was shown that the manifestation characteristics of dimensional sexual dimorphism and the extent of intraspecific weight variation under the conditions of food stress can be used to screen promising speciesproducers among the representatives of the family Coccinellidae.

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Efficiency on pepper (*Capsicum annuum* L.) seed quality of different seed production dates, storage and moisture content

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Peppers (*Capsicum annuum* L.) represent an important part of the fresh and dry vegetable market in Turkey, and are also significant worldwide in the segment of condiments, spices and salt preserves. It is the second most produced vegetable after tomato (*Solanum lycopersicum* L.) in protected cultivation in Turkey. Seed quality is important parameter for pepper and its is affected some applications, especially production dates, periods, regions, time of seed harvest, seed moisture content, storage conditions and seedborne diseases. Therefore, it was investigated efficiency of different seed production dates, storage and moisture content on pepper seed quality. Two cultivar (cv. Serademre 8 and Doru 16) produced by different dates (2007, 2008, 2009, 2010, 2011, 2013) were used. Its initially moisture content (MC) was determined by high constant temperature oven method. Germination rate (GR) and vigor (V) (Accelerated aging, Tetrazolium, Standard germination) were determined. Experiments were established in laboratory in randomized plots design with two and three factors three replications. Results indicated that seed production date and MC affected on pepper seed quality (GR and V) and were statistically detected negative correlation. Each cultivar was statistically showed significant differences on GR and V. Therefore, data suggested that MC of pepper seed was low (6.4 to 8.6 %) for high seed quality and storage capacity.

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In vitro efficacy of some PGPR isolates as biocontrol agent against colony growth of *Pseudomonas savastanoi* pv. *savastanoi* causing olive knot disease on olive

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Olive knot disease on olive (*Olea europea*), caused by *Pseudomonas savastanoi* pv. *savastanoi* (Psv), is distributed in olive-growing regions worldwide. It is responsible for major crop losses on olive and has become more common and serious. Psv produces plant growth regulators at infection sites resulting in plant tissue proliferation and gall development. Plant growth promoting rhizobacteria are bacteria that colonize plant roots, and in doing so, they promote plant growth and/or reduce disease damage. Therefore, PGPR can be alternative for management of Psv. This study was undertaken to assess *in vitro* antibacterial effect of some PGPR isolates (*Bacillus subtilis*, fluorescent *Pseudomonads*) against olive knot of olive. The antagonistic properties of PGPR strains was tested against Psv on King B medium by using spraying technique. Psv culture was inoculated on King B medium, and then, PGPR strains were sprayed on plates. Each treatment was replicated three. Applications of PGPR were reduced disease incidence about 22.5 % in olive. The results suggest that PGPRs would be highly useful to control the olive knot of olive without other pesticides.

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Endophytic Bacteria and their Use in Plant Protection

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All plants are inhabited by diverse bacteria known as endophytes. Endophytic bacteria are referred to as those which can be detected at a particular moment within the tissues of apparently healthy plant hosts. The endophytic bacteria has important roles on plants. Endophytes can be beneficial for plant growth, e.g. help against stress or be importance of nitrogen fixation. Some of them are plant growth promoter with the production of plant growth regulators. Some endophytes are seedborne, but others have mechanisms to colonize the plants that are being studied. They are necessary for the growth for plants. Also, these endophytic microbes play important roles in modulating the physiology of plants, it is of major relevance to understand the composition of populations of these organisms and to understand the molecular basis of interaction between these organisms and the host. In this review, It was presented an overview about bacterial species lived as endophytes. Also, It was tried to explain the functionality of endophytic bacteria in plants and their use in plant protection as PGPB (Plant Growth Promoting Bacteria) and biological agent.

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Evaluation of some pesticides on *Tuta absoluta* (Meyrick) in Iran

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Introduction: *Tuta absoluta* (Meyrick, 1970) (Lepidoptera: Gelechiidae) is a serious pest of tomatoes in many parts of the world. *T. absoluta* was introduced to Iran in 2010. In absence of control measures, it can destroy the whole crop. This insect has several generations a year and is an r selective insect. All of these characteristics make it a devastating pest which has to be controlled in time to save the crop. Chemical control is the main method of controlling this pest.

Objectives: This study was done to assess the susceptibility of *T. absoluta* to different pesticides used for controlling this pest in Iran. Another objective was to determine the best pesticide for reduction or elimination of *T. absolute*.

Materials and methods: The first population was in culture for 2 years in greenhouse of plant protection Department of the University of Tabriz (UTG). The second population was collected from a greenhouse in the vicinity of Tabriz (TG). And the third population was collected from a tomato field in Bilasuvar (BSF). The larvae were reared on tomato leaves in plastic boxes until

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they turned into pupae. The adults were fed with 10% sugar. Bioassays were conducted using leaf-dipping method. After drying of the treated leaves, the larvae were transferred on leaves. Diazinon, dichlorvos, deltamethrin, spinosad, abamectin, Bt., indoxacarb, acetamiprid and imidacloprid were tested on 2nd instar larvae.

Results: Spinosad had the highest toxicity, followed by abamectin and indoxacarb. Except for indoxacarb, the other pesticides had a lower toxicity to TG population compared with BSF population: probably the origin of this population was a greenhouse under a higher pesticide spray pressure. UTG population had the highest susceptibility for the pesticides tested.

Conclusion: It seems that neonicotinoid and organophosphate compounds were not highly effective on *T. absoluta*. Hence it may be concluded that newer insecticides with novel modes of action should be used in rotation with commonly used insecticides in controlling this pest.

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Plant growth promotion and induction of systemic resistance by rhizospheric bacteria in rice

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Introduction: The main goal of agriculture is the production of improved quality, safe and affordable food for increasing world population. With an increase in problems associated with the use of pesticides, it is desirable to use environment friendly means for sustainable agriculture. Recently, biopesticides have been introduced to improve the plant health and productivity ensuring safety for human consumption and the protection of the environment.

Objectives: Keeping in view the importance of rice in Pakistan as food and cash commodity and its yield losses due to Bacterial leaf blight (BLB) disease, the focus of the present work was isolation and identification of antagonistic bacteria from rice rhizosphere, screening the selected antagonists *in vitro* and *in vivo* having potential to suppress *Xanthomonas oryzae* pv. *oryzae* (Xoo), the causal agent of BLB, detection of the mechanisms involved in antagonism and induction of defense related enzymes in rice in response to antagonistic bacteria.

Materials and methods: Healthy and BLB infected rice samples collected from twenty eight different sites were used for the isolation of rhizospheric bacteria. Eight hundred and eleven different morphotypes were screened for the detection of their antagonistic activity against different strains of Xoo using diffusion plate assay. Antagonists were explored for different biocontrol determinants and were evaluated *in vivo* for disease suppression and growth promoting effect in a Net House experiment. Induction of defense related enzymes in rice was studied using spectrophotometer.

Results: Production of siderophores was found to be the common biocontrol determinant among all the selected bacterial antagonists. Bacterial antagonists also showed the phosphate solubilization and indole acetic acid. These bacteria significantly improved plant health in terms of reduced %DLA compared with infected control and induced defense responses.

Conclusion: Inoculum of antagonistic bacteria can be used as potential biocontrol agents for the suppression of BLB as well as for rice growth promotion.

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Cultivar and plant organ specificity of fungal epi- and endophyte taxa in olive tree (*Olea europaea* L.)

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The study of plant phyllosphere, colonized by epiphytic and endophytic microorganisms, has been underestimated in relation to the rhizosphere. In fact, only a few studies have compared both epi- and endophytic communities on plant hosts. Both microflora's may have important implications for plant health and fitness, and protection against pests and diseases. The olive tree (*Olea europaea* L.) is one of the most important crops in the Mediterranean basin.

The main aim of this study is to assess the diversity of epi- and endophytic fungi inhabiting the above-ground parts of the most important olive tree cultivars of the Trás-os-Montes region (Northeast of Portugal), Cobrançosa, Madural and Verdeal-Transmontana.

Endo- and epiphytic fungi were isolated from leaves and stems of seven olive trees of each cultivar, selected in Trás-os-Montes region. The identification of isolates was based on their morphological characteristics, as well as by the sequencing of the internal transcribed spacer region of rDNA.

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Both epi- and endophytic fungal community differ among the three olive tree cultivars. The diversity and abundance of fungal epiphytes was found to be higher on cv. Madural (46 taxa, 1370 isolates), followed by Verdeal-Transmontana (36 taxa, 823 isolates) and Cobrançosa (24 taxa, 750 isolates). Endophytic fungi were most diverse and abundant on cv. Cobrançosa (25 taxa, 135 isolates), than on Madural (16 taxa, 64 isolates) and Verdeal Transmontana (15 taxa, 44 isolates). The olive plant organs studied only differed markedly within the endophytic fungal community, being its diversity and frequency of colonization highest in twigs (48 taxa, 80% colonization) than on leaves (20 taxa, 20% colonization). The differences found on endo- and epiphytic assemblage between olive tree cultivars might be a reflection of plant host preference of individual fungal taxa. In fact, all the olive trees analyzed in this study co-exist in the same habitat but their above-ground parts harbored different microbial species. Knowledge of host range and host specificity is vital for potential applications of these microorganisms as biological control agents.

Acknowledgements: This work is funded by FEDER funds through COMPETE (*Programa Operacional Factores de Competitividade*) and by national funds by FCT (*Fundação para a Ciência e a Tecnologia*) in the framework of the project EXCL/AGR-PRO/0591/2012.

P N-CCO 125

Effect of azadirachtin, chlorantranilprole and some insect growth regulators on vegetable leafminer, *Liriomyza sativae* (Blanchard) (Diptera: Agromyzidae)

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Introduction: Vegetable leafminer is a polyphagous and cosmopolite insect and one of the most important pests of greenhouse and field crops especially cucumbers and tomatoes. The usual method for controlling this pest is the use of insecticides. The wide use of insecticides has caused development of resistance against many commercial insecticides in this pest. The use of suitable insecticides to control this pest and reduce the incidence of resistance seems to be necessary.

Objectives: The objectives of this study were to assess the efficacy of some fairly new insecticides with novel modes of action on *L. sativae* and their sublethal effects on certain life table parameters of this insect.

Materials and methods: The effects of some insect growth regulators namely hexaflumuron, chromafenozide, chlorfluazuron, cyromazine, lufenuron+fenoxycarb and also azadirachtin and chlorantranilprole on *Liriomyza sativae* were evaluated. For four of the insecticides which caused higher mortality of the larvae at field recommended doses, Dose - response lines were constructed.

The bioassays were based on the method described by Cox et al. (1995). The effects of sublethal doses of these insecticides on mean weight of pupae, adult emergence and sex ratio of the adults were also studied.

Results: LC₅₀ values for chlorantranilprole, cyromazine, azadirachtin and hexaflumuron were 0.24, 0.49, 8.51 and 67.6 mg ai/l, respectively. For all compounds except chromafenozide, a significant reduction in pupal weight and adult emergence was observed, but the adult sex ratio did not change significantly compared with control.

Conclusion: Most of the insecticides used in this study were fairly new compounds with unique modes of action and had considerable lethal and sublethal effects on *L. sativae*. If these results also hold true in field and greenhouse conditions, these compounds could be considered as suitable candidates in management programs for vegetable leafminer.

P N-CCO 126

Oviposition deterrency of three botanical insecticides on tomato leafminer, *Tuta absoluta* and vegetable leafminer, *Liriomyza sativae*

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Introduction: *Tuta absoluta* and *Liriomyza sativae* are very destructive and harmful leaf mining insects with a strong preference for tomatoes and other vegetable crops in greenhouses and fields worldwide. Chemical control is the main method of controlling these pests. Botanical insecticides have fewer environmental side effects than chemical pesticides.

Objectives: The study was conducted to assess possible oviposition deterrency of three botanical insecticides on *Tuta absoluta* (Lepidoptera: Gelechiidae) and *Liriomyza sativae* (Diptera: Agromyzidae) with the aim of determining their suitability in integrated management of these pests.

Materials and methods: Potted bean plants and tomato leaflets were used for performing the tests on *L. sativae* and *T. absoluta*, respectively. Recommended doses of Tondexir[®] (hot red pepper extract), Sirinol[®] (garlic extract) and Neem Azal[®] (neem

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extract) were used in this study. Treatments were performed by leaf dipping method. Treated plants were placed in infestation cage containing several hundred related insects. The Adults were allowed to feed and lay eggs on the leaves (3h for *L. sativae* and 12h for *T. absoluta*). Then the numbers of eggs on tomato leaflets and feeding stipples on bean leaves were counted. The tests were replicated three times and six plants or leaflets were used in each replication. Percent effective repellency for each compound was calculated using the formula suggested by Xue et al (2001). The experiments were done in greenhouse conditions at 26 ± 2 °C, $50 \pm 10\%$ RH and 16: 8 (L: D) h photoperiod.

Results: Garlic extract had very high oviposition deterrence with 74.6% deterrence rate on tomato leafminer. Red pepper extract with 60.7% repellency rate was less effective than the garlic extract. But, azadirachtin was not oviposition deterrent for *T. absoluta*. In case of *L. sativae* All of the compounds tested were oviposition deterrent. The Repellency rates were 81.2, 75.3 and 66.4 % for hot pepper extract, garlic extract and azadirachtin, respectively.

Conclusion: Based on the results obtained, these compounds have the potential to be used in management of these pests in greenhouse conditions. If similar results are obtained in field conditions and toxicological studies do not prove these compounds unsafe for humans and non-target organisms, they can be implemented in integrated management of these important pests in the field and greenhouse conditions.

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Effect of five pesticides on tomato leaf miner, *Tuta absoluta* (Lepidoptera: Gelechiidae)

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Introduction: Tomato leaf miner is one of the most important pests of tomatoes in many parts of the world. Although some natural enemies of this pest have been identified, the use of insecticides is the main method of controlling this insect. In recent years *T. absoluta* has entered Iran and disseminated in most of the country's tomato producing provinces. It infests tomato plants in both greenhouses and fields.

Objective: This study was done to assess the effects of five pesticides on the 2nd instar larvae and eggs of *T. absoluta*.

Materials and methods: The bioassays and insect rearing were carried out at 26 ± 2 °C, relative humidity of $70 \pm 10\%$, and photoperiod of 16: 8h (light: dark). The insecticides used in this study were: spinosad (48 SC), indoxacarb (15 SC), abamectin (1.8 EC), zeta-cypermethrin (10 EC), chlorantraniliprole (18.5 SC). Tomato leaves were treated with appropriate concentration of the insecticides using leaf dip method. A 2x2 cm piece of treated leaf was put in a glass vial and one 2nd-instar larva was put on the leaf piece. Larval mortalities were assessed 48 hours after treatment. Twenty larvae were used for each concentration and the treatments were replicated three times at different days. The 24 h old eggs were used to evaluate the ovi-larvicidal effect of the insecticides. The leaflets containing *T. absoluta* eggs were immersed in insecticide solutions for five seconds. The number of mines were assumed as live larvae.

Results: The LC₅₀ values for spinosad, chlorantraniliprole, indoxacarb, abamectin, zeta-cypermethrin on second instar *T. absoluta* larvae were 0.08, 0.09, 10.8, 0.29 and 232.2 mg ai/l, respectively. The eggs were treated with LC₅₀ of these insecticides to assess their ovi-larvicidal effects. The mortalities observed in chlorantraniliprole, spinosad, indoxacarb, abamectin, zeta-cypermethrin treatments were 88.8, 76.8, 58.1, 35, 50.5 and 14.6% respectively.

Conclusion: The results showed, chlorantraniliprole and spinosad were the most effective on 2nd instar larvae of *T. absoluta* and eggs, compared with other three insecticides tested. Abamectin and indoxacarb effective on tomato leaf miner and may be used in rotation with other effective insecticides. Zeta-cypermethrin was less effective on this insect.

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Biological control of charcoal rot of mungbean by *Trichoderma harzianum* and shoot dry biomass of *Sisymbrium irio*

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A pot experiment was carried out for biological control of charcoal rot of mungbean [*Vigna radiata* (L.) Wilzeak] caused by *Macrophomina phaseolina* (Tassi) Goid. Pot soil was made sick with inoculum of *M. phaseolina*. Dried powdered leaves of *Sisymbrium irio* L., a weed of family Brassicaceae, were mixed in soil at 1, 2 and 3% (w/w) with and without application of *Trichoderma harzianum* Rifai, a fungal biological control agent. The highest grain yield (3.62 g pot⁻¹) was recorded in combined application of *T. harzianum* and 1% *S. irio* leaves amendment that was 62% and 805% higher than negative and positive control treatments, respectively. Application of *S. irio* leaf amendment and *T. harzianum* generally enhanced leaf protein, sugar and chlorophyll content, and catalase activity. The present study concludes that *T. harzianum* in combination with 1% dry leaves of *S. irio* as soil amendment can be used to achieve maximum grain yield under biotic stress of *M. phaseolina*.

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Is *Colletotrichum acutatum* a merely pathogen or a true endophyte?

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Endophytic fungi reside in internal tissues of living plants without causing any immediate overt negative effects, but may turn pathogenic during host senescence. The fungus *Colletotrichum acutatum*, is the main causal agent of olive anthracnose, however it can also persist on several other plant species without causing disease symptoms. Previous work indicated that this fungus can switch between an endophyte and pathogen lifestyle. The present study aimed to determine the antagonistic ability of endophytic fungi isolated from olive tree against these two isolates of *C. acutatum*. The endophytic fungi studied were *Penicillium commune*, *Penicillium roseopurpureum* isolated from cv. Cobrançosa, and *Penicillium purpurogenum*, *Fusarium oxysporum* and *Macrophomina phaseolina* isolated from cv. Galega. Dual cultures were carried out on PDA medium, and the internal radial fungal growth, sporulation and spore viability were evaluated. The outcome of interaction was completely different among the two *C. acutatum* isolates. All the endophytes tested exhibited highest antagonistic activity against the pathogen *C. acutatum* than to the endophyte ones. Mycelia growth of the pathogen *C. acutatum* was reduced significantly from 29 to 42%, and of the endophyte *C. acutatum* was reduced from 6 to 37%. The highest growth inhibition was displayed by the endophytes *P. commune*, *F. oxysporum* and *M. phaseolina*. Sporulation and viability of the pathogen *C. acutatum* in co-culture with endophytes have similarly reduced significantly (up to 84% and 48%, respectively). Both *P. roseopurpureum* and *P. commune* were the most inhibitory. By contrast, in co-cultures established with the endophyte *C. acutatum* only its viability was reduced significantly when compared to control (up to 75%) by *P. purpurogenum*. The results indicate that the change of *C. acutatum* from a pathogenic to a non-pathogenic lifestyle affects the interaction with other endophytes species inhabiting olive tree tissues. The outcome of these interactions could have important implications in the host plant resistance to anthracnose.

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Evaluation of endophyte mediate effects on olive tree protection against *Colletotrichum acutatum*

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Olive anthracnose, caused mostly by *Colletotrichum acutatum*, is one of the most serious diseases of olive grove, leading to significant yield losses. In olive tree, anthracnose affects flowers, fruits, leaves and twigs. The incidence of olive anthracnose (OA) depend essentially on cultivar susceptibility. In Portugal, the cv. Cobrançosa is moderately resistant to OA whereas the cv. Galega is susceptible. The endophytic fungi are a group of useful microorganisms that have received considerable attention, after they were found to protect their host against abiotic and biotic stress. Therefore, the main aim of this study was to clarify potential fungal endophyte mediate effects on olive tree resistance/susceptibility to OA, under field conditions.

Fungal endophytes were isolated from leaves and twigs of healthy trees from both cultivars and identified by rDNA sequencing. The fungal endophyte frequency and diversity were higher on the OA-susceptible cv. Galega than on the moderately resistant cv. Cobrançosa. Statistical analyses and community ordinations revealed significant differences in fungal community composition between cultivars. The majority of differences associated with the tolerant cv. Cobrançosa were attributed to, *Hypocrea lixii*, *Trichoderma gamsii* and *Phomopsis columnaris*, within twigs, and to *Alternaria alternata* and *A. arborescens* within leaves. OA-susceptible Galega was differentiated by the species *Biscogniauxia mediterranea*, *Pyronema domesticum* and *Ochrocladosporium adansoniae*, isolated from twigs, and by *Chaetomium globosum*, *Pyronema domesticum* and *Biscogniauxia mediterranea* isolated from leaves. These analyses allow the identification of predictable fungal species that could explain the reduction of anthracnose disease infection in cv. Cobrançosa. The features of those species will be further confirmed by using in vitro and in vivo assays.

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Impact of rates of formulated neem seed oil and frequency of spraying on pests damage of improved pigeonpea cultivar, in South eastern, Nigeria

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Research on podsucking bugs (*Riptortus dentipes* Fab., Hemiptera: Coreidae) was carried out at the Postgraduate Teaching and Research Farm, Department of Crop Science and Technology, Federal University of Technology, Owerri Imo State beginning from May 2009, 2010 and July 2010. In this study, the populations of the major pests and pod/seed damages were monitored under control with formulated neem seed oil (F-NSO) and synthetic pyrethroid (cypermethrin) at different rates and frequencies. July planting season often chosen by farmers as the appropriate planting date was also subjected to control strategy with F-NSO to determine plant performance under pest control measures. The experiment was laid out in 3 x 5 factorial comprising three rates of neem seed oil: 2 ml (4.2L ha⁻¹), 4 ml (8.3L ha⁻¹), 6 ml (12.5L ha⁻¹) with 0ml (0L ha⁻¹), and synthetic pyrethroid (cypermethrin) 0.72 ml (1.5L ha⁻¹) as checks plots and three intervals of application: once a week, once in two weeks, and once in three weeks. Results from the research showed that application of F-NSO at higher dosage rate of 12.5L ha⁻¹ and at four regime spraying intervals of once a week significantly (p

In the humid environment of Owerri in South Eastern Nigeria, application of higher dosage rate (12 l ha⁻¹) of formulated neem seed oil (F-NSO) once a week and at four spraying intervals especially during July pigeonpea planting season should be incorporated into pigeonpea *R. dentipes* Integrated Pest Management Programmes (IPM) as the period witnessed low pod/seed damage with enhanced seed quality (percentage wholesome seeds) when compared with May planting season.

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Electrophysiological responses of the male moth antenna to compounds found in the female sex pheromone of *Loxostege sticticalis* Linnaeus

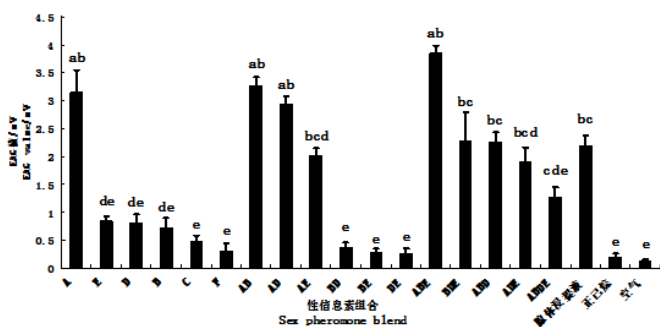
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The EAG responses of the male antenna to different combinations, proportions, doses of sex pheromone of *Loxostege sticticalis* Linnaeus were conducted in laboratory, and the results revealed component A (E11-14: AC), the binary blends of AB (E11-14: AC; E11-14: AL) and AD (E11-14: AC; 14: OH), the trinary blends of ABE (E11-14: AC; E11-14: AL; E11-14: OH) produced the EAG response that was significantly higher than that of n-hexane and air control, and 10 female moth gland extract of EAG values did not differ significantly; The trinary blends of ABC (E11-14: AC; E11-14: AL; 14: AC), when in ratio of 5: 3: 9 and 5: 3: 12, produced significantly higher level of EAG response than those in other ratio of the trinary blends; The dose-response test for ABE blend in ratio of 5: 3: 12 showed that the EAG values climbed quickly after the dose reached 1 µg/µL and totally differ from the EAG values of other doses.

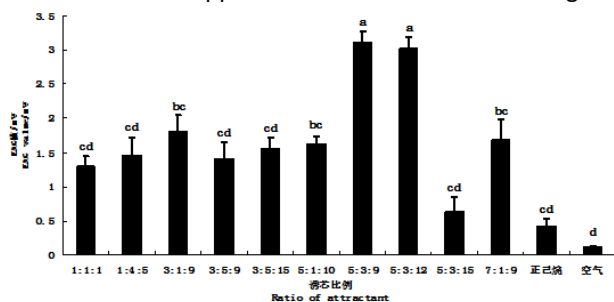
Figure 1: EAG response of male moth to sex pheromone components and their blends of female *Loxostege sticticalis*. Bars topped with different letters are significantly different at P < 0.05 (Duncan's new multiple range test)



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Figure 2: EAG response of male *Loxostege sticticalis* to the blend ABE(E11-14:AC; E11-14:AL; E11-14:OH) of female sex pheromone. Bars topped with different letters are significantly different at $P < 0.05$ (Duncan's new multiple range test)



P N-CCO 133

Autochthonous parasitoids of Algerian Southeast part in the control of the carob moth *Ectomyelois ceratoniae* Zell. (Lepidoptera: Pyralidae)

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The date palm (*Phoenix dactylifera* L.) is one of the most important fruit trees of the Arab countries. In Algeria, the palm trees are located at the South of the country. It generally contributes to stabilize the inhabitants in this area which is characterized by very difficult environmental conditions. It also constitutes a very important product in the nation's economy. Thus, this culture experiences a very fast development these last years and the surface occupied by this speculation does not cease increasing one year to another. The national production on date exceeds the seven million quintals per year of which more half is intended for export. Nevertheless, this culture is confronted with several problems in particular of a plant health nature such as the disease due to *Fusarium* fungus ("Bayoud"), the white cochineal insect (*Parlatoria blanchardi* Targ.), the yellow dust mite (*Oligonychus afrasiaticus* McGregor) and the carob moth (*Ectomyelois ceratoniae* Zell.). The latter is a lepidopteron whose caterpillar attacks the fruits, on foot as with storage, generating considerable losses. In fact, rates of infestation of dates by this pest exceeding the 50% were recorded in fields treated in the area of Oued Righ (Southeastern Algeria). The control of this moth remains limited to the use of the chemicals. Whereas, this method presents a great risk for the man health and the environment. However, safety alternative methods such as the biological control are possible. It is to this goal that a study has been initiated to develop knowledge on natural enemies of the carob moth in this region. The inventory of the autochthonous antagonists of this pest revealed the presence of two parasitoids. There are *Phanerotoma flavitestacea* Fischer and *Bracon hebetor* Say (Hymenoptera: Braconidae). The study of the biological parameters of these hymenopterans was led in the laboratory conditions in order to use them in a possible biological control program of this insect. The results obtained show that the two antagonists can be used in combination, the first with the field and the second for the period of storage.

P N-CCO 134

Influence of *Glomus mosseae*, an arbuscular mycorrhizal fungus, on *Striga hermonthica* incidence and sorghum growth

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Witchweed, *Striga hermonthica* (Del.) Benth., Orobanchaceae, is a debilitating obnoxious root parasitic weed on cereals in sub Saharan Africa. The plant, the germination of which is instigated by stimulants exuded by host roots, is a prolific seed producer and is endowed with a complex life cycle closely cued to that of its hosts. The present investigation was undertaken to study the effects of *Glomus mosseae*; an arbuscular mycorrhizal, fungus, on *Striga* incidence and sorghum, cv wad Ahmed, growth under greenhouse conditions. *Glomus mosseae*; reduced *Striga* emergence by 85.1% and 86.36% at 60 and 75 days after sowing, respectively and *Striga* biomass, at harvest, by 77.7%. Unrestricted *Striga* growth reduced sorghum height by 43.21% and 59.60% 30 and 45 DAS, respectively. *Striga* infested *G. mosseae*; inoculated sorghum, on the other hand displayed 38.51% and 68.41% increase in dry weight over the respective *Striga* infested control. In absence of *Striga* *G. mosseae* increased sorghum dry weight by 6.1% in comparison to the corresponding *Striga* free control. However, in presence of *Striga* the fungus increased sorghum dry weight by over 3-fold in comparison to the *Striga* infested *G. mosseae* free control. The results indicate that *G.*

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mosseae mitigates *Striga* damage to sorghum, mainly, through reduction of parasitism, achieved, possibly, through down regulation of production of germination stimulant(s) by sorghum roots.

P N-CCO 135

Morpho-anatomical and biochemical changes in the roots of rice plants induced by plant growth-promoting microorganisms

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Upland rice is planted in few regions worldwide. However, it presents advantages compared to floodland rice due to its lower production costs and water consumption. However, the average productivity of upland rice has been under 3 ton.ha⁻¹, the low productivity is attributed to water stress, which causes low initial vigor of the seedling root, deficiency in the uptake of nitrogen in the form of nitrate (NO₃⁻) at early stages of rice plant development, lack of plant response to inputs under successive planting, and the occurrence of rice blast. The goal of the present study was to characterize anatomical and biochemical changes in rice plant roots in response to seed treatment with rhizobacteria [*Burkholderia pyrrocinia* (R-46) + *Pseudomonas fluorescens* (R-55)] and *Trichoderma asperellum* (Ta: mixture of strains T-06, T-09, T-12, and T-52). The experimental design was completely randomized, with six treatments (R-46, R-55, R-46 + R-55, Ta+ R-46 + R-55, Ta, and control) and ten replicates. Treatments Ta and R-46 + R-55 increased the root length and diameter as well as the cortex expansion and induced a 2% expansion of the aerenchymal space. Treatments Ta and R-46 increased the vascular cylinder diameter. The number of protoxylem poles and metaxylem vessel elements was increased by R-46 and R-55(Figure 1). The total phenol content increased with treatments Ta, R-46 + R-55, R-46, and R-55, and all the treatments increased the flavonoid content. The lignin content increased with the Ta and R-55 treatments (Table 1). All the root architecture modifications resulting from the interaction between seedlings and bioagents (rhizobacteria and *Trichoderma* spp.) observed in the present study favored the root plasticity of rice seedlings, resulting in greater plant growth due to a better water uptake, resistance to water stress and mechanical impedance. The tested biogents showed to be potential biofertilizer to be inserted in upland rice management, with the goal of minimizing the disadvantages of this system, besides increasing defense response and increasing the productivity levels of genetically improved rice cultivars, without increasing the application of chemicals for fertilization and plant protection.

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Figure 1

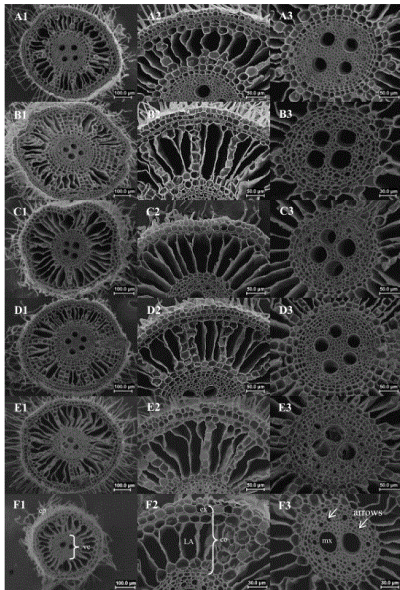


Figure 2. Electronmicrographs of adventitious roots of rice obtained from seeds treated with PGPMs, 21 days following sowing. (A1 – A3) *T. asperellum* (T-06, T-09, T-12, and T-52), (B1 – B3) *B. pyrrocinia* + *P. fluorescens*, (C1 – C3) *B. pyrrocinia*, (D1 – D3) *P. fluorescens*, (E1 – E3) *T. asperellum* (T-06, T-09, T-12, and T-52) + *B. pyrrocinia* + *P. fluorescens*, and (F1 – F3) control. Epidermis with root hairs (ep), cortex (co), aerenchyma space (LA), exodermis (ex), vascular cylinder (vc), protoxylem poles (arrows), and metaxylem vessel elements (mx).

Figure 2

Table 1. Length (cm) and dry mass (g) of leaves, root, and root/leaf, root diameter, cortex and exodermis thickness, lacunas do aerenchyma area (μm^2), vascular cylinder diameter (μm), and number of protoxylem poles and metaxylem vessel elements, phenols, flavonoids, lignin, and lignin monomer contents of roots of rice plants obtained from seeds microbiolized with PGPMs, 21 days following sowing.

Treatment	<i>Trichoderma asperellum</i> ¹	<i>Burkholderia pyrrocinia</i> + <i>Pseudomonas fluorescens</i>	<i>B. pyrrocinia</i>	<i>P. fluorescens</i>	<i>T. asperellum</i> + <i>B. pyrrocinia</i> + <i>P. fluorescens</i>	Control (water)	
Length (cm)	Leaves	33.5 ± 1.5 ² a ³	30.3 ± 1.5a	31.1 ± 1.6 a	33.1 ± 1.5 a	29.7 ± 1.3 a	28.2 ± 1.8 a
	Root	24.3 ± 1.9 ab	22.6 ± 1.8 ab	17.5 ± 2.1 bc	16.3 ± 1.6 c	14.3 ± 1.8 c	14.9 ± 2.5 c
	Root/leaf ratio	0.73 ± 0.23 a	0.75 ± 0.17 ab	0.63 ± 0.26 bc	0.49 ± 0.16 c	0.48 ± 0.25 c	0.53 ± 0.28 c
Dry mass (g)	Leaves	2.527 x 10 ⁻² ± 2.0 x 10 ⁻³ a	1.645 x 10 ⁻² ± 7.0 x 10 ⁻³ c	2.120 x 10 ⁻² ± 1.0 x 10 ⁻³ b	1.500 x 10 ⁻² ± 3.8 x 10 ⁻³ c	1.530 x 10 ⁻² ± 2.0 x 10 ⁻³ c	1.182 x 10 ⁻² ± 2.0 x 10 ⁻³ d
	Root	1.079x 10 ⁻² ± 0.2 x 10 ⁻² a	0.733 x 10 ⁻² ± 0.4 x 10 ⁻² c	0.912 x 10 ⁻² ± 0.7 x 10 ⁻² b	0.680 x 10 ⁻² ± 0.4 x 10 ⁻² d	0.615 x 10 ⁻² ± 0.2 x 10 ⁻² d	0.464 x 10 ⁻² ± 0.3 x 10 ⁻² e
	Root/leaf ratio	0.429 ± 0.32 a	0.464 ± 0.86 a	0.412 ± 0.24 a	0.450 ± 5.33 a	0.402 ± 0.15 b	0.379 ± 0.19 b
Roots	Root diameter (μm)	375.05 ± 59.60 c	373.94 ± 70.98 c	456.63 ± 13.37 a	369.37 ± 49.53 c	411.11 ± 63.51 b	303.81 ± 46.22 d
	Cortex thickness (μm)	159.16 ± 17.03 b	161.13 ± 14.09 b	197.28 ± 11.10 a	155.48 ± 3.43 b	174.32 ± 15.58 b	130.13 ± 12.32 c
	Exodermis thickness (μm)	12.1 ± 1.07 a	13.28 ± 0.48 a	12.3 ± 0.80 a	11.8 ± 1.07 a	12.79 ± 0.80 a	7.38 ± 0.96 b
	Aerenchyma space (μm^2)	9.77 x 10 ⁶ ± 18.99 x 10 ³ a	9.76 x 10 ⁶ ± 0.55 x 10 ³ a	9.70 x 10 ⁶ ± 18.93 x 10 ³ ab	9.72 x 10 ⁶ ± 3.80 x 10 ³ ab	9.68 x 10 ⁶ ± 0.38 x 10 ³ bc	9.61 x 10 ⁶ ± 0.76 x 10 ³ c
	Vascular cylinder diameter (μm)	229.6 ± 26.97 a	221.4 ± 80.92 ab	229.6 ± 26.97 a	213.0 ± 53.95 ab	205.0 ± 26.97ab	180.4 ± 26.97 b
	Number of protoxylem poles	18.66 ± 6.11 a	20.00 ± 1.90 a	19.00 ± 1.90 a	18.00 ± 3.80 a	17.33 ± 2.90 a	13.00 ± 7.60 b
	Number of metaxylem vessel elements	3.66 ± 1.10 a	4.00 ± 1.90 a	4.33 ± 1.10 a	4.66 ± 1.10 a	2.33 ± 1.10 b	2.00 ± 1.90 b
Phenols	Total phenols	9.64 x 10 ² ± 0.15 x 10 ³⁰¹ a ³	8.15 x 10 ² ± 1.21 x 10 ² ab	9.11 x 10 ² ± 1.47 x 10 ² a	8.92 x 10 ² ± 2.27 x 10 ² a	9.48 x 10 ² ± 1.96 x 10 ² a	6.80 x 10 ² ± 0.77 x 10 ² b
	Flavonoids	10.93 x 10 ² ± 0.67 x 10 ² a	8.05 x 10 ² ± 0.62 x 10 ² b	8.16 x 10 ² ± 0.34 x 10 ² b	8.06 x 10 ² ± 0.74 x 10 ² b	7.54 x 10 ² ± 0.37 x 10 ² c	6.79 x 10 ² ± 0.19 x 10 ² d
	Lignin	2.93 x 10 ² ± 1.05 10 ² a	0.40 x 10 ² ± 0.10 x 10 ² b	0.42 x 10 ² ± 1.82 x 10 ² b	2.18 x 10 ² ± 1.82 x 10 ² a	0.90 x 10 ² ± 1.45 x 10 ² b	0.74 x 10 ² ± 0.30 x 10 ² b
	Lignin monomers	1.44 x 10 ² ± 1.37 x 10 ² c	0.49 x 10 ² ± 0.30 x 10 ² c	2.47 x 10 ² ± 1.83 x 10 ² b	4.05 x 10 ² ± 0.56 x 10 ² a	0.38 x 10 ² ± 0.73 x 10 ² c	0.47 x 10 ² ± 8.00 x 10 ² c

¹*T. asperellum* (mix of four isolates: T-06, T-09, T-12, and T-52). ²Standard error ($p \leq 0.05$). ³Averages followed by the same letter within the same column were not significantly different according to Duncan's test ($p \leq 0.05$) at 15 and 21 days following sowing. Measurements were performed using a millimetric eyepiece in a MOTIC BA – 400 light microscope.

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Worth a look: The endophytic fungus *Acremonium alternatum* and its potential in integrated pest management

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Introduction: The fungal endophyte *Acremonium alternatum* controls mildew and tar spot and has been used as biocontrol agent in temperate and warmer climates. Two previous studies from our group successfully applied the fungus to achieve a reduction in clubroot symptoms in Chinese cabbage and *Arabidopsis*. The clubroot pathogen *Plasmodiophora brassicae* is one of the most damaging diseases among *Brassica* crops worldwide. Currently there is no potent chemical treatment available against this protist. This results in crop losses of several million dollars every year worldwide.

Objectives: We aimed to find out whether *A. alternatum* induces resistance mechanisms in *Arabidopsis* and rapeseed and thus reduces clubroot symptoms. Further we wanted to assess the biocontrol potential of this species.

Materials and methods: To test our hypothesis we investigated root tissue by means of a microarray early in the infection in *Arabidopsis* and RT-qPCR in *Arabidopsis* and *Brassica napus* at later time points. *In vitro* tests were applied to the biocontrol potential.

Results: Treatment with the fungus resulted in distinctively smaller root galls and better overall plant performance in *Arabidopsis* and *Brassica napus*. The endophyte primes *Arabidopsis* by inducing resistance genes through the activation of transcription factors (e. g. WRKYs, MYBs) and plant signal response factors (e. g. ERFs, ARFs). RT-qPCR data suggest that *A. alternatum* modulates the plant response to clubroot through specific resistance gene regulation and thus contributes to an enhanced plant health.

In rapeseed the fungus increased the seed production and survival of plants [1]. While the active fungus did not inhibit the disease significantly in oilseed rape, inactivated spores and a crude cell wall extract of *A. alternatum* reduced clubroot severity from 10 to 30 %.

We also found that the fungus promotes the growth of axenically grown plants on normal plant medium by up to 400 % (see fig. 1). First *in vitro* tests revealed that the endophyte can fix atmospheric nitrogen and solubilizes calcium phosphate. No cellulase or chitinase activity was detected.

Conclusion: Overall, this species is worth a closer look to exploit its potential within the context of integrated pest management.

[1] Auer, Ludwig-Müller: Albanian J. Agric. Sci. (2014), 13: 15 - 20 (special edition)

Figure 1

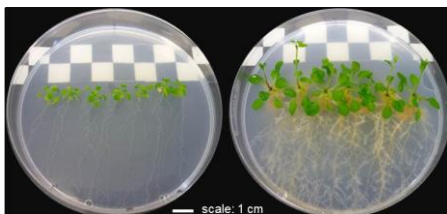


Fig. 1. *Arabidopsis thaliana* on Hoagland medium. Left: control; right: inoculated with *A. alternatum*

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Improving seed treatment methods: A key factor to reduce risks for honeybees and other organisms

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Introduction: Bees and other pollinators have a great importance in maintaining the biodiversity in almost all environments. However these important insects as well as other non target organisms are endangered through the use of pesticides. In late April 2008, dust drift containing insecticide resulted in the largest bee poisoning in Germany for 30 years. The reason for these incidents was the contamination of flowering bee forage plants with dust particles abraded from maize seeds treated with the **neonicotinoid insecticide** Clothianidin.

Objectives: Thus highly specialist techniques should be used when treating seeds with Plant Protection Products to avoid such problem. The aim of the study was to get a better understand of the situation in countries growing cotton and to improve the seed treatment methodology to reduce dust abrasion.

Materials and methods: The current study investigated the amount of drift generated from seeds of two varieties of cotton using two formulation of the **neonicotinoid** insecticide imadocloprid through measuring the fine dust particles from various treatments using the Heubach methods(using a Heubach Dustmeter). Dust drift reduction is not only relevant for bees but also

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for a reduction of risks for people handling treated seeds during the sowing activities and people located in the vicinity of the sowing site.

Results: The increase in percentage of dust abrasion through Heubach Dustmeter of the tested water dispersible powder formulation (WS) formulation of imadocloprid relative to untreated were found to be in the range of 336-378% and 221-287% for the for cotton varieties Hamid and Barakat, respectively. For the Flowable Concentrate (FS) formulation the percentage increase in dust abrasion over the control was ranging 82-95% and 15-445 for Hamid and Barakat varieties respectively. The Heubach vaules (in g/100g) were higher in case of WS formulations. They were ranging between 13.5 - 24.5 for Hamid variety and 23.3-25.4 g/100g for Barakat variety. The values for the FS formulation ranged between 7-8.8 and 2.64-14.7 g/100g for Hamid and Barakat, respectively. The pesticide residues in the Heubach filter dust were higher for the WS formulation compared to the FS formulation for both tested varieties.

Conclusion: The results of the study indicated in general that the flowable concentrate formulation for seed treatment has lower risks than the water dispersible powder formulation by reducing the drift generated from pesticide treated seeds during sowing. Seed treatment improvement can play an important role to save pollinators and other negative side effects of drift of dust.

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Gene transformation and PCR analysis of transgenic Cantaloupes with resistance genes (PR genes) to fungal diseases

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Cantaloupes (*cucumis melo* L., var. samsuri) hypocotyl explants were transformed with strain of *Agrobacterium tumefaciens*, LBA4404 each harboring the recombinant binary vector pBI121 containing the chitinas, glucanase and PR1 genes insert and neomycin phosphotransferase (nptII) gene, as selectable marker. Inoculated tissue sections were placed onto **Cantaloupes** co-cultivation medium. Transformed calli were selected on MS medium containing 50 mg l⁻¹ kanamycin and 300 mg l⁻¹ cepotaxime. Putative calli were subsequently regenerated into **Cantaloupes** plantlets expressing both the kanamycin resistance gene and nptII as a reporter gene. Polymerase chain reaction was used to confirm the integration of chi, glu, PR1 and nptII transgenes in the T1 plants genome. Amplification of these genes showed two Cantaloupes are transgenic.

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Gene transformation and Southern blot analysis of transgenic Cantaloupes with resistance genes (PR genes) to fungal diseases

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Cantaloupes (*cucumis melo* L., var. samsuri) hypocotyl explants were transformed with strain of *Agrobacterium tumefaciens*, LBA4404 each harboring the recombinant binary vector pBI121 containing the chitinas, glucanase and PR1 genes insert and neomycin phosphotransferase (nptII) gene, as selectable marker. Inoculated tissue sections were placed onto **Cantaloupes** co-cultivation medium. Transformed calli were selected on MS medium containing 50 mg l⁻¹ kanamycin and 300 mg l⁻¹ cepotaxime. Putative calli were subsequently regenerated into **Cantaloupes** plantlets expressing the kanamycin resistance gene as a reporter gene. Southern blotting being used to confirm the identity of interesting genes from within Cantaloupes genomic DNA. Integration of chi, glu and PR1 genes into the genome of putative transgenic was further confirmed by Southern blot analysis.

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Study of vertical and horizontal dispersion of *Trichogramma brassicae* on the maize fields using diffusion model

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Introduction: Understanding the dispersal behavior of natural enemies is important for developing effective augmentative release strategies and for evaluating the spread and potential non target effects of an introduced natural enemy (Smith, 1996,

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Orr et al. 2000). *Trichogramma brassicae* (Bezdenko) is an important and dominant parasitoid in the North of Iran. The cotton boll worm, *Helicoverpa armigera* (Hubner) is a polyphagous pest on several crops including cotton, maize, soybean and tomato. Using trichogramma species for management of this pest has reported in different studies.

Question: How well the diffusion model fits vertical and horizontal dispersal of *Trichogramma brassicae*?

Methods: In order to study the vertical and horizontal dispersion behavior of *T. brassicae*, it was reared on the eggs of the grain moth, marked and released from a central point. Trap cards containing the eggs of the grain moth were used to detect the parasitism rate at distances (2, 5, 9, 12, 18, 25m from releasing point) and heights (30, 60 and 100 Cm above the ground). Trap cards were collected and examined in the laboratory 3 days after release. Statistical analysis using SAS software and GLM procedure indicated significant dispersal differences at distances and heights. Also analysis the dispersal of parasitoid wasps carried out based on the well-known diffusion model (Okubo 1980) (See Eq. 1) with a little modification on it.

Results: Primary results of present study showed significant differences in dispersion of parasitoids in distances and heights. Also the appropriate installing place of the trichogramma cards determined as well as the sufficiency and encompass and distance of the cards. In addition, using diffusion model to quantify dispersal of the parasitoid revealed that how well the model fits data ($R^2=0.86$ and 0.97) which always proves that individuals move independently and random. Furthermore, including the effect of the traps heights to the diffusion model despite no changes in diffusion coefficient cause better interpretation of dispersal by decreasing the approximate correlation between the parameters which emphasis on the reliability of the second model.

Conclusion: Using the normal analysis showed appropriate installing place of host egg traps and utilization of the diffusion model indicated is usefulness in quantifying of dispersal as well as fitting dispersal data.

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New Finding of *Ramularia coleosporii* hyperparasite as a Potential Biocontrol Agent in Korea

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Introduction: During the collection of *Coleosporium* rust fungi in Korea, *Ramularia*-like white mould hyperparasitic on rust pustules appeared frequently.

Objectives: This study concentrates on identification of *Ramularia*-like hyperparasite isolated from *Coleosporium* rust fungi through morphological examination. To support the result of identification, molecular phylogenetic analysis was carried out.

Materials and Methods: A total of 43 materials of *Coleosporium* rust fungi infected with the hyperparasitic fungi were collected at various localities in Korea during 2007-2009. Fresh materials were used for morphological observation. The ITS regions of rDNA were amplified using primers ITS1 and ITS4. Phylogenetic relationship was inferred from Bayesian analysis based on the ITS rDNA sequences.

Results: Morphologically, mycelium was internal, and hypha was septate, branched, and hyaline. Conidiophores in loose to dense fascicles were septate, straight to slightly curved or geniculate-sinuous, usually not branched or sometimes branched, and 50-400 x 3-4 μ m in size. Conidial scars were thickened and darkened. Conidia were hyaline, aseptate, cylindrical to ellipsoid, solitary to catenate, and 10-37.5 x 4-6 μ m in size. Hylum of conidia was thickened, darkened, and non-protuberant. Based on the morphological characteristics, the hyperparasitic fungi on *Coleosporium* sori agree with the description of *Ramularia coleosporii*. The ITS rDNA sequence similarity was >99% among 43 isolates of *R. coleosporii* obtained from various myco-/plant hosts, indicating a very low level of genetic variation within the species. In the phylogenetic tree, 43 sequences of *R. coleosporii* formed a distinct clade with high bootstrap value of 96%.

Conclusion: Although 43 *Ramularia*-like isolates were found on urediniosori of 9 species of *Coleosporium* occurring on 10 plant hosts, the hyperparasites were revealed to share identical ITS rDNA sequence homology and morphological features in the present study. Therefore, we confirmed that the *Ramularia*-like hyperparasites represent only one species *Ramularia coleosporii*, having a potential for biological control. This is the first report of *R. coleosporii* as a hyperparasite associated with *Coleosporium* rust fungi in Korea.

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Induction of plant defense mechanisms in *Arabidopsis thaliana* by the PGPR *Paenibacillus alvei* K-165 to control foliar pathogens

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The plant-growth-promoting rhizobacterium (PGPR) *Paenibacillus alvei* K-165 has the biocontrol ability to protect several crops against the soilborne plant pathogen *Verticillium dahliae* in greenhouse and field experiments as well as several other soilborne fungi and bacteria. A direct antagonistic action of strain K-165 against *V. dahliae* was excluded, making it likely that K-165 mediated plant protection results from induced systemic resistance (ISR) in the host. In this study we investigated the ability of *Paenibacillus alvei* K-165 to induce the defense mechanism in *Arabidopsis thaliana* against two foliar pathogens, the bacterium *Pseudomonas syringae* pv. *tomato* and the downy mildew oomycete *Hyaloperonospora arabidopsidis* after application of the biocontrol agent K-165 in the rhizosphere. The experiments performed three times and demonstrated that application of K-165 as root drenching a week before the infection was able to significantly reduce the disease rate caused by the two foliar pathogens and to reduce populations of both pathogens. Further experimentation also showed that K-165 is able to enhance the development of the plant Hypersensitive Response (HR, a form of programmed cell death associated with the rapid death of host cells triggered during the entrance of the pathogen in plant tissues) after the infiltration of *V. dahliae* liquid culture extracts in tomato and tobacco plants. These results suggest that K-165 is receiving a signal from *V. dahliae* to activate the plant defense mechanisms. Additional experiments showed that application of K-165 in rhizosphere, is able to induce the HR in *A. thaliana* plants infected with non-compatible race *Cala2* of *H. arabidopsidis* in leaves. These results demonstrate that the rhizosphere bacterium K-165 has the ability to induce the plant innate immune system even with pathogens that attack aerial parts of the plant that is at a distance from the point of application of the biological antagonist.

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Efficacy of *Bacillus thuringiensis* against *Vanessa cardui* (Nymphalidae, Lepidoptera) and *Ephestia kuehniella* Zeller (Pyralidae, Lepidoptera) larvae

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Local bacterial isolates of (*Bacillus thuringiensis*) (Bt) were isolated from Rhizosphere soil area of host plants (apples, cotton, almonds, forest trees) from different regions for the first time in Syria. Eighteen (Bt) isolates were obtained from 50 soil samples collected during the agricultural season 2010-2011 from Qunaitra, Homs and Raqqa governorates. Efficiency of these isolates were evaluated in their ability in killing the second and third larval instar of colourful butterfly (*Vanessa cardui*) and Mediterranean flour moth (*Ephestia kuehniella*) compared with the biopesticide Antario as positive control. Our experiment was established by adding a suspension of Bt with 10^8 cfu/ml concentration to the larval daily food. The mortality rate % was recorded after 48 hours of treatment. Results showed that 8 Bt isolates were effective and exceeded 50% in mortality rate with black color, and the isolate 212.1 isolated from the soil of cotton crop in Raqqa was surpassed by reaching 75% and 45% of mortality rate in control of *E. kuehniella* and *V. cardui* respectively. Contrary for the isolate 71.2 isolated from the soil of almond trees in Homs reached to 80% and 20% of mortality rate in control of *V. cardui* and *E. kuehniella* respectively. . Although that the isolate 440.4 isolated from soil of cotton crop in Raqqa did not kill *E. kuehniella*, it killed successfully 75% of *V. cardui*. The isolate 342.2 isolated from cotton crop in Raqqa has killed effectively *E. kuehniella* and *V. cardui* larvae and reached to 60% and 80% in mortality rate respectively. Whereas, the control (Antario) killed only 65% and 80% of *E. kuehniella* and *V. cardui* respectively after 72 hours of treatment without changing color of larvae. Our results of biological tests applied on the two species showed that *V. cardui* is more sensitive to be killed by Bt bacteria than *E. kuehniella*.

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Evaluation of *Trichoderma* spp. against different fungicides and their application in integrated disease management

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Compatibility to pesticides in *Trichoderma* is the key area for the successful implementation of IPM schedules as both products are used simultaneously for soil borne diseases. The application of *Trichoderma* is well known for its mycoparasitism and

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growth promotion Pesticide sensitivity in *Trichoderma* had earlier been worked (Sharma and Dureja, 2004) leading to the selection of carbendazim tolerant strain of *T.harzianum* (Th3). The present status of *Trichoderma* has been well reviewed for its ability as a formulation for several crops through IDM strategies which aims mainly on the interaction with fungicides, and raising many fungicide resistant pathogenic isolates. Keeping this in view a study was conducted for the comparison of three species of *Trichoderma* for fungicidal tolerance (mancozeb, carbendazim, carbendazim +mancozeb). The carbendazim tolerance and the effect of tolerant strain on soil microbial biomass composition by using PLFA based methodology in carbendazim and *Trichoderma* amended soils. Soil phospholipid fatty acid (PLFA) was extracted and measured using the modified methods described by Frostegard (1993). The individual PLFAs were expressed as mole percent of total PLFA. An Agilent (Agilent Technologies gas chromatograph (GC) and MIS Sherlock (MIDI, Inc., Newark, DE, USA) and Agilent Chem. Station software was used to measure FAMES generated. Over 25 different types of unique PLFA's signatures were found. There was an increase in *Trichoderma*, *Pseudomonas* and *Bacillus* biomass %with a maximum cfu of 13×10^8 , 2×10^8 and 2×10^8 respectively in both carbendazim and *Trichoderma* amended soil samples. The degradation capacities of selected strains of *Trichoderma harzianum* (Th3), *Trichoderma viride* (Tv9) and *Trichoderma atroviride* (Ta2) were tested at different concentrations 100, 150 and 200 ppm of carbendazim (Fig1 and Table1) and rate of it's reduction was recorded through HPLC analysis. *T. harzianum* (Th3) was able to degrade maximum upto 84.5% of the parental molecule (technical grade) on fourth day of incubation and 100% on fifth day as compared to other species under test where 47 % degradation by *T.viride* and 21% by *T. atroviride* was observed on fifth day. *Trichoderma* spp. were found to be compatible with different concentrations of mancozeb and combination of mancozeb and carbendazim. The additional characteristic of fungicidal tolerance (carbendazim and mancozeb) in *T. harzianum* was utilized in the integrated management of soil borne disease caused by *Sclerotinia sclerotiorum* causing white rot and black leaf spot caused by *Alternaria brassicicola* in cauliflower.

Frostegard, A., E. Baath (1996). The use of phospholipid fatty acid analysis to estimate bacterial and fungal biomass in soil. Biol. Fert. Soils,22:59-65.

Karlinski, L., S. Ravnskov , B. Kieliszewska-Rokicka , J. Larsen (2007). Fatty acid composition of various ectomycorrhizal fungi and ectomycorrhizas of Norway spruce. Soil Biology and Biochemistry.,39:854-866.

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Figure 1

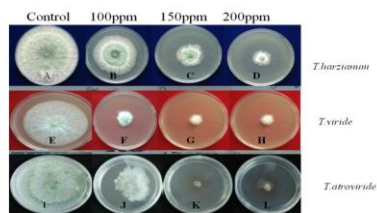


Fig.1 Growth of *Trichoderma* spp. on carbendazim amended PDA plates. (A) *T. harzianum* (Th3) in PDA plate without carbendazim (B+C+D) *T. harzianum* (Th3) in carbendazim amended at (100 ppm; 150ppm and 200ppm) (E) *T. viride*; (Tv9) without carbendazim (F+G+H) *T. viride*; (Tv9) at 100 ppm; 150ppm and 200ppm doses of carbendazim (I) *T. atroviride*; (Ta2) without carbendazim (J+K+L) *T. atroviride*; (Ta2) at 100 ppm; 150ppm and 200ppm doses of carbendazim.

Figure 2

Table 1. Effect of different doses of carbendazim on colony diameter and spore count of *T.harzianum*(Th3), *T.viride*(Tv9) and *T. atroviride*(Ta2)

Isolate name	Name of <i>Trichoderma</i> sp.	Colony Diameter (cms)			Spore count (cfu/ml)			%Inhibition of colony			%Inhibition of spore count				
		100ppm	150ppm	200ppm	Control	100ppm	150ppm	200ppm	Control	100ppm	150ppm	200ppm	100	150	200
Th3	<i>T.harzianum</i>	5.34±0.05	3.34±0.0	1.76±0.1	7±0.09	28x10 ⁸	24x10 ⁸	15x10 ⁸	30x10 ⁸	24	45	60	6.67	20	50
Tv9	<i>T.viride</i>	2.1±0.10	1.7±0.05	1.08±0.1	6.42±0.09	8x10 ⁸	5x10 ⁸	3x10 ⁸	20x10 ⁸	67	73	83	60	75	85
Ta2	<i>T.atroviride</i>	4.9±0.06	1±0.06	0.5±0.02	7.2±0.1	30x10 ⁸	20x10 ⁸	1x10 ⁸	27x10 ⁸	31	83	83	85	80	96

*Figures in parentheses indicate mean fungal diameter with ± SE value. Total number of samples analyzed was N = 30 (3 isolates x 3Replicates and 3 controls).

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Combined application of *Trichoderma harzianum*, *Pseudomonas fluorescens* for suppression of blast and bacterial blight of rice

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The blast of rice pathogen *Maganaporthe oryzae* and bacterial leaf blight pathogen *Xanthomonas oryzae* pv *oryzae* can cause severe economic loss to paddy growers. Efforts are made to develop effective biocontrol system for simultaneous management of these diseases. A combination of biocontrol agents is more likely to have a greater variety of traits responsible for suppression of one or more pathogens. *Trichoderma harzianum* TH3 isolate and *Pseudomonas fluorescens* isolate were selected and identified as potential antagonist against blast of rice pathogen *Maganaporthe oryzae* and bacterial leaf blight pathogen *Xanthomonas oryzae* pv *oryzae*. *T. harzianum* TH3 (1×10^7 cfu ml⁻¹) and *P. fluorescens* (1×10^8 cfu ml⁻¹) were applied singly and in combination with carbendazim (0.2%) as seed treatment, seedling root dip and two sprays with talc based bioformulation and compared with each other for the management of both the diseases (Figure 1). Combined application of both the microbes was found superior in protecting plants from blast of rice disease. Antagonistic effect of *T. harzianum* TH3 and *P. fluorescens* together showed additive effect showed 80.1% and 61.4% reduction in diseases intensity over control. In treatment with *P. fluorescens* bioformulation singly showed significant reduction in disease intensity. The percent reduction in diseases intensity was 51.1% and 64.2% respectively over control. No significance response of treatments in consortia was appeared against bacterial leaf blight of rice. Though experimentally validated but numerical model such as Bliss independence greatly aid in the formulation of consortia formulation of two bio-agents. It assumes that inhibitors bind simultaneously and mutually nonexclusively through distinct mechanism. Our results using Bliss Independence model clearly demonstrates that two bio-agents acts synergistically against blast of rice but antagonistically against bacterial leaf blight of rice. The consortia application of *T. harzianum* Th3 (6gm/kg seeds) and *P. fluorescens* RRb 11 (4g/kg seeds) as seed treatment followed by seedling root dip and two sprays at tillering and panicle initiation stage is very effective in reducing blast of rice (Table 1). Bliss Independence index was also found to be synergistic thus validate the efficacy of consortia of both the bioagents. In case of bacterial leaf blight of rice, application of *P. fluorescens* RRb11 alone reduce the BLB disease more effectively than the other consortia application. Also Bliss independence index were found to be antagonistic for all the consortia applied against BLB. Thus infers that there is no need of consortia application for the management of BLB.

Table 1: Effect of combination of BCAs and carbendazim of synergistic and antagonistic efficiency against blast and BLB of rice

Treatments	Observed efficiency (Mean)	Expected efficiency	Interaction
Blast of rice			
<i>T. harzianum</i> TH3 @ 6gm/lit water	40.55	$E_{TH3,RRb11} = E_{TH3} + E_{RRb11} - (E_{TH3} \times E_{RRb11})$	
Carbendazim @ 2g/lit water	59.9		
<i>P. fluorescens</i> RRb11 @ 4 gm/lit water	21.8		
<i>T. harzianum</i> TH3 + <i>P. fluorescens</i> RRb11 + Carbendazim	64.0	116.9	Antagonistic
<i>T. harzianum</i> TH3 + <i>P. fluorescens</i> RRb11	70.75	53.5	Synergistic
<i>T. harzianum</i> TH3 + Carbendazim	53.4	75.8	Antagonistic
Bacterial leaf blight of rice			
<i>T. harzianum</i> TH3 @ 6gm/lit water	32.6	$E_{TH3,RRb11} = E_{TH3} + E_{RRb11} - (E_{TH3} \times E_{RRb11})$	
Carbendazim @ 2g/lit water	16.5		
<i>P. fluorescens</i> RRb11 @ 4 gm/lit water	56.15		
<i>T. harzianum</i> TH3 + <i>P. fluorescens</i> RRb11 + Carbendazim	53.75	102.2	Antagonistic
<i>T. harzianum</i> TH3 + <i>P. fluorescens</i> RRb11	49.65	70.4	Antagonistic
<i>T. harzianum</i> TH3 + Carbendazim	18.9	43.7	Antagonistic

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Figure 2: Consortial application of *Trichoderma harzianum*, *Pseudomonas fluorescens* for suppression of blast and bacterial blight of rice



P N-CCO 146

Antagonistic potential of selected Bio-Control Agents against *Macrophomina Phaseolina* (Tassi) Goid

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Background: *Macrophomina phaseolina* (Tassi) Goid, a devastating soil-borne pathogen having wide host range cause significant yield losses in crop plants. Bio-control agents are preferred to noxious chemical control as these are eco-friendly and non-hazardous to human health.

Objective: Evaluate the selected agents for their bio-control antagonistic potential and its utilization in seed dressing

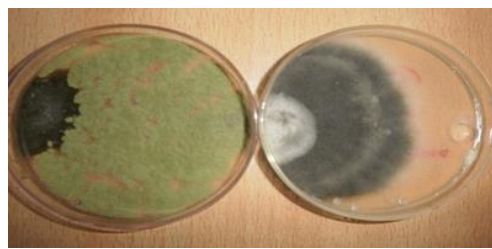
Methods: Dual culture technique was used to test the antagonism of selected microbes by growing both against each other on same petri dish and data regarding zone inhibition was taken on comparing with control. Efficacy was also tested by seed dressing with fungal antagonists.

Conclusion: Native strains of selected biological agents viz. *Trichoderma harzianum*, *Penicillium* spp., *Aspergillus niger* and *Aspergillus flavus* were investigated to assess their antagonistic potential against *M. phaseolina* (Tassi) Goid isolated from the roots of infected mungbean plants. Percentage zone inhibition of pathogenic fungi was recorded when compared against untreated control. *Penicillium* spp. in dual culture technique. Significant growth inhibition (71.6%) was recorded by *Penicillium* spp. followed by *A. flavus* (64.6%), *T. harzianum* (Th2, Th3) (58%) and (57.8%) respectively. *A. niger* (44.5%) was found to be least effective among all antagonists. Seed dressing with antagonistic agents produced positive effect on germination, radical length and vigor index compared to untreated control. Application of environment friendly antagonistic microbes is best alternative to health hazardous chemical formulations.

Figure 1



Figure 2



P N-CCO 147

Biocontrol of Plant Pathogens, Scope and Limitations

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Plant diseases need to be controlled to maintain the quality and supply of food. Different methodologies are being adopted to prevent, alleviate or control plant diseases. Together with agronomic and cultural practices, farmers rely on the use of noxious chemical and fertilizers to get better yield of the produce. Environmental degradation, Insecticide resistance, resource losses,

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and agronomic concerns, have prompted a growing interest in alternate disease management strategies. Biocontrol of plant pathogens being ecofriendly and cost effective can contribute significantly to the improvements in crop productivity. Using Biocontrol agents as insecticides have almost no harmful effects on humans and environment. It leads to the inability of pests to develop resistance however, biocontrol often do not result better in field conditions. Limitations involving research necessary in seeking a biological control solutions to an agricultural problem is often demanding in scientific and technical terms. To adequately practice biocontrol of plant diseases, firm understanding of the host population, pests along with their natural enemies, and their behavioral ecology is necessary as the pest population will continue to exist at a level determined by the host properties, natural enemies and habitat they occupy. The effectiveness of biocontrol agents must always be considered relative to man's economic threshold.

P N-CCO 148

Physiological changes of rice plants induced by plant growth-promoting microorganisms and Silicon fertilization

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Microorganism biopromotores (PGPM) together with the silicon fertilization constitute alternatives for sustainable agriculture, because they can promote higher productivity with reduced use of fertilizers derived from non-renewable source. However it is unclear the physiological changes promoted by the use of PGPM and silicon fertilization on rice plants. This work aimed to determine the physiological changes, during the growth promoted by the interaction among the PGPMs *Burkholderia pyrrocinia* (R-46), *Pseudomonas fluorescens* (R-55), *Trichoderma asperellum* (Ta: mixture of strains T-06, T-09, T-12, T-52) and silicon fertilization. The trial was conducted in randomized blocks, 25 treatments (1, 2, 4 and 8 t Si ha⁻¹ (CaMgSi₂O₆)) x three PGPM (R-55, R-46 and Ta: mixture of strains T-06, T-09, T-12, T-52, control), with 5 replication, under greenhouse conditions. The different doses of silicon were incorporated into the soil 30 days before sowing. Seeds of cultivar "BRS Primavera Clear Field" were treatments with PGPM 24 hours before planting. Biomass, gas exchange, photosynthetic pigments and total sugars were evaluated 21 days after planting. Treatments with 2 and 4 t Si ha⁻¹ and in combination with PGPM promoted the growth of rice plants in 30%. The application of PGPM, alone or in combination with silicon fertilization, increase 100% the photosynthetic rate and water use efficiency (WUE), 176% content of total sugars, and reduced by 55% sweating and by 48% the stomatal conductance. The increase in the content of pigments was positively correlated with increased photosynthetic rate provided by the application only of PGPM. There was a synergistic interaction between the silicon fertilization and PGPM to biomass accumulation in rice plants. However, it was not detected an increase in physiological rate, photosynthesis and water use efficiency and photosynthetic pigments when these elicitors were combined in rice plants.

P N-CCO 149

Impacts of Methyl Jasmonate applications of wheat insect pests, their natural enemies and some agronomic properties of wheat

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Methyl jasmonate (MeJA) is known organic volatile compound, have roles in inducing plant defense and regulation of different plant physiological processes. The aim of present study was to investigate the effects of different MeJA doses (0.88 mM; 1.76 mM; 3.55 mM; 7.1 mM; 14.2 mM; control) on wheat insect pests and their natural enemies by employing three sampling methods (direct count, sweep net, sticky traps) during 2012-2013 under field conditions. Effects of different doses of MeJA on aphid species (Hemiptera: Aphididae), phytophagous thrips species (Thysanoptera: Phlaeothripidae and Thripidae), wheat stem sawfly species (Hymenoptera: Cephidae), Coccinellid species (Coleoptera: Coccinellidae), Syrphid species (Diptera: Syrphidae), *Collyria coxator* (Hymenoptera: Ichneumonidae), *Chrysoperla carnea* (Neuroptera: Chrysopidae) and predatory thrips species (Thysanoptera: Aeolothripidae) were found statistically significant. MeJA treatments had deterrent effects on aphid species, phytophagous thrips species and syrphid species. At the same time, MeJA treatments attracted wheat stem sawflies, coccinellids species, *C. coxator* and *C. carnea*. There was no effect of methyl jasmonate treatments on aphid parasitoids. Effects of MeJA treatments on predatory thrips species population density were not significantly different from control treatments but were varied among MeJA doses. MeJA applications also led to yield reductions and suppressed length of plants. Our results discussed in terms of controlling insect pests and attraction of natural enemies in wheat ecosystem by applications of MeJA and other jasmonate derivatives.

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Effects of CIS-Jasmone treatments on insect pests and beneficial insects fauna of wheat (*Triticum aestivum* L.)

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cis-Jasmone (CJ) is a natural plant volatile, derived from linolenic acid by octadecanoid pathway, which has roles as an insect semiochemical and a plant defence stimulant. In present study, the effects of three different doses of *cis*-jasmone (25 g/ha; 50 g/ha; 100 g/ha) on wheat pest insects and beneficial insects fauna were investigated using three different sampling methods (plant sampling, sweep-net, coloured sticky traps) during 2011-2013 years. It is found that some or all CJ doses have statistically important effects on aphid species (Hemiptera: Aphididae), phytophagous thrips species (Thysanoptera: Phlaeothripidae and Thripidae), wheat stem sawfly species (Hymenoptera: Cephidae), Coccinellid species (Coleoptera: Coccinellidae), Syrphid species (Diptera: Syrphidae), aphid parasitoids species (Hymenoptera: Braconidae), wheat stem sawfly parasitoid, *Collyria coxator* (Hymenoptera: Ichneumonidae) and the green lacewing, *Chrysoperla carnea* (Neuroptera: Chrysopidae). CJ treatments had deterrent effects on aphids, thrips species, wheat stem sawflies and *C. carnea* while it was attractive for coccinellids species and *C. coxator*. Effects of CJ treatments on population densities of aphid parasitoids and syrphid flies were not significantly different from the control treatments but were varied among CJ doses. There were no significant effects of CJ treatments on yield and yield components while CJ-treated plants were taller than those in untreated control plots. It seems that CJ can be used in wheat fields to reduce some insect pest populations and manipulate some natural enemy species.

P N-CCO 151

Growth promoting and antagonistic abilities of Rhizobacterial communities of field-grown potato towards potato soft rot management

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The term antagonism refers to hostility leading to active resistance, opposition, contentiousness. Three rhizobacterial isolates from rhizosphere of freshly harvested potato tubers showed antagonism *in-vitro* and *in-vivo* to potato soft rot caused by *Erwinia carotovora*. Virulent strain of soft rot causing bacteria was tested for its sensitivity against these rhizobacterial isolates using zone inhibition technique and by using whole potato assay along with pot experiment. Among 20 rhizobacterial isolates, 3 isolates (Rb-5, Rb-12 and Rb-18) significantly inhibited the *in-vitro* growth of *Erwinia carotovora* with percentage inhibition recorded as 57% 51.5% and 44.5% respectively. Biochemical, and carbon source utilization tests identified isolate Rb-5 and Rb-18 as *Pseudomonas* sp. while Rb-12 as member of *Bacillus* sp. These isolates were applied to potato tubers kept in humid boxes placed in laboratory tested individually and in consortium revealed variable results. Application of three rhizobacterial isolates promoted overall plant growth as compared to untreated check. Significant reduction of 71.5% soft rot infection was observed in treatment where isolates were applied in consortium followed by Rb-12, 56.7% and Rb-5 44.5%. The results of the study identified Rb-18, Rb-12 and Rb-5 as growth promoters and promising antagonists against potato soft rot.

P N-CCO 152

The Citec Soap, a new additive for *Cassia Nigricans*, *Capsicum Annum* Aqueous extracts used against white flies (*Bemisia Tabaci* (Homoptera: Aleyrodidae) and *Helicoverpa Armigera* (Lepidoptera: Noctuidae) on tomatoes, in Burkina Faso

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A pre extension study of *Cassia nigricans* and *Capsicum annum* aqueous extracts with Citec factory soap and adhesol as additives, was conducted in four locations (Valley kou, Kongoussi, Yako and Diébougou) with farmers, against white flies (*B. tabaci*) and army worm (*H. armigera*) which cause huge tomato yield losses to Burkina Faso' farmers. The experimental design was a factorial experiment with 2 factors (extracts and additives) with additional control in a randomized bloc design in 4 replications. The treatments were:

Untreated plot (control); Decis (deltamethin) 1l / ha; *Cassia nigricans* (250l / ha) + adhesol (30 cm³/100 liters porridge); *Cassia nigricans* (250l / ha) + CITEC soap (52 g / 16 liters porridge); *Capsicum annum* (250l / ha) + adhesol (30 cm³/100liters porridge); *Capsicum annum* (250l / ha) + CITEC soap (52g / 16 liters porridge). Each locality (Kongoussi, Diébougou, kouValley and Yako) was a replication. So, the Number of plots by locality was 6 and the number of replications was 4. The sizes of the plots were 8m

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$x 4m = 32m^2$. The number of white flies were counted using transparent cylindrical cages covering 20 tomato plants with white flies on four lines of the useful plot. The rate of *H.armigera* infestations on tomato fruits, was evaluated on all plants of the useful plot, during the harvest. The coefficients efficiencies of the different formulations were evaluated using Afanaseeva *and al.* (1983) formula. The yield components were estimated after counting tomatoes fruits and weighing it. According to *B. tabaci*, the biological coefficients efficacy of *C.nigricans* (250lporridge/ ha) associated with CITEC soap (52g /16lporridge) varied from 35.48 to 79.38 against 30.48 to 67, 15 for *C.nigricans* (250l porridge /ha) associated with the adhesol (30cc / hl porridge). The biological coefficients efficiencies of *C.annuum* (250lporridge/ha) associated CITEC soap (52g /16l porridge) varied from 35.28 to 74.11. *C.annuum* (250l porridge/ha) formulation associated with the adhesol (30cc / hl porridge) varied from 28.14 to 73.73, in comparison with unthreated plot. According to *H.armigera*, there was no significant difference. These factors, added with the chemical compound of soap, have led aqueous extracts formulations associated with Citec soap to have equivalent yield with the formulation associated with adhesol and to deltamethrin. This shows, that the Citec soap can be an alternative to adhesol in the formulations by farmers, natural substances aqueous extracts, used as natural insecticides, preserving consumers health and the environment.

P N-CCO 153

Isolation and identification of entomopathogenic fungi from the agricultural ground of the region of Mila and application these fungi on the aphids of bean (*Aphis fabae*)

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The present investigation aimed at the isolation and the identification of fungi from the agricultural ground and the aphids of the species *Aphis fabae* of the bean, in the region of Minar Zarza of the wilaya of Mila. Six genera were isolated from the samples: *Fusarium* with the percentage 42,85 %, followed by both genera : *Aspergillus and Penicillium* with percentage 21,43 %, *Rhizopus* with 7,14 %, *Botrytis* with 3,57 %, and finally *Peacilomyces* with 3,57 %. Pathogenicity tests in vitro showed that treatments by *Fusarium sp*, *Penicillium sp*, *Peacilomyces and Aspergillus niger* have a mortal effect on *Aphis fabae* and reproduce natural symptoms of entomopathogenic fungi.

Observations should be considered preliminary because most fungi isolated are not insect -specific pathogens, they can be plant-parasitic as well. It is important to determine the factors affecting the implementation of the fungus at the insects to fight effectively against aphids.

P N-CCO 154

Susceptibility of *Panonychus ulmi* (Koch) (Acari: Tetranychidae) to use of some acaricides in apple orchards in Serbia

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European red mite *Panonychus ulmi* (Koch) is one of the economically most important pests in apple orchards in Serbia. Its control has been and still is largely based on the use of acaricides. However, due to its short life cycle, abundant progeny reproduction, it is able to develop resistance to ppp very rapidly. As a consequence, products may fail to achieve sufficient efficacy.

Materials and methods: Testing the effectiveness of acaricides in suppression of *P. ulmi* was carried out in apple orchards during 2013-2014, at 2 sites in Serbia, Maradić and Bela Crkva.

The following acaricides were evaluated: Masai (tebufenpyrad), Sanmite (pyridaben), Abastate (abamectin), Envidor (spirodiclofen) and Borneo (etoxazole) at dose rate of product 0.15 %, 0.05 %, 0.05 %, 0.1%, 0.06%, 0.05%, *respectively*. The trial features were: experimental design - randomized block; plot size - 5 trees; replications - 4; type of application - spraying until run-off. Motile forms were counted on 25 leaves per plot before and after treatment and the efficacy was calculated according to Henderson-Tilton formula.

Results:

Applied in 2013 at Bela Crkva 14DAT efficacy was for Masai 11%, Sanmite 83%, Abastate 0%, Envidor 96%, Borneo 94%.

Applied in 2013 at Maradić 14DAT efficacy was for Masai, 91%, Sanmite 49%, Abastate 0%, Envidor 60%, Borneo 92%.

Applied in 2014 efficacy was for Masai 91%, Sanmite 84%, Abastate 75%, Envidor 94% and Borneo 97%.

Conclusion: Inconsistent results are more likely caused by extreme weather conditions (heavy rain and high temperature) than change in susceptibility of population of *P. ulmi*. Further studies (field and laboratory) will be conducted in 2015 so more precise conclusion can be made.

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P N-CCO 155

Decreasing infection of European canker in Brazilian apple orchards with fungicides, fertilizers and resistant inductors sprayed during leaf fall

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European canker (*Neonectria ditissima*) was first detected in Brazil in 2002, in a few number of orchards established with nursery plants from Europe. When believed eradicated, a severe and widespread epidemic was seen in 2010. The aim of this study was to compare for the first time in the Brazilian condition with natural infection in Gala apple orchards: 1. The efficacy in decreasing infection of *N. ditissima* by reduced rate of copper compounds, potassium and copper phosphites and methyl thiophanate; tebuconazole, dithianon, chlorothalonil, captan, phosetil-Al and azilbenzolar S-methyl sprayed one time in leaf fall (LF) and in spring or three times during (LF) S-methyl and 2. The performance of six programs that included tebuconazole, dithianon, chlorothalonil, captan, methyl thiophanate and phosetil-Al. The first two trials were performed in randomized blocks, 4 plots of 6 plants and the other, with two blocks with 2 plots of 100 plants. In the evaluation were recorded infected shoots, the presence of conidia or/and perithecia, and infection sites. Results in the first trial with three sprays showed that methyl thiophanate was the more effective treatment and all the protectant tebuconazole and phosetil-Al were equally effective, and the K phosphite was less effective. One spray in LF and one in spring was not enough to control the infection but fruits with K phosphites and phosetil-Al increased fruit firmness. In other hand, programs where captan + methyl thiophanate were sprayed in 50% of leaf fall were more effective and as well as three consecutive sprays in LF with phosetil-Al.

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Mechanism of biological control of Phytopathogenic fungi of wheat using new Biopreparations on the basis of PGP Riso Bacteria Bacillus Subtilis

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Question: The purpose of research - to study the mechanism and the spectrum of antifungal action of new biological products based on PGPR Bacillus subtilis against phytopathogenic fungi genera *Fusarium*, *Microdochium*, *Pyrenophora*.

Methods: We used the standard and original methods: antifungal activity (Montealegre, Reyes, Perez, 2003); bioautography (Sidorova, 2002).

Results: In the laboratory of laboratory for the development of microbiological crop protection products and microorganisms collection of ARRIBPP there were elaborated original experimental samples of biopesticides of multifunctional mode of action based on aboriginal PGPR: *B. subtilis* BZR 336g and *B. subtilis* BZR 517 for winter wheat and other crop protection against economically significant diseases.

Range of antifungal activity of the producing strains in co-cultivation with phytopathogenic fungus was researched *in vitro*: the degree of mycelium inhibition of *Fusarium graminearum* Schwabe was 49-53%, of *Microdochim nivale* (Fr.) Samuels&I.C. Hallett - 43-53%, *F. culmorum* (W.G. Sm.) Sacc.-42-48%, *Pyrenophora tritici-repentis* (Died.) Drechsler - 76-94%.

The mechanism of interaction of strains producers of new biopreparations of the genus *Bacillus* with phytopathogenic fungi of the genera *Fusarium* and *Pyrenophora* is studied. It is established that estimated mechanisms of action of bacterial strains with phytopathogenic fungi include: production of antibiotic substances and synthesis the cell-wall-degrading enzymes, and also exhaustion of exogenous power supplies at the competition for nutrients owing to fast dynamics of growth (fig. 1).

Figure 1: Degradation of the hyphae of the pathogen *Fusarium oxysporum* (X400)

a - hlamidospore type cell of *Fusarium oxysporum*

b - the adsorption of the bacterial cells along the hyphae *Fusarium oxysporum*

With the help of a bioautography method (orig.) with *F. oxysporum* test-culture there were revealed in the culture fluid of the both strains substances with fungicide properties (fig. 2).

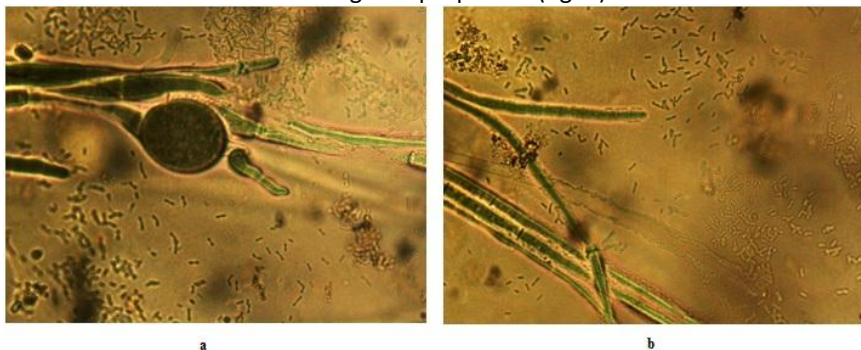
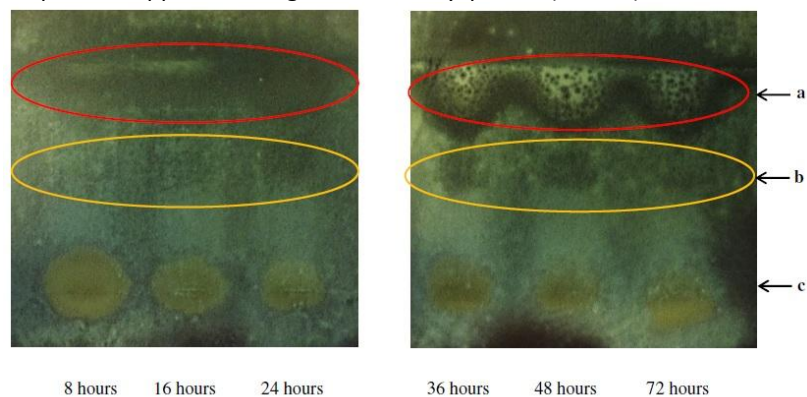


Figure 2: - Fungitoxicity metabolites of *B. subtilis* BZR 336 g in the batch fermentation (method of bioautography (orig.), silica gel plastin, test-culture *F. oxysporum*)

a - spots of suppression of growth of *F. oxysporum* (Rf 0.88)

b - spots of inhibition of growth of *F. oxysporum* (Rf 0.53)

c - spots of suppression of growth of *F. oxysporum* (Rf 0.53)



8 hours 16 hours 24 hours 36 hours 48 hours 72 hours

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Preliminary analysis showed that most of them are presumably cyclic lipopeptides.

Conclusions: Developed biopesticides may be used to form self-defense system of winter wheat and other crops protection or included in the system or integrated protection, reducing the pesticide load on agrocenoses.

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Evaluation of the efficacy of antifungal activity of the heat treated culture filtrate of *Streptomyces philanthi* RM-1-138 against rice sheath blight disease

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Rice sheath blight disease caused by *Rhizoctonia solani* is an economically important disease in rice production and accounted about 20% of the yield loss in Thailand. Biocontrol is a promising method to control the disease and species of *Streptomyces* have a very potential application. They are ubiquitous in the environment and many of them produce various secondary metabolites with diverse antifungal activities. *S. philanthi* RM-1-138 isolated from the rhizosphere soil of chili peppers grown in southern Thailand, was found to produce a strong antifungal metabolite that inhibited a range of plant pathogens both in volatiles and non-volatile forms. In this study, the efficacy of the antifungal activity of the heat treated culture filtrate of *S. philanthi* RM-1-138 against *R. solani* PTRRC-9 *in vitro* and on rice plants was evaluated. Results indicated that the heat treatment on the culture filtrate at 40°C, 60°C, 80°C, and 100°C for 30 min and 121°C for 15 min had no negative effect on the suppression of antifungal activity against *R. solani* PTRRC-9 tested on both solid and liquid culture. The results indicated the thermal stability of the antifungal substance in the culture filtrate. The effective dose (>80% inhibition) of culture filtrate in liquid culture was found to be at 5.0% (v/v) while it was at 10% (v/v) in solid culture. The greenhouse experiment revealed that using either culture filtrate or the autoclaved culture filtrate of *S. philanthi* RM-1-138 could effectively suppressed the rice sheath blight disease up to 65.6 and 60.8%, respective.

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Effectiveness of bee propolis to control bacterial wilt of tomato caused by *Ralstonia solanacearum*

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Bacterial wilt (*Ralstonia solanacearum*) of tomato causes a considerable amount of damage to tomato all over the world. Propolis is a resinous mixture that honey bees collect from botanical sources. Based on antagonistic activity against *R. solanacearum*, three concentrations of aqueous propolis extract (1, 10 and 100 mg/ml) (**PC1**, **PC2** and **PC3**) were selected to study their effect on bacterial wilt of tomato under greenhouse and field conditions. In both greenhouse and field experiments, the **PC3** gave the highest reduction of disease severity than **PC1** and **PC2**, also this concentration, **PC3**, caused higher biomass, 18.27 % than control plants. As recorded after treatment in field experiments, the control efficacy caused by **PC3** were 76.9 and 71.7%, respectively in the two trials seasons. and yield increases of **PC3** was about 82.3% in both trials. This is the first report of using propolis against bacterial wilt of tomato in the world and may be the first in bacterial plant disease management.

P N-CCO 161

In vivo assay to compare efficacies of biotechnological plant protection agents against *Phytophthora infestans*

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Question: In a research project, supported by funds of the Federal Ministry of Food, Agriculture and Consumer Protection (BMELV) based on a decision of the Parliament of the Federal Republic of Germany via the Federal Office for Agriculture and Food (BLE) under the innovation support programme, biotechnological fungicides to control oomycetes are under development.

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These fungicides contain antagonistic bacterial or yeast strains as active ingredients. Artificial inoculations of tomato leaf discs with *Phytophthora infestans* were used as an *in vivo* test system to test the influence of the production processes and formulations on the efficacy of the antagonists.

Methods: For strain selection the toxicity and pathogenicity data from literature were considered. The efficacy of selected bacterial and yeast strains against *P. infestans* were tested on tomato leaf discs in different formulations. For evaluation of the tests the sporangia formation was quantified and compared to the sporangia formation in untreated control and of leaf discs treated with a chemical standard. Furthermore different steps in the production and formulation processes were investigated to compare the efficacy against *P. infestans*.

Results: The efficacy of test preparations based on antagonistic microorganisms was at the same level as the efficacy of copper. In addition the combination of microorganisms and copper allowed a copper reduction to a tenth of the recommended dose compared to copper stand-alone treatments. The influence of various steps in the production processes and formulations on efficacy against *P. infestans* was identified and conclusions for the optimized production and formulation of the antagonists can be realized.

Conclusion: An improved leaf disc assay to test the efficacy of fungicides based on bacterial and yeast strains against *P. infestans* was developed. Regarding the formation of sporangia of *P. infestans* and not only the infected leaf disc area increased the selectivity of the test system. The production procedures and formulations of the antagonists were optimized for efficacy of the preparation.

P N-CCO 162

A New Potential Biological Control Agent for Field Bindweed in Turkey: *Titanio* sp.

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Field bindweed (*Convolvulus arvensis* L.) is a problematic weed species in cultivated areas, especially wheat and cotton fields in Turkey. *Titanio* sp. was firstly seen on this weed species in an organic farm in Şanlıurfa province, which is located Southeast Anatolia region of Turkey. Medicinal and aromatic plants are grown, pesticides have not been used for 15 years, field bindweed is dense and widespread in this farm.

Field bindweed can be suppressed by feeding of *Titanio* sp. according to our field observations. The larvae of *Titanio* sp. feeds between the two epidermis level of leaves, causes a transparent state and swell of the leaves like a balloon and mature larvae gets pupa under the soil. This review presents potential of this biological agent for controlling field bindweed.

P N-CCO 163

Transformation of plants with bacteriocins for pathogen resistance

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Antimicrobial peptides (AMPs) are short peptides produced by plants, animals, and other organisms. They are part of a primitive defence system with a narrow or broad spectrum of activity against plant pathogens. Bacteriocins are the type of AMPs produced by bacteria and archaea. Based on their activity, they can be recruited as defensive tools against resistant pathogens like *Pseudomonas syringae*. In this study, several different classes of bacteriocins with different modes of action have been chosen as suitable candidates for plant transformation. They were synthesised and cloned into suitable binary vectors for plant transformation, including Arabidopsis and tomato. Plants expressing bacteriocins will be challenged with pathogens to find resistant lines. The results will be used to generate crops with multiple disease resistance.

P N-CCO 164

Impact of entomopathogenic bacterial symbionts, *Photorhabdus luminescens*, and *Bacillus thuringiensis* subsp. *tenebrionis* on management of red palm weevil, *Rhynchophorus ferrugineus* (Olivier) in Egypt

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The red palm weevil, *Rhynchophorus ferrugineus* (Olivier), is considered to be one of the most lethal pests affecting date palms in Egypt, where the average rate of annual infestation is about 2.5. After its detection in the Egyptian palm area at 1992, it

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spread quickly to different governorates in the country. Currently, red palm weevil, *Rhynchophorus ferrugineus* (Olivier) management in Egypt is mainly based on chemical treatments. Nonetheless, special emphasis is being developed on implementing environmentally safe strategies. Commercial formulates based on *Bacillus thuringiensis* ssp. *tenebrionis* (Btt) strain NB-176 and the bacterial symbionts, *Photorhabdus luminescens*, which we have isolated from the entomopathogenic nematode, *Heterorhabditis bacteriophora* strain HP88, showed to be a good alternative, as they have been used to control other coleopteran insect pests successfully. The laboratory and open-field experiments presented in this work are evidence that the cumulative effect of both bacterial insecticides is highly efficient in controlling red palm weevil. The first instar larvae were the most susceptible in the laboratory studies, while susceptibility was lower in second, third instar larvae and adults. Our results also, showed that the impact of *Rhynchophorus ferrugineus* (Olivier) can be greatly reduced in the field by spraying and injecting the date palms with a lethal doses of both *Bacillus thuringiensis* ssp. *tenebrionis* (Btt) strain NB-176 and *Photorhabdus luminescens* formulates, at weekly intervals. This technology could reach to 60-80% recovery of date palms, with no need for chemical insecticides. Furthermore, the integration of this technology with other biological control methods such as, predators or parasitoids could reduce the number of bacterial pathogens treatments with the consequent increase in date palm produce.

P N-CCO 165

Augmentative on-farm releases of parasitoids to control the millet head miner in Niger

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Questions: The head miner (MHM) *Heliocheilus albipunctella* (de Joannis) (Lepidoptera: Noctuidae) is a chronic insect pest of pearl millet in Niger. Damage is due to larvae that feed on the panicle and caused up to 85% yield losses. Augmentative biological control with releases of the parasitoid braconid wasp *Habrobracon hebetor* Say is the most promising strategy for controlling MHM. The Objectives of this study was to evaluate augmentative releases of different *H. hebetor* adult numbers per millet acreage for controlling the millet head miner

Methods: Parasitoids are mass reared in the laboratory on larvae of the rice moth *Corcyra cephalonica*. Different numbers of parasitoids were released in 12 villages in Eastern Niger and 12 other villages in Eastern Niger in 2014. In each of the two districts the experimental design includes four treatments: i) three villages each supplied with 400 parasitoids; ii) three villages each supplied with 800 parasitoids; iii) three villages each supplied with 1600 parasitoids and iv) three control villages that did not receive any parasitoids. Data on MMH parasitism by *H. hebetor* were recorded 30 days after releases. For this purpose, 500 millet panicles were randomly selected in each village from five millet farms (100 panicles/farm) and dissected.

Results: The releases of the parasitoids significantly increased the parasitism of MHM as compare to control villages, which did not, received any parasitoid. The highest parasitism was recorded in villages where 1600 parasitoid were released.

Conclusions: Augmentative on farm releases on *H. hebetor* adults is effective in controlling the millet head miner.

P N-CCO 166

Effect of sublethal dose of *Bacillus thuringiensis* subsp. *kurstaki* on cotton bollworm *Helicoverpa armigera* (Lep. Noctuidae)

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Introduction: Cotton bollworm (CBW), *Helicoverpa armigera* (Hubner) (Lep.:Noctuidae) is an important insect pest of cotton that also attacks other crops and ornamentals. A more recent and environmentally friendly strategy for cotton bollworm management has incorporated the use of *Bacillus thuringiensis*. Despite of use of *Bt* for CBW control, there is less knowledge about the biological effects of sublethal *Bt* exposure. To achieve it, the effect of LC₂₀ concentration of *B. thuringiensis* on biological parameters of *H. armigera* was assessed in laboratory.

Materials and methods: Insect: *H. armigera* larvae were obtained from the Department of Plant Protection in the University of Tabriz. A stock culture was established on artificial diet according to the procedure elaborated by Singh (1982).

Formulation of *Bt*: The *Bt* formulation used in the experiments was *B. thuringiensis* sbsp. *kurstaki* Ceba PB 54 32% p/p (32×10⁶ u.i/gr) WP, Belthirul®, Probelte Company, Spain.

Bioassay: Third instar larvae of *H. armigera* were exposed to *B. thuringiensis*. Five concentrations of *Bt* with constant intervals in logarithmic scale within a range causing 10-80% mortality of the larvae were determined based on a preliminary test. Mortality was daily recorded till seven days. For experiment, we used the LC₂₀ concentration of *Bt*.

Experiment: Experiment was assessed in laboratory in 26 ± 1°C, 65 ± 10% RH and 16: 8 h (light: darkness) photoperiod. Life table parameters including intrinsic rate of natural increase (r_m), net reproductive rate (R_0), generation time (T) and doubling time (DT)

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were calculated as Carey (1993). Data analyses and comparisons of means by t- test were carried out using the SAS procedures (SAS 2003).

Results: The LC₂₀ concentration of *B. thuringiensis* subsp. *kurstaki* determined on the 3rd instar larvae of *H. armigera*, to be 9.8×10^5 IU/l diet. The stable population growth parameters estimated for control as well as CBW treated by LC₂₀ concentration of *Bt* at 3rd instar. The value of these parameters was estimated to be 582.22 ± 51.89 and 126.38 ± 27.78 female/female/generation for the net reproduction rate (R_0), 0.1442 ± 0.002 and 0.1028 ± 0.004 d⁻¹ for r_m , 44.16 ± 0.62 and 44.73 ± 0.62 d for T, and 4.80 ± 0.08 and 6.41 ± 0.21 d for DT, respectively for control and *Bt* treatments. The t-test revealed that all stable population growth parameters except generation time were significantly affected in *Bt* treated CBW.

Conclusion: The results suggest that sublethal doses of *Bt* has an effective role on the biological performance of the *H. armigera* that may reduce population levels of the pest survived following field spraying via a 5-time decrease in fecundity.

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P N-CCO 167

Assessing possible effects of host treatment by *Bacillus thuringiensis* subsp. *kurstaki* on *Trichogramma brassicae*, egg parasitoid of cotton bollworm

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Trichogramma spp. are important biological control agents in fruit trees, forests, stored products, and field crops such as cotton, tomato, maize etc. Nowadays, a combination of *Trichogramma* release mainly based on *T. brassicae* Bezdenko and *Bacillus thuringiensis* Berliner application is used in Iran cotton fields. No comprehensive knowledge however is present about possible effects of *Bt* applications upon *Trichogramma* spp. Although egg parasitoids are not in direct contact with larval insecticides, probable physiological effects in oogenesis of host that is a known effect may affect indirectly egg parasitoids. In this study the effect of LC₂₀ concentration of *B. thuringiensis* subsp. *kurstaki* (9.8×10^5 IU/l diet) was assessed on biological parameters of *T. brassicae* in a growth chamber with 26 ± 1 °C, $65 \pm 10\%$ RH and 16: 8 h (light: darkness) photoperiod. Results revealed that there were no significant differences between biological and stable population growth parameters of *T. brassicae* in control and *Bt* treatments. It suggests that LC₂₀ of this compound had no adverse impact on quality of the egg parasitoid *T. brassicae*. So a common mean of net replacement rate, intrinsic rate of population increase and generation time was estimated as 41.22 ± 3.85 female/female/generation, 0.3545 ± 0.009 /d and 10.49 ± 0.19 d respectively as average of the two treatments.

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Biological control bacterial leaf streak of rice with *Bacillus amyloliquefaciens* strain Lx-11

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Introduction: Bacterial leaf streak (BLS) is a destructive rice disease caused by *Xanthomonas oryzae* pv. *oryzicola* (Xooc). The bacterial pathogen infects the host plant at all growth stages. Available reports suggest that yield losses due to this disease typically range from 15% to 25% depending on the rice variety and climatic conditions.

Objectives: BLS of rice is biologically controlled with antagonist bacteria.

Materials and methods: 1173 bacterial strains were isolated from the disease leaves infected by *Xanthomonas oryzae* pv. *oryzicola*, the healthy leaves and the rhizosphere of rice plants sampled in Nanjing, Taizhou, Yangzhou and Suqian in Jiangsu province. Among the tested strains, 12 strains demonstrated high inhibitory activity against *Xanthomonas oryzae* pv. *oryzicola*. The Diameter of inhibition zones of 4 strains were more than 27 mm.

We found that Lx-11 secreted three kinds of lipopeptides including surfactin, bacillomycin D and fengycin, which exhibited antibacterial activity against *X. oryzae* pv. *oryzicola*. The BLAST analysis showed that the *sfp*, *fenB*, *ituA* or *bamA* genes exist in the genome of Lx-11. Three kinds of lipopeptides surfactin, bacillomycin D and fengycin were identified in the antimicrobial extract by the mass spectrum analysis. The antibacterial activity of could be associated with surfactin-lipopeptides, and practically abolished in surfactin-deficient mutants.

The expression of the defense-related genes PR-1a, PR-1b, NPR1 and PAL in the leaves of rice after treated with Lx-11 were concurrently detected by RT-PCR or Real-time RT-PCR

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Results: Control efficiencies of BLS with strain Lx-11 were 62.5% and 60.2% in pot and field trials, respectively, significantly higher than those of 20% bismethiazol treatment (51.2% and 45.8%). Based on morphological, physiological and biochemical characteristics and 16S rDNA sequence analysis, the strain Lx-11 was identified to be *Bacillus amyloliquefaciens*. The surfactin of strain Lx-11 secreted might play an important role in controlling BLS. Strain Lx-11 might triggered a systemic immunization activity of rice.

Conclusion: Strain of *Bacillus amyloliquefaciens* Lx-11 can significantly reduced disease incidence of BLS. Bacterial leaf streak of rice was efficaciously controlled with strain Lx-11.

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Effect of temperature and photoperiod on reproductive behavior of the corn stem borer, *Sesamia cretica* Led. (Lep.: Noctuidae)

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The corn stem borer, *Sesamia cretica* Led. (Lep.: Noctuidae) is the most important pest of maize and sugarcane throughout the world. The egg parasitoid wasp, *Telenomus busseolae* Gahan (Hym.: Scelionidae) is reared and released against the pest in Khuzestan province of Iran as the most important natural enemy of the pest. Because of host specificity, it is only rearable on the natural host (*S. cretica*) in the laboratory or insectarium. In this study the best temperature and photoperiod condition on the reproductive behavior of the adult parasitoid was evaluated in the laboratory. For this purpose different attributes such as percentage of fertilized eggs, oviposition rate, peak of egg laying and adult longevity were evaluated. Temperature treatments were included 24, 27 and 30°C and photoperiod treatments were included 24D,12D:12L, 8L:16D (short day period), 16L:8D (long day period) hours. The result of the data statistical analysis showed that the best temperature for oviposition was 24 to 27°C and in 30°C, the rate of oviposition was significantly decreased. Also the temperature had not any effect on the percentage of fertilized eggs and adult longevity. In addition, different photoperiod duration had significant effect on the rate of oviposition, so that the highest oviposition occurred in 12L:12D hours and after that 16D:8L hours (short day period) and the lowest rate of oviposition was observed in 24D period. According to the results, photoperiod had not effect on the percentage of fertilized eggs and adult longevity. In the all temperature and photoperiod treatments, the peak of egg laying was occurred in second day of female oviposition.

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The traditional use of saharian plant (haloxylon) by Algerian population and their antibacterial affect against the strains responsible for urinary tract infection

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Natural extracts from plants contain a variety of therapeutic compounds, the survey focuses on the importance of *haloxylon* (*Chenopodiaceae*). This plant is traditionally used by the Algerian population against several infections.

The aim of this study is to know the main microbial species responsible for urinary tract infection (UTI) in the South Algeria (Tamanrasset, Bechar and Ain Salah); to adjust the therapeutic and preventive attitudes to prevent the emergence of strains multi-resistant bacteria and monitor the effectiveness of traditional uses by identification of the active constituents of *haloxylon*. We evaluated antibacterial activity of crude extracts, flavonoids and alkaloids of selected plant. Moreover the highlighted separation methods by TLC [1], and GC of the active components (alkaloids, flavonoids and essential oils).

All bacterial strains tested (*Proteus mirabilis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*) have revealed a resistance to alkaloids and interesting sensitivity to crude extracts and flavonoids. Preliminary tests of this plant have revealed the presence of tannins, flavonoids, glycosides, alkaloids and saponins whereas the absence of anthraquinone derivatives and quinones. The crude extract of *haloxylon* at (5-15 and 30) minutes have a remarkable effect to *Proteus mirabilis*, which give different inhibition zones of diameters (10-16 and 20) mm; The high effect is shown in presence of *Escherichia coli* and *Staphylococcus aureus* in 30min that give the zones of 22 mm as diameter. The flavonoids tests confirm that *Haloxylon* has an important effect on *Staphylococcus aureus*, *Proteus mirabilis* and *Escherichia coli* by giving 21mm but a weak one on *Pseudomonas aeruginosa* with 10mm, On the other hand the alkaloids have no effect on the tested strains.

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The GC analysis of the *haloxylon* oils quantifies the seven components where three represent the majority which has the main percentages of 33.96%, 25.23% and 20.63%.

The TLC analysis on a thin layer has allowed to highlight the distribution of major alkaloids. The *Haloxylon* containing isoquinoline alkaloid (β . Carolline) has agreement with our results.

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P N-CCO 171

How different pollens affect the life table parameters of *Typhlodromus bagdasarjani* (Acari: Phytoseiidae)

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Integrated Pest Management (IPM) programmes rely heavily on maximum preservation of predatory insects and mites, particularly phytoseiids. *Typhlodromus bagdasarjani* Wainstein & Arutunjan is an indigenous mite in Iran and several parts of the world. Offering pollen as supplementary food gives rise to improve the predator fast establishment and performance. Considering the fact that the pollen spectrum accepted by this predator is not adequately known, this study aimed towards a comparison of the suitability of different pollens (date palm, almond, maize, sunflower, bitter orange and bee pollen) for *T. bagdasarjani* performance to optimize experimental and commercial mass rearing and food supplementation in greenhouses. All experiments were conducted in a growth chamber at 25 ± 1 °C, 16L: 8D hours photoperiod and 65 ± 5 % RH. Each experimental unit consisted of a green plastic sheet on water-saturated foam and was supplied with fresh pollen in two day intervals, removing the older pollen. Data were analyzed based on two-sex life table procedure. The highest values of the intrinsic rate of natural increase (r), net reproductive rate (R_0), as well as finite rate of increase (λ) were found when the predatory mites were fed on almond pollen (0.160 d^{-1} , 13.46 offspring and 1.174 d^{-1} , respectively). When the predatory mites fed on maize and sunflower pollen, the r and R_0 values were higher than those found on date, bitter orange and bee pollen, but lower than those obtained on almond pollen. Pollens of date, bitter orange and bee were less suitable as food source and caused a poor performance of the predator. Almond pollen can be recommended as supplementary food offered as banker plants in greenhouses and can be used to improve mass rearing of this predator.

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Interaction between *Beauveria bassiana* (Balsamo) and *Metahrizium anisopliae* (Metsch.) and the Host /Parasitoid System *Aphis craccivora* (Koch) / *Aphidius colemani* (Viereck.)

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Laboratory experiments were conducted to evaluate the virulence of two entomopathogenic fungi, *Beauveria bassiana* (Balsamo) and *Metahrizium anisopliae* (Metsch.) against the cowpea aphid, *Aphis craccivora* (Koch). The relationships between the solitary endoparasitoid *Aphidius colemani* (Viereck.) in the cowpea aphid, infected with the same entomopathogenic fungi was also considered. The mortality percentages in *A. craccivora* treated with *B. bassiana* and *M. anisopliae* were increased by increasing the concentrations of fungal conidia and time elapsed after treatment. The LC_{50} of *B. bassiana* and *M. anisopliae* for *A. craccivora* were estimated to be 2.94×10^7 conidia/ml and 1.8×10^7 conidia/ml at three days post treatment and 2.06×10^6 conidia/ml and 1.92×10^6 conidia/ml at eight days post treatment, respectively. Releasing *A. colemani* on fungus-treated seedlings infested with *A. craccivora* resulted in reduction of parasitism percentages with *B. bassiana* and *M. anisopliae*, where it recorded an average 91.9% and 93.5% in control pots and it ranged from 85.4 to 53.1% and from 86.6 to 54.9% with the increase of the concentration of the fungus from 1×10^6 - 1×10^{12} conidia/ml, respectively. It was found that, *B. bassiana* and *M. anisopliae* reduced emergence percentage of *A. colemani* from the mummies at concentrations of 1×10^8 - 1×10^{12} . While the two concentrations 1×10^6 and 1×10^7 were found to have little effect.

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Efficacy of novel compounds derivative from urea and rice straw on some biological aspects, protein profiles and peroxidase of *Spodoptera littoralis*

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A laboratory experiment was carried out to study some biological aspects (larval mortality, pupation, pupal weight, pupal duration, adult emergence and malformation percentage) of 4th instar larvae after treated with three newly compounds extracted from wastes from natural origin, Cyano acetyl urea (CAU), Benzimidazolyl acetyl urea (BAU) from urea and Cyano acetyl hydrolyzate (CAH) from rice straw. In addition, the present study aimed to investigate the effect of the above mentioned compounds on protein profiles and peroxidase pattern. Data indicated that the response was positively-related to concentrations of the investigated extracts and larval mortality increased significantly with the increase in their concentrations. Pupation percentage and pupal weight declined dramatically through successive ascending concentrations. The pupal durations were increased significantly, while the percentage of the adult emergence was reduced significantly with ascending concentrations. The total inhibition was increased with increasing concentrations.

On the other hand, some proteins in treated larvae were missed or expressed with less in density. Also, peroxidase pattern recorded in control and treated larval samples has 27 bands with Rf ranging between 0.04 and 0.84. The genetic distance between control and treated samples at LC25, LC50 and LC70 of (CAU), (BAU) and (CAH) were (0.71, 0.57, 0.71), (0.86, 0.69, 0.69) and (1.00, 1.00, 1.00), respectively.

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Reproductive life table studies on *Trissolcus djadetshkoe* (Hym. Scelionidae)

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Trissolcus djadetshkoe (Hym. Scelionidae) is an inferior egg parasitoid of sunn pest *Eurygaster integriceps* Puton (Hem. Scutelleridae) in Iran wheat fields. This is reason that why this species is less considered in biological control as well as research programs. However a parasitoid with a minor effect is not necessarily an unimportant species. Although this species is a negligible mortality factor in many parts of Iran, it is the second abundant species of northwest of Iran. Its efficacy was studied in terms of life history parameters in seasons 2013 and 2014 in laboratory (26±1 °C, 50±10% RH, and 16: 8 h photoperiod). Developmental time as well as life time fecundity was similar in both years. However a strongly male-biased sex ratio in the first year led to a significant decrease both in net replacement rate and intrinsic rate of increase. The sex ratio, net replacement rate and intrinsic rate of increase were 0.3±0.04, 20.3±3.1 female progeny per generation and 0.141±0.007 d⁻¹ in 2013 and 0.6±0.03, 39.8±2.9 and 0.212±0.004 in 2014. Also a four day decrease in generation time from 21.3±0.5 at the first year to 17.4±0.1 d at the second year occurred due to decreasing reproductive intervals from 1.9±0.2 to 1.5±0.1 d. the reason probably be due to lower light intensity in the first year. This hypothesis need further be examined.

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Searching ability of *Trissolcus djadetshkoe* (Hym. Scelionidae) on different densities of sunn pest *Eurygaster integriceps* (Hem. Scutelleridae) eggs

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Trissolcus djadetshkoe Rjachowsky (Hym. Scelionidae) is an egg parasitoid of sunn pest *Eurygaster integriceps* Puton (Hem. Scutelleridae) in Iran. Searching efficiency of this species was investigated in a functional response context. Effect of female insemination as well as parasitism experience was also investigated. Host densities of 2, 4, 7, 14, 28 and 56 eggs were exposed to a single female in 20, 20, 15, 15, 10 and 10 replications in a 24 h experiment in 1.5×10 cm vials. Virgin vs. inseminated females as well as inexperienced vs. experienced ones were used in two sets of experiments in two successive years (2013 and 2014). All experiments were conducted in a growth chamber (26±1 °C, 50±10% RH, 16: 8 h photoperiod). Logistic regression and nonlinear regression model of random search were used for analysis. All parasitoid responses were type III. Experienced females showed lower parasitism in all densities. Maximum attack rates were 14-22 in experienced females vs. 34 in inexperienced ones. It seems that previous reproductive efforts have depleted energy of the females. A lighter difference was observed in virgin females over inseminated ones (25 vs. 17). This difference probably refers to costs of male and female progeny for mothers.

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Virgin females produce only males. It seems that male progeny is less costly for mothers than females or switching between females and males.

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Response of *Trissolcus vassilievi* (Hym. Scelionidae) crowded in a multi-patch environment with different densities of *Eurygaster integriceps* eggs

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Trissolcus vassilievi Mayr (Hym., Scelionidae) is an important egg parasitoid of sunn pest, *Eurygaster integriceps* Puton (Hem., Scutelleridae) in Palearctic. Little known is responses of *Trissolcus* spp. upon the host egg in terms of density. In this study aggregation of the parasitoid in response to host density and mutual interference arisen by wasp crowding was investigated in laboratory conditions (26±1°C, 50±5% RH, 16L: 8D photoperiod). Five host densities including 1, 2, 4, 8 and 15 clutches were offered simultaneously to 1, 2, 4, 8 or 16 female parasitic wasps in an arena (30 cm diameter × 10 cm height). Patch times also were recorded as wasp-hours spent in each patch or out of patches. Results revealed a strong correlation between parasitism and patch times. Patch time increased up to eight clutches followed by a further decline. The wasp was totally a less mobile species and tended to remain in the patch where initially was chosen even though it was a poor one unless an external disturbing factor intervened. In higher wasp densities, patches were occupied and exploited by competitors as soon as possible and a shorter *per capita* handling time was recorded, while total time of attendance of wasps in patches was similar in all wasp densities. It seems that female wasps respond to presence of the other individuals by further movements and this caused to an increased dispersal and more homogeneous distribution among patches as described by diversity indices and coefficient of variation of wasp numbers in patches. Pseudo-interference; remaining in some patches and leaving the other ones unvisited was however a more important factor than actual interference (patch leaving) in determining final rate of exploitation. A decreasing searching rate and twice higher survival rate of the host was observed as a result of both phenomena. Parasitism rate increased by host density up to two or four clutches and then declined; a typical type III response to host density. Based on the results early season inundative release is recommendable.

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Inhibition of digestive protease and α -amylase enzymes in Colorado potato beetle, *Leptinotarsa decemlineata* (Say) (Coleoptera: Chrysomelidae), by proteinaceous seed extract of pinto bean and white bean

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Introduction: In many cases, interfere with protein and starch digestions of pest insects by defensive protease and α -amylase inhibitors in plants (especially in legumes), is suitable method for the control of pests.

Objectives: In the current study, inhibition of gut α -amylase and protease of Colorado potato beetle, *Leptinotarsa decemlineata* (Say), by seed protein extracts of pinto bean (cv. Talash) and white bean (cv. Daneshkadeh) were investigated.

Material and methods: General protease and α -amylase assays were done using 2% azocasein and 1% starch, respectively.

Results: The maximum inhibitory effect of both extracts for α -amylase and protease was at pH 5 and 6, respectively. The results showed that four proteinaceous fractions (0-30, 30-50, 50-70, and 70-100% saturation of ammonium sulfate) of pinto bean and white bean caused 41, 37, 45 and 46% and 45, 39, 39 and 42% inhibition on the α -amylase activities and less than 15% inhibition on the protease activities of the L4, respectively. The effects of pinto bean fractions on the different stages α -amylase activity were; fraction 0-30% resulted 58, 58, 63, 45 and 63%, fraction 30-50% resulted 54, 52, 62, 49 and 59%, fraction 50-70% resulted 62, 56, 68, 56 and 65% and fraction 70-100% resulted 66, 58, 66, 59 and 65%, Also, in the case of white bean, fraction 0-30% resulted 60, 58, 69, 52 and 65%, fraction 30-50% resulted 60, 53, 58, 49 and 56%, fraction 50-70% resulted 59, 52, 57, 47 and 62% and fraction 70-100% resulted 56, 53, 55, 41 and 57% decreases in the L1, L2, L3, L4 and adult's α -amylase activity, respectively. To gain an insight toward kinetic inhibition of adult's α -amylase by last fraction of pinto bean and first fraction of white bean, Lineweaver-Burk plot was drawn. The type of inhibition was determined partial uncompetitive in the both cases. White bean proteinaceous extract ($K_i=0.039$ mg/ml) had inhibitory activity higher than pinto bean ($K_i=0.103$ mg/ml). In the zymogram, two isozymes for α -amylase were detected and the inhibitory effects were seen as reduction in the intensity of the main band and elimination of the other one. As a whole, gut α -amylase is more sensitive than protease to inhibitory effect of extracts.

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Conclusion: These data revealed that these legume seed extracts can interfere with digestive α -amylase of the Colorado potato beetle present an interesting potential for the development of insect-resistant transgenic plants.

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Determination of life table parameters of *Hippodamia variagata* (Col.: Coccinellidae) by feeding on *Nasonovia ribisnigri*

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The lettuce aphid, *Nasonovia ribisnigri* (Mosley) (Hemiptera: Aphididae), a lettuce specialist herbivore, native of temperate regions, has become one of the most serious pests of lettuce worldwide within the last decades. *N. ribisnigri* reduces the lettuce yield directly by causing leaf distortion, reducing seedling vigour and deforming lettuce heads. Furthermore, the presence of *N. ribisnigri* decreases the percentage of harvested heads that can go to market, which can have serious financial implications for the producers. *Hippodamia variagata* (Goeze), a Palearctic coccinellid species is a widespread aphidophagous predator. It has been a native species in Iran. The study of performance of biocontrol agents is critical for using these species in the biocontrol decision-making process. In this research, we studied the life table parameters of *H. variagata* on *N. ribisnigri*. The development, reproduction and life table parameters of *H. variagata* were studied at constant temperatures ($25 \pm 1^\circ\text{C}$). To approach this purpose, 100 eggs with less than 6 hours old were selected and maintained individually on leaf disc in Petri dishes. Newly emerged larvae were transferred to individual dishes and larval development and mortality were assessed every 12 h. After the emergence of adults, males and females with less than 6 hours old were paired and transferred to new dishes. They were checked daily to record survival and fecundity until death. The data showed that the duration of total pre-adult stage of ladybird beetle and the oviposition period lasted 15.19 and 50.06 ± 0.32 days, with females laying an average of 1253.61 ± 17.8 eggs. The mortality rate was observed 14%. Life table data were analyzed using an age-stage, two-sex life table. The intrinsic rate of increase (r_m), finite rate of increase (λ), net reproductive rate (R_0) and mean generation time (T) were recorded 0.2413 ± 0.0039 , 1.239 ± 0.0048 (d^{-1}), 819.03 ± 68.92 (offspring) and 31.3 ± 0.28 (day). Male adult *H. variagata* lived an average of 57.33 d, which was longer than that of the female adults (54.33). According to these results, it seems that *H. variagata* will be a suitable candidate for biocontrol of *N. ribisnigri* in Iranian lettuce fields.

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The biology of *Dicrodiplosis manihoti* Harris (Dip.: Cecidomyiidae) and its interactions with *Nipaecoccus viridis* (Newstead) (Hem.: Pseudococcidae)

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The spherical mealybug, *N. viridis* (Hem.: Pseudococcidae) is native to Asia and widespread throughout the tropics and subtropics. The pest has been recorded attacking over 100 plant species in more than 30 families. It is a common pest of ornamentals such as citrus. On citrus, the pest frequently causes curling and dwarfing of young growth. Heavy infestations result in deterioration of the crown, which turns yellow, wilts and eventually dies. Over 25 species of predators have been recorded from this pest populations, such as cecidomyid larvae (Dip.: Cecidomyiidae). One species of *Dicrodiplosis* were collected in mealybugs colonies, *N. viridis*, on citrus trees in different parts of Fars Province, during 2012-2014. Since the most common fly species was *D. manihoti*. *N. viridis* were reared on *Solanum tuberosum* tubers in an incubator ($27 \pm 1^\circ\text{C}$, 16:8 (L:D) h and $65 \pm 5\%$ RH). Cultures were maintained in clear plastic boxes (30*20*7 cm). *D. manihoti* was reared on the mealybug onto sleeve cage at growth chamber. The biology and some predator - prey interactions of the predator were studied on *N. viridis* as prey. The mean developmental times of *D. manihoti* from egg to adult at 15, 20, 27 and 30°C were 23.78, 14.5, 13.14 and 12.71 days, respectively. By increasing temperature from 15 to 37°C , the daily mean feeding rate of larvae increased. The fly larvae consumed maximum number of prey at 37°C (4.5 ± 1.49 preys per day). The life table constructed for the midges fly at $26 \pm 1^\circ\text{C}$ showed that the female's longevity from emerging until death time was about 56 days and the probability of death increased during last 10 days of the adult's life expectancy. The age specific life table revealed that each female produced 21.76 offspring during all over life time. The net reproductive rate (R_0), intrinsic rate of increase (r_m) and innate rate of increase (λ) were estimated, 18.5, 0.109 and 1.11, respectively. The results of the study suggest that this species show promising qualities as a predator of mealybugs. The results showed that *D. manihoti* is able to control the mealybug in the special conditions.

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Effects of chitosan oligosaccharides on improving cold resistance ,yield components and production quality of different wheat cultivars (*Triticum aestivum* L.)

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Introduction: Chitosan oligosaccharides (COS) have been shown a wide range of biological applications in plant defense induction and growth promotion, however, little information is known about the effects of COS on improving cold resistance in plant.

Objectives: In Northwest China, the wheat suffered severe production reduction after late spring coldness. This study was designed to investigate the effects of COS on improving cold resistance in wheat, and provide detailed information about the effects of COS on wheat in large-scale field production.

Materials and methods: COS (degree of polymerization = 2-10; degree of deacetylation > 95%) was obtained by enzyme hydrolysis degradation of chitosan. In the lab research, wheat seedlings was treated with COS before transferred to low temperature chamber, the electric conductivity, MDA level, total chlorophyll and water soluble sugar were assessed. In test field, seeddressing and foliar spraying at different growth stages with COS were applied to four wheat cultivars. The yield components and production quality of the COS treatments were measured.

Results: Our results showed that COS has the effect on improving cold resistance in wheat. The concentration of COS at 75 mg/L show the most effective results. In the lab research, the COS group showed a lower growth in electric conductivity and MDA level to control, while total chlorophyll, water soluble sugar increased. In test field, the wheat seedlings treated with COS suffered less damage after late spring coldness compared to the control. The total chlorophyll content of COS group increased by 10% over control. COS impacted the grain yield in all irrigated cultivars, whereas no significant COS effects on rainfed cultivar were observed. In the irrigated wheat cultivars, grains per spike from the COS seeddressing were significantly improved, and the spike number from COS spraying at tillering stage and returning-green stage increased obviously. Improvements in spikelet sterility, plant height and the first internode length were detected in the COS treated cultivars. In COS spraying treatments, the flour rate and protein content exhibited a decreasing trend in irrigated cultivars, whereas a reduction of the gluten index and farinograph value were found in the rainfed cultivars.

Conclusion: These results from field experiments revealed that COS could affect the yield components and the production quality of wheat by promoting photosynthesis and metabolism at low temperature, and it is economical to apply COS as a seed coating or foliar spraying agent in agriculture production.

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Preliminary evaluation of pest and diseases affecting wild and cultivated *Gentiana lutea* L. subsp. *aurantiaca*

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Introduction: *G. lutea* L subsp. *aurantiaca* M. Lainz. is an endangered medicinal plant endemic from the silicic soils of the northwestern mountains of the Iberian Peninsula, mainly León Province. Due to the over-exploitation of wild stocks, the size of the populations is very small. With this small size, the damages caused, mainly by pest, affect more intensively their reproduction capacity and the wild population survival. Cultivation of gentian is a way to protect wild populations from being decimated. Different assays have being carried out establishing experimental cultivation fields into and outside the habitat of this subspecies.

Objectives: The aim of this study is to identify the principal pest and diseases that affect *G. lutea* subsp. *aurantiaca* in its habitat and cultivated under ecologic farming outside its habitat (from nursery to regular fields).

Materials and methods: Regular trips to mountainous areas were made from 2009 to 2014 during the vegetative period of gentian to identify the damages over the plants, collecting samples of tissues and insects.

Insects causing damages and tissues with evidences of being affected by diseases were collected in the same period from seeds, seedlings and cultivated fields of subspecies *aurantiaca*, carried into and outside gentian habitat in León Province. Also samples from wild populations from mountainous of León Province areas were collected in this period.

Identification of pest and diseases were carried out with the collaboration of the Laboratorio de Diagnóstico de Plagas y Enfermedades Vegetales (University of León).

Results: Observed pest and diseases over *G. lutea* subsp. *aurantiaca* are represented in Table 1:

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Table 1: Pest and diseases affecting cultivated and wild *G. lutea* subsp. *aurantiaca*, and caused damages

	Wild	Cultivated	Damages
Hemiptera	X	X	<i>Aphis</i> sp. and <i>Myzus persicae</i> affecting the development of young plants in nursery and transplanted <i>Aphis orocantabrica</i> a monoecious holocycle species over <i>Gentiana lutea</i> L. in wild populations (not important damages)
Hymenoptera	X	X	Damages by larval stages, mainly in the seeds (wild populations)
Lepidoptera	X	X	Important damages feeding on seeds in wild populations and destroying seedlings in the nursery, feeding on leaves
Acari (Tetranychidae)		X	Important damages by feeding on vegetal tissues of seedlings and young plants in the nursery
Gastropoda		X	Important damages by feeding seedlings and young cultivated plants
<i>Botrytis</i> sp.		X	Damages in seedbeds
<i>Fusarium</i> sp.		X	Causing the death of an important number of young plants in nursery and in the first year after being transplanted

Conclusions: In the natural habitat of *G. lutea* subsp. *aurantiaca*, the main phytosanitary problem over the wild populations is the destruction of the seeds mainly by Hymenoptera and larval stages of Lepidoptera and species. In small populations of gentian it can suppose an impact over their expansion capacity.

When cultivated, the main problems affecting *G. lutea* subsp. *aurantiaca* are: in seedbeds, *Botrytis* sp. causing the death of a high percentage of the seeds. Limaces and larval stages of Lepidoptera defoliate and feed the seedlings. The main problem in cultivated plants is *Fusarium* sp. which affects plants from the nursery to fields in the next two years after being transplanted, both, out and into gentian habitat.

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Multiple Resistance of Abamectin, Spirodiclofen and Fenpropathrin to *Tetranychus urticae*

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Two-spotted spide mite *Tetranychus urticae*, which is a worldwide agricultural pest mites, was controlled by chemical pesticides as the main measurements in field, but the mite has serious resistance to many insecticides in recent years because of using acaricide incorrectly, which lead to the control times increase each year and seriously affect the development of pollution-free agriculture. with the development of study in pesticide structure, activity and resistance mechanism, it was proposed that the program of using mix pesticides at same field, but blind, a lot, alternating and mixed pesticides, brought multiple-resistant of the mites. In this study, three kinds of insecticides, spiroadiclofen, avermectin, fenpropathrin, were selected to indicate multiple resistance mechanism and resistance monitoring to *Tetranychus urticae*. through breeding by the three mixed insecticides to obtain multiple resistant strains of *T. urticae*, then the major detoxification enzymes MFOs, CarEs and GSTs and gene expression changes were analyzed between mixed resistance (Mix-R) strains and susceptible strains (SS) in order to clarify the molecular mechanism of multiple resistance, to establish rapid multi-resistant molecular monitoring technology, to provide theoretical basis for multiple resistance management of field.

1. to obtain Mix-R strains of *T. urticae* in laboratory condition. *T. urticae* were treated by mixture of spiroadiclofen, avermectin and fenpropathrin for continuous 59 generations, the LC₅₀ of mixture increased from 2.725 mg/L to 304.327mg/L, resistance ratio reached 110.584 times.

2. Using RT-qPCR technology, ELFn as reference gene, the expression amount of total 10 gene of *T. urticae*, including TuGSTd01, TuGSTd05, TuGSTd06 and TuGSTd09 of GSTs , CYP392D8, CYP392E10, CYP392A6, CYP392A of P450 enzymes, TuCCE35, TuCCE3616 of CarEs Enzymes were analyzed. The result showed the expression amount of Mix-R strain higher than Fe-R, Av-R, Sp-R and susceptible strains, which indicate that these genes were very related to mixture multi-resistant of avermectin, fenpropathrin and Spirodiclofen of *T. urticae*.

3. By combined with allele genotyping technology, multiplex PCR and semi-quantitative RT-PCR method, the mutation sites of F1538I of anti-fenpropathrin and G326E of anti-avermectin of *T. urticae*. By gene expression analysis, F1538I mutations expression is 1.02 times of the SS, 2.33 times of wild homozygotes; G326E mutations expression is 1.48 times the SS, 1.09 times the wild homozygotes. Mix-R strains gene frequencies and expression levels higher than the susceptible strain. So the method can be used to detect sodium ion channel mutation, glutamate-gated chloride channels associated with resistance genes can be used to monitor multi-resistant of *T. urticae* in the field.

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Demography of *Telenomus busseolae* (Hym.: Scelionidae) on two sugarcane stem borers

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The sugarcane stem borers, *Sesamia cretica* Led. and *S. nonagrioides* Lef. are the most important pests of sugarcane in Iran. The egg parasitoid wasp, *Telenomus busseolae* Gahan is the most important natural enemy of *Sesamia* spp. in Khuzestan province that play an important role in regulating populations of sugarcane stem borers. In order to evaluate the efficiency of *T. busseolae* on two hosts laboratory investigations were carried out.

Biology and life table of *T. busseolae* were studied on two hosts at three constant temperatures (20, 25 and 30 °C). Results of regression analysis showed that temperature had a significant effect on adult period and fecundity and there was no effect on progeny sex ratio on both hosts. The intrinsic rate of increase at various temperatures was greater with *S. cretica* than *S. nonagrioides* eggs. The intrinsic rate of increase at these temperatures on *S. cretica* was 0.113, 0.174 and 0.298 d⁻¹ respectively and on *S. nonagrioides* it was 0.103, 0.164 and 0.278 d⁻¹ respectively.

Our results suggest that *S. cretica* eggs are superior host to *T. busseolae*.

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Influence of Inoculation Technic in the Biocontrol Capacity of *Trichoderma harzianum* against *Fusarium oxysporum* from Protected Geographical Indication (PGI) "Alubia de la Bañeza-León"

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The common bean (*Phaseolus vulgaris* L.) is the third most important food legume crop worldwide. *Fusarium oxysporum* Schlecht is a pathogen that affects the bean in the province of León (Spain). It can use biocontrol agents such as *Trichoderma* to control *Fusarium*. *Trichoderma* (Teleomorph: *Hypocrea*) is a fungal genus that is found in the soil. It produces large numbers of spores, enzymes and compounds with antimicrobial activity.

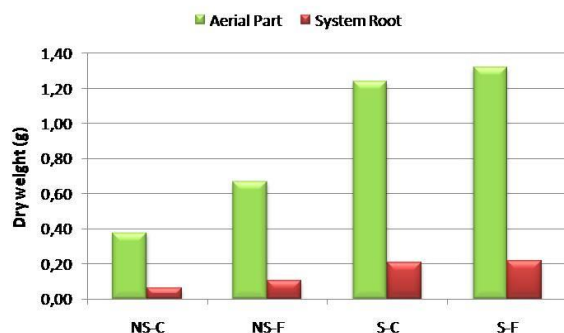
In this study we used two types of inoculation, spore and mycelium suspension of an isolate of *Trichoderma harzianum* to evaluate their effect on the growth of bean plants against *Fusarium oxysporum*.

It was conducted with one isolate of *T. harzianum* and one of *F. oxysporum* collected from the production area of the Protected Geographical Indication (PGI), called "Alubia La Bañeza - León". *F. oxysporum* was inoculated by surface irrigation with 50 ml per pot of a suspension of triturated monosporic culture (5 Petri dishes/litre). The coated seed with a final concentration of 2×10^7 spores/ml spore of *Trichoderma* were sown and the others without spore were sown and inoculated with 500 µl of mycelium suspension of *T. harzianum*. They were sown after 8 days of the inoculation of *F. oxysporum*. The next parameters were evaluated in removed plants after 45 days from sowing: wet weight and dry weight (72 hours in an oven, 82 °C) of the aerial part and root system. The data were compared by analysis of variance (ANOVA) and Fisher least significant difference (LSD) tests using SAS (SAS Institute Inc., 2004, Cary, NC, USA).

When aerial parts were analysed, plant treated with spore of *T. harzianum* had greater weight that plant treated with mycelium suspension. In the case of root system the situation was similar to that observed in the aerial parts (Fig. 1).

In conclusion, when plants are treated with spores suspension of *T. harzianum* were greater grown and were more protected against of *F. oxysporum* that where they were inoculated with mycelium suspension.

Figure 1: Evaluation of the dry weight (g) of the aerial parts and the system root of bean plants grown during 45 days after sowing. [*T. harzianum* no sporulate and without pathogen (NS-C), *T. harzianum* no sporulate and with *F. oxysporum* (NS-F), *T. harzianum* sporulate and without pathogen (S-C), *T. harzianum* sporulate and with *F. oxysporum* (S-F)].



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Fungi associated with cereal cyst nematodes in the Bouira region (Algeria)

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Cereal crops are invaded by cereal cyst nematodes particularly the species *Heterodera avenae* that is causing significant worldwide losses of production. Among the means of struggle, antagonistic fungi are of great interest in biological control against these parasites.

The aim of this study is to investigate parasitic fungi cysts of *Heterodera* sp.

Cysts are disinfected and planted in five culture media: CMA, PCA, PDA, MEA, and CZ- dox.

Identification of isolates is achieved through the key Barnett and Hunter (1998) and we enumerated six fungal genera:

Geotrichum sp., *Torula* sp., *Acremonium* sp., *Cylindrocarpon* sp., *Fusarium* sp. and *Aspergillus* sp.

These types are reported to be antagonists of plant parasitic nematodes especially the genera *Fusarium* sp. and *Aspergillus* sp. associated with cysts of *Heterodera avenae*.

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Effects of Bt cotton on fitness of *Macrolophus pygmeus* Rambur (Hemiptera: Miridae) Solmaz Azimi, Masood Tohidfar,

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One of the most important researches on genetically modified plants is impact of the plants on non-target arthropods. These plants can direct effects (mortality) and indirect (via herbivores) on natural enemies. Improving the use of biotechnological plant resistance for herbivore control critically depends on predictable interactions with nontarget organisms, including natural enemies. Therefore in this research, the effect of Bt cotton and prey (*Bemisia tabaci* Gennadius) on the *Macrolophus pygmeus* Rambur. Feeding four diets including Bt-cotton+*Bemisia tabaci* (first group), non Bt-cotton+*Bemisia tabaci* (second group), Bt-cotton (third group), non Bt-cotton (forth group) were used for experiments. The results showed that Bt-cotton, had direct effect significantly and also through predator on development time and fecundity. Nymph development time in the first and third group was 19.85 ± 0.32 and 29.42 ± 0.45 days respectively but this parameter in the second and forth group (control), showed 16.08 ± 0.24 and 23.11 ± 0.23 days. Also, total lying eggs in treatment of non Bt-cotton+*Bemisia tabaci* was significantly more than other groups (32.778 ± 0.97). While, there was not any significant difference between Bt-cotton and non Bt-cotton fecundity. Thus, the results indicated that the Bt-cotton has potential to affect severely fitness of *Macrolophus pygmeus*, directly or through effects on the prey, and therefore using transgenic plants as one of integrated pest management agent, needs still more experiments and more care.

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In vivo and in vitro models for evaluation of mycorrhizae and root aquaporins participation in response of mature maize plants to low water potential stress

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For purposes of comparative evaluation of different mycorrhizal isolates as protective agents against water stress there is a need for plant growth cultures with standardized parameters.

According to this postulate we developed green cabinet pot culture system with following key components. The AMF inoculum (250 spores per pot) was obtained from monoxenic sterile cultures (TERI, New Dehli). Naturally germ-free coconut/ river sand mixture was used as a nutritionally inert substrate. Its excellent water-air exchange properties allowed to obtain severe (-1,8 MPa leaf water potential) but fully reversible drought stress in 7 days. This minimized the risk of side-effects related to depletion of nutrition by mycorrhizal plants which continued assimilation during gradual drought. Finally, fertilizer with lowered P content (NPKMg 19:6:20:3 and microelements) and clearly defined dosage per week (114mg N & 36mg P₂O₅ /week /plant /4L pot) provided good balance between plant needs and fungal colonisation rate.

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We used this system to investigate protein accumulation of PIP1 and PIP2 type aquaporins during severe drought and recovery in roots of mature maize plants inoculated with arbuscular fungi - *Rhizophagus irregularis*. The level of water stress and recovery was monitored using psychrometric, chlorophyll fluorescence, nitrogen status and leaf gas exchange measurement equipment. Moreover, in order to in vitro reproduction of observed drought stress effects we developed an incubation system on rotary shaker with subsequent microcentrifugation procedure specialized to obtain microsomal proteins from small plant samples. Root fragments were isolated from pots with non-stressed plants or during drought period and incubated with PEG 6000 of $\Psi = -1.37$ MPa or non-PEG solution. This method allowed to replicate profiles of PIP1 and PIP2 proteins accumulation under changing water regimes very similar to pot experiments.

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Investigations on the mechanism of herbicidal properties of aqueous extracts of sunflower shoots, their total phenolic acids and the herbicide trifluralin on seed germination and early growth of some weed and crop species

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Investigations were made of effects of aqueous shoot extract from two month old sunflower plants, the total phenolic acids identified within these extracts and the herbicide trifluralin on sugar content, protein content, proline content, DNA content and gibberellic acid (GA) content of *Brassica napus* (Rapeseed), *Cephalaria syriaca* (Syrian *Cephalaria*), *Triticum aestivum* (Wheat) and *Secale cereale* (Rye) seedlings.

Aqueous shoot extract (3% w/v) was prepared from 2 months old shoots of sunflower. High performance liquid chromatography (HPLC) was used to identify and quantify the phenolic acids present in these extracts. There were twelve phenolic acids - Gallic acid; Syringic acid; Vanillic acid; Protocatechuic acid; Catechol; 4-Hydroxybenzoic acid; P-coumaric acid; Sinapic acid; Ferulic acid; Caffeic acid; Chlorogenic acid; Trans-cinnamic acid. A solution of these total phenolic acids was prepared, with the concentration of each one determined by the HPLC measurements. The effects of this solution and the herbicide trifluralin (100ppm, positive control) on important chemical constituents of weed and crop species grown in petri dishes in a growth chamber were assessed to indicate possible mechanisms of their allelopathic and herbicidal actions .

Sugar content: both trifluralin and sunflower extract significantly decreased sugar content ($P < 0.001$) of the test species, but the magnitude of the effect of trifluralin was much greater than the extract. Protein content: both trifluralin and sunflower extract significantly ($P < 0.001$) increased protein content of *Triticum aestivum* and *Secale cereale*, with the extract having the greatest effect. Proline content: total phenolic acids significantly reduced proline content in *Secale cereale* and *Cephalaria syriaca*; the effect of trifluralin was only significant for *Cephalaria syriaca* ($P < 0.001$); sunflower extract had no significant effects on proline content of any of the test species. DNA content: trifluralin had the greatest significant effect on DNA content of *Triticum aestivum* and *Secale cereale* ($P < 0.001$) and total phenolic acids the least. Gibberellic acid: sunflower extract had the greatest significant effect on gibberellic acid contents. In contrast, total phenolic acids had least significant reduction of proline content in *Brassica napus* ($P < 0.001$). In conclusion, sunflower 2 months old aqueous extract and its total phenolic acids has less significant effect on seed germination and early growth in comparison with herbicide trifluralin.

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P PPI 1

The study of antagonistic activity of *Trichoderma atroviride* and the influence of wheat genotypes in the protection against root rot disease

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Fusarium head blight and root rot are two major diseases that affect wheat, causing yield loss and also grain contamination by mycotoxins. In Algeria as in the Mediterranean area, these last years the most predominant species is *F. culmorum* which can be associated with root and collar rot and head blight. The use of chemical control method against *Fusarium* diseases is still possible but with limited effectiveness. This status involves the use of biological control agent as an alternative method to manage these disease. In this way, the main objective of this work is the study of the antagonistic activity of an isolate belonging to *T. atroviride* species (Ta.13) against *F. culmorum*.

The *in vitro* test of Ta.13 antagonistic activity isolate showed that this isolate effectively inhibited *F. culmorum* isolates growth. Data recorded showed that the percentage of growth inhibition varied between 88.47 and 97.35% in the case of direct confrontation (dual cultures) and 51.95 and 84% by indirect confrontation under the Ta.13 antifungal volatils substances.

By *in vivo* test, seed treatment of seven wheat genotypes (Vitron, Waha, Bousselem, GTA, ARZ, Ain Abid, Hiddab 1220) by Ta13 conidia suspension before the sowing in a soil already infested by *F. culmorum* showed that this strain effectively reduce disease index compared to the untreated control. Significant difference depending on the variety where obtained and the highest percentage of disease reduction was 86% recorded for Waha variety.

P PPI 2

Isolation, identification and seed transmission of ice nucleation active bacteria *Pseudomonas syringae* from dry agrosystems

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Pseudomonas syringae (*P.s.*) is a plant pathogen well known for its capacity to grow epiphytically on diverse plants and for its ice-nucleation activity. This study is demonstrating that large epiphytic populations of ice nucleation active (INA) bacteria could establish on some wheat cultivars without causing disease. This approach led to the evaluation of combinations of bacterial strains and wheat cultivars. 25 samples from leaves of cultivated bread wheat in the Tel Hadya ICARDAs' fields were studied for presence of *P.s.* on semi-selective medium. The biochemical characterizations and Ice nucleation activity had been studied with the ability of the bacterium to transmitting to the seeds and its persistence on the seeds after storing period.

Results showed that only 14 leaves samples exposed presence of the genus *Pseudomonas*, whereas just 5 isolates were the target bacterium *P.s.* after testing its biochemical characterizations and Ice nucleation activity.

The study aimed to obtain non-pathogenic isolates, yet two out of five screened isolates did induce hypersensitive reactions. The reminders (three isolates) did not induce hypersensitive reactions, indicating that they might be non-pathogens or only mildly pathogenic.

Transmission of *P. syringae* from plants to their seeds was studied immediately after seed harvest and again after seed storage for three months. Results indicated that there are 12 strains out of the 25 tested that clearly showed bacterial transmission to the seeds under natural inoculation and it only 6 isolates remained active after 3 months.

P PPI 3

Distribution and Incidence of Apple Powdery Mildew in a Mixed Cultivar Orchards and Relationship to Disease Severity

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Apple powdery mildew epidemics, caused by *Podosphaera leucotricha* (Ell. and Ev.) Salm. can be readily described in terms of the disease triangle. The role of different environmental factors, viz., temperature, relative humidity, leaf wetness, sunshine and rainfall were studied in relation to disease development. The present experiment was conducted during the season 2003 to 2005 to determine a simplified assessment procedure by which apple powdery mildew severity/index could be predicted from

incidence data and develop incidence-severity relationship in apple cultivars under Uttaranchal hilly conditions. The use of percentage scales and keys of visual disease severity, remote sensing, and some indirect methods like spore counts and disease incidence are considered valid approaches for disease assessment. The relationship between increase in incidence of powdery mildew in relation to severity can be established either by making sequential records in one tree during the progress of an epidemic or by assessing many trees with different amounts of disease at one point of time. The combination of several factors like the presence of susceptible host, virulent pathogen, and congenial environment for disease development during receptive phenological stage of apple tree., was responsible for the incidence of the powdery mildew on apple.

P PPI 4

FocVel1* is required for biofilm formation, and virulence in *Fusarium oxysporum* f. sp. *cucumerinum

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Question: In an earlier study by our group, *F. oxysporum* f. sp. *cucumerinum* was found to form biofilms in flat-bottomed polystyrene microtitre plates (1). Biofilm formation not only represents a mere biological coating but also provides important clues for determining appropriate therapeutic strategies against certain microbes. Therefore, a better understanding of the regulatory mechanisms of biofilm formation and virulence will be essential to facilitate the development of efficient control strategies against cucumber Fusarium wilt.

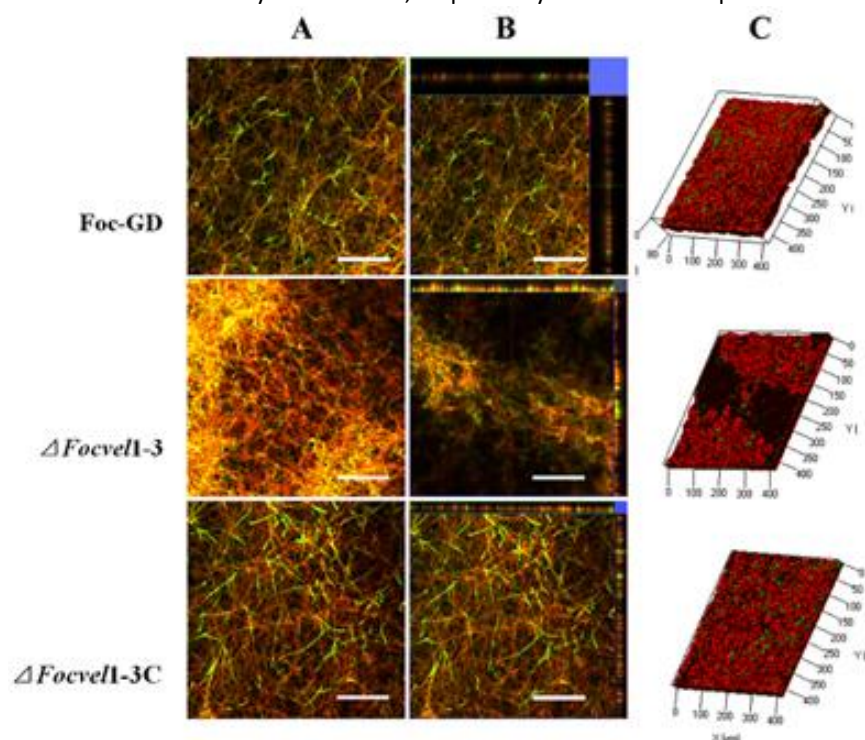
Methods: The deletion vector and the complement plasmid were constructed and transformed into protoplasts of Foc-GD. The architecture of biofilms was observed using a Zeiss LSM710 confocal laser-scanning microscope.

Results: The mutant $\Delta FocVel1-3$ showed significant defects in the thin biofilms, exhibiting heterogeneous hyphae and EPS production (Fig. 1). The disruption of *FocVel1* reduced the virulence of *F. oxysporum* f. sp. *cucumerinum* on cucumbers (Fig. 2).

Conclusions: In conclusion, our study demonstrated that *FocVel1* contributed to the attenuated virulence and biofilm formation in *F. oxysporum* f. sp. *cucumerinum*. Therefore, it will be interesting to elucidate relationships between biofilm formation and pathogenic mechanisms in *F. oxysporum*, which may improve our understanding of the biology of *F. oxysporum* f. sp. *cucumerinum*.

Acknowledgments: This work was supported by the Innovation Project of Guangxi Graduate Education (YCBZ2014017).

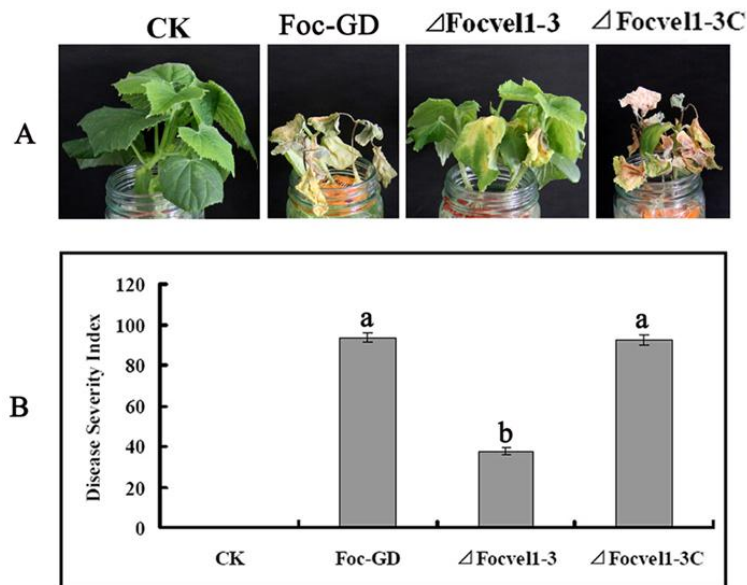
Figure 1: CLSM images of biofilm formation by the wild-type strain (Foc-GD), *FocVel1* deletion mutant ($\Delta FocVel1-3$) and complemented strain ($\Delta FocVel1-3C$) at 48 h. Cell wall-like polysaccharides and heat-killed biofilm cells were marked with green and red fluorescence by ConA and PI, respectively. Scale bar: 100 μ m.



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Figure 2: Effects of *FocVel1* on the virulence of *Fusarium oxysporum* f. sp. *cucumerinum*. Cucumber seedlings were inoculated with 1×10^6 conidia/mL from each strain. (A) DSIs in cucumber seedlings 15 days after inoculation. (B) Symptoms of cucumber plants were photographed 15 days after inoculation.



P PPI 5

Identification of pathogenic races of *Tilletia carries* the agents of wheat common bunt disease in Iraq

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Common bunt disease incited by *Tilletia carries*, is one of the most destructive disease of wheat in Iraq, that can cause sever yield losses when the susceptible cultivars are grown without chemical dressing. Collections of common bunt were prepared from bunt-infected spikes from the main wheat growing areas during 2012/13 seasons. Eighteen isolates from these collections were selected based on high germination percent of the teliospores and used for artificial inoculation of international differential set genotypes at faculty of agricultural sciences field in Sulaimania. Results revealed that there is a wide genetic diversity among *T. carries* isolates representing different locations. 15 races of *Tilletia carries* and *T. foetida* pathogens were identified according to the international nomenclature system. Nine of these races match the international races T1, T2, T4, T9, T11, T17, T18, L1 and L2, while the rest 6 races may be new. Genes Bt2, Bt14 and Bt15 were ineffective against most of the races while Bt4 and Bt8 were ineffective against four races, Bt1, Bt3 and Bt5 were ineffective against three races and genes Bt7, Bt9 and Bt13 were ineffective only against two races. All the identified races were can't overcome the resistance of the known resistant genes Bt11 and Bt12 while only one race was able to overcome the resistant gene Bt10. Confirmation of the identification of these races on the molecular bases is further needed.

P PPI 6

Biocontrol of *Rhizoctonia* disease of potato by *B. subtilis*

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Each environmental condition has its own microbial community. Therefore, it is important to select a biocontrol agent that previously adapted or at least already has growth conditions similar to the environment, where it will be applied. Chitinase (glycanohydrolase) is considered as an important enzyme in various fields including biological control and forms a suitable candidate to evaluate such hypothesis. *Bacillus subtilis* chitinase was optimally synthesized under batch fermentation conditions similar to those in the soil. The maximum chitinase production was obtained at initial pH 8 and 30 °C. Under laboratory

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conditions, chitinase successfully hydrolyzed the cell wall of the phytopathogen *Rhizoctonia solani* (Kühn), suggesting the presence of chitinase enzyme in the bacterial filtrate. However, *B. subtilis* could successfully antagonize the growth of *R. solani*. Under greenhouse conditions, incorporation of a bacterial suspension of *B. subtilis* at 10^9 cell mL⁻¹ in potato production programs reduced the stem canker and black scurf diseases caused by *R. solani*. Additionally, it improved some biochemical parameters, growth and tuber yield. Based on these results, the harmonization and suitability of the soil conditions for the growth and activity of *B. subtilis* guaranteed a high controlling capacity for the target pathogen.

P PPI 7

Cushion gall of cacao, more than *Fusarium decemcellulare* and *Lasiodiplodia theobromae*

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Cushion gall of cacao is a disease with at least five types of symptoms described (green point, flowery, knob, fan and disc). This pathology is globally considered a minor disease, mostly studied in Central and South America. *F. decemcellulare* is the stage recognized from flowery and green point galls, the most common variants of the disease. The aim of this study was to describe the relationship between cultivable fungal species/isolates present in cushion galls of cacao and their pathogenicity. Three key areas of cocoa production in Venezuela were sampled. Cultivable fungal isolates associated with five symptoms of cushion galls were identified by sequencing internal transcribed spacer regions of DNA or morphological observations. To determine gall inducing capacity of the selected isolates, IMC-67 cacao seeds were inoculated, and symptoms were observed at the seedling stage (45d). The whole collections rendered an abundance of 133 isolates (Fig. 1), a richness of 49 taxa, including 23 isolates identified until species level. Nineteen isolates were designated as morphotypes. The dominant species were *F. decemcellulare* and *L. theobromae*; both reached ≈30,8% of all identified isolates. Between 63-44 % of isolates of both species were pathogenic on cacao seedlings (Tab. 1), respectively. From 17 taxa, seven pathogenic and 10 non-pathogenic were observed. Within six of these pathogenic species (*L. theobromae*, *F. decemcellulare*, *F. solani*, *F. incarnatum*, *F. equiseti* and *F. camptocera*), pathogenic and non-pathogenic isolates were detected. Moreover, within a single naturally infected gall, it was found that: i) 2-5 species were identified, indicating a broad diversity of taxa that naturally co-occurred and, ii) co-occurrence of pathogenic and non-pathogenic isolates of the dominant species were also observed. All isolates identified as *Rhizoctonia solani* were able to induce galls. In cushion gall of cacao, the diversity of species and isolates pathogenicity described, together with the endophytic nature of some species, could influence the variety of symptoms associated with it.

Figure 1. Operative taxonomic units identified in five gall types of cacao.

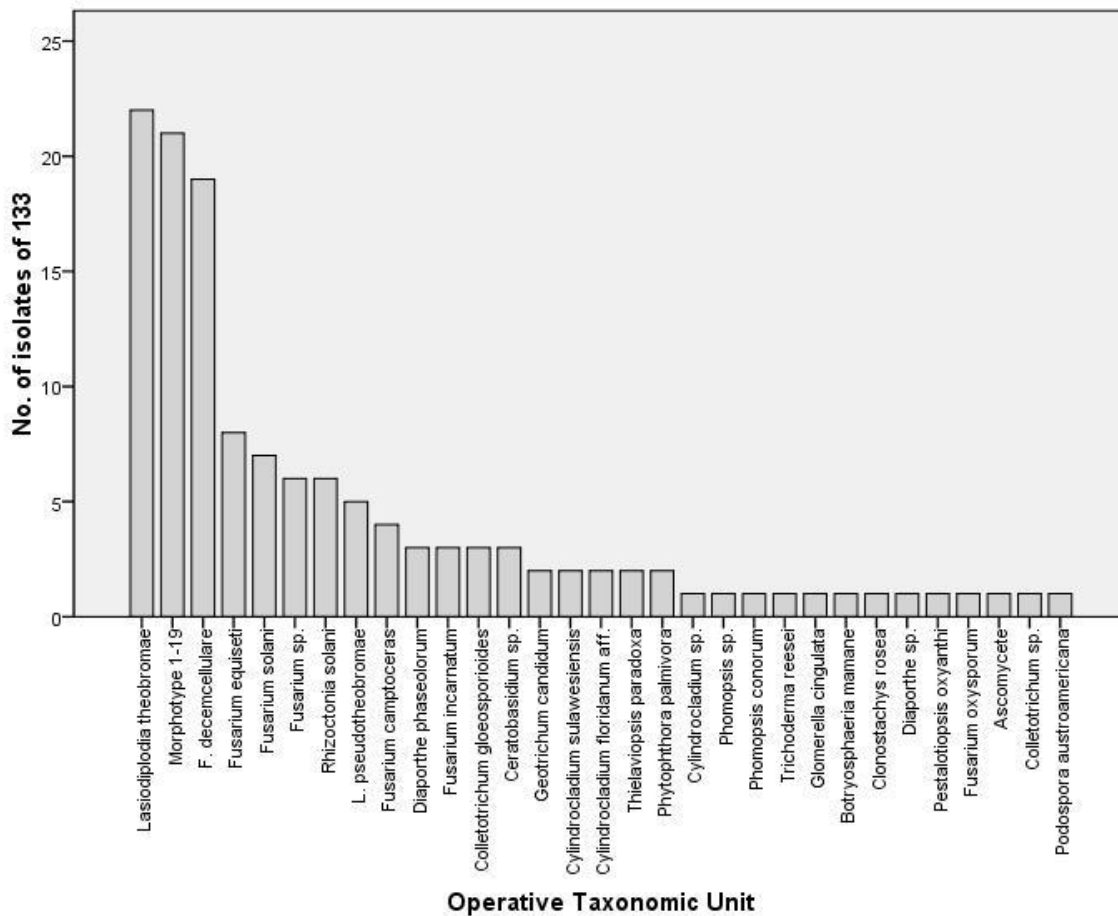


Table 1. Gall inducing capacity of *F. decemcellulare*, *L. theobromae* and other cultivable fungi isolated from galls

Gall types	PATHOGENIC			NOT PATHOGENIC			Total
	<i>F. decemcellulare</i>	<i>L. theobromae</i>	Other Species. & OTU	<i>F. decemcellulare</i>	<i>L. theobromae</i>	Other Species. & OTU	
Fan	2	3	12	3	1	8	29
Flowery	2		1			2	5
Green point	1	1	2	1	3	3	11
Knob			2		1	2	5
Unidentified	2					2	4
Total	7	4	17	4	5	17	54
% total	13.0	7.4	31.5	7.4	9.3	31.5	100
% within sp.	63.6	44.4		36.4	55.6		

P PPI 8

Root rot and leaf spot agents of spring cereals in Mordovia Region, Russian Federation

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The specificity of fungi causing Root Rot and Leaf Spot on cereals was studied in various areas of Mordovia. As a result of mycology researches there were isolated 457 colonies of pathogenic fungi from damaged roots and leaves of spring wheat and barley. There were revealed species of genus *Fusarium*: *F.culmorum*, *F.oxysporum*, *F.heterosporum*, *F.avenaceum*, *F.sambucinum*, *F. redolens*, *F. verticillioides*, *F. tricinctum* and also *Bipolaris sorokiniana*. There was noticed the high frequency of

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occurrence for *F. heterosporum*, *F. sporotrichioides*, *F. oxysporum* and *B. sorokiniana*. Some species as *F. redolens*, *F. verticillioides*, *F. tricinctum* were noted in tests with lower frequency. Twenty-four strains of genera *Fusarium* and *Bipolaris* have been received by monosporous selections. Morphology of these strains was described on the 2% potato agar medium, and pathogenicity and phytotoxicity were tested on seedlings of wheat and barley. The pathogenicity and phytotoxicity of isolates were determined by inhibition levels of seed germination and the coleoptiles and roots lengths after treatment with spore suspensions and cultural liquids filtrates. Intraspecific distinctions on pathogenicity and toxicity among strains of *F. heterosporum*, *F. sporotrichioides*, *F. oxysporum* and *B. sorokiniana* were observed. Strains of *F. heterosporum* and *F. oxysporum* were weak pathogenic and toxic. The majority of *F. sporotrichioides* strains had strong toxicity and pathogenicity for barley and wheat. *B. sorokiniana* strains were the most pathogenic and toxic. Products of a metabolism of these strains suppressed coleoptiles and roots growth of wheat more than for 90%. Characteristics of pathogenicity and phytotoxicity of widespread species of the tested fungi were ambiguous that testifies to their strong intraspecific variability. The strains of seldom found species have possessed toxicity to wheat seedlings, but they were not pathogenic, probably it is possible to explain by their insignificant number in Root Rot fungi populations. Such species as *F. sporotrichioides* and *B. sorokiniana* were the most widespread in a complex of fungi causing cereals Root Rot and Leaf Spot apparently owing to their high variability, plasticity and competitive potential.

P PPI 9

Influence of *Fusarium* isolates on the expression of barley genes related to plant defense and malting quality

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Fusarium head blight (FHB) is a widespread fungal disease of wheat & barley and is a leading cause of economic loss in these crops. Barley grains are commonly made into malt for use in beer and whisky production. Infection and mycotoxin contamination by FHB have been shown to impair the quality of barley grain for use in malting. FHB disease is linked to certain brewing-related problems, including gushing in packaged beer, off-flavors, and reduced fermentation efficiency. Malting quality is a complex trait involving multiple inter-related components. Brewers are able to look at certain biological processes which influence malting quality such as enzymatic activity but not at the underlying gene expression. Favorable conditions for microbial growth are present during malting, enabling microorganisms to interact with the grains metabolically during the process. Subsequently, microorganisms such as *Fusarium* present in the barley grain will have a significant influence in malting performance and final malt quality. The presence of *Fusarium* is likely to impact on barley gene expression both before and during malting, for example through increased expression of genes involved in defense responses. The aim of our research is to improve understanding of barley gene regulation over time, starting from the onset of infection on living grain tissue in the field, right up to preparation of finished malt. As FHB disease is caused by a complex of different *Fusarium* species, we further aim to investigate the impact of different *Fusarium* isolates on barley gene expression over this period. We are currently using quantitative RT-PCR to accurately measure barley gene expression. We will examine the differential expression of malting-related genes, as well as defense-related genes to determine whether these contribute overall to malting quality. This research will increase our fundamental understanding of transcriptional changes in barley in response to different *Fusarium* isolates and produce robust gene markers linked to malting quality for improved brew-monitoring and quality-control.

P PPI 10

Molecular characterization of *Pythium* spp. isolated from tomato seedlings in the Syrian coast

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Tomato seedlings damping-off is a limiting factor in commercial greenhouse production. To determine the causal agents of disease, sampling and fungal isolation were performed during 2012. Samples were collected from infected seedlings growing in greenhouses in the Syrian coastal region, Isolation of fungi was done in the laboratories of the Agronomical Research Center, in Lattakia and the molecular analyses were done in the Biotechnology Center at Tishreen University, Lattakia, Syria, during the years 2012,2013. Eight isolates of *Pythium* sp. obtained were purified using hyphal tip method (named P1, P2, P3, P4, P5, P6, P7 and P8). Isolates were morphologically identified by optical microscope, then molecularly Characterized using genus specific ITS primers. The results of morphological characterization of pathogenic species suggested the detection of *Pythium aphanidermatum*, *P. ultimum*. The analysis of DNAs from the different isolates with ITS primers, recognizing the inter transcript spacer of nuclear ribosomal DNA proved that the eight, isolates were belonging to the species *P. ultimum*. The complete sequences of ribosomal DNA internal transcribed spacers regions of selected isolates were determined and submitted to

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GenBank. The GenBank-BLAST homology search revealed *P. ultimum*. as the most similar sequence (> 99% identity) with GenBank entry AB355596.

P PPI 11

The study of the antagonistic power of four microorgans (Trichoderma viride , Trichoderma harzianum ,Phoma et Camarosporium) on the mycelial growth of Ascochyta pinodella et Ascochyta pinodes. Agents responsible of the aschochyt blight on the pea.

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This present work has the objective of testing the antagonistic effects in vitro of four different microorgans (Trichoderma viride , Trichoderma harzianum ,Phoma and Camarosporium)on the mycelial growth of Ascochyta pinodella and Ascochyta pinodes) Agents responsible of the anthracnoses of the pea . The two technics used to know the direct confrontation and volatile substances, shows a clear antagonistic action of Trichoderma on the mycelial growth of Ascochyta .

In other hands , the antagonistic action of Phoma and Camarosporium stay weak .

This action explains the reduction of the mycelial growth of pathgène .

From these experiences we come up that each of the two antagonistic (Trichoderma viride , Trichoderma harzianum) has a particular aptitude to inhibit agent pathogène and can give positive results in the use of the biologic control .

P PPI 12

Volatile metabolites as markers for *Plasmopara viticola* resistance in two grapevine developmental stages

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Questions: The European grapevine species *Vitis vinifera* is highly susceptible to the obligate biotrophic oomycete *Plasmopara viticola*. Pathogen infection causes heavy yield losses and plant protection is based on high amounts of fungicides. In contrast species like *V. labrusca* or *V. riparia* are resistant against this pathogen.

Comparing the metabolite profiles of resistant and susceptible grapevines will foster the understanding of host-pathogen interactions. The detection of resistance related plant metabolites may also lead to new concepts in plant protection strategies.

Methods: Comparison of metabolite profiles was based on 12 genotypes with different resistance traits against *P. viticola* from the species *V. vinifera*, *V. labrusca*, *V. riparia* and six hybrids. Resistance rates were ascertained by inoculation of leaf disks with *P. viticola*. Grapevine leaves were sampled at developmental stages BBCH 6 and BBCH 9. Volatile organic compounds (VOC) were detected by HS-SPME-GC-MS. Non-targeted chemometrical data processing was used to screen the metabolite profiles. Relationships of metabolites with resistance traits were determined by spearman rank correlation.

Results: This operation resulted in three metabolite markers in BBCH 6 and four markers in BBCH 9. Methyl salicylate and benzaldehyde are highly correlated with resistance in both developmental stages. In BBCH 6 the third marker was metabolite ID86. This metabolite was not detected in leaves of BBCH 9. The two VOCs (Z)-3-hexenol and metabolite ID64 correlated highly with resistance in BBCH 9, but not in BBCH 6, although they were both present in BBCH 6. Identities of ID64 and ID86 are still to be confirmed.

Conclusion: The three identified VOCs are generally related to induced resistance traits. However, in this assay only plants without pathogen inoculation and with no visible symptoms during the trial were analyzed. The methyl salicylate and benzaldehyde concentrations are higher in resistant genotypes than in susceptible ones. (Z)-3-hexenol induces the up-regulation of defence genes, e.g. defence against *Botrytis cinerea* in *Arabidopsis thaliana*. To our knowledge a (Z)-3-hexenol relation to *P. viticola* resistance has never been reported before. In ongoing studies we will analyze, if a (Z)-3-hexenol treatment has an effect to *P. viticola* growth.

P PPI 13

Salicylic acid, a basal resistance component in the interaction of *Verticillium longisporum* and oilseed rape (*Brassica napus* L.)

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Introduction: Oilseed rape (OSR) is the third most important source of vegetable plant oil, however, production may be threatened by a number of constraints including damage caused by diseases. *Verticillium longisporum* (VL) is a soilborne vascular pathogen, which is host-specific on crucifers including OSR. The pathogen has occurred in Europe so far, but recently it was found for the first time in Canadian OSR. Due to a lack of effective fungicides, breeding for resistant OSR cultivars is the main approach against VL. Salicylic acid (SA) was reported in many plants as an essential endogenous mediator of pathogen resistance and therefore we investigated its role in response of OSR to VL.

Objektives: We studied the role of SA in the interaction of VL and OSR, analyzed the correlation between SA levels and disease severity, and tested SA for direct effects on the pathogen.

Materials and methods: *In vitro* and *in planta* experiments were conducted. *In planta* experiments were performed with a transgenic *NahG* line and wild type of spring-type oilseed rape cv. 'Drakkar'. Increasing concentrations of SA were exogenously applied on VL- and mock-inoculated OSR. Disease screenings were done from 7 dpi to 42 dpi. The amount of pathogen and endogenous SA levels in hypocotyls were detected by qPCR and HPLC-fluorescence, respectively. In the *in vitro* experiments, VL was grown on PDA plates with different SA concentrations.

Results: In the *in planta* experiment, the transgenic *NahG* plants were more susceptible than wild type plants to VL infection (higher disease severity and AUDPC, less plant biomass, higher fungal biomass in plants). Infection with VL significantly increased SA levels in wild type OSR but not in *NahG* transformants. While in *NahG* plants SA levels show a strongly negative correlation with infection this is not found in the wild type. Application of SA up to 2.0 mM did not affect infection with VL as it was not taken up by the plants. *In vitro* results demonstrate that SA has no significant fungitoxic effects on VL up to 1 mM.

Conclusions: A basic concentration of SA is required for basal resistance in OSR against VL infection. This is in contrast to previous work on cultivar-specific resistance, which was inversely correlated with resistance, suggesting a second role of elevated levels of SA as factor of susceptibility.

P PPI 14

Potential of rhizobacteria for suppressing *Striga hermonthica* germination and radicle elongation

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The objective of this study was to screen the potential of four plant growth promoting rhizobacteria (PGPR) for suppression of *Striga hermonthica* development. *Bacillus subtilis* Bsn5, *B. subtilis* GBO3, *B. amyloliquefaciens* FZB42 and *Burkholderia phytofirmans* PsJN inocula and their corresponding cell culture supernatants were evaluated in Petri dish and extended agar gel assays (EAGA) for their potential to inhibit germination and radicle growth of *S. hermonthica*. Sorghum root exudates and synthetic stimulant GR24 were used to induce seed germination. *B. subtilis* Bsn5 supernatant, which showed the greatest inhibitory activity, was further separated by ethyl acetate into lipophilic and hydrophilic phases to identify the polarity of the inhibitor. After seven days of growth, effect of PGPR inocula and supernatants on germination and radicle length was analyzed.

Complete *S. hermonthica* germination inhibition (0% germination) occurred in seeds exposed to all PGPR inocula. Similarly, supernatants exhibited inhibitory effect on both germination and radicle length. Compared to the untreated control, germination and radicle elongation was lowest in *B. subtilis* Bsn5 supernatant treatments. There was complete inhibition of germination after exposure to either *B. subtilis* Bsn5 supernatant or 100% hydrophilic fraction of the supernatant. Light microscopy examination of *S. hermonthica* radicles exposed to *B. subtilis* Bsn5 supernatant revealed that stunting of the radicles was due to reduction in cell sizes at the radicle elongation zone. EAGA experiments showed a similar trend of results with *B. subtilis* Bsn5 showing the highest inhibitory activity on germination and radicle elongation compared to other PGPR and control treatments.

This study identified all test strains with promising potential to suppress *S. hermonthica* development. Greenhouse and field experiments are recommended to better understand the efficacy of the strains under natural conditions where other biotic and abiotic factors come into play. Such findings will provide novel information of the effect of PGPR on *S. hermonthica* development and will open new avenues for *S. hermonthica* control.

P PPI 15

Tomato osmotin, NP24 might induce an apoptosis to *Saccharomyces cerevisiae*

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Introduction: Pathogenesis related proteins (PR-Ps) are known to accumulate in plant in response to biotic and abiotic stress. PR-Ps, identified both in dicot and monocot, are classified into 17 different families based on identity of amino acid and immunological homology. Osmotin detected in tobacco cell culture has been categorized as PR-5, which is also called thaumatin like protein because of high similarity to thaumatin from *Thaumatococcus danielli*. Antifungal activity of osmotin against *Fusarium* and *Phytophthora* species has been demonstrated *in vitro*. Further, osmotin was recently found to binds PHO36 in yeast transmembrane and induce cell apoptosis. Tomato (*Solanum lycopersicum*) is one of the important crops in the world. NP24 was isolated as high degree of homology to osmotin from tomato, but most of the function has not been clarified yet.

Objective: The purpose of this investigation was to characterize and identify antifungal function of NP24.

Materials and methods: We amplified full length NP24 cDNA from *Solanum lycopersicum* cv momotaro by PCR using primers designed from the sequence of Heinz1706 osmotin. The open reading frame sequence was cloned into a pET plasmid, expressed in *E.coli*, and resulted protein was purified by affinity chromatography. Antifungal activity of purified NP24 was examined by spot assay against model fungus *Saccharomyces cerevisiae* BY4741 stain.

Results: The NP24 cDNA was 744 bp encoding 247 amino acids. An N-terminal signal peptide of 22 amino acids was present in the protein. Recombinant NP24 protein was purified from crude protein by immobilized metal ion affinity chromatography. NP24 caused significant growth inhibition to viable cells of *S. cerevisiae* BY4741 spheroplast. In contract, growth of ΔPHO36 mutant spheroplast was not inhibited.

Conclusion: Tomato osmotin, NP24 showed growth inhibition to *S. cerevisiae* spheroplast significantly different between wild and mutant strain. These results suggests that NP24 have an influence on cell viability involved in apoptosis via PHO36 similar to tobacco osmotin. Thus, we expect that NP24 might induce apoptosis in pathogenic bacteria by ROS generation via PHO36 like protein.

P PPI 17

Durable resistance to rice blast (*Pyricularia grisea*) in Egypt

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Rice is one of most important food crop in Egypt not only for local consumption but also for exportation. Meanwhile, rice blast disease (*Pyricularia grisea*) is the production constraint. One strategy to improve the durability of blast resistance is to pyramid resistance genes. To do that, extensive studies were conducted on the genetic structure of blast pathogen population at the Rice Research & Training Centre ,Sakha, Egypt, determined composition, distribution and frequency of the avirulence that show race varieties; identified and incorporated resistance genes into commercial rice cultivars using genetic marker; and continuously evaluated and selected breeding lines under high disease pressure and pathogen diversity. Rice differentials with known blast resistance genes have been used to study avirulence gene combinations and frequency in blast pathogen and identify relevant resistance genes. The combination of resistance genes (*Pi-1*, *Pi-2*, *Pi-33*) for which their corresponding avirulence genes are highly conserved in blast pathogen population in Egypt has proven to confer stable blast resistance after several years of testing under high pressure in the field and greenhouse inoculations. Additional pathogen characterization of spontaneous mutations of the blast pathogen allowed the identification of the blast resistance genes (*Pi-b*, *Pi-g* and *Pi-to2*), which will be needed for protecting rice cultivars from potential future change in the avirulence/ virulence genes in the blast pathogen population. Microsatellite markers highly linked to these blast resistance genes have been found from public database facilitating the introgression and pyramiding each of the six blast resistance genes into the Egyptian rice cultivars and elite lines derived from rice breeding programs and aiming at developing rice cultivars with durable blast resistance.

P PPI 18

Current status of chickpea *Ascochyta* blight in Turkey

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Introduction: Turkey is one of the leading chickpea producing country in the world and origin center of chickpea cultivation dating back 10.000-12.000 years (Lev-Yadun et al., 2000). *Ascochyta* blight and *Fusarium* wilt are major diseases that severely restrict chickpea cultivation in Turkey (Kaiser and Kusmenoglu, 1997; Demirci et al., 1999). To perform and breed resistant/tolerant chickpea cultivars against *Ascochyta* blight, it is necessary to define population structure of *Didymella rabiei* in our country.

Objectives: This study was undertaken to explore *Ascochyta* blight incidence, mating type distribution and virulence variations of *D. rabiei* in the chickpea growing areas of Turkey.

Materials and methods: Survey studies to chickpea fields were conducted in 47 chickpea growing provinces covering seven regions of Turkey in 2013-2014 growing season. Altitudes, GPS locations, *Fusarium* wilt occurrence, weed coverage, nodulation and vegetation data's were also collected for each field. A total of 806 (19.536 da) chickpea fields were evaluated and disease incidences were calculated according to Reddy and Singh (1984). Mating types of over 250 *D. rabiei* isolates were analyzed using SP21, COM1 and Tail 5 primers through PCR (Barve et al., 2003). Pathotyping of selected isolates were determined according to Udupa et al., (1998).

Results: The highest disease incidence was observed in the Bosphorus region with %40.11 and followed by Black Sea, Aegean, Central Anatolia, Mediterranean, Southeastern and Eastern regions of Turkey. Disease incidence exhibited negative correlation with elevation ($r = -.314$; $p < .001$) and nodulation ($r = -.076$; $p < .05$). Mat1.1/Mat1.2 ratio was close to 1/1 but differences were observed among regions and provinces. Pathotypes 1, 2, 3, and 4 were detected in all the regions but Pathotype 4 was excessive in amount in institutes where chickpea breeding is being conducted for several years.

Conclusion: *Ascochyta* blight caused by *D. rabiei* was determined to be common and predominant over *Fusarium* wilt in chickpea growing provinces in Turkey. However, *Fusarium* wilt was detected to be an emerging disease in the Aegean, Mediterranean and Bosphorus regions. Occurrence of Pathotype 4 which is a highly virulent group of *D. rabiei* (Imtiaz et al., 2011) was first determined in Turkey through this study.

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P PPI 19

Physiological and morphological changes in cell-wall responses of *Brassica napus* genotypes contrasting in resistance to *Sclerotinia sclerotiorum*

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Introduction: Oilseed rape (*Brassica napus*) is an agronomic relevant host of *Sclerotinia sclerotiorum* (Lib.) de Bary, the causal agent of white mold or stem rot disease. Commercial cultivars exhibit insufficient resistance and fungicide application is the predominant means for disease control. Due to the intensifying cultivation of oilseed rape there is a large interest for the development of resistant cultivars.

Objectives: The major objective of this project is to explore resistance factors in oilseed rape effective against *S. sclerotiorum*. The focus of our analyses was on changes in the cell-wall composition in stem tissue of a set of *B. napus* genotypes varying in resistance to stem rot.

Methods: Two separate greenhouse experiments were conducted with the Chinese oilseed rape line Zhong You 821 and the spring cultivar Loras. For histological observations, the Wiesner staining for detection of G (guaiacyl) lignin and UV light for autofluorescence of phenols were used. Genetic studies on the transcriptome level were performed with quantitative real-time PCR to analyze the expression levels of key genes of the phenylpropanoid pathway in oilseed rape infected with *S. sclerotiorum*.

Results: Histological examination showed an increased accumulation of phenols in the *Sclerotinia* infected tissue as a part of the natural defence responses. The Wiesner staining indicated an elevated constitutive G lignin content in the sclerenchymatous

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sheath of the resistant genotype Zhong You 821. First results of the genetic studies revealed enhanced expression of a cinnamoyl-CoA reductase (*BnCCR2*) in the resistant Zhong You 821, which is suggested to be linked to enhanced G lignin synthesis.

Conclusions: The strong expression of *BnCCR2* and the elevated constitutive deposition of G lignin indicate a potential role of the secondary cell-wall composition and the accumulation of monolignols in the cell-wall as resistance factors against *Sclerotinia* infection.

P PPI 20

Relationship between the alkaloid content of *Lupinus angustifolius* L. genotypes and aphid multiplication and feeding

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Introduction: Narrow-leaved lupins (*Lupinus angustifolius* L.) are an interesting crop rich in proteins, enhancing the soil structure by their deep taproot and the soil quality by symbiosis with nitrogen fixing bacteria, accumulating nitrogen in the rhizosphere. Toxic alkaloids are characteristic for wild lupins, acting as a chemical defence against pathogens and herbivores. Breeding for reduced alkaloid content in the seeds (sweet lupins) led to an increased susceptibility to aphids. For breeding resistant cultivars a better knowledge of the interactions between alkaloid content and aphid development is needed.

Objectives: Therefore, the relationship between alkaloid content, aphid multiplication and feeding behaviour was investigated with selected aphid species on lupins differing in the alkaloid content.

Materials and methods: The alkaloids in the leaves of four selected genotypes were analyzed by GC/MS. To estimate the population growth of *Macrosiphum albifrons*, *Aphis fabae*, *Aphis craccivora*, *Acyrtosiphon pisum* and *Myzus persicae* 10 young plants per genotype (3 replications) were settled with 2 to 4 apterous aphids in a growth chamber. After 1 and 2 weeks the aphids were counted and the average number of aphids/plant and day was calculated. The feeding behaviour was studied by using the EPG (electrical penetration graph) technique and the influence of the alkaloid content on different EPG-parameters was determined.

Results: Multiplication and feeding of *M. albifrons* which is adapted to lupins was not affected by the alkaloid level. For the other aphid species the multiplication was significantly negatively correlated with the alkaloid content. The EPG showed that the time to the first penetration and the pathway of the stylet to the phloem is not influenced by the alkaloid content, however, the time of phloem feeding and related parameters are strongly reduced on genotypes with a high alkaloid content. But, a reduced aphid multiplication and feeding behaviour was detected on cultivar Kalya having a low alkaloid content.

Conclusion: Feeding and multiplication of aphids is generally affected by the alkaloid content, probably in the phloem, but differences between sweet genotypes are present. Breeding of sweet lupins with reduced aphid susceptibility, except *M. albifrons*, is therefore possible.

P PPI 21

The Arabidopsis lipid transfer protein LTPIV.4 enhances resistance to *Pseudomonas syringae* pv. *maculicola*

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Lipid Transfer proteins (LTPs) are small proteins characterized by an internal lipid-binding pocket. LTPs comprise large groups of related proteins in higher plants, with only a small fraction functionally characterized. In Arabidopsis, the lipid transfer proteins DEFECTIVE IN INDUCED RESISTANCE 1 (DIR1), DIR1-like and AZELAIC ACID INDUCED 1 (AZI1) have been shown to play a role in systemic acquired resistance (SAR) or in priming of defense responses. Despite their function in enhancing pathogen resistance, *Azi1* and *Dir1* are not strongly induced by pathogen treatment on the transcriptional level.

An LTP closely related to DIR1, LTPIV.4, on the other hand, showed strong upregulation upon pathogen inoculation and in response to salicylic acid. We are therefore analyzing the role of this LTP in the Arabidopsis pathogen response. In tests with the hemi-biotrophic bacterium *Pseudomonas syringae* pv. *maculicola*, *ltpIV.4* mutant plants were more susceptible, while 35S::LTPIV.4 expressing lines were more resistant. LTPIV.4 promoter:GUS studies revealed that expression of LTPIV.4 is high in leaves, especially in young rosette leaves. Although high GUS levels were detected around the leaf vasculature, a requirement of LTPIV.4 in systemic acquired resistance, as described for the closely related DIR1, was not detected. LTPIV.4 is therefore, different to related LTP's, enhancing local, but not systemic resistance of leaves infected with *Pseudomonas syringae*.

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P PPI 22

First report of *Myzus persicae* as a vector for Carrot motley dwarf (CMD) complex

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Carrot motley dwarf (CMD) is caused by virus complex of *Carrot red leaf virus* (Polerovirus), *Carrot mottle virus* (Umbravirus) and *Carrot red leaf virus* associated RNA virus (Unassigned +ssRNA virus). CMD complex is vectored by *Cavariella aegopodii* in plants in persistent and non-propagative manner. Previous studies have failed to prove *Myzus persicae* as a vector of CMD in plants. We tested *Myzus persicae* for the transmission of CMD complex by analyzing disease symptoms in host plants and by PCR analysis, and found up to 90% inoculated plants showed positive physical symptoms, which later confirmed by RT-PCR results. Sequence divergence with respect to databank sequences of CMD also reported. Obtained sequences of CtRLV, CMoV and CtRLVaRNA were submitted to European Nucleotide Archive with accession numbers LN554261, LN554262 and LN554263, respectively.

P PPI 23

Study genetic variation using DNA molecular markers and Identification physiological races of of wheat stripe (yellow) rust *Puccinia striiformis* f.sp tritici

during 2010-2014 In some regions of Syria

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Wheat, as the most important staple food crop, is grown on approximately 225 million ha⁻¹ worldwide with 600 million tons of wheat produced annually; globally, the most precursory threatening for future wheat production in the third world represented in obligate biographic parasites with complex life cycle like the rust fungi. Rust diseases characterized by mutable physiological races that differ from each other in virulence and pathogenicity ability. Yellow Rust (stripe) rust (*Puccinia striiformis* West. f. sp. tritici) is one of the most epidemic diseases infects wheat in cold and wet regions. In 1988, this disease caused a loss of seasonal production amounted 70% on wheat variety Maxpak in Syria, and recurrent infection in 2010, caused by virulence race called Yr27 caused a considerable loss in bread wheat production (Cham 8 - Cham 6 particularly) amounted 90%. Recently, 15 races of yellow rust had been addressed in Syria for seasons 2010- 2014, 159E256, 166E254, 166E256, 255 E112, 0 E0, 64 E 6, 230 E150, 0 E 18, 198 E130, 166 E150, 102 E160, 128 E0, 126 E150, 214E150

Molecular Variance Analysis of Molecular Variance (AMOVA) of 55 yellow rust of Molecular Variance (AMOVA) of 55 yellow rust *Puccinia striiformis* f.sp tritici isolates examined by Amplify Fragment Length Polymorphism (AFLP) revealed high genetic variation within population. dimensional scale analysis (MSD) and tree diagram showed that the Syrian yellow rust isolates were clustered in three groups. The first group contained isolates derived from durum wheat, the second group contained bread wheat isolates, but the third group was made of mixture of isolates derived from both wheat species

P PPI 24

The transcriptional response of potato tubers to *Pectobacterium* spp. during soft rot infection

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Introduction: Potato (*Solanum tuberosum* L.) is the third most important global staple food source. Potato production is highly regulated to keep the crop free from pathogens that reduce tuber quality and yield. *Pectobacterium atrosepticum* (*Pba*) and *Pectobacterium carotovorum* subsp. *brasiliensis* (*Pbr*) are soil-borne necrotrophic bacterial plant pathogens that cause blackleg on potato stems during the growing season and soft rot of tubers post-harvest. Coronafacic acid (CFA) is a virulence determinant identified in isolates of *Pectobacterium*. In the hemibiotrophic pathogen *Pseudomonas syringae*, CFA is a component of coronatine (COR). COR acts as a molecular mimic of jasmonic acid (JA) during pathogenicity on host plants, suppressing the salicylic acid (SA) signalling pathway which is essential for defence against *P. syringae*.

Objective: The aims of this study were: i) to investigate the early response of potato tubers to soft rot infection and to identify the defence pathways involved in resistance to *Pectobacterium*; and ii) to examine the impact of CFA on the defence response of potato tubers.

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Methods: Total RNA was extracted from susceptible potato tubers (cv. Summer delight) inoculated with either *Pectobacterium* or purified CFA. Global transcriptional profiles during early stages of soft rot infections (6, 12 and 24 hours post inoculation) was studied using Illumina-based RNA sequencing.

Results: Potato tubers inoculated with *Pectobacterium* spp. showed differential expression of genes related to ethylene (ET) biosynthesis and the JA pathway. Genes associated with plastids were also highly differentially expressed, suggesting that tubers had initiated the production of plant phenolics in response to the pathogen. Finally, isoforms of many of these defence related genes were expressed differentially in tubers in response to the pathogen.

Conclusion: The differential expression of genes related to ET biosynthesis and the JA pathway was as expected as these pathways are central to plant defence against necrotrophic pathogens. The production of plant phenolics was also predicted, as phenolics are known to be produced by tubers in response to biotic and abiotic stress. Biochemical analysis of tubers is being undertaken to confirm the differential production of ET and JA as well as the identity of the phenolics.

P PPI 25

Development trend analysis of Gui 22-9 and Gui 22-14 new strains of wheat stripe rust

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New virtual race's occurrence and becoming predominant race are the fundamental reason of the breakdown of resistance to stripe rust and disease outbreak in wheat cultivars. So its mutation monitor research is not only an important area of wheat stripe rust research, but a key link to control. In the early 1990s, "Guinong 22" wheat variety was firstly bred by Professor Qingqin Zhang, who works at Agricultural College of Guizhou University, through the hybridization of *Haynaldia villosa*, and *Triticum Durum* and *Triticum aestivum*. It's a vital resource in China, which is immune to those races earlier than CYR33. Now this new variety has been widely used in disease resistance breeding, playing an important role in wheat production. In 2010, two kinds of new emerging stripes, Gui 22-9 and Gui 22-14 were monitored to have strong toxicity to Guinong 22, which was developed from CYR32 and CYR33. The pathogenetic characteristic of Gui 22-9 is similar to the former, while that of Gui 22-14 is similar to the latter. However, both of them have toxic effects to Guinong 22, Chuanmai 42 (Yr24), Lantian 17 (Yr26) and 92R137 (Yr26). Herein 1681 stripe rust-infected wheat leaves were sampled from Gansu, Qinghai, Shaanxi and Qinghai provinces in 2010-2014, and the results showed that the occurrence frequency of Gui 22-9 increased from 2.8% (2010) to 12.5% (2013), whereas that of Gui 22-14 increased from 7.0% (2011) to 9.0% (2013). And the frequency of Guinong 22 group increased yearly, from 3.5% (2010) to 17.78% (2011) and 23.6% (2012) and 34.1% (2013), and even to 45.2% (2014). These two races and Guinong 22 group emerged especially in Gansu and Sichuan provinces, which are fully correlated to the frequent occurrence of stripe rust and the existence of lots of nurse varieties. The new emerging stripes have great concerns to those varieties grown in several provinces (including Gansu, Sichuan and Shaanxi), such as Lantian 17 and 24, 95-111-3, Zhongliang 29, Mianmai 42, Neimai 8 and 9, Chuanmai 42, Shaanmai 175 and Hanmai 6, in which have blood relationship with Guinong 22, Nannong 92R and Moro. Therefore the target of disease resistance breeding should be rapidly adjusted across the county, and some resistant resources such as Guinong and 92R must be eliminated to avoid more disease outbreak caused by the breakdown of resistance to stripe rust.

P PPI 26

Role of PR-proteins in defense reactions of potato tubers against *Fusarium solani*

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Introduction: β -1,3-glucanase and chitinase are PR-proteins and both are part of the protective mechanism of plant against pathogens. Investigation of the role of these enzymes involved in the response of potato under *F. solani* infection is important for their use as biochemical markers of resistance against fungus cause dry rot of tubers.

Objectives: To investigate the changes of activities and isoenzyme spectrum of PR-proteins in potato tubers under *F. solani* infection.

Materials and methods: Objects - potato tubers varieties Aksor (conditionally resistant), Santa (resistant), strain *F. solani* 0167. Tubers were infected with cultural filtrate and mycelium. The enzyme activity was determined after 0-7 days after infection.

Results: The results showed that the response of Aksor cultivar was earlier than Santa. The glucanase was activated stronger (30-40% increase) by cultural filtrate than by mycelium. Peak of enzymatic activity was observed in 3 days after infection, 5 fold higher than the control. Then in 4 days, there was some decline of activity, but still level of activity in the experimental variants was 3 fold higher than the control. At the 5th day activity in infected samples was the same level as the control. Chitinase

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activation occurs before glucanase (at the 2nd day after infection), but the level of activity was lower (1,5-2,0 fold higher than control). Chitinase was activated only by treatment with CF. The treatment with mycelium inhibited activity of enzyme throughout the experiment. Under *F. solani* infection of potatoes were no changes observed in the qualitative composition of glucanase and chitinase isoforms. Only certain isoforms were activated. So for glucanase at the 3rd day of infection were activated two isoforms - acidic (pI 4,5) and especially alkaline (pI 8,0). The infection was also enhanced the activity of chitinase isoform one acidic (pI 5,3), which correlated with increasing of total activity of the enzyme at the 2nd day after infection.

Conclusion: Under infection in tubers take place the rapid activation of PR-proteins localized on the surface of the tubers to prevent further penetration of the infection into the inner layers of the tuber tissue. In resistant cultivars the activation of enzymes occurs at the earlier stages of infection, which allows more effectively resist attack of the pathogen.

P PPI 27

Identification of QTLs conferring resistance to net blotch (*Pyrenophora teres f. teres*) in barley using a nested association mapping population

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Net blotch, caused by the fungus *Pyrenophora teres f. teres*, is an important foliar disease of barley causing high yield losses. The identification of QTLs conferring resistance to this fungus is the basis for targeted and sustainable breeding approaches aiming to improve net blotch resistance in modern barley cultivars.

Therefore, a SNP-based nested association mapping (NAM) approach was used to map resistance QTL derived from *H. spontaneum*.

To achieve this, the barley nested association population (HEB-25) comprising 1420 BC1S3 lines in 25 families originating from a cross of 25 wild barley accessions (*H. spontaneum*) with the elite cultivar Barke will be screened for resistance in 2-year field trials using a summer-hill-design. Based on first year data the area under the disease progress curve was calculated. Using these and SNP data obtained from the 9k iSelect barley chip a nested association mapping approach was conducted.

First year results indicate a high variability in net blotch resistance between and within families of the NAM-population with some genotypes showing higher resistance than the resistant check line included in the trial. In summary, SNPs associated to net blotch resistance were detected on chromosomes 2H, 4H and 6H. In a next step, field trials will be replicated in a second year and additional markers will be generated by exome capture in order to achieve more detailed information on *Pyrenophora* resistance derived from *H. spontaneum*.

P PPI 28

Effect of proteinaceous toxins produced by *Stagonospora nodorum* on necrosis induction in triticale.

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Introduction: *Stagonospora nodorum* is a necrotrophic pathogen of all assimilative green plant parts of wheat and triticale as well as of other cereals and grasses. Oval or lens-shaped, first chlorotic and later in the season redbrown spots develop along the leaf blade and sheath and affect the entire leaf and/or glume and awns. With development of the disease, called *Stagonospora* leaf and glume blotch, on necrotic lesions appears pycnidial sporulation. Destruction of green plant parts affects adversely photosynthesis, what results in grain yield loss, quantitative and qualitative in nature. In past several years appeared quite a number of reports on proteinaceous host selective toxins produced by *S. nodorum* in affected plant tissue. These play crucial role in induction of tissue necrosis. Toxins interact with specific host genes. Positive recognition with dominant allele in affected plant leads to necrosis induction, while absence of dominant allele causes toxin insensitivity. So far, six pairs of *S. nodorum* toxin/host gene were described. Tests conducted under controlled environment as well as field trials confirmed that protein toxins are important factors in *S. nodorum* leaf and glum blotch of wheat.

Objectives: Evaluation of triticale varieties and breeding lines reactions to semi purified extracts from *S. nodorum* liquid cultures.

Materials and methods: Isolates were grown for 4-5 weeks on **liquid medium** [g/L] : 5g ammonium tartrate; 1g NH₄NO₃; 0,5g MgSO₄ *7H₂O; 3,9g KH₂PO₄; 30g sucrose; 1g yeast extract, pH 5,7. Medium was dialyzed in 8kDa MWCO tubing, precipitated with acetone and dissolved in: 50mM MOPS; 12,5mM sodium acetate; 1,25mM EDTA; pH 7,5. The extract was 50× concentrated and infiltrated to second seedling leaves. Digestion of proteins was conducted with Pronase® 1mg/ml.

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Results: Differential reactions among host genotypes infiltrated with semi purified extracts were observed. Extracts digested by proteases lost their activity.

Conclusion: Losing activity after digestion and particle size in extracts suggest that necrotrophic factor in our extracts is proteinaceous in nature. This finding is in agreement with literature data. Differential susceptible reaction among triticale genotypes shows that proteinaceous toxins have an impact on symptom expression in triticale as well as in wheat seedling leaves.

P PPI 29

Dynamic subcellular changes in glutathione and its precursor levels in plants under different environmental conditions

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Introduction: Glutathione and its precursors are the most important antioxidants in plants. They are involved in the detoxification of reactive oxygen species (ROS), redox signaling, in the modulation of defense gene expression and they are important for the regulation of enzymatic activities. For these reasons levels of glutathione and their precursors are often used as stress markers in plants.

Objectives: In our studies we focused our interest on the dynamic compartment specific changes of glutathione and its precursors, to gain thorough knowledge about the subcellular distribution of these antioxidants in plants and on the importance of these antioxidants in certain cell compartments during stress situations.

Material and methods: Different agricultural plants (*Cucurbita*, *Nicotiana*), as well as *Arabidopsis* were used as model plants under different environmental conditions. For this purpose beside other techniques an immunogold cytohistochemical approach was developed and adapted to different plant material in order to detect and quantify subcellular glutathione and its precursors with computer-supported transmission electron microscopy.

Results: The development and application of these methods to various plants under different environmental conditions revealed that glutathione precursors (especially cysteine) limit the operation of glutathione metabolism. The modification (increase) of cysteine contents in plants resulted in a strong increase in glutathione contents and subsequently in a higher stress tolerance.

Conclusion: These studies and methods can now be used for the development of new defense strategies for agricultural use in the future, and can protect farmers from possible crop losses induced by environmental stress situations in the future.

Acknowledgement: This work was supported by the Austrian Science Fund.

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Detection, Taxonomy and Genetic Variability of Alder Yellows Phytoplasma in Black Alder in Spreewald Habitat

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Introduction: Phytoplasmas are cell wall-less bacteria and obligate parasites colonizing the phloem of plants. Diseases caused by phytoplasmas are associated to more than 1,000 plant species worldwide, including economically important crops and forest trees. Alder yellows phytoplasma (AldY), which frequently infects *Alnus* spp. (alder), is closely related to quarantine pathogen Flavescence dorée (FD) in grapevines. Only few *A. glutinosa* (black alder) trees are exhibiting typical symptoms such as yellowing or decline.

Objectives: This study aims to determine the prevalence of AldY infection in black alder not exhibiting infection-associated symptoms in a riparian forest and the phylogenetic relatedness between determined and previously described strains, based on 16S *rRNA* and methionine aminopeptidase (*map*) gene.

Materials and methods: Leaf samples from fifty-seven black alder at different ages were harvested during summer for DNA-isolation followed by PCR on partial *rRNA*-operon and *map*. RFLP analysis was performed on 16S rDNA using *TaqI* for classification to 16SrV-C on all samples. Sequencing of 16S rRNA and *map* genes from selected strains was performed for phylogenetic analyses.

Results: AldY phytoplasmas (16SrV-C) were detected in all samples by PCR-RFLP and sequence analyses of 16S rDNA. *Map* analyses revealed diversity of the strains present in the analysed samples as well as several samples with mixed infections of closely-related AldY strains. The determined strains were assigned to phylogenetic clusters close to Palatinate grapevine yellows, AldY or FD.

Conclusion: Black alders of the Spreewald area are in general infected with AldY phytoplasma without symptom exhibition. Our results support the presence of an established common balanced phytoplasma infection in black alder.

P PPI 32

Study genetic variation using DNA molecular markers and Identification physiological races of of wheat stripe (yellow) rust *Puccinia striiformis* f.sp tritici during 2010-2014 In some regions of Syria

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Wheat, as the most important staple food crop, is grown on approximately 225 million ha⁻¹ worldwide with 600 million tons of wheat produced annually; globally, the most precursory threatening for future wheat production in the third world represented in obligate biographic parasites with complex life cycle like the rust fungi. Rust diseases characterized by mutable physiological races that differ from each other in virulence and pathogenicity ability. Yellow Rust (stripe) rust (*Puccinia striiformis* West. f. sp. tritici) is one of the most epidemic diseases infects wheat in cold and wet regions. In 1988, this disease caused a loss of seasonal production amounted 70% on wheat variety Maxpak in Syria, and recurrent infection in 2010, caused by virulence race called Yr27 caused a considerable loss in bread wheat production (Cham 8 - Cham 6 particularly) amounted 90 %. Recently, 15 races of yellow rust had been addressed in Syria for seasons 2010- 2014, 159E256, 166E254, 166E256, 255 E112, 0 E0, 64 E 6, 230 E150, 0 E 18, 198 E130, 166 E150, 102 E160, 128 E0, 126 E150, 214E150

Molecular Variance Analysis of Molecular Variance (AMOVA) of 55 yellow rust of Molecular Variance (AMOVA) of 55 yellow rust *Puccinia striiformis* f.sp tritici isolates examined by Amplify Fragment Length Polymorphism (AFLP) revealed high genetic variation within population. dimensional scale analysis (MSD) and tree diagram showed that the Syrian yellow rust isolates were clustered in three groups. The first group contained isolates derived from durum wheat, the second group contained bread wheat isolates, but the third group was made of mixture of isolates derived from both wheat species

P PPI 33

Zoospore exudates suppress plant defense gene expression in *Arabidopsis thaliana*

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Introduction: *Phytophthora nicotianae* (syn. *P. parasitica*) is an agriculturally important pathogen that attacks hundreds of plant species. Our previous studies showed that this pathogen uses zoospore exudates to coordinate homing and plant infection. However, it is not clear whether and how zoospore exudates may affect plant defense during infection.

Objectives: This research used model plant *Arabidopsis thaliana* and zoospore free fluid (ZFF) from *P. nicotianae* to examine whether zoospore exudates affect plant defense systems.

Materials and methods: Four to five week old plants or plant parts of *A. thaliana* Col-0 and its mutants *eds16-1*, *npr1-1* and *pad4-1* were flooded with a zoospore suspension of *P. nicotianae* at 2×10^4 /mL, ZFF that was prepared from 5×10^5 /mL or higher concentrations of zoospore suspensions, or sterile distilled water in a growth chamber at 23°C for 72 hours. Plant responses were assessed by disease severity rating, microscopy, and qRT-PCR analysis of the expression of plant defense genes *PR1* and *PDF1.2* in periodically sampled plants from treatment.

Results: ZFF induced severe symptoms on *eds16-1* plants. It also induced mild symptoms on *npr1-1* and Col-0 but not *pad4-1* plants, indicating that plant defense to ZFF required salicylic acid (SA) conferred by EDS16 or NPR1 but not phytoalexin camalexin conferred by PAD4. Meanwhile, expressions of *PR1* and/or *PDF1.2* were similar in plants treated with ZFF and the zoospore suspension: they were downregulated for the entire time course or at the late times of treatment, indicating that ZFF played same role as zoospores in attacking SA and jasmonic acid (JA) signaling-dependent defense mechanisms. However, expression levels of ZFF treated plants most time were higher than those of zoospore inoculated plants. In addition, in ZFF treated *eds16-1*, expression of *PR1* was upregulated even at late time, suggesting that ZFF may also act as pathogen-associated molecular patterns for a SA independent plant defense.

Conclusion: Zoospore exudates suppress SA and JA signaling-dependent plant defense system, in addition to coordinating zoospore homing and plant infection as reported previously.

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Cereal leaf beetle proteases and their reaction to dietary protease inhibitors

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Cereal leaf beetle (*Oulema melanopus* L., Chrysomelidae, Coleoptera) is one of the most important pests in Poland. Adults and larvae cause cereal leaf damage what leads to losses in crop yield quantity and quality, but larvae are considered as the most damaging stage. Insects use range of proteases which play an essential role in insect growth, reproduction, and many other important processes. Recently, we reported that *O. melanopus* possesses proteases of four mechanistic classes, cysteine, serine, aspartyl and metalloproteases. Plants have developed a number of defense mechanism to protect themselves against biotic and abiotic stress factors. They synthesize also antinutritional proteins, such as protease inhibitors (PIs) which belong to group 6 of pathogenesis - related proteins (PR6). Anti-insect activity of plant PIs results in drastic effects on insect growth and development. But insects also have evolved some strategies to deal with PIs in their diet. In this study we analyse how various dietary PIs delivered both *in vivo* and *in vitro* influence cereal leaf beetle proteases

Methods: Composition of proteases was determined using in-gel activity assay, spectrophotometric method and application of specific PIs. Selective PIs were added to the larval extracts followed by the separation in a polyacrylamide gels containing gelatine as a substrate. In order to verify the effect of PIs on protease activity, insects were fed with plant leaves covered with PIs: E-64, pepstatin A, cocktail of PIs and AEBSF. Expression of plant protease inhibitors was examined by real-time PCR.

Results: The presence of E - 64 and cocktail of inhibitors caused significant reduction of the most proteolytic activities, contrary to PMSF which resulted in the disappearance of one out of eight activities observed in gel. However, when effect of PIs on protease activity was analysed *in vivo* and insects were fed with plant leaves covered with PIs, AEBSF caused increase of protease activities and novel isoforms of serine protease expression. On the other hand, real - time PCR analysis of insect-treated plants showed up-regulation of plant PIs in response to cereal leaf beetle larvae feeding.

Conclusions: Understanding the principles of plant-pest interaction is necessary to establish effective systems for plant protection.

P PPI 35

Insights into the population structure complexity and the interactions with hosts of 'Candidatus Phytoplasma phoenicium', the etiological agent of almond witches'-broom disease

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Introduction: 'Candidatus Phytoplasma phoenicium' is the etiological agents of a lethal devastating disease of almond trees (almond witches'-broom, AlmWB) in Lebanon and in Iran. AlmWB was also identified on peach and nectarine. Due to complex ecology of 'Ca. P. phoenicium', it is necessary to evaluate its genetic diversity and to investigate the interactions with the hosts.

Objective: The aim of this study was to obtain a draft genome of 'Ca. P. phoenicium' to identify genes suitable for distinguishing closely related strains and to acquire information on its metabolism and mechanisms of interaction with hosts.

Materials and methods: During the spring season in 2012, samples from almond, peach, and nectarine plants showing AlmWB symptoms were collected in Lebanon. 16S rDNA PCR-based amplification and nucleotide sequence analysis were utilized to identify phytoplasmas. DNA extracted from almond infected by 'Ca. P. phoenicium' strain SA213 was used for Illumina sequencing. Bioinformatic tools were employed to assembly and annotate the draft genome, and to predict the presence of transmembrane and secreted proteins. *tufB*, *groEL* and *inmp* genes were selected for investigating the genetic diversity among 20 'Ca. P. phoenicium' strains identified in examined plants.

Results: Sequence-based typing and phylogenetic analysis of the gene *inmp*, coding an integral membrane protein, distinguished AlmWB phytoplasmas from diverse host plants, whereas their sequences of 16S rRNA, *tufB* and *groEL* genes were identical. Moreover, dN/dS analysis indicated a positive selection acting on *inmp* gene. Draft genome analyses suggest a parasitism based on the import of glycerol-3-phosphate, a critical mobile inducer of plant systemic immunity. Moreover, the identification of a putative inhibitor of apoptosis-promoting Bax factor suggested its potential role as a phytoplasma fitness-increasing factor by modification of the host-defense response.

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Conclusion: Even the incompleteness of the draft genome, the results obtained revealed important insights into both the gene repertoire of the pathogen and its population structure, revealing useful information with regard to the candidate determinants of pathogenicity and highlighting genetic diversity among '*Ca. P. phoenicium*' strains associated with the disease.

P PPI 36

Temperature modulation of plant gene expression at early stages of plant-pathogen interactions

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Plant-pathogen interactions are modulated by environmental factors. Temperature is known to affect plant immunity to pathogens and change plant disease resistance. Understanding the variation of plant responses to pathogens under environmental conditions may lead to new methods to increase plant health and productivity. In this study, we have determined the effect of frequent temperature fluctuation in the daily cycle on plant R-mediated resistance to biotrophic pathogen. The study was carried out on a parasite system "potato (host plant) - potato cyst nematode *Globodera rostochiensis* Woll. (highly specialized endoparasite of plant root system)". A susceptible and a resistant cultivars were chosen for this study. Fourteen-days-old plants were subjected to a temperature drop from 23 to 5°C for 2 h at the end of the night for 6 days (DROP-treatment). After that, the plants were infected by the nematode (20 cysts per plant, pathotype Ro1) and kept under optimal growth conditions for 2 months. Tissues (roots, leaves) were harvested at 0, 2, 6, 20 days after nematode invasion. We focused on the effect of DROP-treatment on key components of plant immune response: resistance genes (*H1* and *Gro1-4*) and defense-related genes (*PR1*, *PR2*, *PR3*, *PR5*, *PR10*, *PAL1*, *PAL2*, *PI*). Gene expression was analyzed by PCR in Real Time. Susceptible potato plants had inactive resistance genes (*H1* and *Gro1-4*) and most of defense-related genes. These plants were unable to recognize nematode invasion and initiate the cascade of defense reactions. Upregulation of *H1* and *Gro1-4* genes was observed in the roots of susceptible plants after DROP-treatment. The expression of *PR* genes, *PAL* and *PI* genes of the plants rose by the 20th day after nematode invasion, and had tissue-specific manner. Importantly, patterns of gene expression in DROP-treated plants were similar to those in resistant plants. This fact indicates that the plants can recognize nematode effectors and activate defense responses resulting in plant disease resistance. Thus, daily temperature drop is an important step in turning compatible host-parasite interactions into incompatible ones, and that could be the basis for a strategy to enhance plant resistance to the pathogen. The study was partly supported by the Russian Foundation for Basic Research (N 15-04-04625).

P PPI 37

Biocontrol efficiency of *Trichoderma harzianum* against chickpea wilt pathogen *Fusarium oxysporum*

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Introduction: Chickpea is an important field crop in Mediterranean region including Kurdistan. Wilt diseases caused by soil-inhabited fungi are considered as main constraints limiting production of this crop. *Fusarium oxysporum* is a major fungal pathogen cause wilt disease in chickpeas. Biological control using antagonistic fungal species displayed great efficiency against several plant pathogens. *Trichoderma harzianum* is the most important species used in the field of biological control of diseases.

Objectives: Due to the important of the disease and because the pathogen, *F. oxysporum* is soil-inhabiting fungus that can remain in the soil for several years, and because of limitation of the use of other control measures, the use of antagonistic fungi may play as an alternative and sound method to control and limit such deleterious diseases and avoid the use of eco-harmful chemicals.

Materials and methods: A field experiment conducted in research fields belong to College of Agriculture of Salahaddin University in Erbil. The commercial product of *T. harzianum*, Biocont-T, is applied as a bio-agent to combat the soil-borne pathogen *F. oxysporum*. The treatments included two levels of bio-agent used as seed treatment, two levels of bio-agent added to peatmoss, and two amounts of peatmoss amended with the bio-agent. Both inoculated and amended peatmoss were mixed with the soil before planting.

Results: The results showed that the use of *T. harzianum* as a commercial product (Biocont-T) applied in the field have decreased both disease severity and percentage of seed infection. The results also showed that the use of 5 g Biocont-T used as seed treatment and 250 g peatmoss amended with Biocont-T and mixed with the soil were the most efficient in decreasing the disease incidences by 27.68% and 26.50% respectively. The treatments were also had significant affect on yield parameters. The seed yield per plant, weight of 100 seed, biological yield and harvest index (HI) had increased.

Conclusions: The field application of *T. harzianum* revealed the possibility of application of antagonistic fungi whether used as seed coats or mixed with organic fertilizers such as peatmoss before planting.

P PPI 38

A phytoalexin for controlling Asian soybean rust

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Phakopsora pachyrhizi causes Asian soybean rust (SBR) disease thus threatening global soybean production. If applied swiftly, fungicides help but current fungicide regimes comprise at least three applications, and traditional breeding did not provide soybean varieties with stable resistance to all

P. pachyrhizi isolates. In an attempt to exploit nonhost resistance (NHR)-associated *Arabidopsis thaliana* genes for genetically engineered soybean resistance to SBR, we identified a **POSTINVASION-INDUCED NONHOST RESISTANCE GENE (PING)** with a role in Arabidopsis postinvasion NHR to

P. pachyrhizi. Activation of PING expression accompanies Arabidopsis postinvasion SBR resistance. PING is a key enzyme in the secondary metabolism. Consistently, certain metabolites exclusively accumulated in *P. pachyrhizi*-inoculated *pen2* mutants with postinvasion NHR to SBR. In *in vitro* and *in situ* analyses, those metabolites inhibited germination of *P. pachyrhizi* uredospores and countered fungal penetration and rust symptom development. Furthermore, overexpressing PING in Arabidopsis and *Nicotiana benthamiana* lead to hyperaccumulation of the metabolites. Future work will reveal the potential of the metabolites for providing SBR resistance, either as a natural contact fungicide or by genetical engineering.

P PPI 39

Histopathological assessment of the infection of maize leaves by *Fusarium graminearum*, *F. proliferatum* and *F. verticillioides*

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Maize plants were inoculated by hand spraying *Fusarium graminearum*, *F. proliferatum* and *F. verticillioides* suspension on the 4th leaf and adding a droplet of fungal suspension into the whorl of the 6th emerging leaf. Infection of asymptomatic mature leaves and immature leaves with symptoms were investigated. All species were able to penetrate into the tissue through cuticles, epidermal cells, and via stomata. Forming appressoria, infection cushions or direct penetration demonstrated the broad host tissue these species resembled a high potential leading to symptomatic as well as asymptomatic infections. All pathogens showed intercellular and intracellular infection of epidermal and mesophyll cells. Additionally, *F. graminearum* hyphae were found in sclerenchyma cells, xylem and the phloem vessels of detached leaves. The superficial hyphae and re-emerging hyphae of the three species produced conidia. Especially, macroconidia of *F. graminearum* produced secondary macroconidia and *F. proliferatum* formed microconidia inside tissues and sporulated through stomata and trichomes. The infection of maize leaves by the three *Fusarium* species and their sporulation indicated an inoculum contribution to cob and kernel infection which may lead to reduce yield, quality and increase in potential mycotoxin contamination on maize.

P PPI 40

The impact of plant volatiles on the migration behaviour of *Cacopsylla pruni*, the vector of the European Stone Fruit Yellows (ESFY).

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Introduction: The European stone fruit yellows (ESFY) is caused by a phytoplasma and the economically most important disease of stone fruit. Its vector is the plum psyllid *Cacopsylla pruni*. This univoltine jumping plant louse (Hemiptera: Psyllidae) overwinters as adult insect on conifers. In early spring the remigrants migrate to *Prunus ssp.*, e.g. *Prunus spinosa* and *P. cerasifera*, but also to the cultivars apricot (*P. armeniaca*) and peach (*P. persica*) for reproduction. At the end of May the adults of the new generation (emigrants) hatch and migrate after a short feeding period on their reproduction host to conifers.

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Objectives: The impact of plant volatiles on the migration behaviour of *Cacopsylla pruni* was investigated during the different migration periods from conifers (spruce) to *Prunus* spp. and *vice versa*. Additionally, the influence of the ESFY phytoplasma on volatile production was studied.

Materials and methods: We identified highly attractive and less attractive *Prunus* species for the vectoring insect over a period of 4 years in a field survey. We analyzed the emitted volatile organic compounds of both reproduction host plants and overwintering host plants during the different migration periods by GC-MS. We statistically compared differences in the emitted volatile blends and identified attractive and repellent compounds in olfactometer bioassays.

Results: The relation of α -pinene, bornylacetate, eucalyptol und camphene in the headspace of spruce was higher during the migration of the remigrants from spruce back to *Prunus* spp., while the relation of these compounds was reduced at the time the emigrants migrated to spruce. A mix of these compounds was repellent for remigrants in olfactometer bioassays. Additionally, α -farnesene was emitted by conifers during migration of emigrants, which was proved to be attractive for emigrants in olfactory bioassays.

Conclusion: The plum psyllid uses host plant odours for the orientation during its migration periods. It is not influenced by phytoplasma induced volatiles like the close related species *C. picta*. We could identify both attractive and repellent compounds which shall be used in future for the control of this pest insect.

P PPI 41

Rice response to simultaneous stress of bacterial blight and drought: Evidence from two major rice R genes mediated resistance to bacterial blight

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Xanthomonas oryzae pv. *oryzae* (*Xoo*), the causal agent of rice bacterial blight (BB), causes severe economic yield losses in rice. Plant response to one type of stress is affected by further stress factors, as observed under simultaneous exposure to abiotic and biotic stress. Evidences from plant morphology, bacterial blight development and *in planta* bacterial spread from different rice genotypes under two drought stress levels and a well-watered control were used to demonstrate the challenge rice is facing under simultaneous stress of drought and bacterial blight.

Low drought stress (70% soil moisture) and moderate drought stress (50% soil moisture) were imposed to ten rice genotypes (genotypes with bacterial blight *R* gene; genotypes with drought QTLs and BB *R* gene and bacterial blight susceptible check) progressively from seven days after sowing until 21 days. The plants were inoculated with *Xoo* (strains PXO99 and PXO145) at 21 days and the stress level was maintained. The disease evaluation was carried out at 11 days post inoculation.

Plant height and shoot biomass were highly negatively affected by drought stress. Drought stress reduced bacterial blight disease development; however, the pathogen spread *in planta* was increased under moderate drought stress conditions. Rice genotypes IRBB61 and IRBB67 with BB *R* genes combination showed less BB development under drought stress and rice near isogenic line IRBB7 carrying the *Xa7* gene showed lowest bacterial spread and a reduction in bacterial population under drought stress. IRBB4 (*Xa4*), IR87705-6-9-B (*Xa4*; *DYT2.2*) and IR87707-445-B-B-B (*Xa4*; *DYT2.2*, *DYT4.1*) showed an inverse reaction of BB spread *in planta* compared to IRBB7.

Our results suggest that rice genotypes' with BB *R* gene *Xa4* response to BB is affected by drought stress reducing the effectiveness of the *R* gene, while *Xa7* effectiveness is more enhanced under drought stress. Gene combinations, especially *Xa4* + *Xa7* showed a good tolerance to simultaneous BB and drought stresses, while *Xa4* together with drought QTLs revealed less effective, demonstrating that a combination of *Xa4*+*Xa7* with drought QTLs in rice varieties will be suitable under the future climate situations.

P PPI 42

Effect of Volatile Organic Compounds against fungal and bacterial plant pathogens

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Barley is threatened by various edaphic fungal diseases. Common root rot, caused by *Fusarium culmorum* and *Cochliobolus Sativus*, is one of the major fungal diseases of barley, causing between 9 and 23 % of yield losses. Since most of chemicals used

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for crop protection are being forbidden, new ways of protection are needed. In a previous study, we showed that barley roots infected by common root rot emitted 23 Volatile Organic Compounds that were not emitted by healthy barley roots. In precedent studies, we have shown that, among these, Methyl propionate and methyl acrylate reduced significantly the development of the barley diseases.

The main objective of this study is to evaluate the antifungal and antibacterial activity of both VOCs on a wider range of plant pathogens: *Fusarium culmorum*, *Fusarium graminearum*, *Penicillium expansum*, *Penicillium digitatum* and *Penicillium italicum* (as fungal pathogens) and *Pectobacterium carotovorum carotovorum* and *Pectobacterium atrosepticum* as bacterial pathogens.

Methods: The evaluation has been made through ELISA microplates with PDB or V8 media. The growth of the pathogen (bacteria and conidia) in the presence of the VOCs was evaluated and compared to a control (same media without VOCs).

Result: Methylpropionate showed interesting antimicrobial activity with 40% of inhibition of *Pectobacterium carotovorum carotovorum* and more than 96% of *Pectobacterium atrosepticum*. In the same way, methylacrylate inhibits more than 77% of *Pectobacterium carotovorum carotovorum* and 97% *Pectobacterium atrosepticum* development.

Methyl acrylate inhibited the growth of all tested fungi. The lowest growth inhibition (70%) was observed for *P. digitatum*. In a similar way, inhibition of methylpropionate was also good with a minimum of 50% growth reduction for *F. culmorum*.

Conclusion: Further studies are undertaken to understand the cellular and molecular mechanisms underlying the production of methylacrylate and methylpropionate and their effect in genetic mechanisms of barley.

P PPI 43

Maize leaf trichomes: an entry point for infection with *Fusarium* species

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Maize leaves were inoculated with *Fusarium graminearum*, *F. proliferatum* and *F. verticillioides*. More than 90% *F. proliferatum* and *F. verticillioides* conidia formed one germ tube and 50% *F. graminearum* conidia formed one and the rest of *F. graminearum* conidia formed two to three germ tubes. The germ tubes of *F. graminearum* conidia were longer than that of *F. proliferatum* and *F. verticillioides*. Three *Fusarium* sp. infected bi-cellular trichomes by adhering and growing along the trichomes or fastened around the cap cell of trichomes 48 hours after inoculation (hai). Hyphae penetrated into the trichomes at the base or the side or top of the cap cells. The hyphae colonized the cap cells and then spread to base cells. Prickle trichomes were infected later, 72 hai. The hyphae either wrapped around prickle trichomes or formed a mass of hyphae around the top of prickle trichomes or formed appressorium. Macro trichomes were infected by *F. graminearum* 7 days after inoculation. Following penetration, the fungus spread to adjacent epidermal cells and the subcuticle. This investigation provides the first assessment of *Fusarium graminearum*, *F. proliferatum* and *F. verticillioides* infection via trichomes of maize leaves.

P PPI 44

Biochemical and physiological features of *Clavibacter michiganensis* PF008 isolated from the canker symptom in pepper

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The bacterial species *Clavibacter michiganensis* (Cm) causes severe diseases in important crops like potato, tomato, corn, wheat, alfalfa, and soybean. *Cm* subsp. *michiganensis* (Cmm) is one of six subspecies of *Cm*. It is a Gram-positive bacterium and causes bacterial canker disease in tomato. Cmm invades host plants and eventually moves to xylem and colonize in xylem. Cmm damages pith tissues in xylem that causes the canker symptom. *Cm* bacterial pathogen that is very closely related to Cmm has been isolated from the canker symptom in pepper. The purpose of this study is to determine biochemical and physiology characteristics of *Cm* isolated from pepper in Korea. *Cm* strain PF008 as a representative strain was used in this study and also the Cmm type strain was included as a control. Several biochemical and physiology characteristics such as NaCl tolerance, reaction to methyl red, levan production, maximum temperature for growth, production of diverse enzymes, usage of diverse carbon sources, fatty acid composition and so on. Most of experiments were performed according to Bergey's manual and API 50CH kit and API ZYM kit were used for examining utilization of carbon sources and enzyme activity. Colonies of *Cm* PF008 showed orange color, whereas Cmm showed yellow. In addition, colonies of *Cm* PF008 were stickier than those of Cmm. Both produced levan and could grow until 34°C. *Cm* PF008 also showed some differences from Cmm in terms of fatty acid

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composition, utilization of certain carbon sources, and three enzyme activities. Based on these results, Cm PF008 isolated from the canker symptom in pepper looks different from Cmm originated from the canker symptom in tomato.

P PPI 45

LC-MS-based Proteomics Reveals Proteins Involved in Grand naine-*Meloidogyne incognita* interaction

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With a diverse host range, *Meloidogyne incognita* (root-knot nematodes) is listed as one of the most economically important obligate parasites of agriculture. This nematode species establishes permanent feeding sites in plant root systems soon after infestation. The establishment of these feeding sites will eventually result in the formation of multinucleated cells, manifested as root-knots/galls on plant roots. Compatible host-nematode interaction triggers a cascade of morphological and physiological process disruptions of the host, leading to pathogenesis. Such disruption is reflected by altered gene expressions in affected cells, detectable using molecular approaches. We employed LC-MS proteomic approach to understand the events involved in plant- *Meloidogyne incognita* interaction. This is due to the fact that proteomics is seen as a rapid strategy to isolate differentially-expressed genes involved in the interaction, reflected as protein abundance changes in isolated tissues. This study served as the first crucial step in developing natural plant resistance for the purpose of biological-based nematode management programme. Using banana (Grand Naine) as our experimental plant model, we had successfully recovered 9307 peptides and 2065 banana root proteins. Out of 2065 proteins, only 94 proteins from 60 days old treated root fragments showed significant abundance changes compared to the control root fragments. One percent and 12% of these significant abundance different proteins were involved in defense and stress responses, respectively. Our result showed that as opposed to tomato plants, banana defense mechanism against *M. incognita* did not involve *NBS-LRR Resistance* gene, but *Lipoxygenase* gene instead.

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Evaluation of resistance to *Phytophthora citrophthora* in citrus somatic hybrids using a fast screening test

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Phytophthora gummosis is a serious disease that threatens citrus production in many areas around the world, including Morocco. However, the use of resistant rootstocks may be considered as the most suitable approach to face this problem. Nowadays, somatic hybridization is an important tool for providing new genotypes that could be candidates for use in citrus breeding programs. For testing the inheritance of the resistance to *Phytophthora gummosis*, ten somatic hybrids obtained from different parental combinations (Chios mandarin + Rangpur lime / Chios mandarin + Carrizo citrange / Chios mandarin + citrumelo 4475 / Chios mandarin + citrandarin / Chios mandarin + citrange C35 / Chios mandarin + C. volkameriana / Valencia + C. Macrophylla / Kinnow mandarin + C. Macrophylla) were subjected to a fast screening test for tolerance to *Phytophthora citrophthora*. The inoculation was made by applying an agar disc containing the mycelium of the fungus on the center of injured leaves using a needle. The percentage of infected tissue was estimated for each leaf of the hybrid plants and compared to that of parents. All somatic hybrids showed a sensitive reaction to the isolat used, except for seven genotypes, .i.e. (Chios mandarin + Carrizo citrange), 985 et 983 (Kinnow mandarin + C. Macrophylla), H et 5 (Chios mandarin + Rangpur lime), 970 (Chios mandarin + citrumelo 4475, 979 (Valencia + C. Macrophylla) which showed a high resistance.

P PPI 47

Effects of host-resistance to *Verticillium longisporum* on the performance of winter oilseed rape (*Brassica napus*) under drought stress

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Introduction: Intensive oilseed rape (OSR) production has resulted in the emergence of a new pathogen, *Verticillium longisporum* (VL), which causes premature ripening leading to substantial yield losses. Improving cultivar resistance is the sole control strategy. Previously, major VL resistance mechanisms (vascular occlusions, lignin and phenolics) were identified. However, it is not known whether these mechanisms are associated with reduced plant performance or yield penalties particularly during drought periods.

Objectives: The stability of VL resistance under drought was investigated. Furthermore, the effects of VL resistance factors on drought tolerance and performance of OSR under drought stress were determined.

Materials and methods: In a controlled pot experiment, seedlings of a VL tolerant OSR line were inoculated with VL and exposed to optimum watering and different levels of drought stresses. Disease assessment was performed by visual disease severity evaluation and quantification of VL DNA via qPCR. To assess changes in agronomic traits, plant developmental stage, plant height, branching, stem diameter and dry matter (DM) yields were evaluated.

Results: AUDPC analysis in the resistant genotype showed significantly lower levels of disease severity and low quantities of VL DNA across all watering regimes, indicating stability of VL resistance irrespective of water supply. Furthermore, stem thickness and plant height evaluations confirmed consistent resistance response of this genotype under drought conditions. Interestingly, DM yield analysis also revealed no yield penalty associated with VL-induced resistance. Production of excessive side branches was induced by infection but not affected by water supply. In contrast, unlike drought which inhibited or delayed flowering, VL had no significant effect on plant development.

Conclusions: The present comprehensive study not only proved that VL-resistance mechanisms have no additive negative consequence on the response of OSR to drought stress but also demonstrated effective functioning of the quantitative VL resistance even under conditions of severe drought. Nevertheless, despite the stability of VL resistance during drought, simultaneous exposure of OSR to both stresses may cause a considerable yield loss.

P PPI 48

Biochemical characterization of the resistance of olive tree to *Verticillium* wilt caused by *Verticillium dahliae*

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In Morocco, olive cultivation is one of the important fields of social and economic sector. The "Moroccan Green Plan" provides for the area of olive an extension to 1.2 million hectares and production of 2500000 tons of olives by 2020(1). However, the Moroccan Picholine has been proved sensitive to dangerous fungal disease especially to *Verticillium* wilt (VW) caused by *Verticillium dahliae* (VD). Our research is designed to study the defense mechanisms involved in the resistance of the olive tree and particularly oxidative events happening right after the inoculation in two varieties: Moroccan Picholine (sensitive) and Frontoio (resistant). The inoculation of olive tree by VD conidial solution induces an important modification in the oxidative metabolism. In the resistant cultivar, the inoculation was accompanied by an early fast and intense increase of the peroxidase (PO) and the superoxide dismutase (SOD) activities. In the sensitive cultivar, we observe a late increase of the activities of polyphenol oxidase (PPO) and SOD. These results suggest that the fast and localized reaction in resistant cultivar was related to the intervention of the PO, whereas the late and generalized reaction in the sensitive one was associated to the PPO.

References: (1) <http://www.agriculture.gov.ma/pages/acces-fillieres/filiere-oleicole>.

P PPI 49

SBTX Expression Profile During Soybean Development and Its Role in Plant Defense

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Soyabean toxin (SBTX) is a protein composed of two subunits (17 and 27 kDa) isolated from soya seeds. *In vitro*, SBTX has antifungal activity against fungi of agricultural interest such as *Cercospora sojina* and *C. kikuchii*, which are natural pathogens of soybean. Such property raises the question whether SBTX has a defense role in soybean. Therefore this study was conducted in order to evaluate the gene expression profile of the two SBTX subunits during the different stages of the plant development and in soybean leaves inoculated with the *C. kikuchii* (CK) spores or treated with salicylic acid (SA), as an attempt to answer the above question. Soybean seeds were grown in a greenhouse and cotyledons, unifoliolate and trifoliolate leaves, hypocotyls, epicotyls, roots, pods and flowers were harvested at different days and assayed. Leaves treated with SA and inoculated with *C. kikuchii* were harvested at 0, 2, 6, 12, 24 and 48 h after treatments. RNA was extracted from all these above tissues and also from mature seeds. Based on the N-terminal polypeptide sequences of the SBTX subunits, primers were designed and their gene expression evaluated by quantitative real-time PCR technique. Transcripts were detected for both SBTX subunits, but their expression levels were different. The highest transcript levels were found for the 27 kDa subunit in mature seeds, cotyledons and unifoliolate leaves. Gene expression of the 17 kDa subunit was as well observed in the same above tissues, but to a much less extent. Furthermore, the leaves treated with SA showed induction of the corresponding 17 and 27 kDa subunit transcripts with maximum at 24 and 12 h, respectively. Challenge with *C. kikuchii* revealed similar expression profiles of both SBTX transcripts that accumulated at 6 h after inoculation and slightly decreased onward. These data support the role of SBTX in plant defense as its transcript expression was induced by both the SA treatment and challenge by the fungus *C. kikuchii*. Moreover, besides its function in plant defense, SBTX also appears to play additional physiological role as its genes were expressed in healthy tissues at different stages of development.

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TALEs from *Xanthomonas citri* have Additive Effects on Bacterial Growth and Canker Development, and Differentially Transactivate Host Target Genes

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Transcription activator-like effectors (TALEs) of *Xanthomonas* spp are among the best-studied pathogen effector proteins. The *X. citri* strain 306, the causal agent of citrus canker, contains four variants of the TALE named PthA (PthAs 1-4). To assess the contribution of each PthA in canker development, simple, double and triple *pthA* knockout mutants were generated and used to infiltrate sweet orange and lemon plants to evaluate bacterial growth and canker symptoms development. In addition, RT-qPCR analyses were performed to validate potential TALEs targets in citrus, previously identified by gene expression analyses. We found that although PthA4 is necessary and sufficient for canker formation in sweet orange *Pera* and lemon *Tahiti*, deletions of *pthA1* and *pthA3* significantly affected canker development in *Pera* leaves, suggesting an additive or synergistic role in the activation of canker susceptibility genes in this host. In *Tahiti*, only PthA4 was essential for canker development; however, we noticed that deletion in any of the *pthA* genes caused a small but significant reduction in bacterial growth in planta. Moreover, deletions in two or more *pthAs* caused even greater reduction on bacterial growth, again indicating an additive effect of the PthAs 1, 3 and 4 on pathogen growth. We also analyzed the activation of LOB1 (Lateral Organ Boundaries), a citrus susceptibility gene that is a direct target of PthA4. LOB1 expression in *Pera* leaves was not abolished but significantly reduced in the *pthA4* mutant, compared to wild type bacteria, suggesting that LOB1 is also activated by other PthAs. However, in *Tahiti*, LOB1 expression was drastically reduced in response to *pthA4* mutant infection. Furthermore, we assayed the expression levels of Apyrase gene, a potential PthA3 target. We found surprisingly higher levels of Apyrase expression in *Pera* leaves infiltrated with the *pthA3* mutant, relative to the wild type bacteria, suggesting that PthA3 represses the Apyrase gene. In contrast, Apyrase expression levels in *Tahiti* leaves were not affected by the *pthA3* mutation. In conclusion, our data indicate that TALEs of *X. citri* strain 306 have additive effects on pathogen growth and canker development, and show distinct pattern of target activation in different citrus hosts.

P PPI 51

Fusarium wilt of Chickpea (*Cicer arietinum* L.) in North-west Algeria :pathogenecity variation and vegetative compatibility of isolates

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Fusarium oxysporum (Schletend: Fr) f.sp. *ciceri* (Padwick) (*Foc*) is a soil fungus that is a permanent threat to the chickpea (*Cicer arietinum* L.) causing wilt syndrome. The presence of the disease was found in all sites chickpea visited in north-western Algeria .The special shape *ciceri* is determined by inoculation with the chickpea cultivar ILC482, a highly susceptible cultivar to *Fusarium* wilt . 49 isolates of *Foc* from different chickpea growing areas were evaluated for the pathogenic variability and physiologic races .Complementation test of various nit mutants among 22 isolates representing race 0 and race 5 from different regions was realised . The pathogen *F. oxysporum* f.sp. *ciceri* was isolated from infected plants harvested. Majority of the isolates were less virulent to moderately virulent (83%) and only (7%) were virulent and caused gradual yellowing. 5 isolates induced vascular wilt and 44 isolates induced vascular yellowing.In pathogenic isolates, disease incidence varies between 22.33% and 98.80% . Among isolates of *Foc* tested, for race from 7 regions, races 0 and 5 consisted of 34 and 4 isolates. it is a positive correlation between the distribution of races and geographic location.Complementation test with isolates revealed that the wilt causing isolates (race 5) were compatible with the yellowing isolates causing (race 0) and belonged to vegetative compatibility group 0280.

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Subcellular Localization and Functional analysis of Blast Effectors in Rice Cells

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Elucidating of the molecular mechanism of effector-mediated suppression of plant immunity is an emerging topic and challenging task in plant-pathogen interaction. Rice blast disease, caused by the fungus *Magnaporthe oryzae*, is one of the most damaging factors in rice production worldwide, affecting global food security. To cause rice blast disease, the biotrophic invasive hyphae of *M. oryzae* will secrete cytoplasmic effectors, which preferentially accumulate in biotrophic interfacial complexes (BICs), and eventually are translocated into the rice cytoplasm to facilitate disease development by suppressing plant defence responses. Although the *M. oryzae* genome consists of a large number of secreted proteins, only few of them have been functionally characterized. Bioinformatic analysis of the in planta expressed secretome of *M. oryzae* revealed that about 90 secreted proteins are predicted to contain conserved plant organelle target motifs. Four (MoNLE1-4) of them were confirmed to localize in the plant nucleus. Moreover, both MoNLE1 and MoNLE2 were found to suppress Bax-induced cell death in *Nicotiana benthamiana*. The *MoNLE1* gene knock-out mutant was compromise in virulence on rice, whereas *MoNLE2* mutant cause normal disease comparing to wild-type strain, indicating MoNLE1 may play an important role in facilitating fungus colonization and fungal growth during infection. Any targets to Chloroplast or mitochondria? Further survey of the localization and functionally studies of those candidate effector genes will provide insight into the function of blast effector targets in regulation of plant immunity.

P PPI 53

Cyclophilin: A factor explaining major differences of aggressiveness among the blackleg causing fungal species *Leptosphaeria maculans* and *L.biglobosa*?

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Introduction: Oilseed rape is challenged by a number of fungal pathogens. One of the economical most important diseases is stem canker caused by the fungal species complex *Leptosphaeria maculans* and *L. biglobosa*. However, several studies have shown that *L. maculans* is much more aggressive on oilseed rape (Shoemaker and Brun, 2001). This phenomenon can only in small parts be explained by its attribute to produce the host-unspecific toxins sirodesmins (Sock and Hoppe, 1999), which are not produced by *L. biglobosa*. However, it is still unclear which main factors underlie these huge differences of aggressiveness. Similarly, it has been shown that cyclophilins may contribute to the virulence of certain fungal pathogens (Viaud et al., 2002).

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Objective: Comparative analysis of cyclophilin gene family in *L. maculans* and *L. biglobosa* and to determine their precise role in virulence.

Material and methods: *In silico* analysis cyclophilin gene family, fungal isolates and growth conditions, RNA isolation and cDNA synthesis, qRT-PCR and cloning of *Cyp4*.

Results: Through comprehensive whole genome analyses of *L. maculans* and *L. biglobosa*, we identified seventeen and fifteen genes respectively encoding cyclophilin in these fungi. Further to gain more insight, *in silico* analysis followed by cloning of *Cyp4* (sequencing) showed the compelling differences among two species at sequence level as well. In addition, expression levels of the cyclophilin gene (*Cyp4*) found to be relatively high in *L. maculans* as compared to *L. biglobosa*. However, the expression analyses not only demonstrated a significant difference among species but also significant intraspecific variation.

Conclusions: Taken together our finding shed light onto the significant differences among the two species. In addition ongoing *ad planta* studies may further support our hypothesis that cyclophilins and their expression may explain the difference in virulence on oilseed rape of *L. maculans* and *L. biglobosa*.

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P PPI 54

Necrotrophic effectors and sensitivity genes - gene-for-gene interactions in the wheat-*Stagonospora nodorum* pathosystem under Norwegian field conditions

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Leaf blotch diseases in wheat can cause significant yield losses and reduce grain quality. In Norway, *Stagonospora nodorum* is the dominant causal agent. The mechanisms of the pathosystem of this necrotroph have been thought to be mostly quantitative and nonspecific. More recent research suggests that very specific, inverse gene-for-gene actions are involved. Many host-selective necrotrophic effectors (NEs) and corresponding sensitivity (*Snn*) genes in the host have already been identified. The effectors induce cell death in the host, which enables the pathogen to invade the dead tissues. The core of this research has been to "Mendelize" the host-pathogen system by deconstructing the components of a single interaction between the host and the pathogen. Single effector molecules are purified from culture and infiltrated into wheat lines from a segregating mapping population. The chromosomal location of the corresponding sensitivity gene in the host can then be found using linkage mapping and DNA markers. The focus of our project is to identify and map NE/*Snn*-interactions in the Norwegian pathogen population and wheat material, and to determine the importance of these interactions under field conditions. Preliminary results from field and controlled environments in Ås, Norway and Fargo, USA, indicate that several novel QTL are found in our material, and that some are detected under both field and seedling experiments. Also, sensitivity and resistance to confirmed NEs, i.e. Tox3, is confirmed. Further work will include fine mapping of the chromosomal regions of interest and development of diagnostic markers for practical breeding.

P PPI 55

Determination of the pathotype of *Alternaria alternata* strains isolated from sunflower fields in Northwest region of Iran

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Introduction: *Alternaria alternata* has been reported as the most prevalent species causing leaf spot on sunflower in Northwest region of Iran. The disease reduces crops yield and quality by affecting photosynthetic capacity of plant and production of toxic secondary metabolites.

Objectives: Very little is known about the host specificity of *Alternaria alternata* infection sunflower. The main aim of the present study was to determine if *A. alternaria* strains isolated from sunflower are pathotypes of sunflower or not.

Material and methods: *A. alternata* isolates were recovered from sunflower plants with leaf spot symptoms. Five cultivars of sunflower, ornamental sunflower and other plant species such as squash, cucumbers, melon, watermelon and safflower were inoculated by spraying of *A. alternata* spore suspension and crude metabolites, harvested from fungal cultures. Diseases progress and severity was evaluated on inoculated sunflower cultivars and other plant species as well.

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Results: The results of inoculation of plants with fungal spore suspension showed that only five cultivars of sunflower were susceptible. The severity of disease, however, differed between cultivars. No symptoms were observed in other plant species. In inoculation with fungal crude metabolites severe symptoms were observed in compare to spraying with spore suspension. All five cultivars of sunflower were completely necrotized and other plant species showed (slight) symptoms.

Conclusion: The obtained results reveal the role of host specific toxins in inducing sever necrotic symptoms on sunflower cultivars. Such that, *A. alternaria* isolates originated from sunflower might be considered as sunflower pathotype.

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Can susceptibility to net blotch in barley be explained by sensitivity to necrotrophic effectors?

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Question: Net blotch is a major barley disease in Norway caused by the necrotrophic fungus *Pyrenophora teres* leading to yield losses of up to 40%. *P. teres* and other closely related wheat pathogens such as *Parastagonospora nodorum* secrete necrotrophic effectors (NEs) which act as virulence factors in order to gain entry into and nutrients from the host. Upon recognition by corresponding host susceptibility factors (SF), NEs trigger programmed cell death, thereby creating a suitable environment for the pathogen.

The main objective of this study is to examine the potential role of NEs and corresponding host receptors in explaining susceptibility to net blotch in Norwegian barley. This knowledge together with an understanding of the genetic background of the Norwegian net blotch population will be utilized to speed up resistance breeding.

Methods: 350 isolates have been collected from naturally infected barley plants and will be genotyped in order to assess genetic diversity and population structure of the Norwegian net blotch population. Selected isolates and their culture filtrates will be screened for specific reactions against an association mapping panel, a set of differential lines and two biparental mapping populations to characterize novel NE-host susceptibility interactions and to map the corresponding sensitivity loci. Effector protein candidates will be purified and further analysed to verify their effect on disease development.

Results: In the 2014 field testings, all current Norwegian cultivars lacked sufficient resistance against net blotch. The most resistant current variety showed 28% susceptibility while all other current varieties were above average susceptible. The field data also indicated recent changes in the pathogen population. For instance 'Heder' was the most susceptible of all entries 2013 and averagely susceptible in 2014, while it previously had been considered to have good resistance. 'Arve' was widely grown in the 1990s and considered highly susceptible, but was rated moderately resistant during the field testings in 2013 and 2014. Preliminary screenings with a small number of isolates revealed a resistance/susceptibility QTL on barley chromosome 3H in the Hector x NBD112 mapping population and two resistance QTL on 3H and 6H in the CI5791 x Tifang mapping population under greenhouse conditions.

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Temporal progress of anthracnose in a 'Niagara Rosada' vineyard in Brazil

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São Paulo State is the largest Brazilian producer of rustic table grapes. Niagara Rosada (*Vitis labrusca*) is the main cultivar planted with 89% of total plants. However, the weather of this region is highly conducive to the occurrence of anthracnose (*Sphaceloma ampelinum*). Thus, the aim of this study was to evaluate the temporal progress of anthracnose incidence in leaves, shoots and fruit. The experiment has been carried out in a 12 years-old vineyard of 'Niagara Rosada' in vertical trellis system in Brazil (23°07'24.53"S, 46°51'47.33"W). The experimental vineyard was divided in five blocks (each block with 118 plants). Pruning was performed in August 22nd, 2014. The incidence of plants with diseased leaves, shoots and fruit was evaluated weekly from the development of primary shoots until the beginning of fruit ripening. Exponential, monomolecular, logistic and Gompertz models were fitted by non-linear regression to the disease incidence for the different plant organs. The first symptoms on leaves were observed 32 days after pruning (DAP) with incidence 1.41%. After 7 days, 54.8% of the plants had diseased leaves and 53 DAP all plants had symptoms. The first symptoms on shoots were observed 46 DAP and the maximum incidence was 25%. Approximately 77 DAP incidence of plants with symptomatic shoots remained constant due to outset of lignification of these organs. Monomolecular model showed the best fit to the disease progress on leaves and shoots with coefficients of determination (R^2) of 0.99 and 0.90, respectively (Table 1). The maximum asymptotes estimated by the monomolecular model were 1 and 0.64, and the progress rates (r) were 0.09 and 0.08 for leaves and shoots, respectively. The

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exponential model showed the best fit to the disease incidence in plants with symptomatic fruit ($R^2 = 0.82$). Less than 4% of plants showed diseased berries until 88 DAP. This low incidence probably occurred because of low rainfall (125 mm) during the period of berries development (Figure 1). At 101 DAP the incidence increased to 13.8% due to heavy rains (92 mm) on days 95-98 DAP and then stabilized due to fruit ontogenetic resistance. According to the results, the primary inoculum is very important for the disease progress in leaves and shoots and the secondary inoculum in fruit. Acknowledgments: this research was supported by FAPESP (2013/24003-9).

Figure 1

Table 1. Parameters and respective standard errors estimated by non-linear regression of logistic, monomolecular, Gompertz and exponential models fitted to the anthracnose incidence in 'Niagara Rosada' plants with symptoms in leaves, shoots and fruit in experimental vineyard in Brazil, 2014.

Organ	Model*	Parameters				
		R ² (a)	b ₁ (b)	b ₁ SE ^(c)	r ^(b)	r SE ^(c)
Leaf	Logistic	0.9872	26.2769	2.7941	0.4226	0.0441
	Monomolecular	0.9939	-0.1123	0.0193	0.0935	0.0074
	Gompertz	0.9962	5.2528	0.6174	0.2689	0.0139
	Exponential	0.7724	0.2642	0.0536	0.0648	0.0107
Shoot	Logistic	0.6533	1.9051	0.2026	0.0253	0.0030
	Monomolecular	0.9049	0.1549	0.0246	0.0804	0.0102
	Gompertz	0.6895	1.1312	0.0763	0.0197	0.0021
	Exponential	0.5687	0.3840	0.0235	0.0097	0.0014
Fruit	Logistic	0	0.65887	11.0148	-6.6799	0
	Monomolecular	0.7074	-0.0276	0	0	0
	Gompertz	0	0.3576	0.9530	-287802	0
	Exponential	0.8212	0.0051	0.00017	0.0525	0.0055

(a) Coefficient of determination. (b) b₁ and r are parameters estimated by models, b₁ is related to the amount of initial inoculum and r is progress rate. (c) SE: standard error estimated for each parameter.

Figure 2

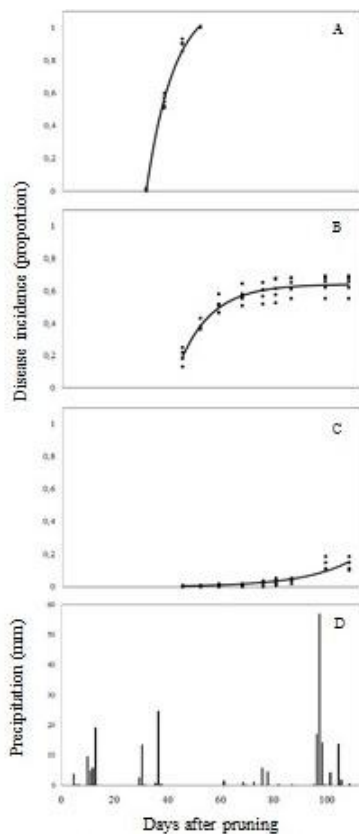


Figure 1. Cumulative incidence (proportion) of anthracnose in grapevines cv. Niagara Rosada with symptoms (dark circles) in leaves (A), shoots (B) and fruit (C) in experimental vineyard in Brazil, 2014. Solid lines indicate the monomolecular model fitted to the disease incidence in leaves (A) and shoots (B), and exponential model fitted to incidence of anthracnose in fruit (C). Total amount of rain during the experiment (D).

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Efficiencia da adubação com silício e rizobactérias na supressão de arroz da brusone em condições de sequeiro.

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Silicon (Si) is considered as a beneficial element for increasing plant growth and development with corresponding increase in grain yield, besides controlling different rice diseases. The main aim of the present investigation was to evaluate the efficiency of bioagents singly and in combination with different doses of silicon fertilization in controlling leaf blast, in upland rice, under no tillage and in rotation with soybean. A field experiment was conducted during two consecutive years in Embrapa Rice and Bean Research Center. The lay-out was a randomized block design with four repetitions. The treatments totaling 10 consisted of 5 doses of calcium and magnesium silicate (0, 1, 2, 4 and 8 ton.ha⁻¹) singly or in combination with a mixture of two PGPR's (*Pseudomonas fluorescens* + *Burkholderia pyrrocinia*). The calcium and magnesium silicate was applied by broadcasting in the field 30 days before planting soybean. The bioagents were applied by seed microbialization and soil drenching. Leaf blast was assessed 60 days after planting and the data were submitted to analysis of variance. The results showed statistical differences among Si doses and in combination with bioagents in relation to the suppression of leaf blast in both years. The doses 4 and 8 tons.ha⁻¹ of (SiCaMg) in combination with bioagents reduced leaf blast by 85.34% and 99.88% in the first year and 59.10% and 50.48% in the second year, respectively, compared to control.

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Evaluation of resistance to Ramularia leaf spot in different German barley cultivars under field conditions

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Ramularia leaf spot (RLS), caused by *Ramularia collo-cygni*, has recently become a more frequent problem in barley cultivations not only in Germany but also worldwide. When temperature and humidity are favorable, infection can happen any time from the beginning of flowering stage to the maturation of barley. Control of RLS needs application of several different disease management strategies. Using resistant cultivars should be a main aspect of an integrated access to reduce the destruction of RLS. Previous studies have shown that the resistance to RLS varies widely among barley genotypes and cultivars, but any cultivar has yet been identified that exhibits complete immunity or resistance. At the present study, replicate field trials with natural infection on 3 different locations in Germany were carried out in 2013 and 2014 to evaluate susceptibility and resistance of 86 winter barley and 37 spring barley genotypes under natural field conditions. The amount of disease severity (%leaf necrotic area) and area under the disease progress curve (AUDPC) was assessed during growing season. Additionally, spore traps were used to determine the timing of spore dispersal and spore concentration during growing season.

Our results showed significant differences ($P \leq 0.05$) between the cultivars and between the locations. Disease severity was relatively lower in 2013 especially in Ahlum, Region of Braunschweig. A few barley cultivars have a relatively higher level of RLS resistance. Cultivars Lomerit and Hobbit showed a low resistance in whereas cultivars California and Lonni were found to be highly resistant. Very low numbers of Rcc conidia were found from the beginning of growing season to end of April. The number of spores increased slightly from beginning until mid of May almost one week before the first symptoms were detected in winter barley cultivations. Unfortunately we could not find the main source of inoculum around the field trials. Spore concentration increased strongly three weeks later when the plants were completely covered with necrotic spots and bunches of conidiophores carrying conidia emerging on the leaves. These spores should be the primary inoculum for spring barley cultivations. The results of this study could be useful for developing better strategies for more effective control of the disease.

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P PPI 60

Evaluations of nano- imidacloprid against rice insect pest under laboratory and field conditions

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Nanotechnology is a promising field of interdisciplinary research. It opens up a wide array of opportunities in various fields like medicine, pharmaceuticals, electronics and agriculture. The potential uses and benefits of nanotechnology are enormous. These include insect pests management through the formulations of nanomaterials-based pesticides and insecticides, enhancement of agricultural productivity using bio-conjugated nanoparticles (encapsulation) for slow release of nutrients and water, nanoparticle-mediated. *Chilo agamemnon* and *Hydrellia prosternalis* are the main rice pests they cause a lot of damage to the rice crop. The nano- imidacloprid were tested against these two insect pests. LC50 recorded 221×10^4 and 178×10^4 spores/ml for *Chilo agamemnon* and *Hydrellia prosternalis*, respectively under laboratory conditions. Results showed that the percentage of infestation were significantly decreased to 12 and 12% under field conditions in the plots treated with nano- imidacloprid. The yield significantly increase to 6735 ± 31.50 and 6221 ± 13.10 ton/ feddan in the plots treated with nano- imidacloprid.

P PPI 61

The role of transcription factor Thc6 of *Trichoderma harzianum* in the induction of maize resistance against foliar pathogen *Curvularia lunata*

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Methods: *Trichoderma harzianum* mutant T66 was constructed with *Agrobacterium tumefaciens*-mediated transformation method. qPCR analysis of JA/ET genes of leaf was conducted. Leaf proteome was also profiled by 2-D gel and MS/MS analysis.

Results: To investigate *Trichoderma harzianum* Thc6 function, we obtained knock-out, complementation, and overexpression mutants of Thc6. The Thc6 overexpression mutant can reduce the disease index of maize inbred line Huangzao 4 against the leaf spot pathogen (*C. lunata*). Meanwhile, The Thc6 mutants were found to affect the resistance of maize leaf against *C. lunata* by enhancing the activation of jasmonate-responsive genes expression. Thc6 protein is localized in the cell nucleus, consistent with the prediction that it is a transcription regulator. Liquid chromatography mass spectrometry data further confirmed that the concentration of jasmonate in the induced maize exhibits a parallel change tendency with the expression level of defense-related genes. Yeast one-hybrid assay found two DNA fragments interaction with Thc6 protein. The two gene were belong to polysaccharide hydrolase family, named Thph1 and Thph2. Electrophoretic Mobility Shift Assay (EMSA) confirmed that Thc6 regulated both gene expression. Comparison of cellulase activity and the mRNA level of revealed obvious decrease in the Δ Thc6 strain cultivated on inducing compound. The same trend of the cellulase activity is observed in the Δ Thph1 and Δ Thph2 strain, respectively. Further research indicated both of Thph1 and Thph2 protein triggers differential expression of a group of JA/ET signal genes in leaf. The response of leaf comparative proteome corresponding to *Trichoderma* colonization on root after challenging inoculation of pathogen was profiled to unveil what happen along the long distance transduction of signals from root to leaf.

Conclusion: Hence, it was concluded that the Thc6 and cellulase genes in *Trichoderma harzianum* are closely involved in the induction of maize leaf resistance against *C. lunata* infection due to *Trichoderma harzianum* interaction with maize root.

Reference: Zhang J, Zhou J-M. Plant immunity triggered by microbial molecular signatures. *Molecular plant* 2010;3:783-93.

P PPI 62

Influence of elevated atmospheric CO₂ on *Fusarium* wilt (*Fusarium oxysporum* f sp. *ciceris*) compatible and incompatible interactions in chickpea

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Nowadays the dynamic climate change has become a challenge that needs to be checked as it is likely to affect most of the pathosystems. Despite this trend, the extent and mechanisms through which elevated CO₂ affects plant diseases remain uncertain and limited research has been done on the influence of elevated CO₂ on plant pathogens and diseases. An important example of a potential increase in disease as a result of climate change is the risk of *Fusarium* wilt (FW) caused by *Fusarium oxysporum* f. sp. *ciceris* (Foc). This disease is of major consequence because of its widespread distribution and significant yield loss under favourable conditions. To understand if elevated CO₂ plays a role in FW, the levels of antioxidant enzyme activities

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were monitored. Therefore, in this study we examined morphological and biochemical changes in FW resistant (WR 315) and susceptible (JG 62) chickpea at various post-inoculation times under ambient (350 ppm) and elevated (550 ppm) CO₂ levels. The study was conducted in specifically designed facility called Open Top Chambers (OTC) and measurable changes in the morphology of the host in elevated CO₂ concentration was observed compared to ambient CO₂ concentration. Plant height and plant biomass increased significantly in elevated CO₂ concentration. Incubation period was advanced under elevated CO₂ in susceptible cultivar as compared to ambient. Biochemical studies reveal differential expression of antioxidant enzymes in compatible and incompatible interactions under elevated CO₂. APX and GPX activities increased in susceptible plants whereas, CAT and SOD activities induced in resistant plants. DAO activity decreased in susceptible cultivar but increased in resistant cultivar. GR activities were induced in susceptible plant but CO₂ dependent increase was non-significant. Therefore, it appears that, under elevated CO₂, susceptible plants succumb to pathogen infection more easily than the plants grown in ambient condition which is directly correlated with induction of plant defense enzymes.

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Timing of inoculation and *Fusarium* species affect the severity of Fusarium head blight on oat

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Fusarium head blight (FHB) is a destructive disease of oats in Canada. To assist the development of FHB-resistant cultivars, the influence of timing of inoculation and pathogenicity of four *Fusarium* spp. (*F. culmorum*, *F. graminearum*, *F. sporotrichioides*, and *F. avenaceum*) causing FHB were examined on 12 oat genotypes under controlled environmental conditions in two separate sets of experiments. In the first set, early inoculations with *F. graminearum* at or before the complete emergence of ears resulted in little or no visible FHB symptoms but deoxynivalenol (DON) contents ranging from 0.4 to 2.6 ppm were detected in the harvested grain. Severe levels of FHB were observed on these genotypes with infected spikelets ranging from 30 to 74% and DON concentrations, from 6.6 to 10.0 ppm, when plants were inoculated at or after the 50% anthesis stage. Inoculation at 50% anthesis was considered the most appropriate as it allowed sufficient time for disease development and assessment prior to the physiological maturity of the plants. In the second set of experiments, *F. culmorum* and *F. graminearum* were equally highly pathogenic, having areas under the disease progress curve of 45.3 and 47.3, respectively. *Fusarium sporotrichioides* was significantly less pathogenic than the two highly pathogenic species. The pathogenicity of *F. avenaceum* was intermediate and was not significantly different from those of either the highly pathogenic or the weakly pathogenic *Fusarium* species.

P PPI 64

Study of Chitinase activity on banana seedling that induce with *Trichoderma* spp. inducer as resistance response of *Fusarium Oxysporum f.Sp.cubense*

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An experiment was conducted to investigate the chitinase activities of banana seedling that induce with *Trichoderma* spp. (*T. Koningii* isolat S6sh, *T. Viride* isolat T1sk, *Trichoderma* sp isolat P4sh and control.) The study consists of two parts: 1. Testing chitinase activity in banana seedlings induced by *Trichoderma* spp., Using a factorial in a completely randomized design with two factors: a. types of inducers (biomass, liquid culture and filtrate), b. Isolates of *Trichoderma* spp. with 4 replications. Parameters measured were chitinase enzyme activity, the specific activity of the chitinase enzyme were detected in stems, leaves and roots of banana seedlings. 2. Testing of several types of inducers of *Trichoderma* spp. in inducing resistance in banana seedlings to *Foc*, together with the study design 1. Parameters measured were: incubation period, the percentage of symptomatic leaves and percentage discoloration of vessels. Result showed that the inducer liquid culture *T. viride* isolat T1sk and *T. koningii* isolat S6sh were effective in increasing the activity of chitinase enzyme and cause induction of resistance of banana seedlings to *Foc*. The increase in the specific activity of the chitinase enzyme liquid culture *T. viride* isolat T1sk on stems 280% and leaves 594% with the effectiveness of suppression of Fusarium wilt disease 42.98%, while the liquid culture inducers of *T. koningii* isolat S6sh an increase in the specific activity of the chitinase enzyme on the root 410% with the effectiveness of disease suppression intensity of 27%. The increase in the specific activity of chitinase enzyme in leaves more determine the incidence of induced resistance than with an increase in the stem.

P PPI 65

Characterization of ribosome-inactivating protein (RIP) of oil palm (*Elaeis guineensis*) and antifungal effect on *Ganoderma boninense* in vitro

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Oil palm is the most valuable oil crop in Malaysia. The major obstacle hampering palm oil production is basal stem rot disease caused by a basidiomycete, *Ganoderma boninense* due to lack of effective control measures. A more sustainable option would be genetic improvement of oil palm using defense-related genes such as ribosome-inactivating proteins (RIPs). RIPs inhibit protein synthesis by ribosomes in the cells and were shown to exhibit antimicrobial effect on fungal and viral pathogens, and insect pests. However, neither RIPs have been isolated from oil palm, nor their antifungal effect on *G. boninense* has been investigated. The objectives of this study were to, 1) isolate and characterize RIP transcript(s) from oil palm root and basal stem tissues, and 2) to determine the antifungal activity of RIP proteins against *G. boninense* in vitro. Total RNA was extracted from root and basal stem tissues of 6-month old oil palm seedlings 2 weeks post inoculated with *G. boninense*. Specific primers targeted to RIP were designed based on ESTs of oil palm RIPs and multiple sequence alignment using RIP nucleotide sequences of monocot plants. PCR amplified products were sequenced and identified based on similarity search using BLAST tool. Crude proteins were extracted and RIPs of oil palm were partially purified using ion exchange spin column. The partially isolated RIPs were subjected to rRNA depurination assay using yeast ribosomes and dual culture assay to evaluate antifungal activity on mycelial growth of *G. boninense*. Two transcripts were isolated from oil palm tissues and showed highest similarity to RIP type I of *Populus trichocarpa* (98%, XP_002328056.1) and *Mirabilis expansa* (98%, AAN65450.1). They were designated as EgRIP-1a (GenBank: KJ885619) and EgRIP-1b (DDBJ: AB968223.1). The partially purified RIP proteins demonstrated rRNA depurination activity and inhibited *G. boninense* mycelial growth in vitro by 44.1% at 5 days post inoculation (DPI). It is concluded that the isolated RIP transcripts from oil palm are type I RIPs demonstrating rRNA depurination activity and antifungal effect on *G. boninense* mycelial growth.

P PPI 66

Biological Functional Analysis of *tatB* in *Acidovorax citrulli*

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In order to explore the relationship between Twin-arginine Translocation system (Tat) and pathogenicity of *Acidovorax citrulli*, a *tatB* disruption mutant (Δ *tatB*) and its complementary strains were constructed by homologous recombination, and five factors (virulence, motility, growth ability, extracellular polysaccharide and cellulase producing ability and biofilm formation) were tested to illustrate Tat system's influence on *Acidovorax citrulli*(Ac). By comparing the expression of *TrbC*, *hrcN*, *Aave_1810*, *Aave_0034* and *hrpE* gene with real-time fluorescent quantitative PCR, we explored the relationship between *tatB* genes associated with type III secretion system, Tat system related genes and cell toxicity related genes. The results showed that Δ *tatB* could trigger the hypersensitive response to non-host tobacco. The deficiency of *tatB* weakened *Ac* virulence, motility and growth ability, while had no effect on the production of extracellular polysaccharide (EPS), extracellular cellulase and biofilm formation. Real-time fluorescent quantitative analysis showed that the deficiency of *tatB* made the expressions of *TrbC* and *hrcN* genes in mutant strain significantly increase, while the expressions of other genes notably decreased. Consequently, the deficiency of functional gene *tatB* in Tat affects some biological characteristics of *Acidovorax citrulli*, and ultimately weakens pathogenic ability. We researched the key gene *tatB* of Tat in *Ac*. for the first time, and we found that the mutant's pathogenicity was weaker than that of the wild type strain, which showed that *tatB* had close relationship with pathogenicity. We found that *tatB* could affect the bacterium's formation of flagella. In the mobility test, compared with the wild type strain, the mutant's mobility was weaker than the former's. In the growth test, Tat had close relationship with formal growth. At the early stage of logarithmic phase (4~14 h), the mutant grew more slowly than wild type strain, which was different from what was reported in *Pseudomonas syringae* pv. *tomato* DC3000. Therefore, the deficiency of functional gene *tatB* in Tat system affects some biological characteristics of *Acidovorax citrulli*, and results in the decrease of Pathogenic ability.

P PPI 67

New phytoplasma identified from grapevines and leafhoppers collected in vineyards of Quebec

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Grapevine Yellows, caused by phytoplasmas, are economically important diseases that have been detected in most grape-growing regions of the world. Phytoplasma are mainly transmitted by leafhoppers that are phloem feeders. In order to better understand the epidemiology of phytoplasma diseases in grapevines grown in Quebec (QC), it is important to know phytoplasma strains occurring in leafhoppers and in grapevines. Leafhoppers and grapevines were sampled from 2009 to 2012 in QC vineyards. PCR tests were used to identify phytoplasma strains in grapevines and leafhopper species. In grapevines, strains of Aster Yellow phytoplasmas belonging to the subgroups 16SrI-A, -B, and -C were detected. In leafhoppers, phytoplasmas belonging to subgroups 16SrI-A, -B, -C, -S, -W, 16SrVII-A and 16SrIII were detected. In Quebec vineyards, we found 5 new strains of phytoplasmas in grapevines and 2 new strains in *Erythroneura* species that belonged to the AY group. RFLP analyses showed that 5 of the new strains belong to known AY subgroups 16SrI-A, -C / -R, or -F; Phylogeny and biodiversity of phytoplasmas in QC vineyards are discussed.

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Expression and function analysis of metacaspase gene family during pathogen-triggered PCD in grapevine

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1. What is the role of grapevine metacaspases in plant defence immunity?

2. Can we use the promoter of grape metacaspase to generate a tool that helps us to see at an early stage of programmed cell death?

Hypersensitive response (HR) is a form of programmed cell death (PCD) localized at the site of attempted pathogen invasion, which is an indispensable and ultimate mean to block the spread of pathogens in innate immunity and defence. HR is also a common response accompanied with the second layer of plant immunity, which is called effective triggered immunity (ETI). Metacaspase, one type of cysteine-dependent protease, was reported to play an essential role in plant PCD processes. However, its interplay with other HR factors and specificity is far from being understood. *Plasmopara viticola*, one kind of biotrophic pathogen, could induce hypersensitive cell death-like phenomena (necrotic spots) on pathogen resistant cultivars of grape, such as *V. rupestris* and *V. riparia*. We intend to make use of the differential response of those genotypes to examine the function of metacaspases in PCD.

Using BLASTN against the 9 known Arabidopsis metacaspases, all 6 grapevine metacaspases could be identified and grouped into Type I and Type II. Each phylogenetic branch has a similar exon/intron structure. The gene expression of two metacaspase genes on *V. rupestris*, *VrMC2* and *VrMC5*, were up-regulated on leaf discs after 24h with 40,000 sporangia/mL of *Plasmopara* infection, but not happen on *Müller Thurgau*, suggested that differentiation of metacaspase expression could be used as marker to measure if the host-pathogen battle is won or lost on the level of cell-death related ETI immunity (Fig 1). The pathogen related cis-elements of *MC2* and *MC5* promoters were analyzed in *Müller Thurgau* and *V. rupestris* respectively. There are different cis-element distributions between *Müller Thurgau* and *V. rupestris* in the same gene promoter region. Most interestingly, we found one disease resistance response cis-element called BIHD1OS, which exists 3 repeats in the *VrMC5* promoter, but not find in the *VvMC5* promoter from *Müller Thurgau*. That could be a possible reason to explain the different expression trend between these two grape cultivars. Furthermore, Subcellular localization results showed that *VrMC2* was located around the nuclear zone, and *VrMC5* was located in the cytoplasm. That suggested two different types of grape metacaspases probably play the role of PCD in different cell subcellular location (Fig 2).

Figure 1

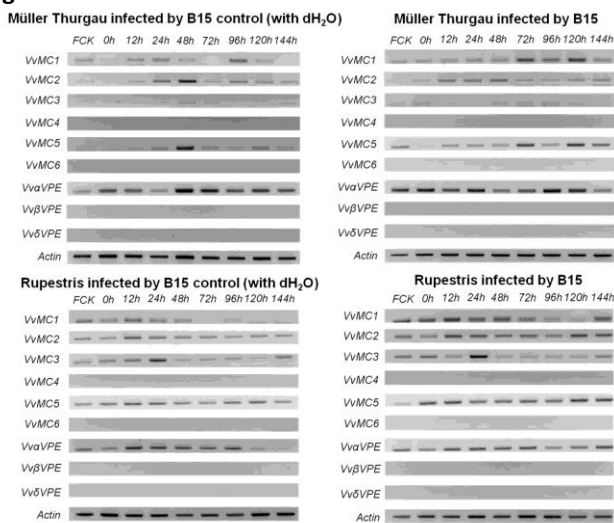


Figure 1 Expression of caspase-like genes induced by B15 infection

Figure 2

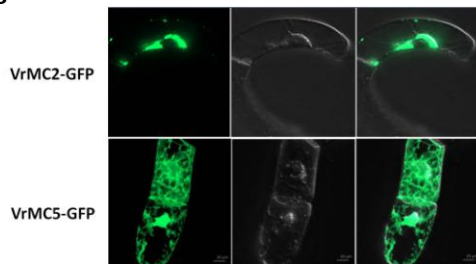


Figure 2 Subcellular localization of VrMC2-GFP and VrMC5-GFP(C terminal) transient transformation in BY-2 cell culture

P PPI 69

Virulence of population of *Blumeria graminis* f. sp. *tritici* and resistance in wheat cultivars at seedling stage in Gansu province, China

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Powdery mildew caused by *Blumeria graminis* f. sp. *tritici* (Bgt) is one of the most important diseases that damages wheat production in China. It is important to the control of powdery mildew with analysis of virulence structure and effectiveness of resistance genes. This study was conducted to test virulence structures of 91 isolates collected from Gansu province in 2013. The result of virulence survey reveals that the virulence frequencies to the resistance genes *Pm1*, *Pm2*, *Pm3a*, *Pm3b*, *Pm3c*, *Pm3d*, *Pm3e*, *Pm3f*, *Pm4a*, *Pm4b*, *Pm5*, *Pm6*, *Pm7*, *Pm8*, *Pm19*, *Pm5+Pm6*, *Pm4+Pm8*, *Pm4b+Pm5*, *Pm4+Pm2X* and *PmEra* have reached 70% or higher, respectively, indicating that these resistant genes cannot be used as mildew resistance sources any more. The virulence frequencies of the mildew population tested to resistance genes *Pm13*, *Pm16*, *Pm17*, *Pm18*, *Pm21* and *Pm24* were lower than 15%, and these genes can still be used in breeding for mildew resistance. By means of artificial inoculation 91 Gansu Bgt isolates which had different virulent spectrum at seedling stage, the resistance to 35 wheat varieties which come from Gansu and other province were evaluated. The results showed that only 'Mianmai37' was immune to all isolates, about 85% wheat varieties and its virulent frequency above 60%, such as 'Dingxi40'. The resistant wheat cultivars absent in Gansu province and China.

P PPI 70

Interaction between the biocontrol phylloplane bacterium *Bacillus mojavensis* A-BC-7 and the pathogen *Pseudomonas savastanoi* ITM317 in olive knots

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Olive knot disease, caused by *Pseudomonas savastanoi* pv. *savastanoi*, is one of the most important biotic constraints for olive cultivation. *Bacillus mojavensis* A-BC-7, a natural colonizer of symptomless olive phylloplane, was examined as potential BCA against olive knot disease. Bioassays in *in vitro* micropropagated and on one year olive plants were carried out to assess the antagonism of A-BC-7 controlling knot development and pathogen populations when co-inoculated with the pathogen in stems with different ratios. Results showed that A-BC-7 was able to decrease knot weights and pathogen population size, producing less necrotic tumours, and altered the localization of the pathogen in the hyperplastic tissue, which may pose epidemiological consequences. Confocal laser scanning microscopy combined with fluorescent tagging of bacteria revealed that when the pathogen was inoculated alone, tended to be localized at the knot surface. However, presence of the BCA seemed to confine *P. savastanoi* at inner regions of the tumours. This approach has also enabled to prove that the pathogen can move systemically beyond the hypertrophied tissue.

P PPI 71

Transcriptome profiling of potato cultivars under *Pectobacterium carotovorum* subspecies *brasiliense* challenge using RNA-seq

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The soft rot enterobacteriaceae (SRE) are important phytopathogens infecting a wide range of economically important crops worldwide. One of the best studied host of the SRE is potato, the 3rd most important crop plant after rice and wheat. There are currently no chemical control options available for soft rot pathogens. In South Africa, *Pectobacterium carotovorum* subsp. *brasiliense* (*Pcb*) has emerged as the most aggressive SRE infecting potato plants causing major economic losses. Previously, we reported differences in the colonization patterns of *Pcb* in a susceptible vs. tolerant potato genotype¹. Hence, the aim of this study was to determine global transcriptional changes in the two potato cultivars in response to *Pcb* infection using RNA sequencing (RNA-seq). To this effect, two potato cultivars, a tolerant (cv. BP1) and susceptible (cv. Valor) were stem-inoculated with *Pcb* strain 1692. Total RNA was harvested at 0, 6, 12, 24 and 72 hours post inoculation (hpi) followed by mRNA enrichment, cDNA library preparation and sequencing on the Illumina HiSeq2000 platform. RNA-seq analysis generated more than 1 billion clean 90-bp pair end reads from 30 libraries derived from inoculated and mock-inoculated tolerant and susceptible potato stems. Over 25,000 potato genes were expressed in each library and pathway enrichment analysis against the Kyoto Encyclopedia of Genes and Genomes Pathway database (KEGG) assigned 16,820 genes to 124 pathways. Differentially expressed genes (DEGs) were identified at all 5 time-points, however, the large differences in the transcriptome profiles were observed at 12 hpi. At this time point, 4178 and 2925 DEGs were up regulated and down regulated, respectively. The DEGs identified were involved in plant-pathogen interaction, biosynthesis of secondary metabolites, cell wall modification, signaling and other functions, indicating a strong activation of defense-related genes in the tolerant cultivar. A substantial volume of potato transcripts was generated providing valuable insights into responses of potato plants to *Pcb* infection. This work contributes to the identification of key genes in plant defenses against necrotrophic bacteria adding to the current understanding of plant-microbe interactions.

1 Gugulethu C Kubheka, Teresa A Coutinho, Ntsane Moleleki, and Lucy N Moleleki, 'Colonization Patterns of an Mcherry-Tagged *Pectobacterium Carotovorum* Subsp. *Brasiliense* Strain in Potato Plants', *Phytopathology*, 103 (2013), 1268-79.

P PPI 72

Reproductive performance of two vector aphids of *Turnip mosaic virus* on the infected and non-infected plants

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Introduction: Potyviruses belong to the family *Potyviridae*, together with seven other genera, and represent one of the most economically important and widely distributed groups of plant viruses. Non-persistent viruses were considered not to have close interactions with their vector aphids because of their extraordinary short interaction periods compared to persistent viruses. However, several recent studies have revealed various interesting interactions between non-persistent viruses and their vector aphids. Clarifications of such interactions may contribute to establish novel control measures against the viruses.

Turnip mosaic virus (TuMV) is a Potyvirus that causes diseases mostly in Brassicaceae plants. The virus is usually spread by 40- 50 species of aphids in a non-persistent manner. In Japan, three phylogenetic groups of TuMV, world-B, basal-BR and Asian-BR, as well as their recombinants, are distributed. After 2000, basal-BR population was suddenly appeared and the shift from world-B group to basal-BR predominant was observed.

Objectives: To clarify effects of TuMV infection on the reproductive performance of vector aphids.

Materials and methods: Periodical investigations were performed from October 2014 to February 2015 to reveal the seasonal occurrence of vector aphids in a Japanese radish field. Laboratory experiments were also conducted to compare reproductive performance of vector aphids on TuMV-infected and non-infected turnips.

Results: In the periodical investigation, two vector aphids *Myzus persicae* and *Lipaphis erysimi* were found in the census field. Among them, *M. persicae* was rather high density in the early stage of Japanese radish, but soon *L. erysimi* was became predominant as the development of plants. Although the TuMV infection did not affect the population growth of *M. persicae*, it significantly promoted the reproduction of *L. erysimi*.

Conclusion: The present study revealed that, among two vector aphids, *L. erysimi* is considered to have more mutualistic relationships with TuMV than *M. persicae*. Based on this as well as other results, we will discuss possible mechanisms of the predominant genotype shift in the field.

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Genetic diversity of *Pyrenophora tritici-repentis* in Algeria as revealed by amplified fragment length polymorphism (AFLP) analysis

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Introduction: *Pyrenophora tritici-repentis* (Died.) Drechs. (anamorph *Drechslera tritici-repentis*) is the causal agent of tan spot disease of wheat, which is found in major wheat growing areas worldwide. This disease is very destructive and can cause high yield losses. Breeding resistant wheat cultivars seems to be the best option for managing this disease. Therefore, knowledge of the structure of pathogen populations is essential for an efficient breeding approach to utilize host resistance.

Objectives: This study's objective were to assess genetic diversity among the Algerian population of *P. tritici-repentis* using AFLP markers and to decipher the relationship between molecular markers and race classification on one hand, and between molecular markers and geographic origin and host plants (durum and bread wheat) on the other hand.

Materials and methods: DNA of 61 isolates of *P. tritici-repentis* from different cereal growing areas in Algeria were analyzed. DNA of each isolate was digested using the restriction enzymes *EcoRI* and *MseI*. Initially, 78 primer combinations were tested, of which 12 were selected and applied to the 61 isolates. Amplification products were separated by capillary electrophoresis in an ABI PRISM 3100 and analyzed. Genetic similarities between all isolates were computed using the Jaccard coefficient while a dendrogram was generated from clustering with the unweighted pair-group method with arithmetic averages (UPGMA).

Results: A high level of genetic variability was demonstrated between *P. tritici-repentis* isolates. The 12 primer combinations used produced an average of 104.25 DNA fragment per primer combination. Of the 61 isolates comprising the analyzed population, the AFLP markers have revealed the presence of 61 different haplotypes. Primer combination C70 and C11 showed the most informative values (0.156 and 0.149, respectively). All of the bands obtained were polymorphic; no monomorphic band across all isolates was detected. However, one primer combination amplified a 154 bp DNA fragment that was found in 58 of the 61 isolates evaluated, similarly, the same primer combination amplified 317 bp and 351 pb DNA fragments in 57 of the isolates. The Jaccard similarity index range was 1.43 to 68.37%. Cluster analysis showed that, clustering of isolates was independent of their race classification, geographic origin, or host plant. However, one isolate (Ptr24) that showed a new virulence pattern in our previous race analysis study was clearly distinguished from the rest of the population studied. This isolate had not only new

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virulence but also different genetic makeup to other *P. tritici-repentis* isolates and requires additional studies to decipher complete knowledge of host-pathogen interactions for tan spot of wheat.

Conclusion: This work is the first study of genetic diversity of *P. tritici-repentis* in Algeria. It provides information about genetic structure of this population which should be used by plant breeders. In fact, pathogen populations with large genetic variation gain advantage as they can rapidly respond to changing environments and overcome host resistance and fungicide treatments.

P PPI 74

Influence of abiotic factors on the development of mango malformation disease (MMD)

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Mango malformation disease (MMD) caused by a predominant and virulent fungus, *Fusarium nivale* (Fr.) Ces. is one of the most devastating disease in mango orchards of Sindh province of Pakistan. In the present study, *in vitro* and *in vivo* attempts were made to analyze the influence of abiotic factors on the development of MMD. *In vitro* results comparing growth media, revealed that radial mycelial colony growth of *F. nivale* was significantly increased in potato-dextrose agar medium (34.07 mm) followed by V-8 juice agar (32.17 mm) as compared to Mango flower extract agar (27.61 mm); while it was decreased in Richard's agar medium (9.068 mm). Increase in intensity was observed in trees of Desi (local) variety (6.25-11.42%) when the trees were kept under water stress in the month of July as compared to June, August, and May, respectively under the field conditions. The correlation of different environmental conditions i.e., temperature, relative humidity (%), evapotranspiration, sunshine hours and rainfall was studied to see their effect on the incidence of MMD. The overall incidence of the disease was highest in July (70.0%) when the average minimum and maximum temperature was 28.10-36.30 °C with average relative humidity of 55.50-75.50% having 19 cloudy days, followed by June (56.66%), August (33.33%), and May (30.0%), respectively. The intensity of the disease was lowest in November and December with cool temperatures (16.10-31.20 and 8.68-25.10 °C) having intensity values of 10.00%, respectively. Based on the findings, it is concluded that higher temperature with moderate relative humidity favoured the development of MMD, however, in the months of June and July, the frequent application of irrigation can reduce the extent of temperature and increases the humidity under the microclimatic conditions that ultimately be helpful in reducing the severity of MMD. This preliminary investigation also provides the basis for further in depth investigations on the issue, leading to prioritization for the improvement of a particular variety and interventions at optimal time limits. It is also suggested that Integrated MMD Management maybe adopted to overcome this obstacle from future outbreaks.

P PPI 75

Studies on mechanism of high temperature-induced disease resistance in *Arabidopsis*

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Introduction: Systemic acquired resistance (SAR) is a potent innate immunity system in plants and has been used in rice fields. Development of SAR, involving priming, is achieved by activation of salicylic acid (SA)-mediated pathway. The induction of disease resistance by high temperature treatment has been reported in cucumber, in which SA is likely to take part.

Objectives: To determine whether heat shock (HS) treatment can induce SAR, we analyzed the effects of HS on *Arabidopsis*.

Materials and methods: *Arabidopsis thaliana* was grown in sterilized potting soil in pots in a growth chamber. HS treatment was applied by immersing 3-week old plants in hot water preheated to various temperatures. After HS treatment, the plants were cultured in a growth chamber under normal conditions. For the *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst*) infection assay, *Pst* was inoculated by dipping the plants in the bacterial solution (2×10^5 cfu/ml).

Results: HS treatment induced disease resistance, expression of SAR marker genes, and SA accumulation in wild-type but not in SA-deficient *sid2* and *NahG* plants, indicating that HS treatment induced SAR by activating SA-mediated signaling pathway. Time course analysis of the effects of HS indicated that SAR was activated transiently, differently from biological induction, with a peak at 2-3 d after HS, and that it ceased in several days. Production of reactive oxygen species was observed before SA biosynthesis, which might be a trigger for SAR activation.

Conclusion: The data presented here suggest that HS can induce SAR, but there exist unknown regulation mechanisms for the maintenance of SAR. Clarification of the regulation mechanisms of HS-induced SAR at the molecular level is expected to yield useful information in the effort to exploit this resistance for various crops.

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Role of ethylene signaling in induced disease resistance in rice

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Introduction: Induced disease resistance, activated by some microbes and chemicals, protects the whole plant from the attacks by various types of pathogens such as bacteria, fungi and viruses. Systemic acquired resistance (SAR) induced by pathogen infection through salicylic acid (SA) accumulation has been practically utilized in rice fields by exploiting the plant activators capable of inducing of SAR. Another induced resistance activated by symbiotic bacteria has been speculated to involve unknown signaling pathway.

Objectives: In this study, we analyzed the requirement of ethylene signaling for induction of these resistances.

Materials and methods: SAR was activated by treatment of rice plants with a plant activator benzisothiazole 3 to 5 days prior to challenge inoculation. The induced resistance by symbiotic bacteria was activated by cultivating rice plants with *Azospirillum* sp.

Results: To analyze the influences of lack of ethylene signaling, EIN2 (ethylene-insensitive2) RNAi transgenic plants were established. The transgenic plants lacked the ethylene response when they are treated with 1-aminocyclopropane-1-carboxylic acid (ACC). SAR induction in the transgenic plants was analyzed by phytopathology and molecular biology techniques with a plant activator benzisothiazole, which revealed that ethylene signaling was not necessary for SAR. On the other hand, the symbiotic bacteria-induced resistance was not developed in the transgenic plants, suggesting an unknown signaling pathway for induced resistance other than SA-mediated one. To clarify the role of ethylene signaling in disease resistance in rice, the interaction between ethylene signaling and other phytohormones during the induction of induced resistance is under investigation.

P PPI 77

Role of ACC deaminase in plant infection by the soilborne pathogen *Verticillium dahliae*

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Ethylene biosynthesis in microorganisms can occur via three different pathways. Two pathways start from the amino acid methionine and involve the intermediates 1-aminocyclopropane-1-carboxylic acid (ACC) and the α -keto- γ -methylthiobutyric acid (KMBA), whereas the third pathway starts from 2-oxoglutarate (OXO). Roles of ethylene produced by microorganisms, including phytopathogenic fungi, have not been well characterized. *Verticillium dahliae* is a soilborne fungal pathogen with a broad host range, causing disease to many economically important crops. Even though disease symptoms caused by *V. dahliae* are similar to those caused by ethylene exposure, whether ethylene or other compounds produced through these pathways is involved in pathogenicity remains unknown. Overexpression of the endogenous *V. dahliae* gene encoding ACC deaminase, which breaks down ACC into α -ketobutyrate and ammonia, resulted in significantly higher disease severity in tomato plants. In this study the role of ACC deaminase in pathogenesis is further investigated by disrupting the gene and by evaluating the effect on morphology, growth characteristics and virulence of resulting mutants. Results from this study will help understand the role of ethylene in the pathogenicity of *V. dahliae*.

P PPI 78

Effect of tomato pathogen *Fusarium oxysporum* MR93 and its biocontrol by the bacterium *Pseudomonas putida* PCI2

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Introduction: Tomato is the vegetable plant with the highest economic value. Production and commerce of tomato are continually increasing. Consequently, the study of control methods of tomato plant pathogens is of great importance for a more efficient production. The present work was undertaken to study the potential of *Pseudomonas putida* PCI2 as a biocontrol agent in the tomato-*Fusarium oxysporum* strain MR193 system and to detect production of bacterial compounds associated with increased plant resistance to fungal pathogens.

Materials and methods: We evaluated root rot by this pathogen in tomato plants pre-inoculated with strain PCI2. In addition, we carried out Polymerase Chain Reaction (PCR) studies to detect the presence of antifungal compounds encoding genes in

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PCI2. We also quantified the production of ethylene and salicylic and jasmonic acids in PCI2 by gas chromatography-flame ionization detection and liquid chromatography-electrospray tandem mass spectrometry, respectively.

Results: The obtained results showed a reduction of disease severity in the root of tomato plants pre-inoculated with PCI2 and an increase in shoot and root dry weight of plants over the untreated pathogen control. No fragments for the encoding genes of 2,4-diacetylphloroglucinol, phenazine-1-carboxylic acid, pyrrolnitrin, pyoluteorin or hydrogen cyanide were amplified from the DNA of PCI2. On the other hand, PCI2 produced $0.7 \text{ ng ml}^{-1} \text{ h}^{-1}$ of ethylene in King's B broth plus L-methionine and 6.95 and $0.091 \mu\text{g ml}^{-1}$ of salicylic acid and jasmonic acid, respectively, in Luria Bertani medium.

Conclusions: This study shows that *P. putida* PCI2 applied to tomato seeds increases the resistance of plants to root rot caused by the fungus *F. oxysporum* MR193 and that PCI2 produces compounds that may be involved at different levels in triggering an induced systemic resistance. Certainly, this work suggests that PCI2 represents a non contaminating management strategy potentially applicable in different agro-ecosystems, particularly in vegetable crops such as tomato.

P PPI 79

Quantitative yield loss of coffee (*Coffea arabica*) caused by Antestia Bug (*Antestiopsis spp.*) in Rwanda

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Coffee is the most important cash crops and major source of rural income in Rwanda. The antestia bug is a major pest causing 30% -47% loss. The study aimed to establish yield loss at mid altitude of Rwanda. The data was collected at the farm, washing station, and cupping laboratory. We selected randomly five fields in the Maraba sector; and at each field, six trees (four from border and two from middle). We assessed bugs population from each tree using knockdown method. After harvesting, we hand sorted the cherries and grouped them into damage categories: (i) insect damaged, (ii) abiotic factors and (iii) clean cherries; and recorded weight for each category. We repeated hand sorting at washing station before floatation. The hand sorted cherries were floated in water, and again sorted out according to the three categories. The seeking and floating cherries were de-pulped and dried separately for further assessment of quality during cupping. The middle trees had one bug/tree, while border had 4 bugs/tree, and the average was three bugs/tree; which is above ET of two bugs/ tree. The loss of cherries by hand sorting was 6%, of which 4% was antestia damage. After floatation, the loss was 24.8% (18.6% by antestia damage), making a total yield loss of 30.8% (22.6% by antestia bug). The yield loss by hand sorting alone was not effective. Yield loss due to poor quality will be reported after cupping is completed. The data were recorded from fields where farmers applied pesticide as recommended.

P PPI 80

Rhizobacteria promoting growth and defense in rice plants against *Magnaporthe oryzae*.

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Plant growth-promoting rhizobacteria (PGPR) are bacteria that lives around the plants roots and are involved in the induction of resistance of plants to diseases. This study investigated the effect of PGPR in promoting growth of rice roots and suppressing leaf blast (*Magnaporthe oryzae*). The experiment was conducted in completely randomized blocks with six treatments, consisting of microbiolized seed with rhizobacterias (T1=235; T2= 82R, T3=235 + *M. oryzae*, T4=82R + *M. oryzae*, a negative control (T5=*M. oryzae*) and a positive control (T6=microbiolized seeds water only)). The rice cultivar BRS Primavera was sown in trays containing fertilized soil (FTE 1g / kg soil, Zn 1g / 2kg and NPK - 5/30 / 15 g / kg). Twenty one days old plants were spray inoculated with *M. oryzae* suspension (3,105 conídios.mL⁻¹). At the same time, rice seeds (with the same treatments) were sown in test tubes containing water-agar (0.8%), kept under controlled conditions in a growth chamber (25 ° C) during 14 days for of roots and shoots growth measurement. The leaf blast severity index (SBF) in microbiolized plants with rhizobacteria 235 was suppressed by 80% compared to the control (Duncan, $p = 0.05$). In plants treated with the PGPR isolate 235, the increase in leaves and roots length was 60.86 and 101.24 mm, respectively (Duncan, $p = 0.05$). These results suggest that rhizobacterium 235 promoted the growth of root and shoot of rice plant, suppressed the leaf blast and can be explored as a potential biological agent.

P PPI 81

The date palm inflorescence rot fungus *Mauginiella scaettae* can infects the model host *Arabidopsis thaliana*

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Mauginiella scaettae is a fungal pathogen causing inflorescence rot of date palm: one of the most devastating diseases in date palm growing regions worldwide. This disease was first reported by Cavara (1925) in Libya but the infection mechanisms remain obscure. To characterise the infection mechanism it was attempted to develop the model plant species *Arabidopsis thaliana* as an alternative host. Ten strains of *Mauginiella scaettae* were isolated from different oases of East Algeria. Ouargla01 (OU1), Ouargla02 (OU2), Ouargla03 (OU3), Hadjira (HA), Touggourt01 (T1), Touggourt02 (T2), Oued01 (OE1), Oued02 (OE2), Ghardaïa (G), Biskra (Bis). Sequencing the internal spacer (ITS) regions of indicated the phylogenetic position of *M. scaettae*. Upon drop-inoculation with conidia of *M. scaettae*, *Arabidopsis* exhibited white necrotic lesions with marginal yellowing appeared on leaves at 06 days post inoculation and subsequently expanded throughout the entire leaves. It was noted that strain G was the most and T2 is the least virulent. Microscopic investigations demonstrated that a preference for fungal penetration via stomata, accompanied by hyphal tip swelling which may represent a primitive haustorium. Both of these features were also observed on *M. scaettae* infected date palm spadices. On *Arabidopsis* initial biotrophic growth was followed by a necrotrophic lifestyle, similar to what happens on date palms. To investigate how host resistance mechanisms could modify the interaction were examined *M. scaettae* interactions with *Arabidopsis* mutants *cpr1* and *sid2* respectively increased and compromised in biotrophic resistance mechanisms, and *eto2* which displays elevated resistance against necrotrophic pathogens. Interactions with *eto2* appeared to be particularly affected, suggesting that anti-necrotrophic mechanisms - here linked to ethylene - could be effective in conferring resistance to *M. scaettae*. This study demonstrated the value of using model species in providing novel insights into previously cryptic interactions.

P PPI 82

Evaluating the Virulence of *Metarhizium anisopliae* (Deuteromycotina: Hyphomycetes) and *Beauveria bassiana* (Ascomycota: Hypocreales) Isolates to Arabian Rhinoceros Beetle (*Oryctes agamemnon arabicus*)

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Virulence of entomopathogenic fungi *Metarhizium anisopliae* and *Beauveria bassiana* might be more efficient tested against *Oryctes agamemnon arabicus* larvae. Four concentrations (1×10^5 , 1×10^7 , 1×10^9 and 1×10^{11} conidia/mL) of two locally isolated entomopathogenic fungi, spore suspensions were used in this study via larval direct spraying. Results revealed that both isolates can cause high mortality rate reaching 100% after 29 days. However, each isolate behaved differently: *Beauveria bassiana* scored higher mortality rate in short time especially at the concentration 1×10^{11} conidia/mL with LT50 12.75 and LT90 20; while, the isolate *Metarhizium anisopliae* caused higher percentage of malformed adults. Moreover, both isolates affected insect's life cycle particularly pupal stage which was reduced remarkably by almost 50% in comparison with the control treatment.

Materials and methods: Two entomopathogenic isolates locally isolated were used in these studies which are MARD 34 and 46 (Table 1); these were selected from Entomopathogenic Fungal Isolates Bank at the Agricultural Research Directorate, Iraqi Ministry of Science and Technology.

Bioassay test: Spore suspension was prepared by adding 5ml of sterile distilled water to pure full growth isolate Petri dish; then, by using a sterile metal scraper, fungal mycelia were scraped and the solution was poured into a 50ml Falcon tube after filtering the solution through sterile miracloth. The spore concentration was determined using haemocytometer and adjusted to 1×10^5 , 1×10^7 , 1×10^9 and 1×10^{11} conidia/mL.

Result: Survival percentages of date palm larvae after treating them with entomopathogenic fungal isolate spore suspensions MARD 46 revealed that the concentration 1×10^{11} scored the fastest mortality among larvae reaching 93.33 % after 19 days, followed by the concentration 1×10^9 that recorded mortality of 66.66% at the same time

Table 1: Malformation scale among date palm borer adults treated with the isolates MARD 46 and 34.

Isolate MARD 34				
Days after treatment	Concentration			
	1×10^5	1×10^7	1×10^9	1×10^{11}
20 Days	-	-	+	+
23 Days	+	++	++	++
27 Days	++	+++	+++	++++
Isolate MARD 46				
20 Days	-	-	-	+
23 Days	+	-	++	++
27 Days	++	+	+++	-

Scale: +: Low malformed adults; ++: Moderate malformed adults; +++: High malformed adults; ++++: The highest malformed adults

Figure 1

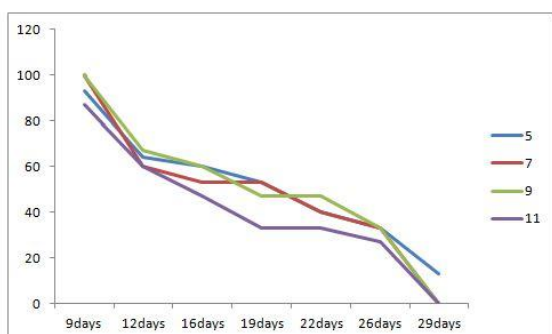


Figure 2: Survival percentages among date palm borer larvae treated with the isolate MARD34 during the experiment duration (29 days).

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Host plant resistance (HPR) traits of watermelon [*Citrullus lanatus* (Thunb.) Mansf.] against melon fruit fly (*Bactrocera cucurbitae* (Coquillett)) in hot arid region of India

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The significant differences were found in tested varieties/ genotypes for fruit infestation and larval density per fruit. The varieties/ genotypes, Asahi Yamato (12.73%), AHW/BR-16 (15.10%) and Thar Manak (18.27%) were found resistant; Durgapura Lal (23.03%), Sugar Baby (26.67%), AHW/BR-12 (29.73%), Arka Manik (34.15%), Charleston Grey (38.70%), AHW-65 (35.80%), AHW-19 (48.97%) were found moderately resistant and IC 582909 (53.18%), AHW/BR-60 (55.52%), BSM-1 (59.10%), AHW/BR-137 (60.58%) and AHW/BR-9 (67.37%) were found the susceptible varieties/ genotypes to fruit fly infestation. The significant positive correlation ($r = 0.99$ $p < 0.01$) was observed between per cent fruit infestation and larval density per fruit. The percent fruit infestation and larval density had significant positive correlation with fruit length ($r=0.57$ & 0.55) and days to first fruit harvest ($r=0.75$ & 0.76) and negative correlation with length of ovary pubescence ($r= -0.91$ & -0.91), rind hardness ($r= -0.86$ & -0.87) and rind thickness ($r= -0.77$ & -0.75). Maximum variation in fruit infestation and larval density was explained by length of ovary pubescence (82.50 and 83.60%, respectively) followed by fruit length (4.3 and 3.0% respectively) and rind thickness (3.2 and 2.0%, respectively). Free amino acid was lowest in resistant (Asahi Yamato) and highest in susceptible variety/ genotype (BSM-1) whereas phenols, tannin, total alkaloids and flavonoid contents were highest in resistant and lowest in susceptible varieties/ genotypes. Flavonoid and total alkaloid contents explained (88.4 and 92.0%, respectively) of the total variation in fruit fly infestation and in larval density per fruit. Thus, from the foregoing account, it can be argued that reduction in fruit fly infestations on resistant varieties/ genotypes could be due to antixenotics (biophysical) and antibiosis (allelochemicals). Certain biophysical traits (e.g. length of ovary pubescence, rind hardness and rind thickness) and biochemical traits (e.g. flavonoid, tannins, phenols, ascorbic acid and total alkaloids) were linked to resistance of watermelon against *B. cucurbitae* and therefore, can be used as marker traits in plant breeding programmes to select resistant varieties/ genotypes.

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Antifungal activity of novel allyl-sulfonamides and synthetic precursors against *Colletotrichum acutatum*.

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This study evaluated the antifungal activity of eighteen novel allyl-sulfonamides, the synthetic precursor methyl (Z)-2-(bromomethyl)-3-(phenyl)acrylate, and six primary sulfonamides and against *Colletotrichum acutatum*, the causal agent of anthracnose of strawberry. The new allyl-sulfonamides derived from Morita-Baylis-Hillman adducts were synthesized and characterized in the Chemistry Department. They were prepared by the reaction of ten different primary sulfonamides with methyl (Z)-2-(bromomethyl)-3-(phenyl)acrylate via two different methodologies, affording methyl (E)-3-phenyl-2-(R-sulfonamidomethyl)acrylates and methyl 2-[(R-sulfonamido)(phenyl)methyl]acrylates, where R = phenyl, 4-fluorophenyl, 4-chlorophenyl, 4-bromophenyl, 4-iodophenyl, 4-nitrophenyl, methyl, ethyl, butyl or octyl groups. The evaluation of the antifungal activity was done at the Plant Pathology Department by the Poison Food technique. The positive control used was the dithiocarbamate fungicide ziram, being 100% active at all doses tested. The most active allyl-sulfonamides, methyl (E)-2-(butylsulfonamidomethyl)-3-phenylacrylate and methyl 2-[(4-chlorophenylsulfonamido)(phenyl)methyl]acrylate, inhibited the growth of *C. acutatum* in 54 and 51%, respectively, at the concentration of 1,5 mmol.L⁻¹. These allyl-sulfonamides were at least twice more active than their parent primary sulfonamides. As all allyl-sulfonamides presented higher log P values than their respective precursors, these results suggest that the greater lipophilic character of the allyl-sulfonamides in comparison with the primary sulfonamides is partially responsible for the best results observed. The (Z)-2-(bromomethyl)-3-(phenyl)acrylate was even more active than the allyl-sulfonamides, presenting an IC50 value of 0.34 mmol.L⁻¹. This compound exhibited the most promising antifungal activity of all tested compounds, showing 100% of inhibition of *C. acutatum* growth at 0.45 mmol.L⁻¹.

P PPI 85

Study of antioxidant and antimicrobial activity of the leaves and pulp of *Argania spinosa* L. in the region of Tindouf (Algeria).

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Our study aims to assess the ability of the extract of the leaves and pulp of *Argania spinosa* L. Skeels to scavenge free radicals, and to test its antimicrobial activity against various bacterial strains and a fungal strain: *Candida albicans*.

A phytochemical screening allowed knowing the phytoconstituants and targeting by a selective extraction the principal families of these metabolites, including tannins whose yield was significant (0.6%), flavonoids and saponins based on their biological activity.

The reading of the results of the antibacterial and antioxidant activities showed that the extracts of the leaves were more active as antioxidants and antibacterial agents.

On testing the antioxidant activity of the methanolic crude extract and tannins extract by HPLTC, the result was positive by the appearance of yellow spots in both TLC plates sprayed with DPPH. The antioxidant activity of both extracts by a spectrophotometric quantification of DPPH showed a = 0.093mg/ml, CI50= 0.12 mg/ml for the ethyl acetate fraction and tannins. The extract flavonoids (ethyl acetate fraction) was found to be more potent against all bacteria strains and gave an interesting antibacterial activity against multi-resistant strain of *Pseudomonas aeruginosa*.

P PPI 86

Change Proteome of *Solanum Tuberosum* tubers under the influence of signaling molecules infected by *Phytophthora Infestans*

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Important role in the relations between plants and pathogens belongs to reactive oxygen species (ROS), including hydrogen peroxide. Jasmonic acid (JA) is a signaling molecule for induced generation of ROS in plant tissues. *Bacillus subtilis* culture filtrate has a protective effect on plants.

Potato tuber discs of Leoni cultivar treated with a solution of JA 10⁻⁷ M or culture filtrate *B. subtilis* were inoculated with *Phytophthora infestans* (10⁶ spores/ml). Isoelectric protein focusing was performed on Protean IEF (Biorad, USA). For the

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separation of proteins by isoelectric point used ready-to-use 7-cm strips (Biorad, USA), the range of pH 3-10. For separating proteins according to molecular weight was carried out SDS-PAGE in a 12% PAAG at Laemmli.

Infection leads to changes in the spectrum of proteins potato: there were synthesized *de novo* proteins with pI 8.0 and Mr 41 and 43 kD, protein expression with pI 5.5 and Mr 24 kDa was significantly reduced. Processing JA and infection resulted in a spectrum similar to the control, indicating that the plant hardly undergoes stress. Treatment of *B. subtilis* culture filtrate led to the appearance *de novo* protein with pI 4.3 and Mr 26 kDa, but to a lesser extent as compared with the infected control.

Identify the impacts of *B. subtilis* and JA on the activity and range of protective proteins may be associated with increased resistance of wheat plants to the *Ph. infestans*.

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P PPI 87

Characterization of pathogenic variability and mating types of *Ascochyta rabiei* population in Morocco

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Ascochyta blight, caused by *Ascochyta rabiei* Lab. (teleomorph : *Didymella rabiei*), is an economically important fungal disease on chickpea in Morocco, and other parts of the world. Significant progress has been made in breeding chickpea for Ascochyta blight resistance in Morocco, but the plant resistance is typically overcome by the evolution of virulent pathotypes. It's assumed that the sexual recombination of *D. rabiei* ascospores could increase genotypic diversity in *A. rabiei* populations and evolve increased virulence that can overcome resistant cultivar. In this study pathogenic variability of forty one Moroccan *A. rabiei* isolates, collected from four different chickpea growing regions, was assessed by screening over a set of four chickpea differential genotypes. The identification and the distribution of mating types of *A. rabiei* were determined using a *MAT*-specific PCR assay to assess the risk of sexual reproduction of *A. rabiei*. The pathogenicity test showed that highly significant variability of AUDPC (Area under the disease curve) had occurred between genotypes and pathogens and their interactions. The isolates were classified into three pathotypes groups according to their level of virulence. The aggressive pathotype PIII has been present in the majority of surveyed regions, however the least aggressive pathotypes PI and PII were the most prevalent. *MAT*-type analysis of *A. rabiei* isolates showed non-significant deviation from equal distribution of *MAT*1-1 and *MAT*1-2 with a predominance of isolates with a profile *MAT*1-1 in the overall populations. It appeared that conidial reproduction is more frequent in Moroccan population of *A. rabiei*, and there is a competitive advantage associated with *MAT*1-1. Nevertheless in two regions, the mating ratio of *MAT*1-1 and *MAT*1-2 were not significantly depart from (1:1) ratio, based on chi-squared tests ($p < 0.05$). Therefore random mating could occur under natural conditions in these regions and can contribute to enhance virulence of pathotypes. The eventual occurrence of sexual reproduction of the pathogen has to be taken into account while designing suitable disease management strategies, including the deployment of resistant germplasm.

P PPI 88

Chemical changes induced by Witches' broom disease in acid lime (*Citrus aurantifolia*)

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Witches' broom disease (WBD), caused by *Candidatus Phytoplasma aurantifolia*, is a very serious disease of acid limes. The disease killed over one million lime trees in Oman and Iran. WBD results in production of small, clustered leaves in some branches of lime trees. More branches develop symptoms with time and become unproductive, until the whole tree collapses within 3 to 5 years of first symptom appearance. This study was conducted to investigate changes in minerals as a result of disease symptom development. Findings from this study revealed that the level of sodium and potassium increased by 4.0 and 1.5 times, respectively, in the symptomatic branches compared to the non-symptomatic branches. On the other hand, the levels of magnesium and calcium were significantly lower by 1.4 and 1.2 times, respectively, in the symptomatic branches compared to the non-symptomatic branches. There was no consistent effect of symptom development on the levels of nitrogen, phosphorus, boron and copper. The study discusses factors affecting changes in the mineral content of symptomatic acid lime branches and the potential to manage the disease through mineral nutrition.

P PPI 89

Nitrate enhances cucumber resistance to *Fusarium* wilt

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Question: *Fusarium* wilt is a major disease that causes severe losses in cash crops (Ajillogba and Babalola, 2013; Flood, 2006; Ploetz, 2006). Mineral nutrition plays a critical role in the management of a broad range of plant diseases (Huber and Thompson, 2007; Marschner, 2012). However, it is still poorly understood how mineral nutrients affect pathogen infection and disease development *via* changes in plant physiology and metabolism, and the plant-pathogen interaction regulated by mineral nutrition is doubted.

Methods: To investigate the effects of nitrogen on *Fusarium* wilt in cucumber, pot and hydroponic experiments were conducted in a greenhouse. The effects of different forms of nitrogen on *Fusarium oxysporum* f. sp. *cucumerinum* infection and root exudate production were investigated.

Results: For cucumber seedlings, nitrate was superior; nitrate significantly suppressed *Fusarium* wilt compared to ammonium (Fig. 1). Plants grown with nitrate accumulated less fusaric acid (FA) after *Fusarium oxysporum* f. sp. *cucumerinum* (FOC) infection. Nitrate-treated plants also exhibited increased resistance to chemical FA by decreasing FA absorption and transportation in the shoots. Root exudates may regulate *Fusarium* wilt in a manner dependent on the form of nitrogen treatment. At lower citrate concentrations, lower citrate synthase (CS) activity was observed (Fig. 2). The expression of CS genes was down-regulated in the root exudates of nitrate-treated cucumbers. Biological tests showed that citrate was preferable for FOC spore germination and infection.

Conclusions: Nitrate nutrition results in superior *Fusarium* wilt resistance in cucumber plants. The lower citrate concentration in root exudates of nitrate-grown plants resulted in a lower wilt disease incidence index.

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Figure 1: Effects of different forms of nitrogen on cucumber *Fusarium* wilt.

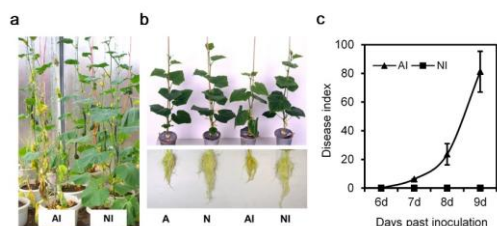
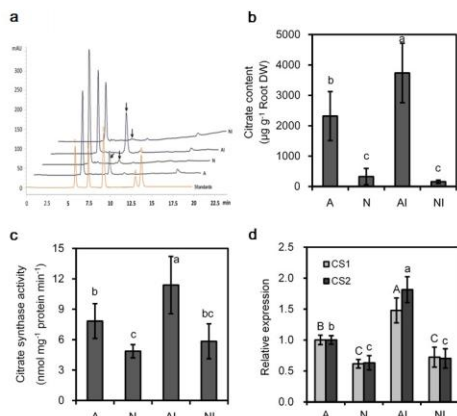


Figure 2: Effects of different forms of nitrogen and FOC infection on organic acids in root exudates.



P PPI 90

TALEN-mediated generation and metabolic analysis of camalexin-deficient *cyp71a12 cyp71a13* double knockout lines.

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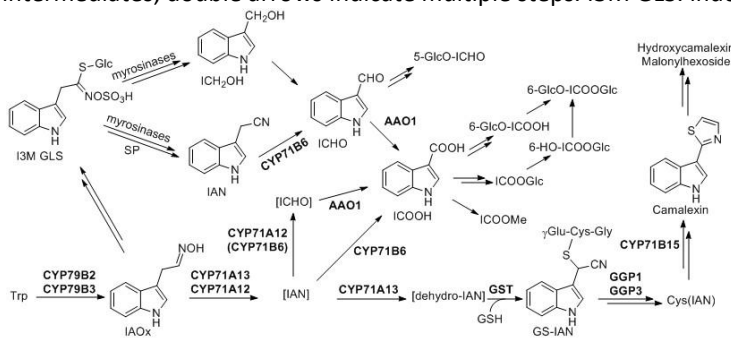
Question: In *Arabidopsis thaliana* tryptophan-derived secondary metabolites play a crucial role in defense against pathogens. A number of these compounds are synthesized via indole-3-acetonitrile (IAN), including camalexin and indole-3-carboxylic acid (ICOOH) derivatives. CYP71A13 is a key enzyme for camalexin biosynthesis and catalyzes the conversion of indole-3-acetaldoxime (IAOx) to IAN. The CYP71A13 gene is located in tandem with its close homolog CYP71A12, also encoding an IAOx dehydratase. However, for CYP71A12 indole-3-carbaldehyde (ICHO) and cyanide were identified as major reaction products. Still, the *in vivo* function of CYP71A12 and its role in the IAN metabolism remained unclear.

Methods: We generated two *cyp71a12 cyp71a13* double knockout mutant lines using CYP71A12-specific Transcription Activator-Like Effector nucleases (TALENs), which were introduced into the *cyp71a13* background. Very efficient somatic mutagenesis was achieved and we observed stable transmission of the *cyp71a12* mutation to the following generations. Challenged leaves of *cyp71a12 cyp71a13* mutants and respective controls were analyzed for their metabolic phenotype by HPLC and UHPLC/ESI-QTOFMS.

Results: In contrast to *cyp71a13* plants, in which camalexin accumulation is partially reduced, double mutants synthesized only traces of camalexin, demonstrating that CYP71A12 contributes to camalexin biosynthesis in leaf tissue. A major role of CYP71A12 was identified for inducible biosynthesis of ICOOH. Specifically, the ICOOH methyl ester was reduced to 12% of wild type level in AgNO₃ challenged *cyp71a12* leaves. In contrast, ICHO derivatives apparently are synthesized via alternative pathways, such as degradation of indole glucosinolates.

Conclusions: Based on these results we present a model for this surprisingly complex metabolic network with multiple IAN sources and channeling of IAOx-derived IAN into camalexin biosynthesis. In conclusion, TALEN-mediated mutation is a powerful tool for functional analysis of tandem genes in secondary metabolism.

Figure 1: Model for the biosynthetic pathways of tryptophan-derived secondary metabolites in *Arabidopsis*. We propose pools of free IAN and ICHO derived from glucosinolate degradation and in addition IAN and ICHO as channelled intermediates of camalexin and ICOOH biosynthesis. Enzyme functions are denoted. Square brackets indicate proposed channelling of intermediates; double arrows indicate multiple steps. I3M GLS: indole-3-methyl glucosinolate; SP: specifier protein.



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P PPI 91

Study on induction of disease tolerance by heat shock treatment of Brassicaceae

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Introduction: Heat shock (HS) treatment has been found to be effective way for disease management in green-houses. HS has not only the direct effects on suppression of disease development but also the indirect effects on systemic resistance responses against pathogen attacks through activation of some defense related-genes, eventually leading to building up or enhancing defense mechanism in plants (Schirra et al., 2000).

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Objective: The present study aims to investigate a molecular mechanism on disease tolerance of *Brassicaceae* against *Colletotrichum higginsianum*, which is induced by HT.

Materials and methods: One-week-old Komatsuna (*Brassica rapa* var. *perviridis*) and 5-week-old *Arabidopsis thaliana* (Col-0) plants were heated under moisture conditions in a growth chamber. Plants were then inoculated by drop or spray inoculation with a spore suspension (5×10^5 spores ml⁻¹) of *C. higginsianum*.

Results: HT at 38°C for 1-7 days was effective on reduction of disease development on Komatsuna (*Brassica rapa* var. *perviridis*). The resistance was fully maintained at least for 6 days after HT, suggesting that the prolonged resistance is likely associated with enhanced defense response(s). The phenomenon was mutually applicable to Col-0 to gain insight into the molecular mechanism on the HT-induced resistance. After HT at 38°C for 1 day, the *PAD3*-mRNA started to accumulate constantly in *Arabidopsis* seedlings, which was accompanied by accumulation of camalexin, one of defense responses against invading pathogens. Furthermore, the HT could not inhibit pathogen development on phytoalexin-deficient mutant *pad3-1*. Interestingly, in Col-0 seedlings with HT, O₂⁻ generation increased significantly by 6 to 12 hpi when challenged subsequently with *C. higginsianum*. This result is well consistent with cytological observation with DAB staining, that strong accumulation of H₂O₂ was frequently evoked at the attempted site of penetration on the HT-treated leaves.

Conclusion: On the basis of these results, it is more likely that appropriate HT of *Brassicaceae* conditions the seedlings toward resistance or a physiological state to respond to subsequent infection by pathogen.

P PPI 92

Protection against anthracnose disease on *Arabidopsis thaliana* induced by volatile compound limonene

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Introduction: D-limonene is one of the monocyclic monoterpenes and is a major constituent in essential oils of several citrus species. Some of *Citrus* species and other plants emitting terpenes have been reported as a potential source of agricultural fungi- and insecticides, raising the question which compounds protect plant disease effectively.

Objective: Our study is thus to evaluate the protective effect of pure D-limonene, a major component in orange oil, on fungal disease under laboratory conditions, using *Arabidopsis* and *Brassica rapa* var. *perviridis*. Especially, we show that limonene activates jasmonic acid (JA)-regulated signaling pathway and effectively reduces the severity of anthracnose disease on *Arabidopsis* and *B. rapa* var. *perviridis* plants.

Materials and methods: Five-week-old seedlings of *A. thaliana* Col-0 and *jar1-1* (*jasmonate resistant 1-1*) were placed in a plastic container where D-limonene was vaporized. The control plants were treated similarly, in the same container without D-limonene.

Results: When *Arabidopsis* Col-0 plants were exposed to limonene (10 or 100 µmol/L) for 6 or 24 h, disease symptom induced by *Colletotrichum higginsianum* was evidently suppressed. Microscopic analysis showed that on limonene-treated leaves, the conidia of *C. higginsianum* failed to penetrate into epidermal cells. To evaluate whether limonene acts as an inducer of induced resistance, we analyzed the expression of defense-related genes by qRT-PCR. Expression of *PDF1.2* gene was enhanced in Col-0 plants exposed to limonene, whereas no prominent expression of *PR-1* was observed. However, limonene-induced expression of *PDF1.2* was diminished in the *jar1-1* plants, indicating that the response of *Arabidopsis* to limonene is mediated through the JA-regulated signaling pathway. A similar protective effect was also observed on *B. rapa* var. *perviridis*, accompanied by a significant increase of penetration failure by *C. higginsianum*.

Conclusion: We demonstrate that *Arabidopsis* plants respond to gaseous limonene, eventually squaring off the upcoming pathogen attack. D-limonene is generally recognized as safe (GRAS) in the Code of Federal Regulations. The use of limonene, by-product of orange juice production, thus also could be one of eco-friendly approaches for improving plant resistance safely.

P PPI 93

Cytological responses of *PsAPY1*-silenced pea to host-adapted and nonadapted fungal pathogens

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Poster Presentations

Plant Pathogen Interactions

Introduction: Our previous studies focusing on plant cell wall have discovered the ecto-type ATPase as a key player of extracellular defense. The elicitor from a pea pathogen *Mycosphaerella pinodes* directly binds to and enhances ATPase in extracts from pea cell wall, leading to O₂⁻ production by extracellular peroxidase. However, the concomitant presence of the suppressins A and B, virulence factors secreted by the same fungus, inhibits the catalytic activity in a host-specific manner, indicating that *M. pinodes* may target the ATPase to counter the host extracellular defense(s).

Objective: To clarify the role of the ecto-ATPase during plant-pathogen interactions, we operationally silenced *PsAPY1* gene, which encodes the pea ecto-ATPase, using an *Apple latent spherical virus* (ALSV)-based virus-induced gene silencing.

Materials and methods: Ten-day-old seedlings of pea (*Pisum sativum* L. cv. Puget) were inoculated mechanically with saps of ALSV containing a fragment of ecto-ATPase gene.

Results: The *PsAPY1*-silenced peas exhibited enhanced disease susceptibility against infection by *M. pinodes*, which is accompanied by a significant increase in the successful penetration. Microscopic analysis showed that callose deposition beneath penetration attempts was impaired in the *PsAPY1*-silenced pea. By contrast, when challenged with a non-adapted *Colletotrichum higginsianum*, the control pea plants restricted the fungal growth, which is associated with the penetration failure. Notably, on the surface of *PsAPY1*-silenced pea, the frequency of a hyphal tip-based entry (HTE), a recently discovered way to enter the plant cells without formation of appressoria (Hiruma et al., 2010), was significantly enhanced, eventually causing necrotic spots. This phenotype is similar to that in the *pen2* and *pen3* Arabidopsis mutants challenged with nonadapted *C. gleosporiodesm* (Hiruma et al., 2010).

Conclusion: Silencing of *PsAPY1* reveals the role of ecto-ATPase in interaction with host-adapted and nonadapted fungal pathogens. In our separate study (see a poster by Miki et al.), we showed that *PsAPY1*-silenced pea attenuated the peroxidase-dependent oxidative burst induced by PAMP treatment. Together, it is likely that the ecto-ATPase modulates extracellular defense(s) during the early stage of infection.

P PPI 94

Extracellular apyrase (PsAPY1) participates in the peroxidase-catalyzed apoplastic oxidative burst in pea

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Introduction: Our research focusing on the plant cell wall where most pathogens first encounter the host surface have discovered the ecto-type ATP-hydrolyzing enzyme (apyrase; EC3.6.1.15) as a key player of extracellular defense. The catalytic activity in extracts from the cell walls of pea is enhanced *in vitro*, with the consequent increase of O₂⁻ generation by extracellular peroxidase(s), when exposed to the fungal elicitor.

Objective: We operationally silenced *PsAPY1* gene encodings the ecto-ATPase in pea, using an *Apple latent spherical virus* (ALSV)-based virus-induced gene silencing. The silenced peas were used to analyze the role of *PsAPY1* in the extracellular defense.

Materials and methods: Ten-day-old seedlings of pea (*Pisum sativum* L. cv. Puget) were inoculated mechanically with saps of ALSV containing a fragment of the ecto-ATPase gene. The silenced peas (Δ *PsAPY1*) were used throughout experiments.

Results: We examined the responsibility of Δ *PsAPY1* to chitosan and lipopolysaccharides (LPS), which is a main component of fungal cell wall and glycolipid components of the outer membrane of Gram-negative bacteria, respectively. When challenged with chitosan or LPS, salicylhydroxamic acid (SHAM)-sensitive O₂⁻ generation was suppressed in Δ *PsAPY1*, indicating that the ecto-ATPase positively regulates the peroxidase-catalyzed O₂⁻ generation. Indeed, our *in vitro* study using extracts from the pea cell walls revealed that, in the presence of NADH, *p*-coumaric acid and Mn²⁺, the SHAM-sensitive O₂⁻ generating activity was enhanced in response to chitosan or LPS. Together, it is likely that plant cell wall is capable of recognizing and responding to PAMPs such as chitosan and LPS, through the oxidation of NADH by an extracellular peroxidase(s). Interestingly, the chitosan-induced expression of *PR10*-mRNA was reduced in Δ *PsAPY1*. On the basis of these results, it seems likely that *PsAPY1* spatially regulates the apoplastic oxidative burst as well as the downstream signaling leading to expression of defense-related genes in pea.

Conclusion: Considering the role of the ecto-ATPase in modulation of the cell wall-based defenses, it is conceivable that some pathogens have evolved strategies to target the host's apyrase to counter the extracellular defense of host cells (see a poster by Yao et al.).

P PPI 95

An evolutionary stable effector in *Venturia inaequalis* modulates apple defense gene expression

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Introduction: The reduction of chemical inputs imposed through the Ecophyto2018 plan underlines that the use of pesticides is still too important in agriculture despite the breeding of resistant varieties. The loss of efficiency of the selected resistance genes along the time is mainly due to the high adaptive diversity of pathogens. Therefore, identification of durable resistance genes is a key point for an efficient breeding strategy.

Objectives: The ROAD MOVIE project (Resistance Of Apple Against Disease: Mechanisms Of Virulence and Identification of Effectors) proposes to identify apple genotypes with durable resistance by focusing on an evaluation of evolutionary constraints on pathogen effectors. This strategy was applied on apple scab caused by the fungus *Venturia inaequalis*. We first screened candidate effectors among 89 fungal genomic sequences. Once putative effectors were identified, we selected those with a low ratio of synonymous versus non-synonymous mutations. Then we focused on the "X1" effector to study its role in plant defense expression and its capacity to be recognized by apple resistant genotypes.

Methods: Apple microarray analyzes were first performed in order to identify the differential responses of a susceptible apple genotype after inoculation with a wild strain of *V. inaequalis* and the null mutant strain ($\Delta X1-A7$). By transient overexpression of "X1" in a partially resistant apple, we also tested its ability to modulate apple defense.

Results: Transcriptomic results revealed metabolic pathways or defenses targeted in the host plant by "X1" effector. We found in particular genes implicated in the jasmonate pathway. Transient overexpression of "X1" in the host plant conferred an increase of resistance to the pathogen. Furthermore, we screened 20 resistant apple varieties in order to find one variety able to recognize this effector.

Conclusion: This study implemented an innovative strategy for the detection of new sustainable resistances to apple scab. The analysis of others conserved *V. inaequalis* effectors is underway.

P PPI 96

Role of extracellular apyrase in cell surface immunity

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Introduction: Apyrases (EC3.6.1.15) are enzymes that efficiently hydrolyze ATP and ADP and function intracellularly and extracellularly. In plants genes encoding the apyrase (NTP/NDPase, NTP diphosphohydrolase) are known to comprise a multigene family. In pea extracellular apyrase (PsAPY1) directly binds and responds to pathogen-derived molecules such as an elicitor and a suppressor from a pea pathogen, *Mycosphaerella pinodes*. Upon treatment with the fungal elicitor, the catalytic activity in extracts from the cell walls of pea is enhanced *in vitro*, with the consequent increase of O₂⁻ generation by extracellular peroxidase(s). By contrast, the suppressins A and B, virulence factors secreted by the same fungus, inhibits the apyrase-dependent ATP-hydrolyzing activity in a host-specific manner.

Objective: Here we used a model pathosystem involving *Medicago truncatula* and *M. pinodes* to assess the role of apyrases in extracellular defense.

Materials and methods: The phylogenetic analysis revealed that *M. truncatula* contains at least seven apyrase genes, five of which (*MtAPY1;1* to *1;5*) are members of a legume-specific family, whereas two genes (*MtAPY2;1* and *MtAPY2;2*) are close to those of non-leguminous plants. Of seven apyrase proteins, three of which (*MtAPY1;1*, *MtAPY1;4* and *MtAPY1;5*) are presumed to be secreted extracellularly, due to the presence of the putative N-terminal signal sequences. Especially, we analyzed the extracellular apyrase *MtAPY1;1* (PsAPY1 orthologue).

Results: Using an *Agrobacterium*-based *in vivo* transient expression in *Nicotiana benthamiana*, combined with a triple c-myc epitope tag technology to define the subcellular location, we found that the *MtAPY1;1* was secreted efficiently. Indeed, the *MtAPY1;1* without the putative N-terminal signal sequence resided intracellularly. Transient expression of *MtAPY1;1* in *N. benthamiana* leaves restricted necrotic disease symptom caused by a virulent pathogen, *Colletotrichum orbiculare*. In our separate study we showed that the pea apyrase (PsAPY1) positively regulates the extracellular defense by modulating the peroxidase-catalyzed superoxide generation (see posters by Miki et al. and Yao et al.). Considering the role of the extracellular apyrase in recognition and modulation for the cell wall-based defenses; i.e. production of O₂⁻, it is no wonder that some pathogens have evolved mechanisms to target apyrase activity to condition susceptibility of the host cells.

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Conclusion: Plant cell wall is well known to act as physical barriers against invading pathogens. However, our current studies have suggested that the plant cell wall employs the perception of pathogen signals, signaling and modulation of cell wall-based defenses (see posters by Miki et al. and Yao et al.).

P PPI 97

Potential traits of the mycovirus MoCV1-A on interaction between rice plant and rice blast fungus

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Magnaporthe oryzae chrysovirus 1 strain A (MoCV1-A) was found in Vietnamese isolates of rice blast fungus (Urayama *et al.* 2010, Le M-T *et al.* 2010). MoCV1-A infected isolates exhibited impaired growth of the host rice blast fungus *M.oryzae*. MoCV1-A-free strains were also obtained by curing through single spore isolation or treatment with cycloheximide. The mycovirus-free strains restored normal mycelial growth and pigmentation, suggesting that the MoCV1-A was causal agent for the impaired growth phenotypes. Spray inoculation tests and leaf sheath inoculation tests with MoCV1-A-infected isolates and MoCV1-A-free strains to 31 international blast differential rice varieties showed that the free strains had more virulence to increase the lesion numbers and to enlarge the lesion sizes on infected leaves. These data suggested that MoCV1-A infection caused hypovirulence to the host fungus. Surprisingly MoCV1-A infection also resulted in the change of pathogenic races in several differential rice lines, namely S (compatible) to R (incompatible) reaction or R to S. When the free strain (Hyg^R gene was introduced as a marker) was re-infected with MoCV1-A by hyphal fusion with the parental strain, the re-infected strain restored the race reactions to the originals in the parental MoCV1-A-infected strain. Probably MoCV1 was a potential factor of epigenetic alteration in pathogenic races and also for the reduced virulence of the host rice blast fungus. The related MoCV1-B is also found recently in Vietnam and causes more severe affects on the host fungus growth rather than MoCV1-A (Urayama *et al.* 2014). The both MoCV1-A and MoCV1-B have 5 segmented dsRNA genomes ranging from 2.8-3.5 kbp and classified into a unique clade in the family of chrysoviridae with phylogenetic analysis using RNA-dependent RNA polymerase (RdRp) conserved domains. Additionally we have found new strains of Magnaporthe oryzae chrysovirus 1 from Japan isolates, MoCV1-C YM-0007-E3 from Yamagata, and MoCV1-C AK199 from Akita, respectively. We have determined the nucleotide sequence of 5 dsRNA segments of MoCV1-C AK199, which showed 75% to 82% sequence identity with MoCV1-A and MoCV1-B. These results suggested that MoCV1 mycoviruses distribute widely in rice blast fungus.

P PPI 98

Functional analysis of the single-copy allantoicase and urease genes *ALA1* and *URE1* in virulence of the fungus *Colletotrichum graminicola* by targeted deletion mutagenesis

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Using ATMT, Münch *et al.* (2011 - Molecular Plant Pathology 12,1, 43-55) obtained a mutant (AT171) of the hemibiotrophic maize pathogen *Colletotrichum graminicola* with a virulence defect, due to the insertion of T-DNA into the promoter region of a gene encoding the enzyme allantoicase. In filamentous fungi purines are converted to allantoate, which is subsequently used as a substrate by the enzyme allantoicase, producing (S)-ureidoglycolate, which is further degraded to urea. Urea is then convert to ammonium by the enzyme urease. Urease was described as a virulence factor in some human pathogenic fungi. In order to investigate the role of allantoicase and urease during vegetative development and pathogenesis, the single-copy genes encoding urease and allantoicase were deleted. In agreement with AT171 mutants, the analyses of deletion mutants revealed that allantoicase and urease are required for differentiation of infection structures and for full virulence. To address the question when allantoicase and urease genes are expressed, we generated and employed *URE1:eGFP* and *ALA1:eGFP* replacement strains. These studies provide information on timing and localization of the purin-degrading enzymes of *C. graminicola*.

P PPI 99

A new biocontrol pathway: the γ -lactone catabolic pathway of *Rhodococcus erythropolis*, is involved in the interruption of the pectinolytic pathogen communication during the potato soft-rot

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Question: The virulence of soft-rot bacteria due to *Pectobacterium* and *Dickeya* is under the control of a quorum sensing process based on the synthesis and perception of *N*-acyl homoserine lactones (NAHSL). We are therefore developing a novel biocontrol strategy for the *Solanum tuberosum* model based on the selective stimulation of NAHSL-degrading bacteria. This approach involves the use of the *Rhodococcus erythropolis* strain R138 as a biocontrol agent, which is able both to degrade diverse γ -lactones effectively *in vitro* and to suppress the maceration of tubers in hydroponic and field culture conditions. However, the mechanism by which this strain controls soft-rot has never been elucidated *in planta*. We recently discovered a lactone assimilation pathway in *R. erythropolis*, involving the lactonase QsdA for ring opening, followed by a β/ω -oxidation of the aliphatic product. The objective of this study consists in analyzing the involvement of this pathway in the control of tuber soft-rot by *R. erythropolis* R138.

Methods: The functions of the key enzyme QsdA were investigated by making a *R. erythropolis* R138 *qsdA* deletion mutant ($\Delta qsdA$), by transferring the *qsdA* gene to an heterologous host (*Escherichia coli*), and by following the *qsdA* transcription by confocal laser scanning microscopy using an *R. erythropolis* strain carrying a plasmid-borne *qsdA::gfp* transcriptional fusion. The NAHSL-breakdown and biocontrol activities of the $\Delta qsdA$ and the QsdA expressing *E. coli* strains were compared to those of the *R. erythropolis* wild type strain in potato tubers infected with the soft-rot *P. atrosepticum*.

Results: The *qsdA* gene transcription was induced in potato tubers by the NAHSL producing pathogens only. The biocontrol and NAHSL-breakdown activities of the QsdA expressing *E. coli* strain were similar with those of the *R. erythropolis* wild type strain. The deletion of the *qsdA* gene partially abolished the biocontrol activity of the *R. erythropolis* wild type strain.

Conclusion: These results demonstrate the involvement of the γ -lactone catabolic pathway both in NAHSL-breakdown and in the control of the disease. This is a novel and unusual biocontrol pathway, based on an antivirulence strategy instead of the pathogen eradication, differing from those previously described as protecting plants.

P PPI 100

Detection of Some Resistance Genes (*Bt-5*, *Bt-8*, *Bt-10*, *Bt-11* and *Bt-12*) against Common Bunt in Ten Wheat Varieties Using Molecular Markers.

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Common bunt (*Tilletia sp.*) is a very dangerous seed-borne disease in wheat and may cause serious economic losses. Wheat cultivars including resistance genes are used as an alternative fight method instead of chemical fungicides against common bunt disease. These resistance genes in wheat are called as *bt* genes. Until today, a little number of *bt* genes in wheat has been detected by some molecular markers. There aren't yet developed molecular markers for detection of all *bt* genes in wheat. In this study, detection of some *bt* genes (*bt-5*, *bt-8*, *bt-10*, *bt-11* and *bt-12*) was carried in registered eighteen wheat varieties (Sertak 52, Bolal 2973, Demir, Kutluk, Harmankaya 99, Pehlivan, Tosun Bey, 4-11, Sönmez 01, Bezostaja-1) using PCR-based molecular markers (Microsatellite and RAPD). PCR-amplified fragments were separated on 1.3% agarose gel containing ethidium bromide (0.5 μ g/ml). Gels were visualized under UV light and digitally photographed. The obtained DNA bands were scored as present or absent for detection of *bt* genes. For comparison, the virulence rates of five *Tilletia foetida* (syn. leaves) isolates against eighteen wheat varieties were obtained from our field results. We observed that wheat varieties including *bt-10* and *bt-11* genes as Kutluk and 4-11 varieties are more resistant to disease in field. We showed analysis results for five *bt* genes in all wheat varieties and resistance rates in field.

P PPI 101

Bio-inspired computational algorithms and their application as a tool for analysis of leaf symptoms

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The incompatible interaction between plants and pathogens leads to the activation of defense reaction with manifestations of typical symptoms. The defense response to necrotrophic (*Botrytis cinerea*) or hemibiotrophic (*Phytophthora parasitica*) pathogens is induction of hypersensitive reaction accompanied by tissue necrosis. The development of disease symptoms directly correlate with development of the pathogen. On this assumption the susceptibility or resistance of the host plant could be directly correlated with proliferation of the disease symptoms.

In order to accelerate and standardize the analysis of tissue necrosis or other visible symptoms, we have created stand-alone software based on combination of certain types of artificial neural networks and common image processing algorithms. The tool has been tested on leaves infected with different common studied plant pathogens and given results compared with conventional analysis methods. Presented software is characterized by very high reproducibility and significant speed-up in data processing compared to results of manual evaluation. After successful training on the trial data sets, classification accuracy has been usually up to 0.01 false positive rate.

Figure 1

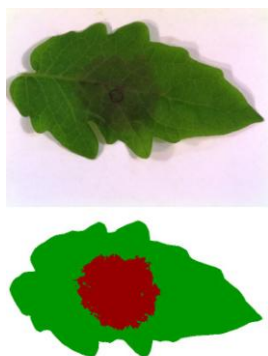
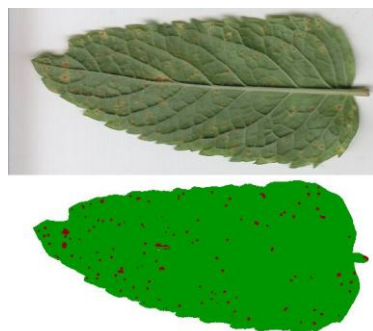


Figure 2



P PPI 102

Potential Cultivars Resistant to Groundnut Rust Confirmed

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Components of resistance to rust were studied among ten groundnut genotypes previously screened in the field. The aim was to study, characterise, and confirm the resistance of these genotypes to rust through detached leaf assay and whole plant assays, conducted under controlled temperature and in a glasshouse, respectively. In both assays, thirty day old leaves or plants were inoculated with urediniospores at 10^6 spore/ mL. Results in all three experiments showed that ICGV-SM 05569 and ICGV-SM 05570 are highly resistant to rust. For these two genotypes, the incubation period (IP) was 31 days, sporulation index (SI)- 4, infection frequency (IF) ≤ 4 , and the percentage leaf area damage (PLAD) ≤ 3 . However, all released cultivars such as CG7, Nsinjiro, Nyanda, and JL-24 were highly susceptible to rust i.e., IP = 14-17 days, SI = 4 - 5, IF = 7 - 35, and PLAD = 3 - 8%, in the detached leaf assay. SI and IP from detached leaf assay were the components highly correlated to AUDPC from the field ($R^2 = 0.9$ and $- 0.7$, respectively). On the other hand, IF and PLAD from whole plants were highly correlated to AUDPC ($R^2 = 0.7$). This indicated that different components are important in characterising and confirming resistance to rust depending on the method used. In both experiments, symptomatic rust-diseased leaves remained attached to the plant in susceptible cultivars, whereas in highly resistant cultivars leaf defoliation was a defence mechanism to rust. The use of components of resistance from detached and whole plant assay was advantageous over scoring in the field since collection of early disease scores was obscured by mud on the leaves from rain splash. Compared to trials where foliar diseases were controlled using fungicides, yield loss due to rust and leaf spot diseases was as high as 67% in the rust inoculated trials and 45% in the non-inoculated. Yield from ICGV-SM 05569 (717 Kg/Ha) and ICGV-SM 05570 (505 Kg/Ha) was higher than those of the controls and other released varieties, e.g., Nyanda

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(270 Kg/Ha) in the rust inoculated trials. A similar trend was observed in the non-inoculated trials. This implies that these varieties are new potential cultivars, highly resistant to rust, as well as high yielding in rust endemic areas. However, their resistance to other groundnut diseases needs to be evaluated.

P PPI 103

Iron as a determinant of virulence and resistance in the *Colletotrichum graminicola* - maize interaction

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In all kingdoms of life iron is an essential microelement. However, iron is hardly soluble in aerobic biogeosphere. Therefore, all living beings evolved strategies for an efficient uptake of iron. On the other hand, the redox mediation of iron can produce highly reactive oxygen species - via the Fenton- and Haber-Weiss-Reaction - damaging bio-molecules and ultimately organisms. Thus, a tight regulation of iron uptake and storage is essential. Pathogenic fungi employ several strategies for iron uptake from the host tissue: (i) reductive iron assimilation (RIA), (ii) siderophore-mediated Fe³⁺ acquisition (SIA), (iii) heme uptake, and (iv) low affinity iron uptake. As free heme is rare in the maize host plant of *Colletotrichum graminicola*, this hemibiotrophic fungus mainly applies RIA and SIA. Saprophytically hyphae growing under iron starvation conditions leads to an up-regulation of both pathways - RIA and SIA. However, during the biotrophic stage of the infection RIA is highly active, while SIA is specifically suppressed. At the later necrotrophic stage it is *vice versa*. Maize leaves pretreated with the *C. graminicola* siderophore Coprogen respond with a strong defense including a respiratory burst when these leaves were infected later on. However, Coprogen alone did not induce a defense response. In conclusion, *C. graminicola* specifically represses the SIA to cover itself. During necrotrophy hiding is not necessary anymore. This strategy resembles the specific repression of the synthesis of β -1,3-Glucane, a pathogen associated molecular pattern (PAMP), during biotrophy. From other fungal species (e.g. *Aspergillus* spp.) the tight regulation of the SIA und RIA pathways occurs on transcriptional level mediated via two transcription factors SreA and HapX, respectively. However, the studied *Aspergillus* spp. are either necrotrophs or saprophytes. Homologues of these genes were identified in the hemibiotrophic fungus *C. graminicola* as *CgSRE1* (GLRG_02856) and *CgHAP1* (GLRG_10059). Functional characterization of these transcription factors during biotrophic and necrotrophic stages will gain the knowledge of iron acquisition in fungal virulence and provide valuable data to develop novel protection strategies. With this, the long lasting question will be elucidated how the switch from biotrophy to necrotrophy operates.

P PPI 104

Characterization of Cell Surfaces of Host Wheat and Pathogens (*Tilletia foetida* and *Tilletia caries*) Using Zeta Potential and FTIR Analyses

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Common bunt is one of the most destructive diseases of wheat. The most familiar species of common bunt disease in wheat are *Tilletia foetida* and *Tilletia caries*. Virulence rate of pathogens changes depending of their interaction with host cell surface. In this study, cell surfaces of wheat and two agents (*T. foetida* and *T. caries*) of common bunt disease were characterized with Zeta Potential and FTIR-ATR analyses. A resistant (M82-2161) and a sensitive (Heinles VI) wheat variety were used for analyzing of *Triticum aestivum* host cell surface. The FTIR-ATR spectrum of *T. foetida*, *T. caries* and *T. aestivum* were measured within the range 4000-400 cm⁻¹ was used to Perkin Elmer-Spectrum100. The FTIR reflectance spectra showed differences between the pathogens and host. Chemical composition of *T. aestivum* et al includes cellulose, hemicellulose, lignin and other extractives [1-2]. N-H , O-H stretching, C-H bonds, carbonyl groups of *T. foetida*, *T. caries* and *T.aestivum* are shown in the FTIR-ATR spectrum. These bands attributed to indicating the presence of amino, amido, hydroxyl, methyl, methylene, aldehydes, and ketones groups. Thus, the poly functionality of *T. foetida*, *T. caries* and *T. aestivum* are obvious. The Zeta potential was defined by a Malvern-Nano ZS tools. These samples Zeta potentials values were defined -43.9 to +0.23 mV. The pathogens charge were measured as more negative according to the host structure. It was found that surface net charge plays an important role for host-pathogen interaction.

1 S.H. Ali, S.M. Asghar, A.U. Shabbir, Neutral sulphite pulping of wheat straw, in:Tappi Pulping Conference Proceedings, Tappi, GA, USA, 1991, p. 51.

2 F. Umar, K. Misbahul Ain, A. Makshoof, K. Janusz A., "Chemical Engineering Journal", 171, 2011, 400- 410.

P PPI 105

Does aboveground herbivory prime *Nicotiana attenuata* plants to better defend against subsequent belowground herbivory?

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Plants are attacked by both above- and belowground herbivores which may indirectly interact with each other via herbivore-induced systemic changes in plant traits. We examined whether the plant *Nicotiana attenuata* can be primed by *Manduca sexta* herbivory (priming stimulus) on its aboveground plant parts and thereby be better defended against subsequent root herbivory by the root-knot nematode *Meloidogyne incognita* (triggering stimulus). Since the defensive alkaloid nicotine is produced in the roots, we hypothesized that aboveground herbivory would reduce nematode performance via leaf-herbivore induced production of nicotine in roots and the plant would benefit. Aboveground herbivory had negative effects on plant shoot and root biomass while only belowground herbivory increased nicotine content in roots. Belowground herbivory decreased seed production and affected seed quality such as seed weight and C/N ratio. Since we have found no effects of the aboveground herbivory treatment on the performance of the belowground herbivore, the hypothesis of priming of plant defense in an above-belowground context was not supported. However, the changes in seed quantity and quality may affect the performance and fitness of the next plant generation.

P PPI 106

Regulation of Calcium-dependent protein kinases in plant disease resistance by heat shock proteins

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Calcium is a long known mediator of fundamental and conserved early signaling processes in plant immunity. Nevertheless, little is known about the activation of calcium-sensing proteins and the mechanism of subsequent signaling to activate defense responses in plants. Calcium-dependent protein kinases (CDPKs) are involved in the early signal transduction upon pathogen attack. Activation of CDPKs upon a pathogen-induced Calcium-influx leads to local and systemic defense responses, including the production of reactive oxygen species (ROS), induction of marker gene expression, enhanced levels of the phytohormone Salicylic acid (SA) and cell death. In accordance, transgenic *Arabidopsis thaliana* lines overexpressing active CDPKs were more resistant against infection with *Pseudomonas syringae* pv. *tomato* DC3000, than kinase-inactive control lines. The biochemical regulation of CDPK activity offers a quick mean to control defense responses under changing stresses and conditions. We have identified a heat shock protein (HSP) as an interactor of CDPKs involved in biotic stress resistance in *A. thaliana*. Biochemical and phenotypical analyses were used to further study the interaction and the functional mechanism between CDPKs and HSPs. Our data suggest a role of HSPs in CDPK-mediated defense responses and implicate a functional link between the ability to mount a robust defense response and environmental stress conditions.

P PPI 107

Temporal progress of grapevine downy mildew in plants with different architecture

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São Paulo State is the largest Brazilian producer of table grapes and cv. Niagara Rosada (*Vitis labrusca*) represents 49% of grape production in the State. Downy mildew (*Plasmopara viticola*) is the most important grape disease in Brazil. This study aimed to evaluate the effect of plant architecture on the temporal progress of downy mildew. The experiment was carried out with cv. Niagara Rosada cultivated in vertical trellis and 'Y' conduction systems in Brazil (22°42'30"S, 47°38'00"W). The vineyard accounted for 3 blocks of 35 plants cultivated in vertical trellis and 3 blocks of 16 plants in 'Y' system. Pruning was performed in August 13th, 2014. After budburst, the number of leaves per shoot, the leaf area index (LAI), disease incidence, and disease severity were assessed weekly. The areas under the curves (AUC) of each variable were calculated by trapezoidal integration and the conduction systems were compared by F-Test ($p=0.05$). The first disease symptom was detected 50 days after pruning (DAP, Fig. 1). Disease incidence reached 100% at 107 DAP in both conduction systems. However disease severity was low, probably due to the low frequency of rainfall in the beginning of the growing season (Fig.1 and Table 1). The AUC values for disease incidence, disease severity and LAI did not show statistical differences between the conduction systems. The AUC values for the number of leaves per shoot were statistically different between the systems, but it did not interfere in the disease progress.

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Figure 1

Table 1. Area under the curve (AUC) for number of leaves per shoot, leaf area index (LAI), disease incidence and disease severity of downy mildew in grapevines cv. Niagara Rosada, cultivated in vertical trellis and 'Y' system.

	Block	Number of leaves per shoot	LAI	Incidence	Severity
Vertical trellis	1 ¹	1434.05	119.79	31.33	0.03
	2	1319.60	121.37	45.78	0.13
	3	1392.60	114.43	45.51	0.08
	Total	4146.25	355.59	122.61	0.08
Y system	1 ²	1551.17	126.39	44.62	0.37
	2	1601.13	121.49	26.95	0.19
	3	1568.58	119.00	25.80	0.16
	Total	4720.88	366.87	97.37	0.72
<u>p value</u>		0.00*	0.28	0.34	0.09

¹48 grapevine plants in each block;

²16 grapevine plants in each block;

*Significant values by F-Test ($p=0.05$).

Figure 2

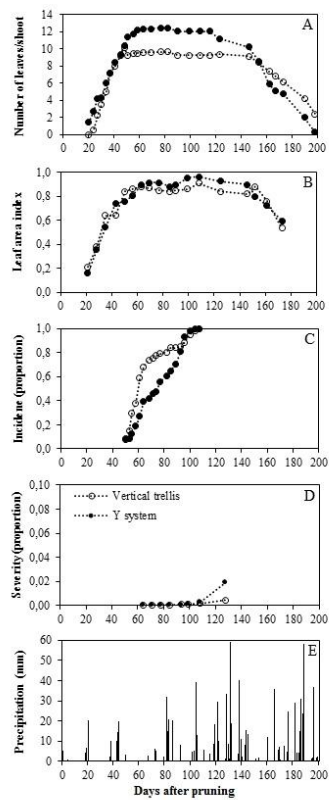


Figure 1. Number of leaves number per shoot (A), leaf area index (B), cumulative downy mildew incidence (C), cumulative downy mildew severity (D) in grapevines cv. Niagara Rosada, cultivated in vertical trellis and 'Y' system, and amount of rainfall during the growing season (E).

P PPI 108

Two-spotted spider mite symptomatology in a F2 strawberry population after wild species introgression

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Observations of susceptibility, tolerance and resistance to the two-spotted spider mite (*Tetranychus urticae* Koch) in the genus *Fragaria* L. is complex and only fragmentary approached so far. The objective of the current study was to determine the symptomatology of a F2 strawberry population after defined spider mite inoculation. The highly susceptible cultivar *F. xananassa* 'Senga Sengana' was crossed with the highly tolerant wild species accession *F. chiloensis* ssp. *lucida* USA. The best breeding clone (P-90999) was selected out from approx. 350 seedlings. After self-pollination of this P-90999 clone a F2 model population consisting of 103 randomly selected genotypes was created. The two-spotted spider mites (*Tetranychus urticae* Koch) used in this experiment were from a greenhouse rearing on clean *F. vesca* seedling plants in cages. The experiment consisted of three blocks of three different inoculation dates and eight replications in total under greenhouse conditions in late summer 2014. The genotypes were evaluated for their symptoms using a score system of nine defined stages. Symptoms were evaluated 21 days after.

It was observed that the infestation occurred in different colonization patterns depending on the genotype. The obtained data of symptom evaluation were compared using cluster analysis. The different clusters could be described with regard to their pathological development. Also the correlation of the expressed symptoms to the climatic conditions and the development of the spider mites respectively could be drawn. A segregation of spider mite resistance in strawberry in a F2 model population after wild species introgression is demonstrated. These results are valuable for strawberry resistance breeding.

This study was funded by the German Federal Ministry of Education and Research (BMBF, FKZ 031A216 A and B).

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Digital Technologies and Modelling/Forecasting

P DTMF 1

Distribution modeling of the carob moth, (*Ectomyelois ceratoniae*, Lepidoptera: Pyralidae) in Iran.

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Carob moth is the most important pest of pomegranate causes variable rate of impacts on its quality and quantity in different climates of Iran. To find out the distribution model of the pest in climates of Iran their current and future distribution models and the suitable habitats based on the records of the species collected from 240 locations was investigated. The geographical information and 6 climatic layers were used in modeling analysis by Maximum Entropy software. The jackknife test determined the contribution rate of the climatic variables in distribution modeling. Results showed northern parts of Iran, besides the Caspian Sea as the most suitable areas for the pest distribution. The pest preferred semiarid to arid localities with warm to very warm summers and moderate to cool winters. The minimum temperature during the cold months of the year and the mean annual temperature with a rate of 51.7% and 40.9% played the most important roles in distribution modeling of the carob moth respectively.

P DTMF 2

A temperature-based phenology model for predicting life table parameters of the sweetpotato butterfly *Acraea acerata* Hew. (Lepidoptera: Nymphalidae)

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The sweetpotato butterfly, *Acraea acerata* Hew., is the third most important insect pest of sweetpotato *Ipomoea batatas* L. Lam, in tropical Africa after sweetpotato weevils (*Cylas* spp.). Currently no models do exist for describing *A. acerata* temperature-dependent development, mortality or fecundity. This study therefore sought to develop a temperature-based phenology model for *A. acerata*. Temperature-dependent development of *A. acerata* was investigated at five constant temperatures of 17.5, 20, 25, 30 and 32 °C in the laboratory and under fluctuating natural temperature conditions (22.12±0.02 °C) on *I. batatas* vines and leaves. Data was collected on development time of each life stage, mortality, adult longevity and reproduction. Data were analysed using the Insect Life Cycle Modeling (ILCYM) software to calculate and predict life table parameters at different temperatures. Development of all *A. acerata* live stages was possible between 17.5 and 30 °C and the development time decreased significantly with increasing temperatures. The upper threshold of development was 32 °C for eggs and 30 °C for larvae and pupae. All immature life stages died at 15 and 35 °C. Egg mortality was quite low (<10 %) at all temperatures where development was possible. Larvae mortality was highest at 17.5 °C and lowest at 20 °C. All pupae died at 17.5 and 32 °C. Oviposition was highest at 30 °C with 131 eggs laid, and decreased with decreasing temperature. The lowest oviposition of 4.5 eggs per female was registered at 17.5 °C. Global warming due to climate change is expected to exacerbate sweetpotato damage by insect pests including *A. acerata*. The developed phenology model can be used for pest risk mapping using Geographic Information Systems (GIS) under different climate change scenarios as implemented in the ILCYM software in order to understand future pest risks and prepare national programs and farmers for IPM adaptation.

P DTMF 3

Dashboards for disease management

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Dashboard - the software application for visually displaying the most important information needed to achieve one or more objectives, and data are combined and arranged on a single screen. For the development the dashboards of disease management of septoria tritici blotch of wheat (*Mycosphaerella graminicola* (*Septoria tritici*)), powdery mildew of wheat (*Blumeria graminis*), stripe rust of wheat (*Puccinia striiformis*), leaf rust of wheat (*Puccinia triticina*), root rots of wheat (*Fusarium* sp.) was used software Xcelsius 2008. One of the main features of the dashboard is a dynamic visualization of the source data (phytosanitary data) and the results of their processing and analysis (forecast phytosanitary situation and safety recommendations) online. The risks are determined by several factors (resistant varieties, predecessor, tillage, weather

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conditions, etc.). Predictive phytosanitary situation is determined by summing the corresponding risk. Recommendations are issued according to the phytosanitary situation and the planned yield. The interface consists of five containers:

- the name of the system;
- the entering phytosanitary information;
- the displays the forecast phytosanitary situation;
- recommendations on possible courses of action, depending on the forecast of the phytosanitary situation. It also contains the module "Control wheat diseases" which contains the text information;
- the fifth consists of auxiliary units of the system. Blocks "Wheat diseases" - text information on the biology of a pathogen, "Wheat pathogens" let you jump to a web page dedicated to the pathogens in the database of the International Mycological Association. Visualization can publish it by exporting to Adobe PDF format.

P DTMF 4

A Microsoft Excel program for Bootstrap estimates of reproductive-life table parameters

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Bootstrap estimates are time-consuming processes that are impossible to use without a computer program. In this study a guideline was offered to prepare a program in Microsoft Excel environment to carry out time-consuming calculations of reproductive life table data in a minute or two by repeatedly pressing a shortcut key.

P DTMF 6

Climatic requirements of a clade of the African maize stem borer, *Busseola fusca* from the Highveld region of South Africa

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The African stem borer, *Busseola fusca*, is one of the major stem borer pests of maize in Africa. It is known that *B. fusca* completes 2-3 generations in the warmer West African countries and also the same number of generations in the cooler areas of Ethiopia and Lesotho. It is therefore suggested that there are different mitochondrial clades with overlapping distribution, different ecological characteristics and different climatic preferences. The main maize production area of South Africa is situated in the Highveld region (>1300 m above sea level). The effect of temperature on the development of *B. fusca* from this area was studied at five different temperature regimes namely 15, 18, 20, 26 and 30 ± 1 °C. Development rate was inversely related to temperature within the range 15 - 26 °C, but remained similar at 26 and 30 °C. The most favourable temperature as well as the upper threshold temperature for larval development was determined to be between 26 and 30 °C. The total development period was 152.6 to 52.6 days, respectively, at 15 °C, and 26 - 30 °C. The thermal constants for *B. fusca* were 99.50, 536.48, 246.25 and 893.66 °D and lower temperature thresholds were 10.36, 8.99, 8.14 and 8.84 °C, for completion of the egg, larval, pupal, and egg to adult stages, respectively. This data was used to model the distribution of *B. fusca* in the South African maize producing area using CLIMEX and to compare it with the species' known distribution. It also provided data on the climatic requirements of this clade.

P DMD 1

Race characterization of *Xanthomonas campestris* pv. *campestris* causing black rot disease of crucifers, their distribution in India and detection from seeds and other plant parts

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Black rot disease of crucifers caused by *Xanthomonas campestris* pv. *campestris* causes and occurs worldwide and damage the crops > 50% under favourable environmental conditions. To study the diversity 217 isolates of *X. campestris* pv. *campestris* (Xcc) were collected from cole crops, mustard, turnip and radish from 19 states of India. The all isolates of Xcc grew yellow, mucoid, translucent, raised circular colonies on YDC medium. They caused black rot diseases on cauliflower and produced typical symptoms 'V' shaped yellow necrotic areas at margins with blackening of veins. The races of 217 isolates of Xcc were identified by testing on a set of seven differential hosts. Out of 217 isolates of Xcc, 100 isolates of Xcc belong to race 1 (46.00%), 89 isolates under race 4 (41.01%), 1 isolate under race 6 (0.46%) and remaining 27 isolates (12.44%) isolates were still unidentified. Genetic diversity of 100 isolates of Xcc was studied using Rep-PCR, indicated the existence of wide range of genetic diversity among the isolates. The strains clustered into five groups at 50% similarity coefficient and designated as Group A, B, C, D and E. Majority of the isolates clustered under Group A and B, which had 26 isolates in each group followed by group C (25 isolates) whereas Group D (22 isolates), and Group E had one isolate (Xcc-C116). In group A, out of 26, 17 isolates belong to race 1, 8 isolates race 4 and 1 unidentified races. In Group B out of 26 isolates of Xcc 8 isolates belong to race 1, 5 isolates race 4 and 13 isolates were unidentified. Multi locus sequence typing (MLST) of 5 isolates of Xcc done using *gyrB*, *rpoD*, *fyuA*, *efp*, *atpD*, *dnaK* and *hrp F* genes to study genetic diversity. The sequence data of all the genes were combined to see the cumulative effect of genetic variability within the strains isolated from different host. Isolate Xcc- C18 isolated from turnip showed genetic variation from Xcc- C131, Xcc-C132, Xcc-C149 and Xcc-C165 isolated from cauliflower. To reduce the time, labour and improve sensitivity and accuracy, the detection of Xcc from artificially inoculated and natural infected seeds and plants parts including root, stem and leaf of cauliflower was done through bio-PCR. The primer was specific to Xcc and did not amplify other *Xanthomonas* species. The Xcc was detected from artificially contaminated seeds up to 0.01 per cent of contaminated seeds by using bio-PCR techniques. The bacteria were also detected from naturally infected leaf, root, stem and seeds of cauliflower.

P DMD 2

Development of Quality Standards of *Ficus Carica* Linn. Leaves

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Introduction: Traditional healing through herbs have been the experienced of many countries since ages, as they were generally whispered to be non toxic natural products. Contemporary medicine is more concern for the cure of diseases but remnants indifferent to health conservation. There is an urgent need to combine the best elements of traditional medicine and modern medicine to improve the health care system of human kind. For the reason that of the rapid progress of herbal drug an increasing need is felt to standardize the herbal products. It is needed to develop the scientific protocols such as SOP and pharmacopoeial standards of the herbal drug. *Ficus carica* Linn. (Moraceae) is commonly known as edible Due to the useful effect of leaves in skin diseases.

Methods: The pharmacognostic standardisation of fresh leaf sample and dried power of the leaf were carried out in terms of organoleptic, microscopic, macroscopic, physicochemical and aflatoxin analysis according to WHO guidelines.

Results: Macroscopic study showed that leaf is long palmate alternate, deciduous, petiolate, subcordate which is rough on upper surface, finely wooly on beneath surface. In the transverse section of leaf lamina of shows upper epidermis which is single layered, cells more or less rectangular with outer walls, cuticularized. Both covering and glandular trichomes emerged from the upper epidermal cell. The upper and lower epidermal layers of lamina are continuous over the midrib. However, relatively more trichomes appear on the epidermal layers of the midrib. A patch of vascular bundle is present in the central portion of the midrib. Powder microscopy shows numerous mucilage granules, starch granules, covering, glandular trichomes and stomata which are anomocytic in nature. Stomatal number 6-10, stomatal index value 17.64, palisade ratio 8.12, vein islet number 86.1, and vein termination value 103.5 were determined by standard method. Successive extractive value was highest (23.606%) in case of aqueous extract. Mean ash values (%) were 23.04 (total), 6.48 (acid insoluble) 12.69 (water soluble). Loss on drying was 5.9107%. Resin content was found 1.33%. Phytochemical screening leaves powder showed the presence of carbohydrates, phenolic compounds, flavonoids, steroids, tannin, resin and acidic compounds.

Conclusions: The present research work was undertaken with a view to lay down standards which could be useful to detect the authenticity of this medicinally useful plant.

Figure 1



Figure 2



P DMD 3

A systematic review of PCR-based specific methods to detect the most important strawberry pathogens

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Strawberry diseases are a major limiting factor that severely impacts plant agronomic performance and lead to economic losses. As traditional detection methods such as baiting or direct isolation are incapable of handling the large volume of material to be tested, researchers have developed more rapid and specific DNA-based tests. Polymerase chain reaction (PCR) methods such as real-time PCR have many advantages over more traditional diagnostic tests. PCR techniques are highly sensitive and rapid, and pathogenic organisms do not need to be cultured prior to their detection and identification. The aim of this systematic review is

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to provide an overview of PCR-based methods used for detection or quantification of the most abundant pathogens on strawberry, including *Xanthomonas fragariae*, *Phytophthora fragaria*, *Verticillium dahliae*, *Fusarium oxysporum* f.sp. *fragariae*, *Colletotrichum acutatum*, *Botrytis cinerea* and *Macrophomina phaseolina* that could be particularly useful for diagnostic laboratories in order to develop a rapid, cost effective, and reliable monitoring technique. So, using appropriate subject headings, AGRICOLA, AGRIS, BASE, Biological Abstracts, CAB Abstracts, Google Scholar, Scopus, Web of Knowledge, Science Direct and Springer Link databases were searched from their inception up to May 2014. Selected articles were included if one of the mentioned strawberry pathogens was investigated based on PCR methods. A total of 259 titles and abstracts were reviewed of which 23 full texts met the inclusion criteria. Our systematic review identified 10 different protocols for *X. fragariae*, eight for *P. fragariae*, four for *B. cinerea*, six for *C. acutatum*, three for *V. dahlia*, and only one protocol for *F. oxysporum* f.sp. *fragariae*. However, no study was found for detection and quantification of *M. phaseolina* on strawberry. The accuracy and sensitivity of PCR diagnostic methods is the focus of most studies included in this review. From a systematic review of the currently available published literature, real-time PCR is shown to be a particularly promising technique for diagnosing and quantifying pathogen populations in strawberry.

P DMD 4

Plant Protection and Data Science: The Normal Distribution is the Log-Normal Distribution

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We advocate a *fundamental change* that will considerably improve data analysis, not only in plant protection but across the sciences¹⁻³.

The *Gaussian or normal distribution* is the most established model to characterize quantitative variation of original data. Accordingly, data are summarized using the arithmetic mean and the standard deviation, by $\bar{x} \pm SD$, or with the standard error of the mean, $\bar{x} \pm SEM$. This, together with corresponding bars in graphical displays has become the standard to characterize variation or confidence ranges.

However, data characterized like this frequently do not fit the normal distribution. This is easily shown with the “95% range check”^{1,3}. Starting from the “normal” characterization, by calculating the range variation $\bar{x} \pm 2SD$, negative values within this range imply that the distribution cannot be normal. We estimate the number of papers with data failing this check to exceed 1'000 per week just for the “Science Edition of the Journal Citation Report”. We found numerous examples also in the leading scientific journals, “Nature” and “Science”¹.

This points to a *basic misunderstanding of variation and science*, based on a fundamental contrast that appears to have been neglected so far. Natural laws and processes creating variation are based above all on multiplication. Thus, intriguingly, the model of the normal distribution based on addition can not fit. It is therefore the multiplicative normal distribution (or log-normal distribution) that we favor and recommend, which is easy to use meanwhile¹⁻³.

The *advantages* for efficiency and data quality including regressions and ANOVA, as well as our ethical responsibility, will be shown and discussed with examples from plant protection.

1 Limpert E, Stahel WA, 2011, Problems with Using the Normal Distribution - and Ways to Improve Quality and Efficiency of Data Analysis. PLoS ONE 6(7):e21403. doi:10.1371/journal.pone.0021403

2 Limpert E, Stahel WA, Abbt M., 2001, Log-normal distributions across the sciences - keys and clues. BioScience 51, 341-352.

3 Stahel WA, Limpert E, The normal distribution is the log-normal distribution. Talk Leibniz-Inst. Magdeburg, Dec 2 2014, <http://stat.ethz.ch/~stahel/talks/lognormal.pdf>

P DMD 5

Cytochrome *b* gene is a reliable tool for detection of fungal species in plant tissue

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The cytochrome *b* gene, the target of QoI fungicides, of thousands of isolates of different plant pathogenic fungal species have been analysed in the last 20 years for detection of mutations causing resistance to QoI fungicides,. Since QoI fungicides are broad active, many species from oomycetes, ascomycetes and basidiomycetes were sequenced. Within a species, data from many isolates from heterogeneous origin (region, time of isolation) were available. This data base showed overall that the cytochrome *b* gene is conserved on intraspecies level but there are differences between species, even within a genus. Such differences are not only manifest in the coding sequence, but also in the presence and localization of introns. Both together

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offer possibilities to develop very easy and reliable PCR assays for species differentiation, as it has been done for *Monilinia*, *Phyllosticta*, *Stemphylium* and other genera. Another advantage of this gene is its high copy number, since it is located on mitochondrial DNA (mtDNA). This high copy number significantly reduces the detection limit, which is favourable for development of detection tools for low amounts of fungal material in diseased plants. Therefore the cytochrome *b* is an appropriate tool and an alternative for ITS sequences for species specific detection and identification of plant pathogens in plant tissue, which is valuable especially for detection of quarantine organisms. Different examples of reliable assays are shown for *M. fructicola*, *P. citricarpa* and other fungal species.

P DMD 6

Metabolomics tools to screen for changes in plant compounds induced by abiotic and biotic factors

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Various environmental factors can cause changes in the concentrations of plant metabolites. Abiotic factors such as ultraviolet (UV) radiation lead to an induction of flavonoids. Using targeted analyses, the influence of different UV exposure on leaf and flower flavonoids of different crop and medicinal plants was determined. We found highly distinct patterns in flavonoid composition in plants from different populations as well as a significant effect of UV on individual flavonoids [1, 2]. Furthermore, we investigated the changes in overall metabolite patterns in leaf tissues in response to biotic challenges, such as the infestation of roots with arbuscular mycorrhiza (AM) [3]. A fingerprinting approach revealed that up to 15% of the polar features are modified in concentrations, depending on the plant species of investigation. Targeted profiling and untargeted metabolic fingerprinting are thus highly useful tools to determine the consequences of the environment on the plant metabolite composition. These changes in plant chemistry could be important determinants to increase plant protection.

1 Reifenrath K, Müller C (2007) Species-specific and leaf-age dependent effects of ultraviolet radiation on two Brassicaceae. *Phytochemistry* 68:875-885.

2 El Morchid EM, Torres-Londoño P, Papagiannopoulos M, Gobbo-Neto L, Müller C (2014) Variation in flavonoid pattern in leaves and flowers of *Primula veris* of different origin and impact of UV-B. *Biochem Syst Ecol* 53:81-88.

3 Schweiger R, Baier MC, Persicke M, Müller C (2014) High specificity in plant metabolic responses to arbuscular mycorrhiza. *Nat Commun* 5:3886.

P DMD 8

Determination of some Biochemical characterization of *Anagasta kuehniella* Zeller (Lepidoptera: Pyralidae) digestive α -amylase and the effects of rye, oat and wild barley proteinaceous extracts on enzyme activity

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Introduction: Mediterranean flour moth is an important storage pest and has been economic importance in Iran. Various chemicals and storage disinfectants may be used to control of this pest, but most of them are inadequate. So integration of different non-chemical methods, such as enzyme inhibitors, could be a suitable way to control this pest.

Objectives: Integration of different non-chemical methods, such as enzyme inhibitors, could be a suitable way to control this pest.

Material and methods: Fourth and fifth instar larvae enzymes were extracted using distilled water, and seeds proteinaceous extracts compounds were extracted using 0.1 M NaCl. The effects of these extracts were studied on α -amylase activity of insect by spectrometry and gel electrophoresis.

Results: The optimal temperature and pH of α -amylase activity was observed in 40 °C and pH equal 10. The results showed that the highest dose of proteinaceous extracts in oat and wild barley

(10 μ g pr) and rye (12.5 μ g pr), was reduced the enzyme activity 55.16%, 44.86% and 70.54% of the L5 and 48.94% 37.31% and 49.2% of the L4, respectively. While the lowest dose of oat and wild barley (0.625 μ g pr) and rye (0.781 μ g pr) species inhibited the enzyme activity 11.74%, 5.34% and 25.42% of the L5 and 11.12%, 7.95% and 8.41% of the L4, respectively. In gel electrophoresis of enzyme without usage of inhibitors, one band were observed. At the highest dose of rye extract, in the L5, amylase band disappeared and in other cases, waned band clearly. Gradually by reduce in protein doses, bands resolution was increased.

Conclusion: The results of spectrometry and gel electrophoresis assay indicated that inhibitory process is dependent to inhibitor dose. Protein extracts of rye compared with oat and wild barley have a strong potential for management of this pest.

P DMD 9

Identification and differentiation of *Monilinia* species causing brown rot of stone fruit using High Resolution Melting (HRM) analysis

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Introduction: Brown rot is a devastating disease of stone fruits caused by several *Monilinia* spp. including *M. laxa*, *M. fructicola*, *M. fructigena*, *M. polystroma*, *M. mumecola* and *M. yunnanensis*. Among them *M. fructicola* is a quarantine pathogen in Europe but has recently been detected in several European countries. Identification of brown rot causal agents relies on morphological differences among different species or the use of molecular methods that require fungal isolation from the infected tissue.

Objectives: The objective of this study was to develop and validate an HRM method for the identification of the *Monilinia* spp. complex and for the detection of *M. fructicola* among other brown rot pathogens.

Materials and methods: Based on the sequence of the *cytb* intron from *M. laxa*, *M. fructicola*, *M. fructigena*, *M. mumecola*, *M. linhartiana* and *M. yunnanensis* isolates, originated from Greece, Spain, U.S.A. and China, a pair of universal primers for species identification and discrimination and a pair of primers specific to *M. fructicola* were designed. The specificity of the HRM analysis primers was verified to ensure against cross-reaction with other fungal species. The sensitivity of the assay was evaluated using concentrations of known amounts of pathogen DNA.

Results: The melting curve analysis using the universal primers generated six different HRM curve profiles, each one specific for each species. The HRM analysis primers specific to *M. fructicola* amplified a 120-bp region with a distinct melt profile corresponding to the presence of *M. fructicola* regardless of the presence of other species.

Conclusions: HRM analysis can be a useful molecular tool for the rapid identification and differentiation of the six *Monilinia* spp. using a single primer pair. This novel assay has the potential for simultaneous identification and differentiation of the closely related *Monilinia* species, as well as, differentiate *M. fructicola*, a quarantine pathogen for Europe from other common pathogens or saprophytes that may occur on the diseased stone fruit.

Acknowledgments: The research was co-funded by a European Union and Greek Secretary of Research and Technology (GSRT) grant. The financial support and the contribution of the enterprises Novacert S.A., ALMME S.A. and K+N Ethimiadis in samplings is also acknowledged.

P DMD 10

Significance of lethal giant larvae gene in *Tribolium castaneum* revealed by RNA interference

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Cell polarization requires the localization of specific proteins to specific regions of the plasma membrane and is controlled by the lethal giant larvae (Lgl) protein. We identified and characterized TcLgl gene in the red flour beetle (*Tribolium castaneum*). The gene is located on chromosome 8, and consists of ten exons and nine introns. Analyses of stage- and tissue-specific expression patterns revealed that TcLgl was expressed throughout all developmental stages and in all pupal tissues examined. The highest transcript levels were found in the mid of pupal stage and in the gut of late pupae. RNA interference (RNAi) of TcLgl by injecting its double-stranded RNA (dsRNA) in 8-day larvae resulted in 100% mortality within three weeks after the injection. The dsRNA injection in 20-day larvae led to a decreased pupation rate. And 80% of injected early pupae were not able to complete their eclosion and died with the exuvia attached to their bodies. These results suggest that TcLgl plays an essential role in insect development, especially during the pupation and eclosion processes.

P DMD 11

Genetic diversity of *Pseudomonas syringae* pv. *aptata* in Serbia determined by pulsed-field gel electrophoresis

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Introduction: Serbia represents major sugar beet production area in southeastern Europe, with 62,895 ha and 3 million tons of sugar beet yield in 2013. *Pseudomonas syringae* pv. *aptata* was identified as a causal agent of bacterial leaf spot disease of sugar beet (*Beta vulgaris* L.). However, there is a lack of information about genetic variability among *P. syringae* pv. *aptata* strains.

Objectives: The main aim of this study was to estimate genetic diversity of *P. syringae* pv. *aptata* collected from sugar beet and contribute molecular characterization of this pathogen.

Materials and methods: During 2013, twenty five isolates of *P. syringae* pv. *aptata* were collected from commercial fields of sugar beet in province Vojvodina (Serbia), in order to determine genetic variability among them. In addition, referent isolate (CFBP 2473) was used for comparison of genetic differences. In order to identify collected isolates biochemical tests were performed followed with the gene sequencing. Genetic diversity of *P. syringae* pv. *aptata* strains was determined by using macrorestriction analysis of genomic DNAs by pulsed-field gel electrophoresis (PFGE).

Results: *SpeI* endonuclease was tested for the total digestion of DNA from *P. syringae* pv. *aptata* and generated bands from 48.5 kb to 582 kb, with the majority of the bands below 194 kb. Results indicate that genetic variability among isolates of *P. syringae* pv. *aptata* was significant, given that among twenty five tested isolates, twenty of them showed genetic differences. Also, all of the isolates from Serbia differed from referent isolate. In order to present obtained results, UPMGA clustering method was used, on the basis of which four different clusters were distinguished.

Conclusions: Genetic differences among tested isolates showed high level of diversity, which is characteristic for *P. syringae* species. The difference in the PFGE patterns from *P. syringae* pv. *aptata* strains could possibly arise as the result of many genomic changes occurred during adaptation of the *P. syringae* species to different host. According to the obtained results, PFGE presents powerful tool for determination of intra-species diversity of *P. syringae*.

P DMD 12

Comparison of Sampling Methods for Onion Thrips, *Thrips tabaci* Lindeman on Onion Crop

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Introduction: Onion crop is an important vegetable in the world. China followed by India is the largest producer of onion. Onion thrips, *Thrips tabaci* Lindeman (thysanoptera, thripidae) is the most global important pest of onion crop worldwide. Yield loss caused by thrips could be 10 to 90 %. Therefore, *T. tabaci* monitoring and detecting their relative abundance are the foundation of an authentic IPM approach.

Objectives: To Compare efficacy of four sampling methods for detection of onion thrips on onion crop.

Materials and methods: Four methods sampling of adults were tested :(1) *Blue sticky trap* (BST) (2) *Yellow sticky trap* (YST), one trap was placed per plot above the canopy and changed weekly by new one. (3) *Plant sampling* (PS): five plants/plots were selected randomly and cut from base of plant (neck of plant) and adults were counted under binocular microscope. (4) *Field sampling* (FS): five plants/plot were selected randomly and counting was done by hand lens in field. Observations were made weekly up to harvesting time.

Results: Density of adults was increased gradually from mid-December upto mid-April with fluctuation peaks then gradual decline (Fig 1 & 2). Almost peaks of population occurred between mid-March until 3rd week-April. Highest number of thrips occurred was recorded by PS at 20th weeks after transplanting (WAT) on Onion Kessar in 2012, while by YST in 2013. Early detection of adults was recorded by BST and YST in both cultivars and PS on Onion Kessar. The highest number of adults was 9.21adults/trap recorded (P<0.05) by BST in 2012, while 2.89 adults/plant (P<0.05) by YST in 2013. The lowest number of adults was 0.87and 0.42 adults/plant (P<0.05) observed on FS in 2012 and 2013, respectively. Interaction analysis showed that the largest capture of adults was 9.68 adults/trap (P<0.05) recorded by BST in 2012, while 3.01 adults/plant (P<0.05) on YST in 2013 on Onion White. The least capture of adults was 0.40and 0.85 adults/plant (P<0.05) by FS in 2013 and 2103, respectively.

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Conclusion: It is conclusion that adults were high on March to beginning of April. Sticky traps were consistently captured and detected adults of thrips earlier than field and plant sampling. Intervention to manage thrips could be in March.

Figure 1

Fig. 1 Density of adult of *T. tabaci* on two cultivars (2012)

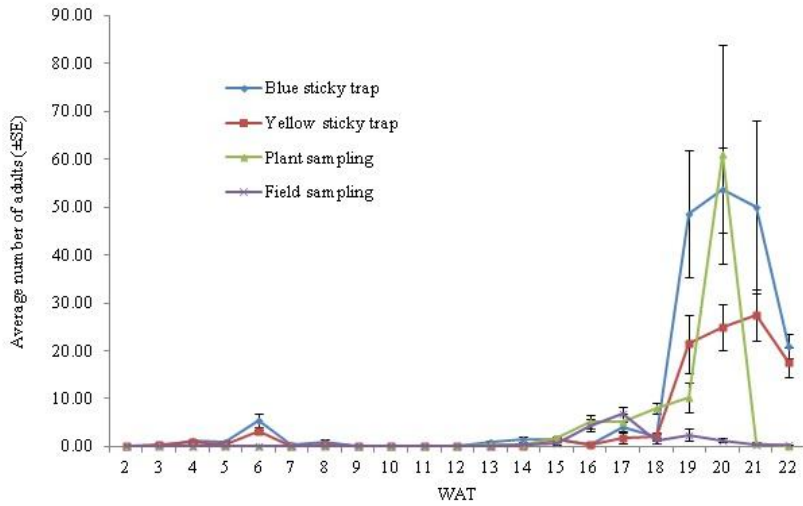
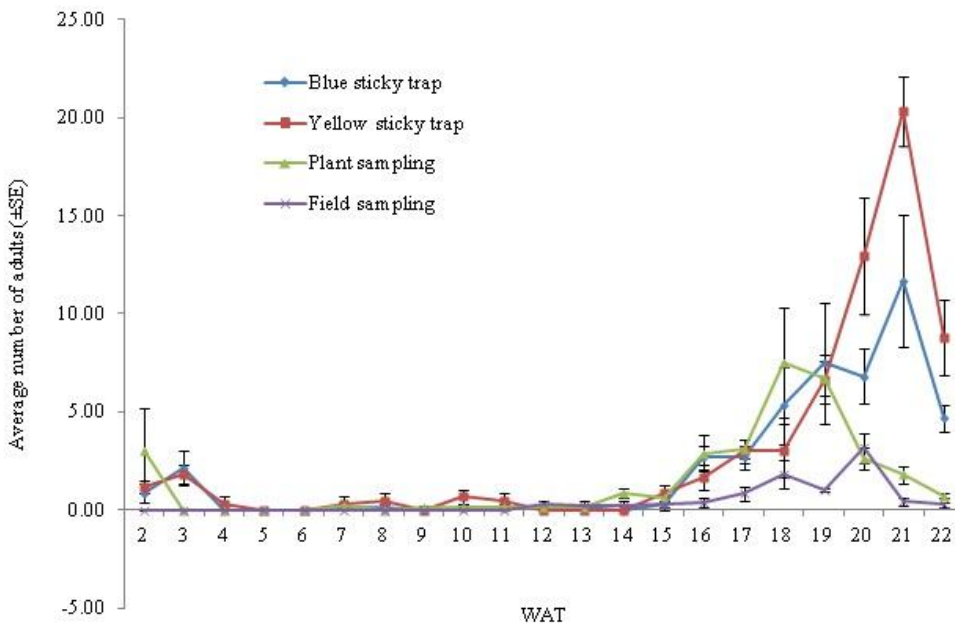


Figure 2

Fig. 2 Density of adults of *T. tabaci* on two cultivars (2013)



P DMD 13

PCR-RFLP assay for distinguishing four *Frankliniella* species

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Introduction: Thrips (Thysanoptera) are tiny, slender insects with fringed wings. Until now more than 200 species were reported in the area of Poland and, among them seven species from the genus *Frankliniella*. Pest of this genus are polyphagous and feed on a number of ornamental and vegetable hosts as well as on many weed species. The most economically important *Frankliniella* species in fauna of Poland are *Frankliniella occidentalis* Pergande and *Frankliniella intonsa* Trybom. Proper pest identification is essential to successful their control. Because of similarity between *Frankliniella* species, especially in larval stadium, and similar host range morphological identification can cause some difficulties. For this reason molecular biology methods could be very useful.

Objectives: The aim of the study was to develop the fast and effective method to distinguish *Frankliniella* species occurring in Europe: *F. occidentalis*, *F. intonsa*, *F. pallida*, and *F. tenuicornis*.

Materials and methods: Material of the study constituted 15 populations of *F. occidentalis*, 6 populations of *F. intonsa*, 1 population of *F. pallida*, 1 population of *F. tenuicornis* and 3 *Thrips* species as negative control (*T. palmi*, *T. tabaci*, and *T. major*). First, the region of 18S-ITS1-5,8S-ITS2-28S rDNA was PCR amplified and sequenced. Received sequences were used to design universal primers, giving PCR products for all analyzed *Frankliniella* but not for *Thrips* species. Received PCR product was then cut by appropriate restriction enzymes to receive bands pattern distinctive for each species.

Results: PCR-RFLP reactions gave positive results for all tested *Frankliniella* populations. Samples containing *Thrips* species as well as no template control did not give any product.

Conclusions: Described protocol proved to be species-specific and sensitive.

P DMD 14

Hermaphrodite and female Papaya Distinction by HR-MAS NMR

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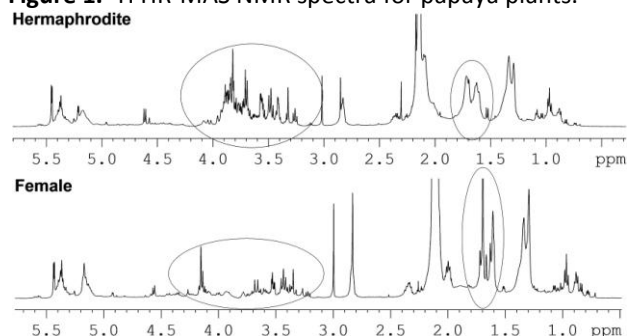
Introduction: Papaya (*Carica papaya* L.) from commercial seed usually produce hermaphroditic and female plants in a ration of 2:1. Males and females plants are usually removed. Flower morphology is the only way to identify visually the sex of the plant.

Objective: In this way this study correlate the sexual expression with chemical profile of hermaphrodite and female leaves through nuclear magnetic resonance spectroscopy (NMR).

Materials and methods: ¹H HR-MAS NMR measurements were performed at 28 °C on a Bruker Avance III 500 spectrometer equipped with a 4 mm HRMAS probe. Noesypr1d and 5 KHz were used as pulse sequence and rotating speed. Powdered leaves (15 mg) and 38 μL of acetone-d6 were added to a 50 μL spherical zirconium rotor. Triplicate experiments were applied for each leaf sample.

Results: The hermaphrodite and female ¹H NMR spectra (Figure 1) are very similar. However, expansions of the 3.1-4.3 and 0.7-1.9 ppm regions clearly indicates different chemical profiles according sex, particularly with respect to the carbohydrates and fatty acids compounds, whose signals are typically observed in this region of the ¹H spectrum. These compounds are observed in all plants however, the hermaphrodite leaves produce in higher concentration.

Figure 1. ¹H HR-MAS NMR spectra for papaya plants.



Conclusions: In this study, we demonstrate that it is possible to distinguish hermaphrodite and female papaya plants by ^1H HR-MAS NMR. The methodology employing demonstrated to be a powerful tool to evaluate chemical profile differences of papaya plants. This study also emphasises the remarkable advantage in using the HR-MAS NMR technique for plant analyses on the basis that the measurement is highly simplified since it does not require any pretreatment of the sample apart from the addition of a small amount of deuterium solvent necessary to produce homogeneous dough and a field frequency lock.

P DMD 15

Digital PCR for Detection and Quantification of Fire Blight and Potato Brown Rot

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Digital PCR (dPCR) is currently the most straightforward absolute quantification of the target nucleic acid copy numbers. Here we present the first assessment of digital PCR (ddPCR) format of qPCR assays for detection and absolute quantification of two quarantine bacteria, *Erwinia amylovora* (1) and *Ralstonia solanacearum* (2).

The evaluation combined the determination of the droplet based dPCR (Biorad) performance parameters of on a defined set of samples with known health status, including: (i) NTCs; (ii) negative plant material; (iii) serial dilutions of target DNA; and (iv) artificially prepared samples with target concentrations relevant to routine testing (in this case, log 3 cells/mL plant extract; Fig. 1) and (v) direct quantification of bacteria prior to DNA extraction.

In the dPCR format, the performance of the *E. amylovora* assay was comparable to its qPCR format, despite the reduced number of replicates (one in dPCR against three in qPCR), which makes the dPCR the first choice for characterisation of in-house reference materials, and for any application where quantification is also required. In contrast, for the *R. solanacearum* assay, where previous data and the data from this study indicate that its design might not be optimal, the dPCR format significantly improved both its analytical and diagnostic sensitivity. In general, the proprietary QuantaSoft analysis required data of high quality, while manual threshold selection was more suitable for non-optimal assays. The R script developed enabled automatic data analysis under different settings, and calculating additional parameters related to the occurrence of “rain” and to the quality of the separation of the negative and positive droplets. The data analysis and interpretation of the dPCR is considerably simpler than in qPCR.

Both dPCR assays accurately determined bacterial concentrations before and after DNA extraction (3). This is of particular importance in the field of plant health where no reference materials are commercially available.

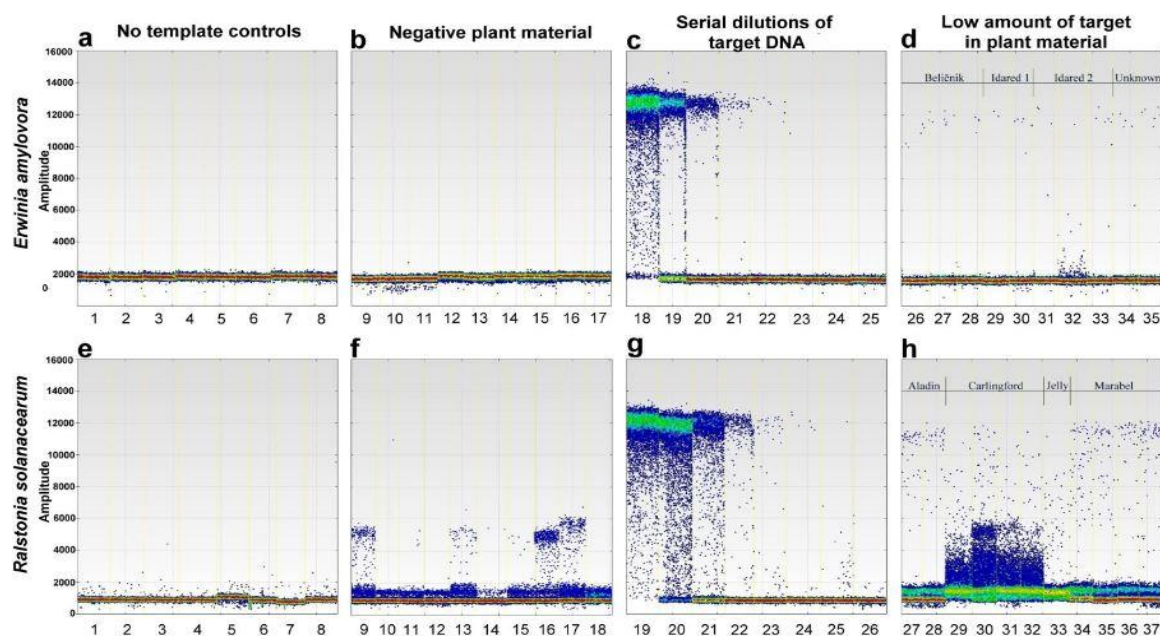
References:

1 Pirc et al., 2009. Plant Pathol 58:872-881.

2 Weller et al., 2000. Appl Environ Microbiol 66:2853-2858.

3 Dreo et al., 2014. Anal Bioanal Chem 406(26):6513-6528.

Figure 1: Heat maps of dPCR amplification of *E. amylovora* (a-d) and *R. solanacearum* (e-h). Samples are numbered sequentially.



P DMD 16

Applying of recombinant protein for developing of serological assays for efficient detection of Iranina isolate of *Citrus tristeza virus*

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Introduction: *Citrus tristeza virus* (CTV) is distributed worldwide and causes one of the most economically important virus diseases of citrus. The virus has long, flexible and filamentous virions (Lee and Bar-Joseph, 2000). The use of serological methods has become an indispensable tool for large-scale diagnosis of CTV worldwide.

Objectives: Developing of serological assays against CTV

Material and method: The bacterial expression vector, pET28a-CP was used for production of recombinant CP. Recombinant protein was purified by affinity purification in columns containing Ni-NTA. The New Zealand rabbits were used for immunization. Antibody purification was performed using protein A column. The purified antibody was subjected to conjugation with AP and HRP. Feasibility of prepared antibody for detection of recombinant and native antigens, infected plants, was evaluated by ELISA and DIBA.

Results: The result revealed the high purity and integrity of recombinant CP with the expected size of about 29 kDa (Figure 1). Total yield of purified protein in the culture medium varied from 8 to 20 mg. ml⁻¹. The antibody titer determined around 1:65000. The concentration of purified IgG was calculated at about 1mg.ml⁻¹.

Applying of DAS-ELISA with AP conjugate led to successful detection and differentiation of infected samples from the healthy ones at a dilution of 1:1000 (Figure 2). Complementary DIBA analysis confirmed specificity of prepared conjugate antibody. Applying of HRP for conjugation to immunoglobulin led to successful detection of infected plant as well.

The prepared antibodies were applied for detection of infected plants gathered from citrus growing area of Iran. The results revealed that samples gathered from northern part are infected with CTV while no positive results received from those gathered from southern area.

Conclusion: Present study described development of serological assays by applying of recombinant CTV coat protein. The produced antibody was successfully used for detection of infected plants gathered from northern area of country.

References: Lee, R., Bar-Joseph, M., 2000. Tristeza. Compendium of citrus diseases 2, 61-63.

Figure1: Expression and purification of recombinant CP in *E. coli*. UNI none induced cells, IN: IPTG-induced cells, S: Supernatant, P: Pellet, W: after washing, E: elution steps, M: prestained protein marker.

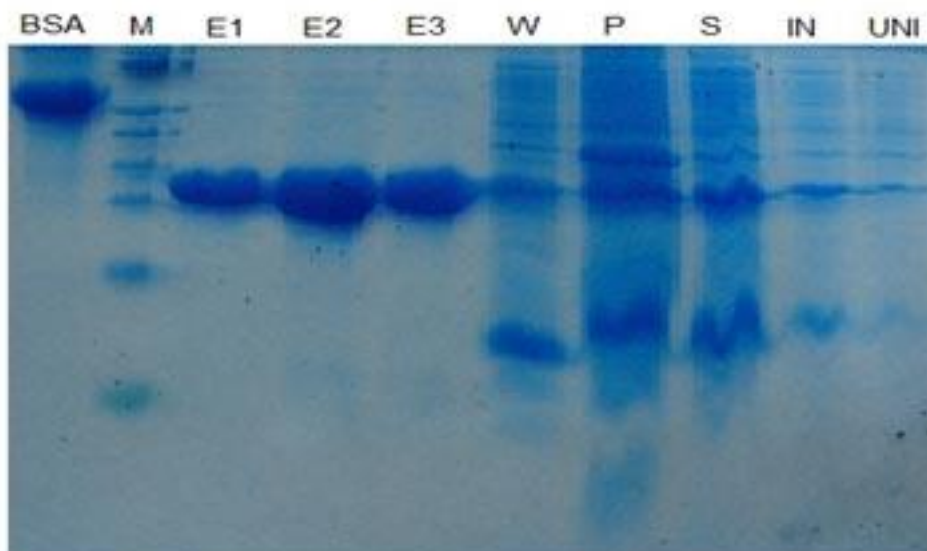
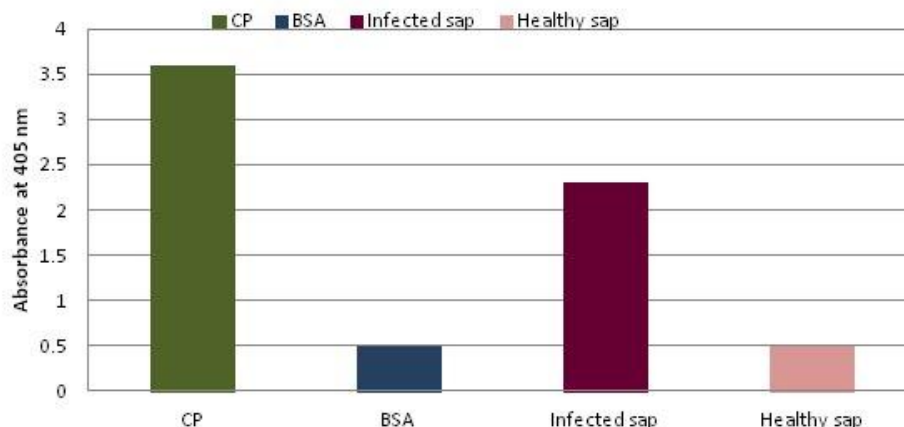


Figure 2: Detection of infected plant samples using DAS-ELISA with polyclonal antibody prepared against recombinant CP protein



P DMD 17

Development of specific recombinant phages against Citrus tristeza virus (CTV) by using phage display technology

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Introduction: Tristeza is one of the most destructive citrus diseases in the world. The disease is caused by a long flexuous closterovirus, Citrus tristeza virus (CTV). Nowadays, new technologies are being applied for developing of diagnosis tools against plant viruses. Among them, phage display has major role in production of specific monoclonal antibodies.

Objective: Producing of recombinant phages against CTV

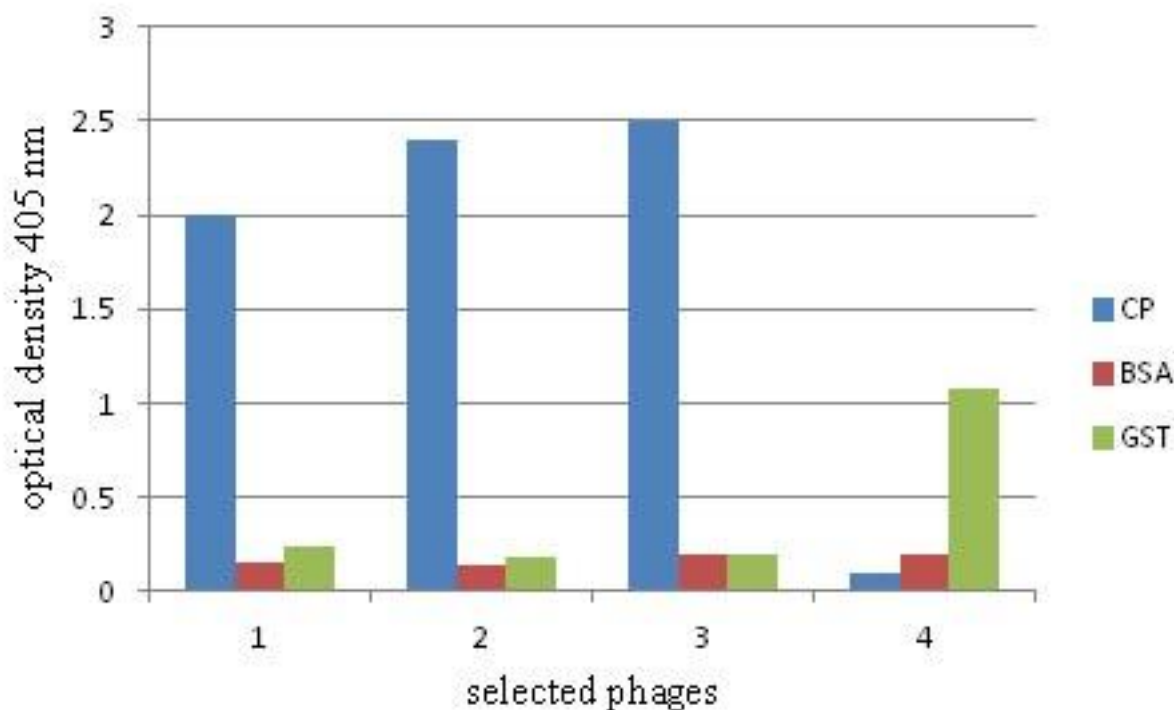
Material and methods: Phage display processes were carried out by performing three rounds of panning against CTV coat protein on Tomlinson I and J scFv phage display libraries as previously described (Safarnejad *et al.* 2008). Phages with affinity for the antigen were eluted and used for infection and amplification of TG1 cells. After the round three, individual colonies producing specific recombinant phages were randomly selected and analyzed for binding activity against CP by ELISA. Bound phages were detected using anti-M13 monoclonal antibody conjugated to HRP.

Results: The CP protein was produced as his-tagged fusion protein and purified by affinity chromatography in nickel-agarose column. The SDS-PAGE results proved integrity and purity of recombinant protein. Three rounds of panning were performed with 10^{13} of recombinant phage in each round. The results obtained after each round confirmed enrichment of CP specific phages throughout the panning processes. After the third round, individual colonies were randomly selected and their ability for production of specific phages was determined by ELISA assay. These results revealed that several phages obtained from Tomlinson I and J libraries could bind to CP (Figure 1). There is no detectable binding to negative control samples including GST and BSA proteins. Complementary analysis applying infected plant samples specificity of recombinant phages as well.

Conclusion: Present article describe developing of specific recombinant phages for detection of coat protein of CTV. This is the first report for obtaining of specific recombinant antibody against CTV by using scFv phage display libraries.

Reference: Safarnejad, M. R., Commandeur U., Fischer R., 2008. Generation and characterization of functional recombinant antibody fragments against Tomato yellow leaf curl virus replication-associated protein. Communications in agricultural and applied biological sciences 73: 311-323.

Figure 1: Screening of specificity of individual phages obtained after panning processes of Tomlinson library.



P DMD 18

Toxicological, biochemical, and histopathological analyses demonstrate that Cry1C and Cry2A are not toxic to larvae of the honeybee, *Apis mellifera*

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The honey bee, *Apis mellifera*, is commonly used as a surrogate species for the regulatory risk assessment of insect-resistant genetically engineered (IRGE) plants. In the current study, a dietary exposure assay was developed, validated, and used to assess the potential toxicity of Cry1C and Cry2A proteins from *Bacillus thuringiensis* (*Bt*) to *A. mellifera* larvae; Cry1C and Cry2A are produced by different IRGE crops. The assay, which uses the soybean trypsin inhibitor (SBTI) as a positive control and bovine serum albumin (BSA) as a negative control, was used to measure the responses of *A. mellifera* larvae to high concentrations of Cry1C and Cry2A. Survival and development duration were reduced when larvae were fed SBTI (1 mg/g diet) but were unaffected when larvae were fed BSA (400 µg/g), Cry1C (50 µg/g), or Cry2A (400 µg/g). The enzymatic activities of *A. mellifera* larvae were not altered and their midgut brush border membranes (BBMs) were not damaged when fed diets containing BSA, Cry1C or Cry2A but enzymatic activities were increased and BBMs were damaged when diets contained SBTI. The study confirms that Cry1C and Cry2A have no acute toxicity to *A. mellifera* larvae at concentrations >10-times higher than those detected in pollen from *Bt* plants.

P DMD 19

Viral metagenomic analysis of sweet potato using high-throughput deep sequencing

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To date, limited studies have been undertaken with regard to the etiology of Sweet potato virus disease (SPVD) in South Africa. In this study, a non-enrichment metagenomic approach was adopted to establish the genetic diversity of RNA viruses infecting sweet potato. Total RNA was isolated from asymptomatic and symptomatic plants collected from the Eastern and Western Cape provinces, and depleted of ribosomal RNA (rRNA) using the Ribo-Zero™ Magnetic Kit. Sequencing was done on the Illumina MiSeq Benchtop platform. Sequence assembly and analysis were done using the CLC Bio Genomics Workbench and Metavir2. *De novo* and reference-guided assemblies generated near full-length virus genomes at high sequence depth. *Sweet potato feathery mottle virus* and *Sweet potato virus C* were detected in the Western and Eastern Cape symptomatic samples, while *Sweet potato chlorotic stunt virus* and *Sweet potato virus G* were detected in symptomatic plants from the Western Cape only. Reads aligning to geminiviruses (*Sweet potato mosaic-associated virus* and *Sweet potato leaf curl Sao Paulo virus*) were detected in symptomatic plants from the Western Cape and two badnaviruses (*Sweet badnavirus A* and *Sweet potato badnavirus B*) were detected in symptomatic and asymptomatic plants from both provinces. This is the first report of sweet potato badnaviruses in South Africa. The sequence data gives evidence of mixed infections of multiple RNA and DNA viruses in individual plant samples. rRNA depletion and deep sequencing of nucleic acids can be used as a diagnostic tool, which detects viruses and differentiates between diverse viral strains.

P DMD 20

Vitellogenin genes in Sunn Pest (*Eurygaster maura* (Hemiptera: Scutelleridae)): Characterizations and Gene Expression Profiles

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Introduction: *Eurygaster maura* is the major harmful pest of cereal crops which undergoes two migrations. During spring migration, overwintered adult insects migrate to cereal fields from mountains for feeding and laying eggs. In late summer, migration is from fields to the mountains where the new adults enter a diapause in overwintering areas. Reproductive success of insects depends on vitellogenin (*Vg*) synthesis. In this study, we characterized and analyzed expression profiles of *Vgs* in sunn pest.

Materials and methods: *E. maura* eggs were collected from wheat fields. Nymphal stages and one-week old adults were obtained from the hatched eggs for developmental expression analyses. Insect dissections were carried out for tissue specific expression analysis. The time course studies were conducted using female and male adults collected from wheat fields or overwintering areas in Ankara Province, Turkey. Three cDNAs encoding *Vg* proteins were characterized in the cDNA libraries generated from the fat bodies of active feeding and overwintering stages. qPCR analyses were performed to examine tissue and stage-specific expression of the transcript as well as expression at biologically-important time points throughout the life of the sunn pest. The 5'- and 3'- ends of the cDNAs were amplified by rapid amplification of cDNA ends PCR. The phylogenetic trees were generated by using the Neighbor Joining method.

Results: Comparative frequency of the cDNAs in the libraries as well as the Real time PCR analyses revealed that the *Vgs* are expressed higher in pre-migration stage. Expressions of the *EmVgs* were profiled for all stages of development and maximum level appeared in the adult stages. Highest *EmVg* transcripts were observed in the head among the adult tissues and organs including fat body, midgut, ovary, Malpighian tubules, head, trachea, flight muscles and nervous system. The full-length sequences of *Vgs* were characterized. Nucleotide and deduced amino acid sequences have similarity to *Plautia stali* *Vgs*.

Discussion: *EmVgs* are expressed higher in pre-migration and adult stages. Since sunn pest lays eggs after the migration to the fields, *EmVg* transcripts were predominant in the pre-migrated and adults. Although sunn pest isn't a social insect, migrations are needed to be well-organized. Also, breaking the hibernation occurs via perception of photoperiodic signals. *EmVg* expression is abundant in the head showing *Vgs* can regulate different mechanisms besides reproduction.

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Novel technique for estimating physical ages of wild male oriental fruit flies using proteomics approach

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Oriental fruit flies, *Bactrocera dorsalis* (Hendel) are recently becoming model research organisms and are frequently reared in research institutions. Research specimens domesticated in a laboratory-reared setting may have different behavioral phenotypes as compared to their wild counterpart. Additionally, it has been determined that the absence of “key” stimuli in the physical environment of captive animals may result in altered behavioral patterns such as the length of life cycle. Our question is whether this behavioral change caused the difference in the age of maturation (physical age). The purpose of this investigation was to establish a regression pattern in the ratio of odorant binding protein 99b (OBPs) of lab-reared fruit flies from 1 to 12-d-old compared to 1-d-old lab males to estimate the physical ages of those outdoor captured male fruit flies. Results demonstrated that a regression pattern was established as $Y = -0.7768 + 0.7205X$; with $R^2 = 0.889868$ from lab-reared flies. We were able to use the regression pattern to estimate the physical ages of those outdoor captured male fruit flies by interpreting the OBPs ratio of outdoor captured males versus 1-d-old lab-reared males to correctly identify the physical age of outdoor captured males. These results indicate that strong behavioral differences between a fly that are descended from laboratory stock and flies that are caught from the outdoors. The characteristic of physical age are identical. It is suggested protein analysis may be a good novel technique to confirm the property of domesticated fruit flies.

P DMD 22

In vitro Infection Conditions of Leaf Spot Disease caused by *Pseudocercospora pistacina* Cr. Qua.&Sarp. in Pistachio

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Introduction: Leaf spot disease is one of the most significant disease that causes yield loss in Pistachio production in Turkey. This disease is mainly seen on the leaves, then attacks to the nuts. Diseased leaves drop off early, photosynthesis reduces, fruit buds are affected and finally whole tree gets weaken. Due to disease severity, following years yield can be also affected which can be changed by 3-100%.

Objectives: It is aimed in this study that to determine the effects of temperature and rain for Leaf Spot Disease formation on Pistachio.

Materials and methods: 1-year-old pistachio seedlings were used in this study. It is sprayed water to seedlings with durations (2, 6, 12, 24 and 48 h). Then, seedlings were incubated in climatized rooms in different temperatures degrees (17, 24 and 30 °C). Observations were made 30, 60 and 90 days and disease severity was calculated according to 0-5 scales. Each topic was composed 3 replicates and 3 seedlings were used in each replicates. Regression analysis was made on results statistically.

Results: According to the results, for disease formation, at least 12 h leaf wetness is necessary at any temperatures. So, leaf wetness is determinative factor for Leaf Spot Disease on Pistachio trees.

Conclusions: Incubation period of causal agent fungus in leaves is very long, 15-25 days. So, it is very important to know how climatic factors effect disease formation. It can be helpful to control disease and these data can be used disease forecasting. Previous studies were made on total rainfall by months, April, May and June, so they were not specialized.

P DMD 23

Fungal pathogen surveillance using metagenomics approach requires one fungus one name

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Introduction: Metagenomics using next generation sequencing (NGS) allows broad-range detection of pathogens at low quantities, but interpretation of such data can be misleading due to insufficient sampling effort, low discriminatory power of DNA barcodes, imperfect computational tools, error-prone reference databases and the confusing dual nomenclature of fungi.

Objectives: Our underlying hypothesis is that pathogen dispersal pathways can be monitored using NGS, yet accurate assessment of richness and structure of fungal communities greatly depends on taxonomic breath and quality of DNA reference databases.

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Materials and methods: To monitor and detect pathogens from agri-ecosystem and agri-food value chain, we applied amplicon-based NGS to c. 1500 samples collected from spore traps, commodity washes, agricultural soil and watersheds. Close to 50 million fungal ITS amplicons were classified by comparing to either GenBank or UNITE ITS reference sequences.

Results: ITS barcodes are sufficient for documenting the occurrence of fungal pathogen-containing genera but often less accurate in discerning species. The dual naming of Pleomorphic fungi caused inflated richness of fungal communities and underestimated abundance of some pathogens. Up to Feb. 06 2015, 550 of 956 fungal generic names proposed for protection, identified from publications and reliable online resources, have at least one synonym. Over 150 such names, some of which may be officially suppressed, are still used in the UNITE+INSD database (2014-12-20 release). Many GenBank sequences linked to at least two taxonomic nodes at the genus level. As a result, classification of NGS data based on such references would assign sequences of the same organism to different fungal names causing confusion when examining community structure.

Conclusion: While NGS is a powerful tool for pathogen surveillance, its reliability suffers from inconsistent naming of fungi deposited in sequence databases. It is important that reference databases only use one name per fungus with the suppressed and synonymous names being cross-referenced to the protected or accepted names. We are documenting synonyms of c. 7532 proposed protected fungal generic names (Kirk *et al.* 2013) among other sources, which will be developed into an open resource for reliable naming of fungi based on DNA sequencing data.

References: Kirk, P.M., Stalpers, J.A., Braun, U., Crous, P.W., Hansen, K., Hawksworth, D.L., Hyde, K.D., Lücking, R., Lumbsch, T.H. & Rossman, A.Y. (2013) A without-prejudice list of generic names of fungi for protection under the International Code of Nomenclature for algae, fungi, and plants. *IMA fungus*, **4**, 381.

P DMD 24

What's in a name? The dilemma of diagnostic identifications and databases.

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Introduction: The National Plant Diagnostic Network (NPDN) is a consortium of diagnostic laboratories supported by a partnership of the United States Department of Agriculture National Institutes for Food and Agriculture (USDA-NIFA) and individual universities and state departments of agriculture. This network's mission focuses on early and accurate detection and diagnosis of plant pests. Those diagnoses are entered into a network database known as the NPDN National Repository, which can be searched for epidemics or new or emerging pests and diseases. However, definitions of microbial species are rapidly evolving with molecular genetic data increasingly influencing taxonomy and the identification of microorganisms. This evolution of taxonomy presents challenges to the interpretation of data from databases such as the NPDN's through time. We describe the occurrence of a new bacterial disease that exemplifies these challenges and warrants dialog concerning the interpretation of diagnostic data over time.

Objectives: We detected a new bacterial disease of *Loropetalum* in Florida in 2012. Incidences of similar disease had been identified as *Pseudomonas savastanoi* by standard diagnostic techniques. However, we undertook further testing that is far beyond the scope of most extension diagnostic laboratories to fully identify the organism and promote discussion regarding the accuracy and utility of diagnostic data.

Materials and methods: We conducted a comparative analysis of Florida and Alabama strains of the organism and related type specimens using LOPAT, fatty acid analysis, Biolog, pathogenicity testing, and MLSA.

Results: Our tests indicated we had identified a new pathovar of *Pseudomonas syringae* that causes cankers on loropetalum. Previous reports of the disease were entered into the NPDN database as *P. savastanoi*. Management recommendations made to clientele were similar, but were incomplete without a known host range.

Conclusion: Diagnosis of a disease is generally guided by the need for management, thus tests are often limited to the most expeditious and cost-effective. Additionally, the diagnostic data are contemporaneous, an issue of which anyone mining the databases will need to be cognizant.

P DMD 25

RAPD-PCR analysis of *Etielia zinckenella* populations and some pyralidae insects within Egypt

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It is the first attempt, to discover the genetic diversity of *Etielia zinckenella* populations within Egypt. The characteristic pattern of RAPD-PCR using some selected primers to examine ten populations of *E. zinckenella* ♂ moths collected from different governorates and the dual sex of *E. zinckenella*, *Euhniella kuehniella*, *Galleria mellonella* and *Achroia grisellawere*.

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OPK-13, OPB-20 and OPH-9 primers produce positive PCR products for dual sex

E. z. and negative products for dual sex of *E. k.*, *G. m.* and *A. g.*, while OPB-5, OPC-3 and OPB-14 gave positive PCR products for *E. z.* ♂ and negative products for *E. z.* ♀. OPJ-15 was positive for ♂ *E. k.*, *G. m.* and *A. g.* and negative for ♀ *E. k.*, *G. m.* and *A. g.* OPJ-13 showed positive product for ♂ *A. g.* and negative for ♀ *A. g.*. OPG-16 was a positive PCR product for dual sex of *E. z.*, *E. k.*, *G. m.* and *A. g.*. OPG-11 was a negative PCR product for all tested insects.

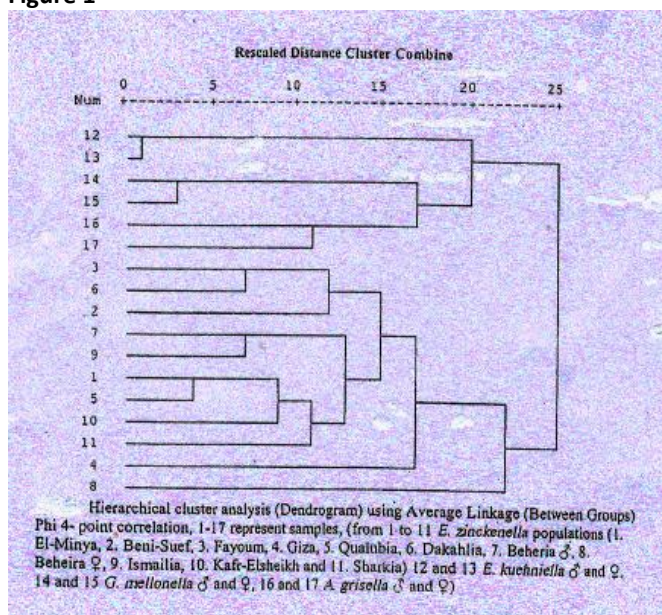
The dendrogram shows that the ten tested populations of *E. z.* ♂ and dual sex of *E. z.*, *E. k.*, *G. m.* and *A. g.* are divided into two main groups with a linkage distance of 25. The dendrogram indicate that the Dual sex of *G. m.*, *A. g.* and *E. k.* represented a first sister group (the linkage distance at 20), the members of 2nd sister group are *E. z.* populations (a linkage distance of 22).

The 1st sister group is divided into 2 subgroups. The 1st subgroup includes *E. k.* ♂ and ♀ in the linkage distance of 11. The 2nd subgroup is divided into two clusters. The 1st cluster includes *G. m.* ♂ & ♀ at the linkage distance of 3.5 and the 2nd cluster includes *A. g.* ♂ & ♀ at the linkage distance of 11.

The dendrogram further showed that, the 2nd sister group were divided into two subgroups. ♀ collected from Behira governorate was in single subgroup (with a linkage distance of 22) and ♂ collected from the same locality represented another position in the dendrogram (with a linkage distance of 13).

Looking into the interchangeability among *E. z.* ♂ populations from the ten considered governorates clearly indicates that the ancestors of *E. z.* ♂ (with a linkage distance of 17) collected from Giza governorate seemed to be the source of all collected populations. From Giza governorate *E. z.* population, the rest populations divided into two main clusters (with a linkage distance of 15). The 1st cluster (with a linkage distance of 12) included two branches. The 1st branch represented Fayoum *E. z.* and Dakahlia *E. z.* ♂ populations. While the 2nd branch represented Beni-Suef *E. z.* ♂ populations. The second cluster (with a linkage distance of 13) was also divided into two branches, the 1st branch included Behira and Kafr El-Sheikh *E. z.* ♂ populations. The ancestor of the 2nd branch is Sharqia *E. z.* ♂ populations. Sharqia *E. z.* ♂ populations are divided into two sub branches. The 1st sub branch represents Ismailia population only while the 2nd sub branch is divided into two clades. The 1st clade represents *E. z.* ♂ populations from Qualubia Governorate and the 2nd clade represents El-Minya *E. z.* ♂ populations.

Figure 1



P DMD 26

Use of simple isothermal assays for real-time and endpoint detection of phytopathogenic microorganisms

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Introduction: Conventional nucleic acid based amplification techniques and serological tests are time consuming, complex and expensive. Routine on-site applications for the detection of phytopathogenic microorganisms are therefore impaired. Isothermal amplification techniques are proved to be a rapid, simple and cost-effective alternative for the detection of a wide range of pathogens [1].

Objectives: We tested and compared simple methods to visualize isothermal amplification reactions exemplified by Loop-mediated isothermal amplification (LAMP) for the detection of potato spindle tuber viroid (PSTVD) [2], *Clavibacter michiganensis*

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ssp. sepodonicus and *Ralstonia solanacearum* [3]. The assays are based on turbidity, hydroxynaphthol blue (HNB), calcein, SYBR® Green I, EvaGreen® and berberine.

Materials and methods: LAMP [4] is characterized by high specificity, sensitivity, speed (< 60 min.), low reaction temperature (60-65°C) and high robustness. Due to carry-over contamination, “one-pot” reactions for field applications are strongly recommended. Therefore we tested several assays which can be evaluated by naked eye (endpoint) or in real-time by a portable fluorometer (ESE Quant Tube Scanner).

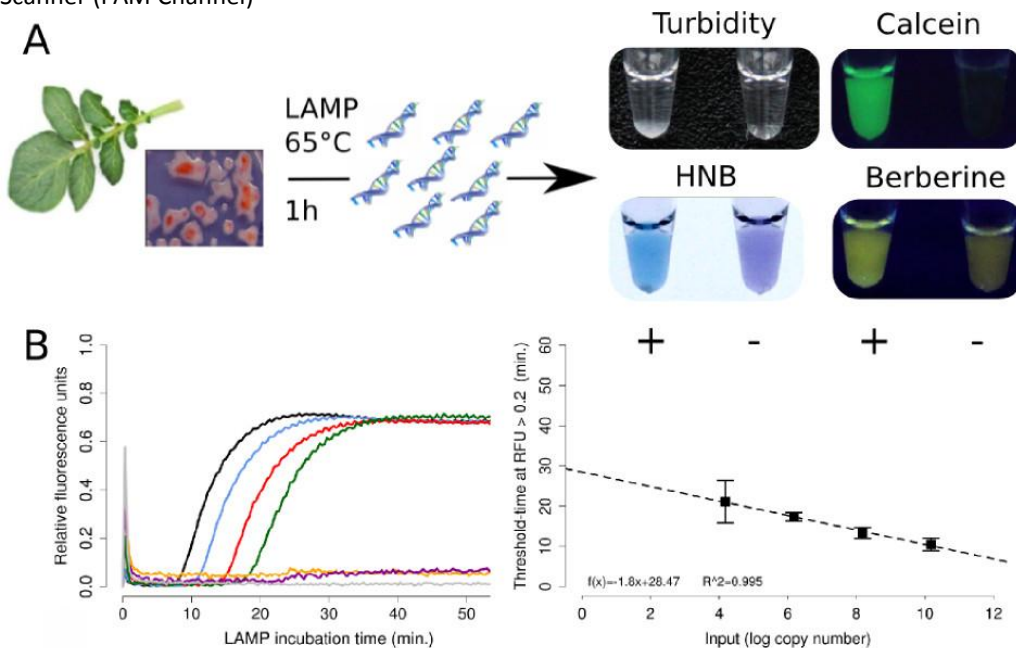
Results: All LAMP assays showed clear differences between positive and negative reactions in endpoint- and real-time analysis after amplification of all three phytopathogens. The evaluation of turbidity is more cumbersome. The use of HNB improves the visual discrimination of positive (sky blue) and negative (violet) samples. When using calcein and manganese, the positive samples show a bright green color, whereas the negative control remains orange. The double-stranded DNA-intercalating dyes SYBR® Green I and EvaGreen® resulted in increasing fluorescence signals in less than 10 minutes. We also investigated berberine (DNA binding plant alkaloid) in both assays for the first time. UV light can be used to show the amplification by naked eye and in real-time [Fig. 1]

Conclusions: We were able to detect relevant phytopathogens by simple “one-pot” LAMP assays. Besides our experimental results facilitate the proper choice of the optimal detection format with respect to the desired application (endpoint or real-time).

References:

- 1 Gill, P., and A. Ghaemi(2008). Nucleic acid isothermal amplification technologies: a review. *Nucleosides, Nucleotides and Nucleic Acids* 27
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- 3 Lenarčič R. et al. (2014). Loop-mediated isothermal amplification of specific endoglucanase gene sequence for detection of the bacterial wilt pathogen *Ralstonia solanacearum*. *PLoS ONE*
- 4 Notomi, T. et al.(2000). Loop-mediated isothermal amplification of DNA. *Nucleic Acids Res.*28

Figure 1: Illustration of LAMP assay for PSTVd detection. A: Endpoint assays B: Real-time assay with berberine in ESE Quant Tube Scanner (FAM Channel)



P DMD 27

Usage SDS-PAGE technique to interpret pod borer, *Etiella zinckenella*, infestation rate in cowpea, soybean and some their varieties.

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In an attempt, to explain infestation rate of *E. zinckenella* larvae for Cowpea and Soybean pods and its varieties, SDS-PAGE tools were used. The three seed maturation stages of tested varieties of cowpea and soybean and feces of *E. z.* were subjected to SDS-PAGE. The three maturation stages of pods were immature, mature and harvested mature. Cowpea varieties were Dokki 331, Cream 7, Kafr-EL Sheikh 1 and Kaha 1 and soybean varieties were Giza 111, Crawford, Giza 82 and Giza 83. These varieties were obtained from Horticulture Research Institute, ARC, Ministry of Agriculture.

SDS-PAGE of the tested cowpea varieties indicated that, the pattern bands changed according to the development stage of the pods. SDS-PAGE further showed that, no protein bands appeared in larval feces during the mature stage of seeds. This refers to, the essentiality of protein content in cowpea seeds throughout seed maturation stage for the development of *E. z.* larvae. It seems that *E. z.* larvae require all of the components of proteins for their metabolic processes.

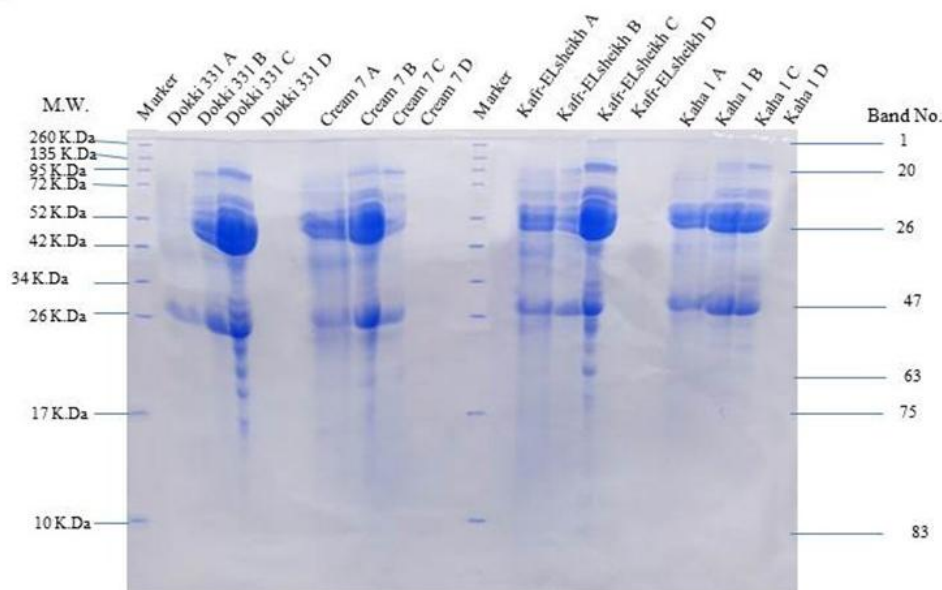
Every soybean variety had a specific SDS-PAGE pattern at each of the 3 developmental stages of pod. This may explain the wide differences in the degree of damage caused by the larvae to the different varieties.

Field observations indicated that the larvae of *E. z.* do not feed on soybean seeds during the harvest stage, (i.e. they do not require proteins in that particular stage). SDS-PAGE of feces larvae indicated that, the larvae change and /or convert certain protein bands in the immature and mature stages of seed development to the same molecular weight of some bands in mature harvest stage). Hence, not all proteins content in soybean were essentially to *E. z.* larvae compared with cowpea. This may be explain the highly infestation rate of soybean compared with cowpea, where *E. z.* larvae in soybean look for its quality and quantity protein needing at many seeds causes great damage in pods.

Refractionation protein fragments of the feces produced by the immature and mature seed stages of soybean indicated 2 categories. The 1st category had protein bands that were not present in the 3 stages of pods development such as the band no. 55 (M. W. 23.5 K. Da.) in Giza 111, bands no. 52, 61, 66 and 68 (M.W. 24.5, 17.7, 13.9, 11.8 K. Da., respectively) in Crawford, bands no. 50, 60 and 77 (M.W. 25.8, 18.2, under 10 K. Da , respectively) in Giza 82 and band no. 62 (M.W. 17.4 K. Da.) in Giza 83. This suggests that the metabolic system of *E. z.* larvae were able to digestive certain kinds of proteins and not able to digest another.

Figure 1

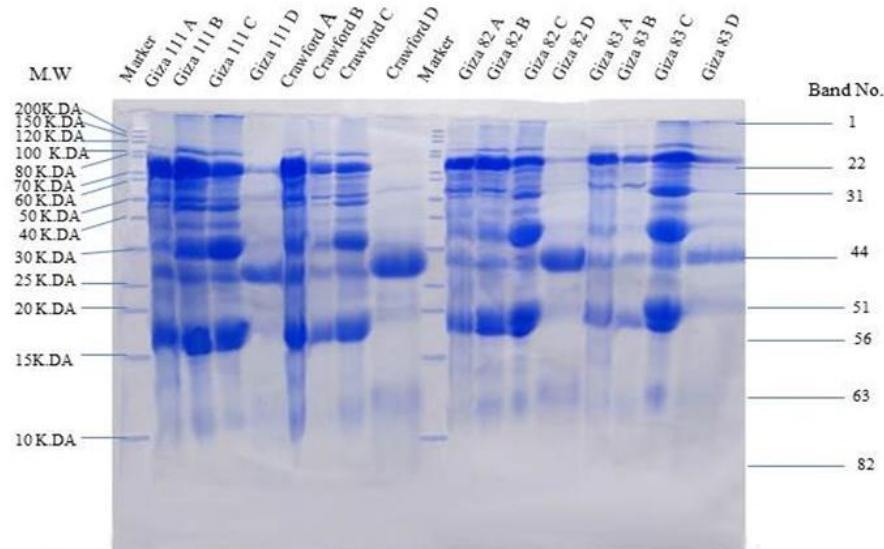
Fig. 11. SDS-PAGE fingerprints of the 4 tested cowpea varieties during the three developmental stages of seed maturation and in the faeces of *E. zinckenella* larvae.



- A: All pods have seeds filling the cavity of the majority of pods.
- B: Mature seeds increase in size and have green color, pods contain ripe seed.
- C: Harvest mature seeds, the seeds and pods become dry and hard, beans have a ripeness color.
- D: Faeces of *E. zinckenella* larvae.

Figure 2

Fig. 12. SDS-PAGE fingerprints of tested soybean varieties during 3 developmental stages of seed maturation as well as in the faeces of *E. zinckenella* larvae.



A: All pods have seed filling the cavity of the majority of pods.
B: Mature seeds increase in size and have green color, pods a ripe seed.
C: Seeds and pods become dry and hard, beans have a ripeness color.
D: Faeces of *E. zinckenella* larvae.

P DMD 28

Detection method and genetic analysis of anthracnose on sorghum in Korea

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Sorghum (*Sorghum bicolor*) is a standout among other cereal crops because of its nutritional status and functional health outcomes. Thus, sorghum's demand has been increasing in Korea. Sorghum anthracnose (*Colletotrichum graminicola*) is known as red leaf blight and, when it occurs on seedlings, it is called seedling blight. Typical symptoms are small, circular, elliptical, or elongated spots usually 5 mm or less in diameter. These spots develop gray to straw-colored centers with wide margins that are tan, orange, or red to blackish purple, depending on the cultivar and pathogen population. For detection of the sorghum anthracnose, PCR conditions and specific detection primer were searched. Thereafter, PCR process at 92°C for 3min and then at 92°C for 1min, 56°C for 1min, 72°C for 1min (35 cycles), and finally at 72°C for 1 min was found to be the best for detection. Detection of anthracnose from locally collected sorghum was possible by the primer set of co.gr 1 (AY622785, Figueiredo, J. E. F. 2004) and co.gr 3 (AY622780, Figueiredo, J. E. F. 2004). co.gr 1 and co.gr 3 are based 18S ribosomal RNA. Sequence of co.gr 1 is (5'→3') GGGTTTACGGCAAGAGTCCC, CCTTCCGTGGGTGAACCTGC and product size is 578bp. Sequence of co.gr 3 is (5'→3') AATGGCTCATTATATAAGTT, TCCGGGTTGAGCCCTAACC and product size is 290bp. In 2014, two isolates were collected at Yeongwol area of Korea. Analysis of the genetic relationship showed a genetic relationship of 85% between isolate 1458 and isolate 1728 of Yeongwol. Genetic relationship with Japanese isolate (AB439813) was 98.6, Germany isolate (AJ301978) was 98.6, Mexican isolate (JQ658887) was 96.6, Indian isolate (KC821517) was 99.8, and American isolate (NR_111191) was 94.0. In future, isolated strains will be assayed for disease symptom development and pathogenicity on major sorghum varieties.

P DMD 29

Detection of soybean major viruses by RT-LAMP

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Soybean mosaic virus (SMV) is a prevalent pathogen that causes significant yield reduction in soybean production worldwide. SMV belongs to *potyvirus* and causes typical symptoms such as mild mosaic, mosaic and lethal necrosis. SMV is seed-borne and also transmitted by aphid. Eleven SMV strains, G1 to G7, G5H, G6H, G7H, and G7A were reported in soybean varieties. Although *Soybean yellow common mosaic virus* (SYCMV) and *Soybean yellow mottle mosaic virus* (SYMMV) have been recently reported, they have occurred a lot with SMV in soybean field. SYMMV is a new member of the genus *Carmovirus* in the family *Tombusviridae*. SYMMV has a single stranded RNA genome of 4009 nucleotides with six putative open reading frames. SYCMV has a single stranded RNA genome of 4152 nucleotides with four putative open reading frames, the entire nucleotide sequence showed 31.2-71.3% nucleotide identity with the previously known eleven species of *Sobemovirus*. In this study, we designed RT-Loop mediated isothermal amplification (LAMP) primers named F3/B3/FIP/BIP from coat protein gene sequence of SMV, SYCMV, and SYMMV. After the reaction of RT-LAMP, each product was identified by electrophoresis and with the detective fluorescent dye, SYBR Green I. under daylight and UV light. Optimal reaction conditions were at 58, 63, and 58°C for 60min and the primers of RT-LAMP showed the specificity for each SMV, SYCMV, and SYMMV tested in this study.

P DMD 30

Current impact and future directions of high throughput sequencing in plant virus diagnostics: the drivers of COST Action 1407

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The ability to provide a fast, inexpensive and reliable diagnostic for any given viral infection is a key parameter in efforts to fight and control these ubiquitous pathogens. The recent developments of high-throughput sequencing (also called Next Generation Sequencing - NGS) technologies and bioinformatics have drastically changed the research on viral pathogens. It is now raising a growing interest for virus diagnostics. This review provides a snapshot vision on the current use and impact of high throughput sequencing approaches in plant virus characterization. More specifically, this presentation highlights the potential of these new technologies and their interplay with current protocols in the future of molecular diagnostic of plant viruses. The current limitations that will need to be addressed for a wider adoption of high-throughput sequencing in plant virus diagnostics are

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thoroughly discussed. This paradigm change gave rise to the COST Action 1407 which is currently launched. This Action, its objectives and expected impacts will be presented.

P DMD 31

Anti-quorum sensing activity of some medicinal plants in Iran

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In recent years, application of antibiotics against microbial pathogens has resulted both in microbial resistance against antibiotics and environmental pollution. Consequently, new therapeutic modalities and agents have received increased attentions. Bacteria often use small diffusible molecules called autoinducers to communicate between each other, also known as quorum sensing (QS) that regulate the target gene expression and results in bacterial pathogenesis activity. In this work, 30 medicinal plants from Iran were screened for anti-QS activity using *Chromobacterium violaceum* CV026 as a biomonitor strain. Three of these plants showed QS inhibition including *Cuminum cyminum* L. (Apiaceae) zire sabz, *Thymus vulgaris* L. (Lamiaceae) avishan and *Rhus coriaria* L. (Anacardiaceae) somagh. These findings introduces a new mode of action and possible validation for traditional plant use, and also a potentially new therapeutic direction for the treatment of bacterial infections.

P DMD 32

Evaluation of mating disruption for controlling the grapevine moth, *Lobesia botrana* (Denis & Schiffermüller) (Lep.:

Tortricidae) in Qazvin vinyardes

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Grapevine moth, *Lobesia botrana*, is a primary insect pest of vineyards. Hazards of insecticide application against this pest have encouraged the development of alternative control methods specially mating disruption by sex pheromone in the recent years. The efficiency of mating disruption with two kind of dispensers: Isonet-L dispensers, Shin-Etsu Co., Japan and dispensers of Russell, U.K., was compared with insecticide treatment (control). The capture rate of monitoring traps and the number of infested bunches were monitored in all treatments. The percentages of infestations were statistically compared. The monitoring-trap capture rate in mating disruption by both pheromone dispensers was 97% less than that in control. The number of infested bunches in mating disruption by Isonet-L and Russell dispensers were respectively 100% and 32% less than that in control throughout the experiment. The percentage of infested bunches in the mating-disruption by Isonet-L (0%) was significantly less than those in the mating disruption by Russell dispensers (2.72%) and control (4%).

Analysis of variance showed that the rates of damages were statistically different ($P \leq 0.01$) between central and marginal places of Russell treatment and control. The percents of infested bunches in three locations: southern margin, center and north in Russell-treated blocks were 4.5, 2.17 and 1.5 respectively. More likely the movement of adult moths from adjacent grape gardens toward our experimental plots resulted in difference of infestation. The results showed that the mating disruption by Isonet-L was more efficient for reducing the damage of the pest in vineyards, when pest population level is low.

P DMD 33

Re-purposing bridging flocculation for on-site, rapid, qualitative DNA detection in resource-poor settings

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Introduction: On-site, quick and cheap pathogen detection is the holy grail of disease diagnostics. Here we describe Single-Drop Genomics (SDG), a novel method to cheaply visualize amplified disease-specific DNA/RNA with minimal equipment via bridging flocculation. A key characteristic of flocculation is the abrupt transition from solution phase to flocculate which makes this phenomena ideal for binary yes/no applications. To the best of our knowledge, the detection of DNA/RNA has not yet been demonstrated via a DNA-mediated bridging flocculation mechanism which can be readily observed by the naked eye (Fig 1).

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Methods: Recombinase polymerase amplification reactions were performed at 37°C for 30 mins using 1 µL of the nucleic acid extraction and 480-600 nM of each primer. For RNA applications, 50 units of MMuLV reverse transcriptase were added to the RPA reaction. Following amplification, 5 µL of the RPA reaction was verified by gel electrophoresis. Another 5 µL was used in the flocculation assay by incubating with 1.5 - 1.8 volumes of SPRI bead solution for 5 minutes. After bead separation with a magnet and an 80% ethanol wash, 30 µL of flocculation buffer (100mM sodium acetate, pH 4.4, 1% v/v Tween20) was added to the beads and gently agitated.

Results: SDG successfully detected fungal (*Fusarium oxysporum*, *Botrytis cinerea*), bacterial (*Pseudomonas syringae*) pathogens (Fig 2) and RNA-based viruses (cucumber mosaic virus) (Fig3B) in diseased plants, including commercial bananas (Fig3A). SDG also detected pathogens in farm animals (bovine herpesvirus 1) (Fig3C) and environmental samples (*E. coli*-laced water) (Fig3D). Finally, SDG's universality was established by detecting causal pathogens for human diseases including HIV (Fig3E), malaria (Fig3F), tuberculosis (Fig3G) and influenza (Fig3H).

Conclusions: In summary, we have described a novel bridging flocculation assay for naked eye qualitative evaluation of amplified DNA. The combination of RPA with the flocculation assay then forms the basis of a simple strategy for on-site nucleic acid diagnostics with minimal equipment. Considering the wide range of pathogens and samples demonstrated here, we believe the assay has the potential for on-site, low resource applications.

Figure 1

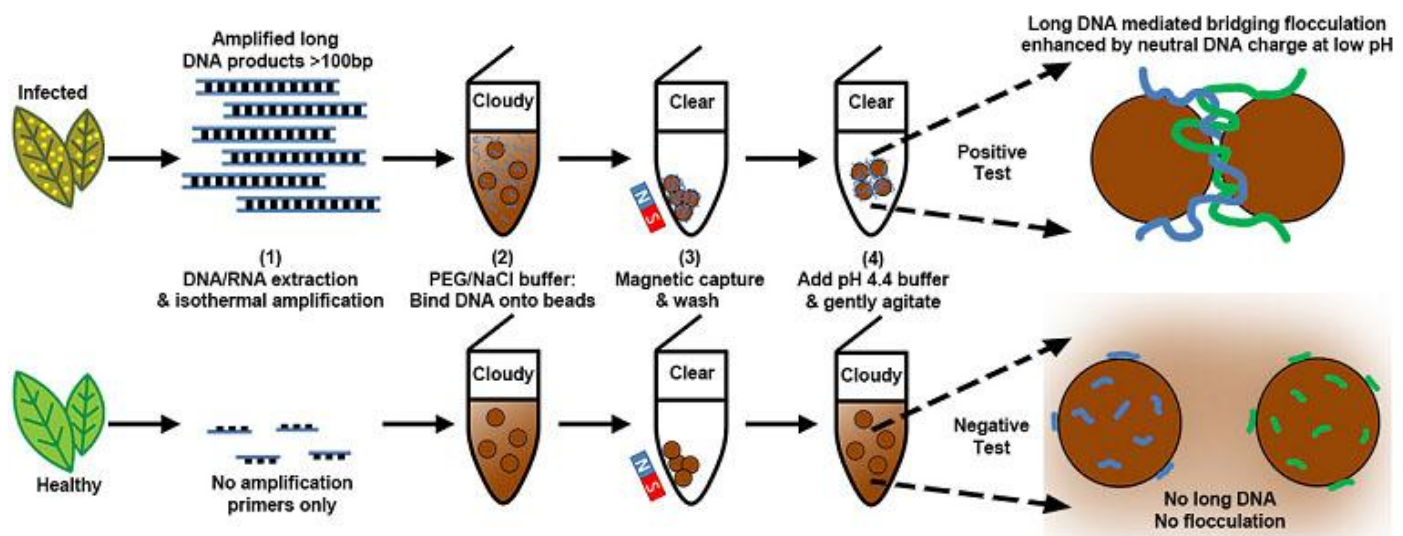


Figure 2:
Figure 2:

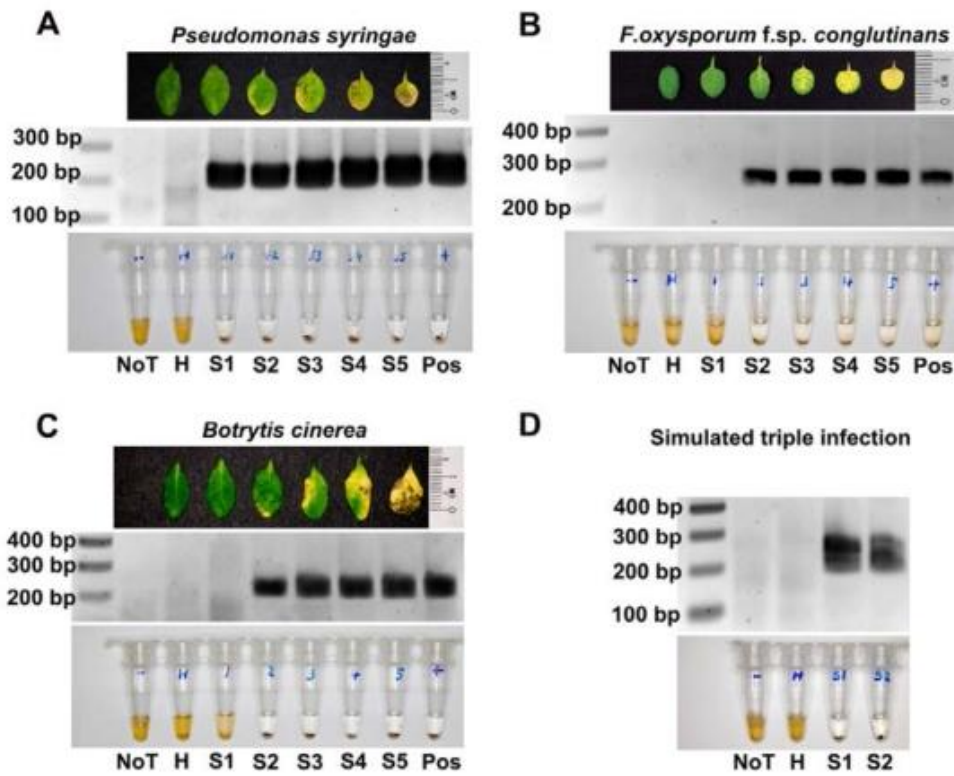
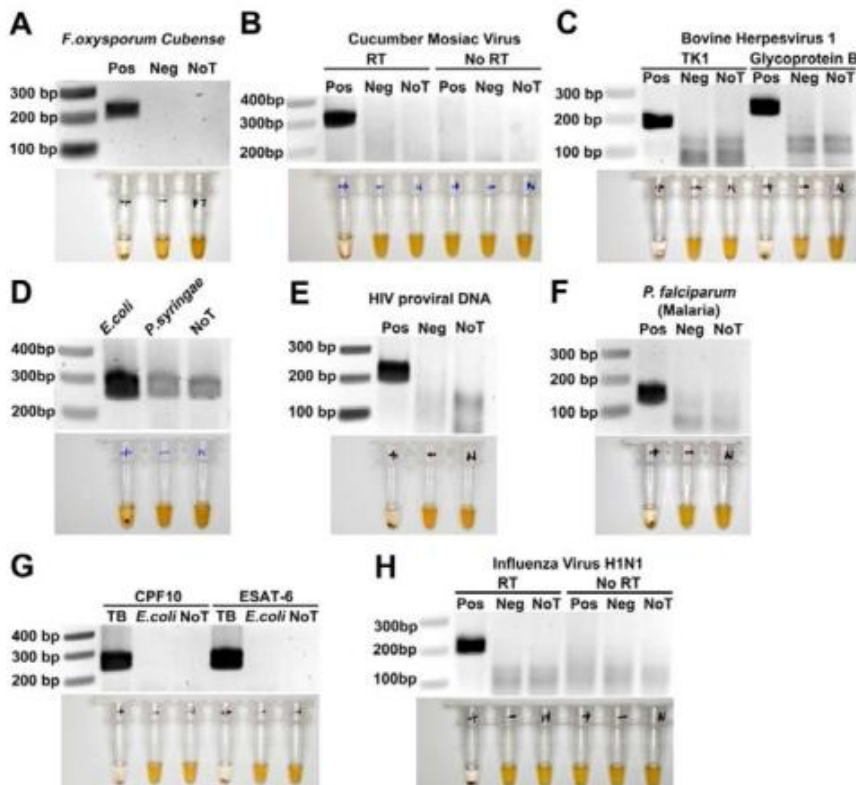


Figure 3:



P DMD 34

Molecular phylogenetic investigation of Loranthaceae based on nuclear DNA ITS and chloroplast DNA trnL-F sequences

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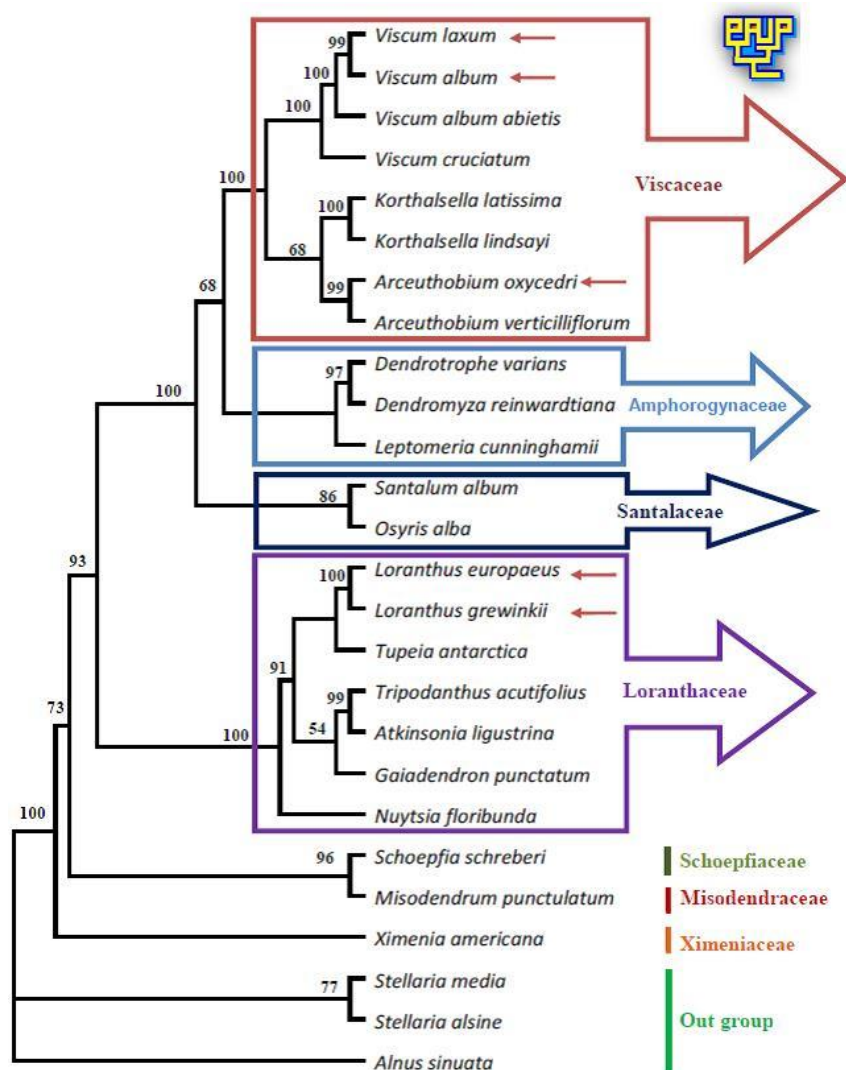
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Loranthaceae comprises three hemiparasitic genera with five species in Iran. The genera of this family occur mainly in tropical and subtropical areas worldwide. These genera family are distributed in two geographical regions of Alborz and the central parts of Iran. However, one species, *Viscum album*, is also found in the temperate and cold habitats of Iran. The taxa existing in Iran which are included in this analysis are: the genus *Loranthus* Jacq., which includes two species: *L. europaeus*, which lives on *Quercus*, and *L. grewinkii* is found on *Pyrus* and *Amygdalus*; the genus *Viscum* L. with two species: *V. album* and *V. laxum* attacking different trees; and *Arceuthobium oxycedri* which is found on *Juniperus spp.* in jungles of Semnan. The total DNA was extracted from fresh or herbarium specimens with a modified CTAB method. The regions of nrDNA ITS and cpnrnL-F were amplified and sequenced. In this study, 27 taxa were studied as ingroups and 3 taxa as outgroups. The sequences of the regions of ITS and trnL-F of some specimens were derived from GenBank. To reconstruct the phylogenetic relationships in Loranthaceae and to compare the affinities of this family with the other members of Santalales, the molecular data were analyzed. The analysis were carried out using Maximum Parsimony approach implemented in PAUP*, Bayesian method and Maximum Likelihood method. Our results indicated that *Arceuthobium* and *Viscum* are in closer relationship than *Loranthus* and therefore we can place *Arceuthobium* and *Viscum* in the separate family from *Loranthus*. Also the strict consensus tree from Maximum Parsimony analysis of the concatenated two gene dataset showed two monophyletic clades with 100% bootstrap support for two families Loranthaceae and Viscaceae.

Figure 1



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P DMD 35

Tools for alternative seed treatment evaluation

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Introduction: New alternative seed treatment methods are currently developed. To be able to evaluate the efficiency of these treatments, tools have been developed in the pathology laboratory of GEVES in order to meet the needs of the solutions providers.

Objectives: Develop tools to evaluate alternative seed treatments efficacy.

Materials and methods: Capacity of transmission of the pathogen from seed to plant or from soil to plant (pathosystem) is being evaluated and the effectiveness of these alternative seed treatment methods is being assessed. Protocols have been set up to obtain seeds infested by different pests, evaluate the percentage of infection and germination of these seeds. Depending of the pest and treatment used, viability of the pest after treatment is assessed by grow-out, vital staining or germination.

Results: Two examples are presented, one on *Tilletia caries* with a PCR approach and primers developed by Arvalis and the other on *Ditylenchus dipsaci* with a biotest.

Conclusion: New biotests and experimental design have been developed. More than ever before, the presence of pathogens on seeds needs to be detected and their damage potential needs to be assessed.

P DMD 36

Biological indexing and detection of viroids from hop (*Humulus lupulus* L.) in Slovenia

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Viroids are the smallest plant pathogens. They consist of a circular, self-replicating, non-coding RNA molecule, with a size ranging from 239 to 475 nucleotides. Diseases caused by viroids are a significant factor in reducing yield quality and quantity, as well as the production life of hop fields. Viroids that can infect hop (*Humulus lupulus* L.) include *Hop latent viroid* (HLVd), *Hop stunt viroid* (HSVd), *Apple fruit crinkle viroid* (AFCVd) and the recently discovered *Citrus bark cracking viroid* (CBCVd). CBCVd has been recognized as the main causal agent of a new disease in Slovenia named severe hop stunt disease, which is still completely unexplored.

With the aim of developing a reliable bioassay for CBCVd for hop genotype resistance screening, we tested the infection efficacy of 4 different types of inoculum based on RNA and sap extract obtained from infected plants. Each inoculum was mechanically introduced using stem injections or rubbing the inoculum onto leaves treated with carborundum. The assay was performed on four indicator plants: cucumber (*Cucumis sativus*), tomato (*Solanum lycopersicum*), eggplant (*Solanum melongena*) and purple passion plant (*Gynura aurantiaca*), with 10 repetitive plants per treatment. Analysis after mechanical inoculation showed that the best bioassay for CBCVd is rubbing the sap extract inoculum onto leaves treated with carborundum, using tomato as an indicator plant. Additionally, we have started the development of one-step multiplex RT-PCR for simultaneous detection of all four viroids from hop. This technique enables shorter analysis of high throughput samples, reduces the use of reagents and, consequently, the cost of analysis.

In conclusion, reliable biological indexing of CBCVd for resistance screening and methods for simultaneous detection of viroids are essential in viroid disease management.

Acknowledgement: This work was supported by the Slovenian Research Agency (J4-4153, P4-0077) and by the Czech Ministry of Education (LH14255).

P DMD 37

Purification of inhibitor protein from rapeseed and its characterization on *Leptinotarsa decemlineata* (Say) gut specific proteases

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Introduction: Protease inhibitors (PIs), broadly distributed in nature, are small protein molecules that form very stable complexes with proteolytic enzymes and have the ability to inhibit the action of them. Production of PIs that interfere with the digestive biochemistry of insect pests is one of the naturally occurring defense mechanisms in plants.

Objectives: The aim of the present work was to purify and characterize protease inhibitors from rapeseed, with potential for the control of the Colorado potato beetle [CPB; (*Leptinotarsa decemlineata* Say) (Coleoptera: Chrysomelidae)].

Material and methods: The crude protein extract from the rapeseed (*Brassica rapa* L. cv. Karaj3), 30-50% ammonium sulfate precipitated fraction which showed the highest protease inhibition on the last instar larvae of CPB was selected and partially purified by using various chromatography techniques (MPLC); ion-exchange by means DEAE column, affinity using silicon dioxide nano powder and gel filtration by means Ultrogel®. For the preparation of affinity matrix CPB larval gut enzyme mix was chosen as ligand.

Results: Three peaks of protein were eluted from ion exchange chromatography using 300, 600 and 1000 mM salt step gradient (NaCl). Similarly three peaks were achieved from the gel filtration chromatography. The fractions related to each peaks with the highest inhibitory activity were pooled, dialyzed and concentrated, also purity and the molecular weight of them were estimated by SDS-PAGE. When used Z-Ala-Arg-Arg-4mβNA as cysteine protease substrate and azocasein as general protease substrate; the purification fold of second fraction of ion exchange chromatography was obtained 24.80 and 4.16, also the yield were 59.09 and 9.89% respectively, and the third fraction of gel permeation resulted 25.60 and 3.29 fold purification with 28.53 and 3.66% of recovery. The fraction of affinity chromatography obtained 22.72 and 5.2 fold purification and yielded 36.35 and 8.29%, respectively. However, ammonium sulfate precipitated fraction did not inhibit the serin protease activity of larval gut protease enzyme of CPB. Apparent molecular mass of purified proteins from ion exchange were 34 and 32 kDa, gel permeation was 14 kDa and affinity chromatography were 24 and 22 kDa.

Conclusion: The data shown here suggest that the proteins present in rapeseed have potential to enhance the defense mechanism of potato against the attack of CPB. At this point, the use of inhibitors in insect control strategies using transgenic crops is good technique, because insect digestive proteinases are promising targets in the control of various insects.

P DMD 38

Reconsidering the Normal Distribution - Benefits from Replacing Plus/Minus by Times/Divide

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The Gaussian or normal model is usually accepted in analysing data in plant protection, biology, and across the sciences. However, there is one basic contrast remaining. Whereas the justification of the normal model to describe variation is based on *addition*, natural laws and phenomena governing variation are mainly based on *multiplication*.

For instance, life is chemistry, and the velocity of chemical reactions is governed by the *product* of the individual concentrations. This means that the (additive) normal distribution cannot be the normal one to apply. Instead, data sets will commonly be skewed and rather fit the log- or multiplicative normal distribution¹.

What is *the difference of additive and multiplicative variation*? Both forms have in common that they are based on a number of forces acting independently. With the help of two ordinary dice some basic principles can be demonstrated. *Adding* the numbers according to most games leads to values from 2 to 12, with a mean of 7. The distribution is symmetric. The total range can be described as 7 plus / minus 5 (7 ± 5) where, in this case, 5 is not the standard deviation. *Multiplying* the two numbers, however, leads to values between 1 and 36. The distribution is highly skewed. The total variability can be described, analogous to the former case, as 6 times / divide 6 ($6^x / 6$). The symmetry has moved to the multiplicative level.

As *another example*, cell numbers are frequently described by $\bar{x} \pm SD$, with the arithmetic mean and the standard deviation as, e.g., 130 ± 100 . The "95% range check"¹ then leads to 130 ± 200 , ranging from -70 to 330. Of course, cell numbers have to be positive. Moreover, they are caused by cell division, *multiplication*, according to the sequence 1-2-4 etc. Transforming 130 ± 100 to the multiplicative level leads to the characterization $100^x / 2$. The 68% range then extends from 50 - 200, with a 95% range, $100^x / 4$, from 25 - 400. This fits the data and is a plausible description.

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In summary, replacing additive signs and thoughts for variation by multiplicative ones and, accordingly, using logarithmic x axis and the multiplicative or geometric standard deviation will be beneficial for our comprehension and results.

¹Limpert E, Stahel WA, 2015, this IPPC, abstract 172

P FUNGI 1

Antifungal potential of essential oils of *Cupressus* sp and *Cupressus lusitanica* against three life stages of *Phytophthora colocasiae*: causing agent of Taro Leaf Blight

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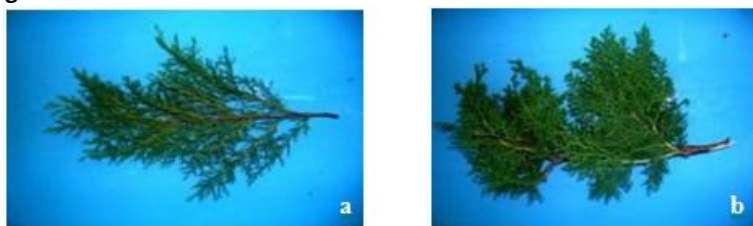
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Taro Leaf Blight caused by *Phytophthora colocasiae* Racib is a major limiting factor in taro growing field worldwide and particularly in Cameroon where since 2010, this disease has severely constrained taro production, lowering the production by more than 80 % (MINADER, 2010). In the search for alternative treatment against TLB because of the limits of chemical method mostly used, the antifungal potential of essential oils isolated from fresh leaves of *Cupressus* sp and *Cupressus lusitanica* was carried out against three life stages of the pathogen and compare with Ridomil. Essential oils were obtained by Clevenger type water distillation. The major compounds in these essential oils were identified using gas chromatography (GC) and gas chromatography coupled with mass spectrometry (GC/MS) (Adams, 2007). *In vitro* antifungal potential of essential oils was tested against mycelial growth by food poisoning technic (Lahlou, 2004). Sporangia and zoospores germination were tested by liquid dilution method (adapted from Sharma and Tripathi, 2006). The results obtained showed that yields of extraction ranged from 0.15 to 0.5 %. Essential oil of *Cupressus* sp was contained mainly 3-allyl-6-methoxyphenol (81.5 %) and α -linalool (4.9 %) whereas essential oil of *C. lusitanica* contained mainly α -citral (72.8 %) and α -myrcene (13.4 %). *In vitro*, sensitivity of *P. colocasiae* varied according to the essential oils tested. The essential oil of *Cupressus* sp was more efficient than the essential oil of *Cupressus lusitanica*. Antifungal activity of this essential oil was recorded with Minimal Inhibitory Concentration and Minimal Fungicidal Concentration of 2.5 mg/ml on mycelia growth whereas the complete inhibition of sporangia and zoospores germination was recorded respectively at 0.625 mg/ml and 0.312 mg/ml. These results demonstrated that, the essential oil of *Cupressus* sp has high ability to inhibit the development of *P. colocasiae*, and might be used for controlling TLB.

Figure 1



Photography 1: Fresh leaves of plants used.
a) *Cupressus* sp. b) *Cupressus lusitanica*

Figure 2

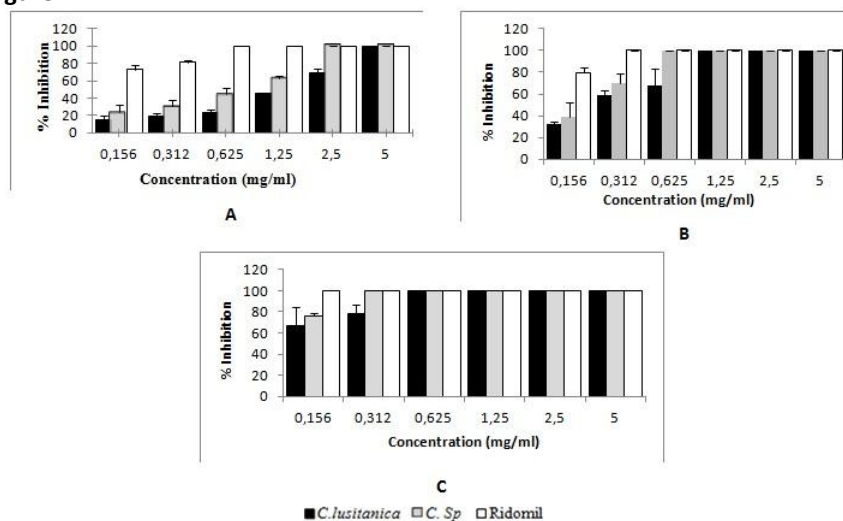


Figure 1: Inhibition percentages of essential oils and Ridomil at various concentrations.
A= Inhibition of mycelia growth.
B and C = Inhibition of sporangia and zoospore germination.

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P FUNGI 2

Morphological and molecular identification and fungicide sensitivity assay of pathogens attacking Guyabano (*Annona muricata* L.) Vell

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This study was conducted to characterize the plant pathogens attacking guyabano fruits and leaves through morphological and molecular approaches and to determine the fungicides where these pathogens are sensitive. Three fungal pathogens were found to be pathogenic to guyabano fruit, these pathogens are: *Colletotrichum gloeosporioides*, *Colletotrichum acutatum* and *Fusarium chlamydosporum*. *Colletotrichum gloeosporioides* are from leaves. Molecular identification of the *Colletotrichum gloeosporioides* species was carried out through amplification of rDNA ITS regions by using species specific primers (CgInt) for *Colletotrichum gloeosporioides* and (CaInt2) for *Colletotrichum acutatum* in combination with ITS4 universal primer. Ver ITS primer was used on *Fusarium* species. All fungal pathogens were found to be highly pathogenic to guyabano fruits and leaves. Among the fungicides tested, *Colletotrichum gloeosporioides*, *Colletotrichum acutatum* and *Fusarium chlamydosporum* were found to be highly sensitive to Captan, Tebuconazole and Difeconazole + Propiconazole.

P FUNGI 3

The Role of FRAC in Fungicide Resistance Management

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The Fungicide Resistance Action Committee (FRAC, www.frac.info) is a technical industry group reporting to Crop Life International, of the main manufacturers developing and supplying fungicides to the market. The FRAC member companies are: ADAMA, BASF, Bayer CropScience, Dow Agrosciences, Du Pont, FMC, Isagro, Sumitomo and Syngenta. Several other companies are represented in regional FRAC groups. Its purpose is to identify potential and existing resistance problems, evaluate scientific knowledge and data, support further studies, and provide resistance management guidelines to prolong the effectiveness of "at risk" fungicides and limit crop losses should resistance occur. FRAC also provides educational material and training in fungicide resistance and its management.

FRAC members are recognised experts and scientists in fungicide resistance. FRAC does not work in isolation - there is excellent communication with country Resistance Action Groups, research and advisory bodies, EPPO and many regulatory authorities. This is a network to help make the best practical recommendations. Although it cannot be claimed that resistance management strategies defined and supported by FRAC and other experts in the field have prevented the occurrence of fungicide resistance, they have clearly reduced the impact of resistance in practical situations, and have limited or slowed down the rate of spread of field resistance.

Fungicides recently introduced represent major advances in technology, potency against diseases, selectivity, safety and rate reduction. They tend however to have single site modes of action which makes them potentially affected by target site resistance. Examples of recent introductions include the QoI and SDHI fungicides. Thus it is very important to proactively design and implement resistance management strategies and recommendations for new fungicide classes, as well as maintaining existing products. Despite many cases of resistance to fungicides occurring over the past thirty years, effective disease and resistance management strategies have ensured the continued usefulness of many fungicides.

Although notable successes have been achieved, continuous efforts are needed to communicate and educate on fungicide resistance and to protect the efficacy of available fungicides worldwide.

P FUNGI 4

MyIPM, A New Smartphone App for Disease and Fungicide Resistance Management in Strawberry and Peach

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We developed a new smartphone application, MyIPM, to promote Integrated Disease Management for sustained peach and strawberry production in the southern United States (Figure 1). The app is available in the Google Play Store for Android phones and in the Apple Store for iOS devices. It features about a dozen of the most important diseases of the two fruit crops. For each disease there are pictures of signs and symptoms, descriptions of the causal agent, and a 2-min audio from the regional

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specialist. The app features chemical and biological control options, including a list of registered active ingredients for each disease that are sortable by FRAC codes and southeastern spray guide-published efficacy (Figure 2). The app also features field toxicity values as published by the Cornell IPM Program. The active ingredients are linked to registered trade names. MyIPM also features some audio recordings from regional specialists on peach and strawberry IPM issues. Our vision is that this app provides a valuable tool for growers and specialists alike that supplements current spray guides. The unique display of active ingredients, color-coded by chemical classes, provides a useful tool to promote resistance management. The app requests and allows for feedback, which should help keep the information up to date at all times. MyIPM is fed by a database that can be updated through an authoring tool. The app is currently free of charge. It is expandable to more crops and may also have potential for other disciplines, such as entomology.

Figure 1

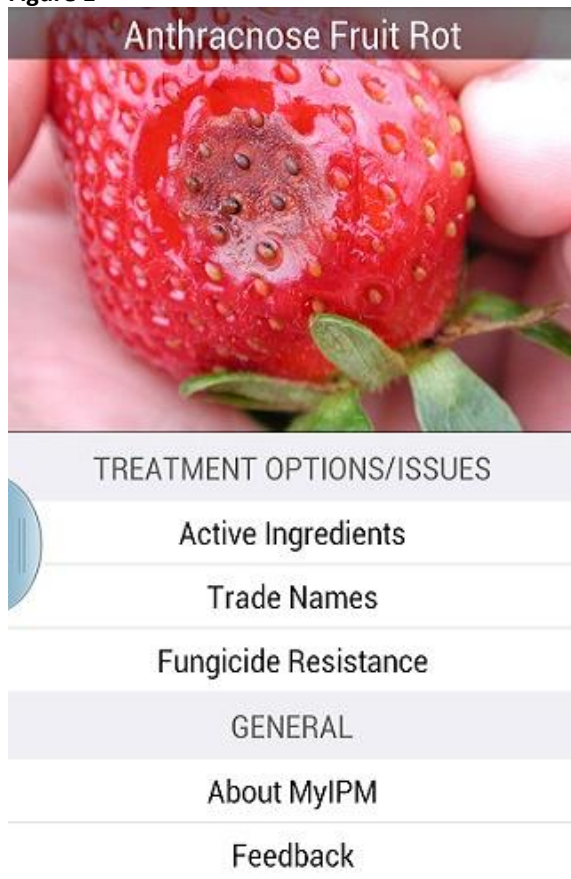


Figure 2

Conventional		Organic	
Active Ingredient	FRAC Code	Efficacy	
Azoxystrobin	11	+++++	
Boscalid; Pyraclostrobin	7; 11	+++++	
Captan	M4	++++	
Cyprodinil; Fludioxonil	9; 12	++	
Difenoconazole; Azoxystrobin	3; 11	++++	
Difenoconazole; Cyprodinil	3; 9	++	
Fenhexamid; Captan	17; M4	+++	
Fluxapyroxad; Pyraclostrobin	7; 11	+++++	
Propiconazole	3	+++	
Propiconazole; Azoxystrobin	3; 11	++++	
Pyraclostrobin	11	+++++	
Thiophanate-Methyl; propiconazole	1; 3	+++	
Thiram	M3	+++	

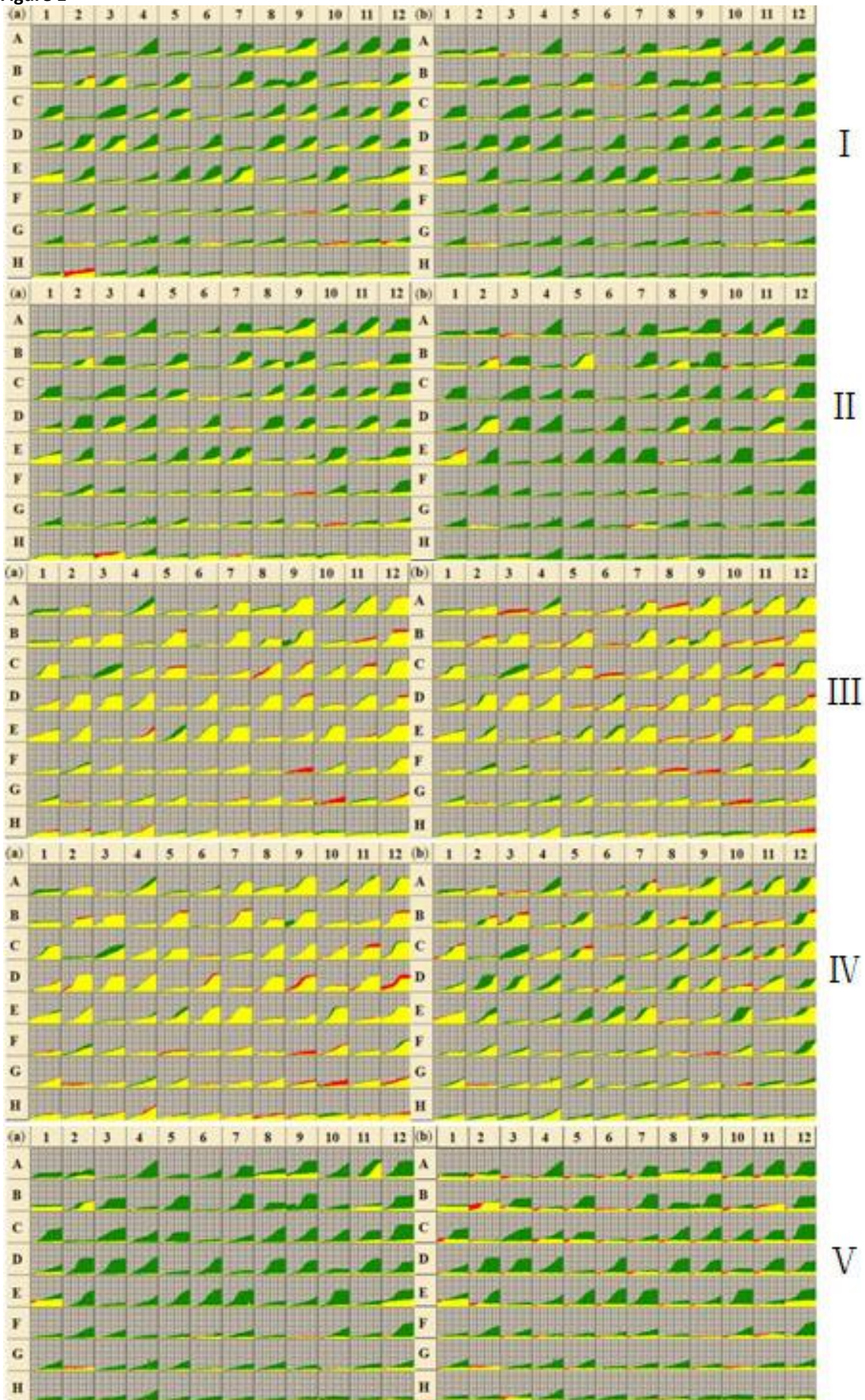
P FUNGI 5

Metabolic effects of five fungicides against *Botrytis cinerea* examined using the Biolog FF MicroPlate

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Tobacco grey mold caused by *Botrytis cinerea* is an important fungal disease during seedbed and maturation periods. Sensitivity of *B. cinerea* to boscalid, carbendazim, iprodione, pyrimethanil and propiconazole were determined by mycelial growth assays on fungicide-amended PDA plates, and the EC₅₀ values were, respectively, 0.50, 0.02, 0.11, 0.79 and 0.13 µg·ml⁻¹. The Biolog FF Microplate was used to study the activity of five fungicides against *B. cinerea*. In absence of fungicide, *B. cinerea* metabolized 96.8% of tested carbon sources, including 29 effectively and 33 moderately. Under the selective pressures of each of the five fungicides, *B. cinerea* showed different metabolic fingerprints, and these activities were greatly inhibited compared to the non-fungicidal control containing carbon sources and a basal medium. With increased concentrations of each fungicide, the metabolic activities of *B. cinerea* was even more greatly inhibited. With boscalid at 0.8 or 8 µg ml⁻¹, the pathogen was unable to metabolize many substrates related to tricarboxylic acid cycle, such as succinic acid, bromosuccinic acid, fumaric acid, α-ketoglutaric acid, D-malic acid, succinamic acid, L-asparagine, L-glutamic acid, L-proline, L-pyroglutamic acid, L-serine, L-threonine and putrescine (Fig. 1 I). With carbendazim at 0.05 or 1 µg ml⁻¹, most carbon sources related to glycolysis were inhibited. While mycelia of *B. cinerea* could not grow at 1 µg ml⁻¹ of carbendazim *in vitro*; there were still 12 carbons utilized by the pathogen, including D-arabinose, arbutin, β-cyclodextrin, D-fructose, D-galacturonic acid, D-glucosamine, maltose, D-mannose, β-methyl-D-glucoside, D-raffinose, D-ribose and D-tagatose (Fig. 1 II). With iprodione at 0.1 or 5 µg ml⁻¹, the metabolic profiling of *B. cinerea* were similar to control, although utilization of most carbon substrates was weakly inhibited (Fig. 1 III). With pyrimethanil at 1 µg ml⁻¹, three substrates related to hydrolytic enzymes were significantly inhibited, including N-Acetyl-β-D-glucosamine, D-glucuronic acid and L-sorbose (Fig. 1 IV). With propiconazole at 1 and 10 µg ml⁻¹, no carbon substrates were metabolized by *B. cinerea* and the physiology and biochemistry pathways of the pathogen were totally inhibited (Fig. 1 V). These new findings would provide some foundations for tobacco gray mould management in China.

Figure 1



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P FUNGI 6

Controlled Release Formulation of licorice extract as a new biological fungicide against grapevine diseases

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The increased public concern regarding the controversial effects of agrochemicals on food safety and environment drives the development of alternative strategies in crop protection. Such novel strategies should be effective, environmental-friendly and preferably based on renewable substances. In the last years we provided the proof that the plant extract of licorice (*Glycyrrhiza glabra*) possesses the potential to be such a new and sustainable alternative. It could reduce and/or replace copper-based products used today in European organic and low input fruit, grapevine and tomato production systems to regulate Oomycetes disease. In our former research project funded by the Federal Ministry for Economic Affairs and Energy (BMWi; Central Innovation Program SME; ZIM) the development a new controlled release (CR-) formulation- targeted to control Oomycetes in tomato and grapevine- was realized.

Objectives: Our main objective was to demonstrate successfully a microencapsulation method that could be subsequently used for field applications. A general aim was the controlled and delayed release of the active biological ingredients given the desired activity and efficacy against Oomycetes in semi-field conditions and green-house trials.

Material and methods: CR-formulations were developed and evaluated for efficacy and UV stability in green houses against copper-relevant fungal pathogens on potted plants.

Results: Overall, the presented results clearly demonstrate (disease reduction up to 90%) the high potential of licorice-based caps for the efficient control of downy mildew and late blight disease on grapevine and tomato plants.

Conclusion: Trifolio-M developed an innovative plant protection test product obtained from by-products of the food industry. The most promising formulation types will be tested in field conditions in the season 2016 as part of our new research project funded by the Federal Ministry of Food and Agriculture (BMEL, Deutsche Innovationspartnerschaft Agrar; DIP).

P FUNGI 7

Enrichment of mutations responsible for azoxystrobin resistance in *Botrytis cinerea* field isolates

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Fungicides belonging to the family of quin oxidase inhibitors (QoIs) e.g. strobilurins, like azoxystrobin play an important role in the protection against several fungal plant pathogens. These fungicides act by binding to the cytochrome *bc1* complex in the respiratory electron transport chain so they block electron transport and inhibit the ATP synthesis. However a point mutation in the cytochrome *b* gene (*cyt b*), results in high-resistance. In addition, a functional alternative oxidase (AOX) may also play a role in the survival of the fungus. *Botrytis cinerea* is the causal agent of grey mould on a wide range of many economically important crops. Fungicides like azoxystrobin is the most frequently applied approach to reduce grey mould disease, but resistance has often reported.

The azoxystrobin resistance and related cytochrome *b* gene genotypes was studied in Hungarian *B. cinerea* field isolates with allele-specific PCR reaction and PCR-RFLP method for detecting the G143A mutation resulting azoxystrobin resistance of the fungus. The development of the QoI resistance was induced by using mediums with increasing concentrations of azoxystrobin. We also developed a method to track heteroplasmy by using real time PCR. Amplified PCR product containing G143A mutation and single copy gene located in the mitochondrial genome (*cox1*) were used as standards.

Field isolates from Hungary were tested for azoxystrobin resistance. In a few cases, we were able to detect the mutation with allele-specific PCR but not with PCR-RFLP, and these strains were sensitive to azoxystrobin. This would indicate marginal presence of the resistance-conferring, mutated mtDNA and these strains may well develop resistance rapidly when faced with QoIs in the field. *In vitro* we managed to induce the development of the resistance toward azoxystrobin. Using the developed real time PCR method it was proved that in the presence of azoxystrobin the ratio of the mitochondrial genome which carries the resistance is increased compared to the sensitive sequence. We related the change in the ratio of the mitochondrial DNA containing the G143A point mutation compared to the sensitive, "wild" sequence.

This research was supported by the TÁMOP-4.2.2.A-11/1/KONV-2012-0043 project.

P FUNGI 8

Biological performance of isofetamid, a novel fungicide

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Introduction: Isofetamid, N-[1,1-dimethyl-2-(4-isopropoxy-o-tolyl)-2-oxoethyl]-3-methylthiophene-2-carboxamide, IKF-5411 (development code), KENJA[®], is a novel fungicide discovered and under development by Ishihara Sangyo Kaisha, LTD. Its molecular structure is shown in Figure 1. Isofetamid is a new chemical class based on thiophene carboxamide moiety. Isofetamid is a member of the succinate dehydrogenase inhibitor (SDHI) fungicide group. It has a good toxicological and ecotoxicological profile to mammals and other non-target organisms, such as birds and fishes. Isofetamid exhibits excellent activity against a broad spectrum of Ascomycota (such as *Botrytis* spp., *Sclerotinia* spp., *Monilinia* spp., *Venturia* spp.) and Deuteromycota (such as *Alternaria* spp., *Mycovellosiella* spp.) at low use rates.

Objective: This study describes the biological properties of isofetamid and results of field trials.

Materials and methods: Isofetamid 400 g a.i./L suspension concentrate (400SC) was used in this study. Biological properties of preventive activity, residual activity, rainfastness, translaminar activity, and curative activity against *B. cinerea* on cucumber were investigated in pot tests. Field trials of isofetamid were conducted for the evaluation of efficacy against gray mold on grape in European countries.

Results: Isofetamid at 16 µg/ml exhibited excellent preventive activity. Isofetamid at 31 µg/ml exhibited stable residual activity (7 and 14 days after application). It also exhibited stable rainfastness and excellent translaminar activity. In curative activity tests, we confirmed that this fungicide inhibited expansion of disease symptoms and sporulation.

In field trials, isofetamid at 600 g a.i./ha exhibited excellent activity against grape gray mold. Control level of isofetamid was equal to superior to that of standard fungicide.

Conclusion: Isofetamid is a novel fungicide that exhibits excellent preventive activity along with good residual activity, rainfastness, translaminar activity and curative activity. These properties lead to a high level of field control of fungal infestation by isofetamid.

Figure 1

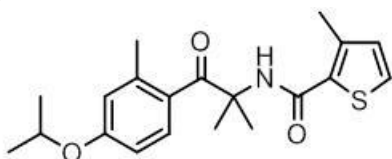


Figure 1. Molecular structure of isofetamid

P FUNGI 9

Discovery of the Novel Fungicide "Pyriofenone"

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Introduction and objective: We have paid a lot of attention to a group of compounds containing the CF₃-pyridine moiety for many years because of their remarkable biological activities, as seen in compounds such as fluazinam, chlorfluazuron, flonicamid, and so on. Various types of CF₃-pyridine derivatives have been synthesized and evaluated. During our continuing efforts, compound 1 bearing the benzoyl group at the 3-position of CF₃-pyridine was selected as a potential lead compound that showed good fungicidal activity against wheat powdery mildew.

Method: Modifications of compound 1 were made by introducing many different types of substituents on the pyridine ring. The substitution position of the benzoyl group on the pyridine ring was also explored. The general synthetic scheme is shown in eq. 1. Intermediate 3 was prepared from CF₃-pyridine and the corresponding benzaldehyde in the presence of LDA. Subsequent oxidation of 3 using MnO₂ afforded compound 4. In addition, compounds having a methyl moiety on the pyridine ring instead of

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a CF_3 group were also synthesized for comparison purposes as shown in eq. 2. The methyl group was introduced in the later stage of the synthesis by means of a transition-metal catalyzed coupling reaction.

Results: Based on the QSAR study of the synthesized derivatives, several compounds exhibiting excellent activity were found. In particular, compound 7 showed high activity in field tests.

Conclusion: We synthesized many different types of pyridine analogues and selected compound 7, called pyriofenone, as a development fungicide candidate.

Figure 1

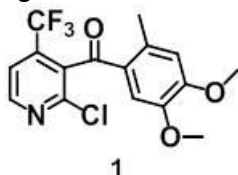
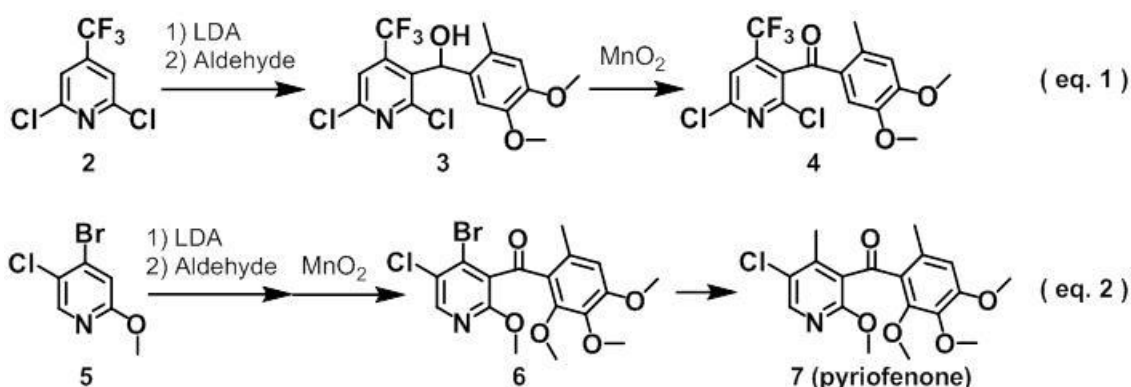


Figure 2



P FUNGI 10

Evaluation of alternative *Plutella xylostella* control by two *Isaria fumosorosea* conidia formulations, oil-based formulation and wettable powder combined with *Bacillus thuringiensis*

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The diamondback moth (DBM), *Plutella xylostella*, is the most destructive pest on cruciferous vegetable worldwide. A strain of *Isaria fumosorosea* with high pathogenicity against *P. xylostella* was screened in our laboratory, which median lethal concentration (LC_{50}) on day 7 and median lethal time (LT_{50}) at 1×10^7 conidia/mL to 2nd instar larva of DBM were only 1.17×10^4 conidia/mL and 1.72 days, respectively, suggesting promising of this strain in the biological control of *P. xylostella*.

In order to make full use of this strain the control efficacy of two *I. fumosorosea* conidia formulations, wettable powder and oil-based formulation, combined with *B. thuringiensis* against *P. xylostella*, was tested. In the laboratory, the combined application increased larval mortality either in an additive or a synergistic way. For pot and field experiments, each formulation was applied alone or combined with *B. thuringiensis* $668 \mu\text{g mL}^{-1}$, then larval mortality, pupation rate, adult emergence rate, female longevity and fecundity of DBM were recorded. In pot experiment, the combined treatments of *B. thuringiensis* with $5 \times 10^5 \text{ mL}^{-1}$ oil-based formulation and 1×10^6 conidia mL^{-1} wettable powder of *I. fumosorosea* resulted in higher mortality (84.4% and 86.2%) with minimum pupation (15.6% and 11.9%) and adult emergency rates (8.7% and 7.0%). Female longevity and fecundity were significantly decreased by two formulations compared to the control. Similar results were also observed in field experiment.

Our results demonstrated that the combination of sublethal doses of *B. thuringiensis* with two *I. fumosorosea* formulations not only reduced the present population of *P. xylostella* effectively, but also has a sustainable control efficacy on its next generation. Therefore the combined application of *I. fumosorosea* and *B. thuringiensis* is a promising alternative strategy for *P. xylostella* control in the field.

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P FUNGI 11

Development of a selective crystallization route to obtain the fungicide (*S*)-Fenamidone

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It is a well-known fact that every organism constitutes a specific chiral environment hence there are many examples for enantiomeric and enantiotropic selectivity to organisms in applications as agrochemicals [1].

Apart from enantioselective synthesis of agents the enantioseparation of chiral active ingredients provides an opportunity to obtain pure enantiomers [2]. One of those separation methods focuses on partition via crystallization as an efficient, flexible & economically attractive technique for enantioseparation in agrochemical or pharmaceutical industry [2,3]. Since the three different types of racemates regarding their solid phase behavior [4] are connected with specific characteristics in the phase diagram the importance of analyzing solid/liquid equilibria is important.

Fenamidone constitutes a commercial fungicide possessing an azole group [5]. Biological & biochemical tests offer the fungicidal activity of the (*S*)-enantiomer [6]. With a low risk of bio-accumulation Fenamidone exhibits a high level of activity controlling diseases as for instance downy mildews or late blight [6,7].

Combining various analytical techniques (e.g. HPLC, XRPD) the solubility and stability properties of the different phases in the system Fenamidone/Ethanol were determined.

Having investigated phase transitions & solid/liquid equilibria in the explored system subsequently a ternary solubility diagram could be constructed. A selective crystallization process can be conducted with an excess of (*S*)-enantiomer > 80%, thus, requiring a preceded enrichment step. XRPD analysis revealed a stable solid phase behavior of Fenamidone in Ethanol, which is of advantage to control the crystallization process.

Utilizing the knowledge of the different phase behaviors a crystallization process to achieve pure (*S*)-Fenamidone was designed.

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P FUNGI 12

Fungicide resistance in *Microdochium nivale* isolated from golf greens in the UK and Ireland

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Introduction: Fusarium patch, caused by *Microdochium nivale*, is the most common and widespread fungal disease affecting cool season amenity turf grass. In the USA and New Zealand, reduced efficacy of iprodione has been seen in the field. Although there have been no reported incidences of field resistance to fungicides within the UK, reduced sensitivity to iprodione has been reported in a small number of UK isolates tested in the laboratory.

Aim: To determine the sensitivity of *M. nivale* isolated from golf greens in the UK and Ireland existed to the most commonly used fungicide products.

Method: Five products (Banner Maxx, Throttle, Heritage Maxx, Surpass Pro and Medallion) were assessed against 15 *M. nivale* isolates collected from golf greens, across the UK and Ireland, using an amended agar test to calculate mycelial growth EC₅₀ values for each fungicide.

Results: Banner Maxx (propiconazole) and Throttle (tebuconazole and prochloraz) gave the lowest EC₅₀ values with results averaging 0.2 and 0.05ppm respectively. Values for Heritage Maxx (azoxystrobin) ranged from 0.7 to <10 with an average of 2.4ppm. EC₅₀ values for Surpass Pro (iprodione) fell into 2 groups, those with a value of approximately 2.5 ppm and those greater than 26 ppm. Isolates tested using Medallion (fludioxonil) again fell into two groups ones where EC₅₀ values ranged between 0.03 and 0.06 ppm and those greater than 5 ppm. showed the greatest variance in EC₅₀ values ranging from 0.01 to 100ppm.

Conclusion: Reduced in vitro sensitivity to iprodione and fludioxonil had developed in some *M. nivale* isolates collected in this study. Isolates which were more resistant to iprodione were also more resistant to fludioxonil indicating possible issues of cross resistance. EC₅₀ values for all isolates tested with azoxystrobin were equivalent to those for *M. nivale* isolates isolated from

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winter wheat which showed field resistance to azoxystrobin. This suggests the potential for widespread field resistance to azoxystrobin in the UK and Ireland. Only propiconazole and tebuconazole/prochloraz produced EC₅₀ values suggesting there was no shift in sensitivity. However, the possible removal of these active ingredients in the future, means a limited number of effective chemical options will be available for control of Fusarium patch infections in the future.

P FUNGI 13

Positioning Tebuconazole 430 SC in rice ecosystem against management of blast and sheath blight Vis-à-Vis impact of abiotic weather factors in West Bengal, India

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Introduction: Blast and Sheath Blight of rice caused by *Magnaporthe grisea* and *Rhizoctonia solani* are the important diseases of rice which accounts for serious yield losses in all rice growing country. Over last two decades a lot of focus has been shifted towards developing new molecules that can be used for controlling rice diseases. The new fungicide Tebuconazole 430 SC was evaluated in the field for management against the diseases blast and sheath blight of rice.

Objective: Rice blast and sheath blight diseases hit large areas causing huge monetary loss. The objective of the study was to evaluate the dose of Tebuconazole a new fungicide for controlling the diseases.

Materials and methods: The field trial was conducted during 2013 and 14 Kharif season laid out on a RBD with six treatments four replications consist of Tebuconazole with three different doses and two recommended fungicides Folicur & Hexaconazole. Uniform plant populations were maintained. Three sprays of each fungicide with desired concentrations were applied starting from 32nd DAT at ten days interval. Disease severity, incidence & rate of spread of the diseases were recorded

Results: It was found that Tebuconazole @ 215 & 187.5 g ai/ha significantly reduced the diseases both incidence & severity significant higher yield was recorded in those doses. From the meteorological relation with the disease development it was found that max RH, vapour pressure and wind speed were negatively and combined influence on disease progression and maximum temperature and wind speed negatively and maximum relative humidity positively influence to increase the disease progression, it was observed that the rate of spread of the disease was decreased with increasing the application of fungicides

Conclusion: Tebuconazole @ 187.5 g ai/ha may be recommended for controlling the blast and sheath blight diseases of rice. The minimum rate of spread of the diseases may be due to minimum rate of multiplication of inoculums potential due to application of fungicides for both the diseases maximum relative humidity positively influence to increase the disease progression

Figure 1

Table 1: Effect of Tebuconazole 430 SC against incidence and severity of Leaf Blast and sheath blight Diseases of Rice. (Two years pooled)

Treatment	Dosage g a.i./ha	Blast				Sheath Blight				Yield kg/Plot
		Disease Incidence%	Disease Control	Disease severity%	Disease Control	Disease Incidence%	Disease Control	Disease severity%	Disease Control	
Untreated control	-	29.87(33.13) *	-	31.01(33.84)	-	29.53(32.90)	-	30.52(33.53)	-	2.99
Tebuconazole 430 SC	161.25	12.60(20.79)	57.81	16.22(23.74)	47.69	16.49(23.95)	44.15	16.67(24.09)	45.34	5.78
Tebuconazole 430 SC	187.5	8.58(17.02)	71.27	10.20(18.63)	67.10	8.05(16.45)	72.73	8.76(17.21)	71.29	8.96
Tebuconazole 430 SC	215	8.53(16.96)	71.44	9.68(18.12)	68.78	7.64(16.00)	74.12	8.34(16.78)	72.67	9.14
Folicur 250 EC	430	12.65(20.83)	57.64	12.89(21.01)	58.43	15.95(23.54)	45.98	15.65(23.29)	48.72	6.31
Hexaconazole 5% EC	187.5	12.68(20.86)	57.54	14.21(22.12)	54.17	16.33(23.83)	44.70	15.84(23.44)	48.09	6.03
SEm(±)	-	0.158	-	0.157	-	0.209	-	0.150	-	0.052
CD(P0.05)	-	0.456	-	0.453	-	0.604	-	0.433	-	0.150

* Figures in the parentheses are angular transformed values.

Figure 2

Table. 2 Correlation matrix of blast disease severity with weather variable and rate of spread of the disease (Two years pooled)

Parameters	PDI (2012)	PDI (2013)	Treatment	Rate of Spread of Blast & Sheath Blight					
				Between 32-42 DAT		Between 42-52DAT		Between 52-62DAT	
Percent disease index(PDI)	1.000	1.000		Blast	SB	Blast	SB	Blast	SB
T _{max}	.112	-.544	T ₁	0.193	0.157	0.031	0.047	0.015	0.012
T _{min}	-.615	-.955	T ₂	0.125	0.113	0.025	0.035	0.014	0.019
RH _{max}	.393	.800	T ₃	0.120	0.066	0.017	0.041	0.010	0.012
RH _{min}	-.507	.088	T ₄	0.090	0.050	0.015	0.042	0.009	0.015
Rainfall (mm)	-.793	.777	T ₅	0.136	0.108	0.018	0.019	0.020	0.029
Vapour pressure 1	-.191	-.650	T ₆	0.153	0.118	0.020	0.022	0.013	0.027
Vapour pressure 2	.254	-.737							
Wind Speed Km/hour	-.946*	-.890							

Multiregression equation

$$2012 \quad Y = 291.543 - 1.25(RH_{max}) - 5.1(\text{Vapour pressure 1}) - 436.3(\text{Wind Speed Km/hour})$$

$$2013 \quad Y = 185.7 - 5.4(T_{max}) + .884(RH_{max}) - 32.7(\text{Wind Speed Km/hour})$$

P FUNGI 14

Investigation of biological efficiency of *Rheum rhaponticum* L root extract in protecting of *Cucurbitaceae* seedlings against powdery mildew

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The most of plant secondary metabolites protect plants from pathogens. Polyphenols and their derivatives play the important role in protection of plants from the fungal diseases. We studied the *Rheum rhaponticum* L. (f. Polygonaceae) alcoholic extract in order to reveal the anthraquinone derivatives which induce resistance to plant phytopathogens and act as an antimicrobial agent with fungicidal activity. An alcoholic extract of *Rheum rhaponticum* L was analyzed using HPLC.

The aim of the work was to determine the biological efficiency of extracts obtained from the *R. rhaponticum* L. The estimation of the *R. rhaponticum* L alcoholic extract effect was conducted against the target pathogen conidial germination of *Sphaerotheca fuliginea* on *Cucurbitaceae* plants *Cucumis melo* L and *Cucurbita pepo* L. at the 4th leaf stage

Methods: Conidia of *S. fuliginea*, collected from the *Cucurbitaceae* cotyledons, were suspended in distilled sterile water. Suspensions were plated onto the nutrient media and incubated at 210C. Germination was determined after 24 h. *Cucurbitaceae* plants at the 4th leaf stage grown in pots were arranged in a complete randomized design with 4 replicates with *R. rhaponticum* L extract (concentration in an aqueous solution for foliage treatment - 0,6; 0,8; 1; 1,5; 2%) and were inoculated with conidia from the infected *Cucurbitaceae* leaves. Disease severity (% infected leaf area) was determined at 14 days postinoculation.

Results: Biological efficiency of extract at the indicated concentrations on seedlings of *C. pepo* L. were 47.3, 75.2, 77.9, 89.4, 97.1%; and of *C. melo* L were 56.9, 74.7, 80.0, 87.4, 93.7%. The additional effect of *R. rhaponticum* L extract foliage treatment should be noted. We observed changes in the green color intensity of the treated leaf blades in comparison with the control. Index of chlorophyll of the experimental plants leaves was measured using CM 1000 Chlorophyll Meter. At the highest concentrations of extract solution the chlorophyll index of treated leaves was 38.2% higher in comparison with the control leaves.

Based on the obtained data we can conclude that:

1. High concentrations of *R. rhaponticum* L extract have fungistatic effect against powdery mildew.
2. Treatment of plants with *R. rhaponticum* L extract enhances their flexibility by increasing the chlorophyll index

P FUNGI 15

Search for alternatives to copper in organic farming: Fungicidal activity of a *Juncus effusus* medulla extract and its active constituent, dehydroeffusol, against downy mildew and apple scab

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Introduction: Copper has been used since the 19th century for the control of plant diseases, and is still permitted in organic agriculture out of this tradition. In recent years, the utilization of copper has been criticized due to an unfavourable ecotoxicological profile. Even though the amounts of copper have been significantly reduced, copper input is still above uptake by plants, and results in accumulation in the soil. Therefore, considerable efforts have been made in organic agriculture to identify ecologically safer substitutes.

Objectives: The aim of this study is the search for copper substitutes of natural origin.

Materials and methods: An in-house library of plant and fungal extracts was screened *in vitro* for an inhibitory effect against several plant pathogens (fungi, oomycetes, bacteria). Hits were further assessed on grapevine and apple seedlings. Active constituent(s) were identified by a procedure referred to as HPLC-based activity profiling which combines biological activity data with chemoanalytical information. Structure elucidation was performed by a combination of ESI-MS and NMR spectroscopy.

Results: As one of the hits, the ethyl acetate extract of *Juncus effusus* L. (Juncaceae) medulla showed strong inhibitory activity against *Venturia inaequalis* (apple scab) and *Plasmopara viticola* (grapevine downy mildew), with mean minimal inhibitory concentrations (MIC) (100%) of 35 µg/mL and 25 µg/mL, respectively. In a secondary assay on grapevine leaf discs inoculated with *P. viticola*, 94% inhibition was observed at a concentration of 0.5 mg/mL. When tested on grapevine and apple seedlings at a concentration of 0.5 mg/mL, the growth of these fungi was, on average, inhibited with 98% and 84% efficacy, respectively. The active constituent was identified as dehydroeffusol, and showed mean MICs of 12 µg/mL against *V. inaequalis*, and 4.1 µg/mL against *P. viticola*, *in vitro*. Subsequent *in vivo* assessment of the pure compound revealed inhibition rates of 82% on grapevine seedlings, and 86% on apple seedlings at a concentration of 32 µg/mL.

Conclusion: The ethyl acetate extract of *J. effusus* showed potent activity *in vivo* against major fungi affecting food plants. Our results demonstrate that plants can provide promising opportunities for the replacement of copper in organic farming.

P FUNGI 16

Control of Apple Scab (*Venturia inaequalis*) by Trunk-injected Fungicides and SAR Inducing Potassium Phosphites with Residue Profiles in Apple Fruit and Leaves

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Trunk injection of biocides is a target precise and eco-friendly approach for control of tree pathogens with minimal risks of applicator exposure, drift and impact on non-target organisms and environment. We showed that injected fungicides reduce apple scab (*Venturia inaequalis*) and that injected potassium phosphites (PH) induce Systemic Acquired Resistance in mature apple trees, reducing fire blight (*Erwinia amylovora*) (Aćimović et al. 2013, 2015). To optimize number and timing of injections for season-long control of apple scab, we evaluated 1-2 seasonal and cross-seasonal injections of difenoconazole + cyprodinil (DC) or PH and compared them to their sprays (Fig. 1). We injected DC and PH in Mac Spur apple trees using treatments: 1 injection Fall 2012 (F), 1 injection Spring 2013 (S), combined injections Fall 2012 + Spring 2013 (F+S), 2 injections Spring 2013 (S+S) (Fig. 1). We rated leaf and fruit scab incidences and quantified compound residues with HPLC-MSD. Apple scab control is shown in Fig. 2.

PH fruit residues peaked at 2.8 ppm, declining at the end of the season in all treatments except in S+S and sprays. PH injection and spraying had similar fruit and leaf residues. D and C fruit residues peaked at 0.02 and 0.07 ppm, declined sharply towards the end of the season, and were ~10-fold lower after injection than after spraying. They were far below the USA, Codex, and EU MRL-s of 1, 0.8 and 0.5 ppm for D, and 1.7, 2 and 1 ppm for C, respectively. PH accumulated in leaves up to 3.4 ppm, aligning better with the leaf scab control than DC with residue maximums of 0.06 and 0.14 ppm. DC leaf residues were higher after sprays than after injection. Higher transpiration of shoots and lower of fruits and spurs, along with higher xylem mobility of PH versus DC, severely affected the accumulation and apple scab control by injected compounds.

References:

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Figure 1

Treatments	Active ingredient	Dose	Dates of injection(s) or sprays		
			in 2012	in 2013	
F		5.17 ml/ 25.4 mm of DFH*	11 October	-	-
S	PH (mono- and di-potassium salts of phosphorous acid 45.8%: Phosphojet)	5.17 ml/ 25.4 mm of DFH	-	21 April	-
F+S		2 x 5.17 ml/ 25.4 mm of DFH	11 October	21 April	-
S+S		2 x 5.17 ml/ 25.4 mm of DFH	-	21 April	22 May
<i>Sprays</i>		9 x 1892.71ml/ 0.405 ha on 1, 8, 16, 21, 31 May and 5, 11, 19, 26 June 2013			
F	DC (difenoconazole 8.4% + cyprodinil 24.1%: Inspire Super)	7 ml/ tree**	11 October	-	-
S		7 ml/ tree	-	21 April	-
F+S		2 x 3.5 ml/tree	11 October	21 April	-
S+S		2 x 7 ml/ tree	-	21 April	22 May
<i>Sprays</i>		5 x 354.84 ml/ 0.405 ha on 1, 8, 16, 21, 31 May 2013			
Water injected control	-	500 ml/ tree	11 October	21 April	22 May

*DFH, trunk diameter at one foot height (30.5 cm). **All DC treatments were injected with 500 ml of water per tree.

Figure 2

Treatments	Active ingredient	Apple scab control (%)		
		Spur leaves	Shoot leaves	Fruit
F	PH (mono- and di-potassium salts of phosphorous acid 45.8%: Phosphojet)	21.3 c*	25.3 d	18.4 b
S		25.5 c	47.4 c	62.8 a
F+S		46.3 ab	49.9 bc	64.6 a
S+S		41.0 b	66.5 a	69.7 a
<i>Sprays</i>		28.2 c	60.9 ab	28.8 b
F	DC (difenoconazole 8.4% + cyprodinil 24.1%: Inspire Super)	5.4 d	0.4 g	- 0.6 c
S		1.5 d	17.2 ef	6.3 c
F+S		5.7 d	2.4 g	0.0 c
S+S		- 0.11 d	10.8 f	- 1.4 c
<i>Sprays</i>			58.9 a	25.1 de
		Disease incidence (%)		
Water injected control	-	88.3	94.4	95.5

*Means followed by different letters are significantly different ($p < 0.05$, *t*-test).

P FUNGI 17

Antifungal potential of *Euphorbia hirta* L. against Anthracnose Disease of Mango

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The present study was intended to evaluate antifungal compounds potential of *Euphorbia hirta* L. against *Colletotrichum gloeosporioides* Penz., the casual agent of anthracnose disease of mango. Methanolic extract of *E. hirta* was tested *in vitro* against the test fungus. Different applied concentrations (1.5-3%) showed pronounced affects in retarding colony diameter of *C.*

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gloeosporioides. Maximum reduction (28%) was showed by 2.5% concentration; however 3% concentration was also effective in reducing the test fungus growth upto 22%. Phytochemical analysis of the methanolic plant extract of *E. hirta* indicated the presence of glycosides, flavonoids, alkaloids, phlobatannins, tannins and coumarins. This methanolic extract was subjected to bioassay guided fractionation due to the presence of these secondary metabolites. *In vitro* bioassays with different organic fractions showed that chloroform fraction was found the most effective as its two concentrations causing 73% and 77% growth inhibition. This effective fraction was then selected for GC-MS analysis. Fourteen phytochemical compounds were identified from the chloroform fraction of *E. hirta*. The major constituents namely linoleic acid, oleic acid and stearic acid along with some minor constituents were detected. These compounds might be responsible of antifungal activity of *E. hirta*.

P FUNGI 18

Sensitivity to isofetamid and fitness of SDHI resistant isolates in fungal pathogen populations

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Introduction: Isofetamid is novel fungicide developed by Ishihara Sangyo Kaisha, Ltd. It possesses excellent efficacy against a number of fungal diseases of vegetable and fruit crops and on turf. Isofetamid inhibits succinate dehydrogenase (SDH) activity, and is a member of the SDHI class (FRAC group 7) of fungicides. Although several SDHI fungicides are currently in use, multiple mutations in fungal SDH genes confer resistance to many members of this fungicide class, thereby reducing their effectiveness.

Objectives: This study describes the efficacy of isofetamid on several fungal isolates that possess resistance to common SDHI fungicides and its competitive attributes.

Materials and methods: SDHI resistant *Podosphaera xanthii*, *Corynespora cassiicola*, *Passalora fulvum*, and *Botrytis cinerea* used in this study were field isolated from Japan, USA and Germany. *B. cinerea* isolates possess the mutations H272R, H272Y, N230I, H272L, and P225F in SdhB. The sensitivity of each strain was examined by plate assay and/or pot test. Competitive fitness was evaluated for *B. cinerea* isolates containing the mutations H272L and P225F in SdhB. SDHI resistant and susceptible isolates were co-cultured at a 1:1 ratio for evaluation of competitiveness.

Results: Isofetamid had activity on all isolates from Japan and USA, but lacked effectiveness on select SDHI-resistant isolates from Germany. The SDHI-resistant isolates unaffected by isofetamid that contained the mutations H272L and P225F appeared to be overall less fit than susceptible isolates based on the competitive ability test.

Conclusion: While some *Botrytis* isolates have resistance to isofetamid, our data suggests that those isolates lack competitive advantage to sensitive populations. We therefore consider that the risk of resistance development to isofetamid is likely low and this new control method is expected to maintain good efficacy in the field, especially those where isolates resistant to SDHI fungicides are present.

P FUNGI 19

Effect of different fungicides and meteorological factors on severity of powdery mildew and fruit rot of chilli in field

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Introduction: Chilli is one of the most important spices crop grown throughout the year in West Bengal. The diseases like fruit rot & Powdery mildew are the major constraints for the cultivation of the crop and they cause heavy loss in yield. Several fungicides are used by farmers to control this disease.

Objective: The diseases causing huge monetary loss hence farmers incorporate tremendous volume of fungicides & due to regular use of same fungicides the pathogen develop resistance so to combat these problems a new pre mix fungicides formulation Lustre 37.5% SE (Flusilazole 12.5% + Carbendazim 25%) was evaluated with others in field against these diseases

Method: The field trial was conducted during Kharif and Rabi season 2014 laid out on RBD with eight treatments four replication consist of Lustre with three different doses and four recommended fungicides Flusilazole Azoxystrobin Difenconazol & Myclobutanil uniform plant populations were maintained two sprays of each chemicals with desired concentrations were given starting from 40 DAT at ten days interval. Disease severity & rate of spread of the diseases were recorded

Results: It was found that Lustre @ 150 & 112.5 g ai/ha significantly reduced both the diseases significant higher yield was recorded in those doses it was observed that both the diseases were significantly and negatively correlated with Wv, Tmin and RHmax. The two prediction equations were developed through MRA. The partial regression co-efficients showed that Tmin and wind velocity were negatively and RHmax positively and their combination effect increase the development of powdery mildew & in fruit rot Tmax, Tmin, RHmax and wind velocity were negatively & in combination influence the disease progression The rate

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of progress of the disease also influence on application of fungicides and was minimum on Lustre @ 112.5 and 150 g a.i./ha applied plots than other fungicides.

Conclusion: Lustre @ 112.5 g a.i./ha may be recommended to control Powdery mildew and fruit rot diseases. Rate of spread of the disease was also low in every fungicide application and minimum in Lustre for both the diseases. Tmin RHmax Wv were the main meteorological factors for disease progression and the prediction equation was different for two diseases also confirmed the above contention.

Figure 1

Table 1: Efficacy of fungicides against powdery mildew (severity) and fruit rot (%) of chilli (Two years Pooled)

Treatments	Dosage (g a.i./ha)	Powdery mildew		Fruit rot		Yield (q/ha)
		Disease severity%	Disease control	Disease severity%	Disease control	
Lustre 37.5% SE	75	13.42 (21.48)	52.63 (34.30)	11.25 (19.59)	63.51 (41.89)	25.50
Lustre 37.5% SE	112.5	8.79 (17.24)	68.97 (46.38)	8.29 (16.73)	73.11 (50.37)	30.83
Lustre 37.5% SE	150	8.75 (17.20)	69.11 (46.50)	7.96 (16.38)	75.16 (51.41)	30.38
Flusilazole 40% EC	60	9.63 (18.07)	66.00 (43.79)	10.50 (18.89)	65.94 (43.96)	27.83
Azoxystrobin 23% SC	125	9.75 (18.19)	65.58 (43.42)	10.79 (19.17)	65.00 (43.13)	28.08
Difenoconazole	37.5	11.46 (19.78)	59.54 (38.48)	11.93 (20.18)	61.30 (40.14)	26.58
Myclobutanil	20	12.75 (20.91)	54.99 (34.96)	13.38 (21.43)	56.60 (36.43)	25.92
Untreated control	--	28.33 (32.15)	--	30.83 (33.71)	--	17.50
S Em±		0.18		0.25		0.24
CD at 5%		0.52		0.73		0.70

The figures in the parenthesis are angular transformed values

Figure 2

Table 2 Correlation matrix of powdery mildew and fruit rot severity with weather variable and rate of spread of the disease (Two years pooled)

Parameters	Powdery mildew	Fruit rot	Treatment	Rate of Spread of Powdery mildew and Fruit rot					
				Between 50-60 DAT		Between 60-70 DAT		Between 70-80 DAT	
				Powdery mildew	Fruit rot	Powdery mildew	Fruit rot	Powdery mildew	Fruit rot
Percent disease index(PDI)	1.000	1.000							
T _{max}	-0.495	-0.890	T ₁	0.053	0.053	0.026	0.024	0.019	0.019
T _{min}	-0.898	-0.890	T ₂	0.040	0.052	0.029	0.036	0.022	0.034
RH _{max}	0.888	0.868	T ₃	0.046	0.052	0.030	0.036	0.019	0.030
RH _{min}	0.110	0.113	T ₄	0.046	0.038	0.019	0.032	0.024	0.024
Rainfall (mm)	0.361	0.318	T ₅	0.039	0.040	0.027	0.032	0.021	0.024
Vapour pressure 1	0.199	-0.149	T ₆	0.048	0.034	0.019	0.026	0.016	0.022
Vapour pressure 2	-0.324	-0.340	T ₇	0.044	0.034	0.020	0.027	0.019	0.023
Wind Speed Km/hour (Wv)	-0.921	-0.957	T ₈	0.072	0.050	0.029	0.039	0.013	0.021

Multiregression equation

Powdery mildew: $Y = 321.44 - 1.21(T_{max}) + 2.20(RH_{max}) - 431.50(Wv)$
 Fruit rot: $Y = 272.33 - 2.21(T_{max}) - 1.25(T_{min}) - 1.10(RH_{max}) - 373.13(Wv)$

P FUNGI 20

Mutations in the succinate dehydrogenase gene of *Botrytis cinerea* field isolates and their impact on fungicide sensitivity, cross-resistance behavior and fitness

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Inhibitors of the complex II of the fungal respiratory chain, also known as succinate dehydrogenase inhibitors (SDHI), are of prime importance for crop protection worldwide. This calls for sound resistance research and management, in order to sustain the efficacy of this important chemical class. Recent research on SDHI resistance mechanisms revealed a plethora of different *sdh* mutations in various plant pathogenic fungi, leading to different levels of SDHI insensitivity. *Botrytis cinerea*, the causal agent of grey mold, is not only of high economic importance in fruit and vegetable production, but also serves as a valuable model for research on fungicide resistance and the entailed fitness costs. The objective of the present study was to (i) monitor the SDHI sensitivity status of *Botrytis* populations, (ii) to investigate the molecular mechanisms of resistance, and (iii) to characterize *sdh* mutations with regard to cross-resistance and associated fitness costs. For our extensive monitoring campaigns we used a standard *in vitro* EC₅₀ assay, which has recently been complemented by a pyrosequencing assay for mutational analyses. Between 2012 and 2014, we analyzed a grand total of more than 1200 field isolates from 340 different sites. We identified more than 300 strains resistant to Boscalid of which 40 % displayed resistance to Fluopyram, indicating incomplete cross-resistance. For 90 % of the isolates with aberrant SDHI sensitivity, we detected known mutations in the *sdh* gene; the resistance mechanism of the remaining isolates is currently under investigation. We report furthermore about initiated studies to evaluate the impact of the identified mutations on the fitness of these field isolates.

P FUNGI 21

On the track of Fusarium head blight - a phylogenetic approach

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Fusarium head blight (FHB, "scab") is a devastating disease on wheat, barley and other grains. It is mainly caused by five major *Fusarium* species: *Fusarium graminearum*, *Fusarium culmorum*, *Fusarium avenaceum*, *Fusarium poae* and *Microdochium nivale*. Often associated with these ascomycetes are other, less pathogenic or opportunistic fungi like *Fusarium equiseti*, *Fusarium sporotrichoides* or *Fusarium tricinctum*. The disease causes high economic losses due to yield reduction, damaged kernels and mycotoxins burden in grains.

This study was conducted to estimate the evolutionary history of Fusarium head blight over two years in order to give insights into the evolutionary potential of the disease.

First, the main causal agents of FHB collected in France, Germany and Switzerland in 2012 and 2013 were identified with real-time PCR based on several locations with total of many isolates. Afterwards the phylogenetic relationship between the identified *Fusarium* species was analysed based on several marker genes. Additional investigations of amino acid mutations in the protein sequence of the candidate genes for succinate-dehydrogenase (SDH) and 14- α -demethylase (CYP51C) has been performed to reveal indications for sensitivity differences and selectivity to the fungicides Sedaxane (SDHI) and Prothioconazole (DMI), respectively.

The phylogenetic analysis of five candidate genes (CYP51C, SDHB, SDHC, SDHD and β -tubulin) revealed clear and well-structured separation of the *Fusarium* isolates according to their species affiliation, their mycotoxin production and *Fusarium* section. Furthermore the identified *Fusarium* species clustered independently from their locations indicating a strong species affinity over a broad geographical area. In general, this study provides insight into genetically different species causing Fusarium head blight and suggests a high evolutionary potential of the disease.

P FUNGI 22

Characterization of Emerging and Fungicide-Resistant Fungal Plant Pathogens Causing Postharvest Apple Decay Using Conventional and Molecular Methods

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The United States is the second largest apple producer in the world behind China. In 2012, twenty-five percent of the US apple crop was exported to Canada, Mexico, and Taiwan with an approximate value of \$1.1 billion dollars (USD). Apples are stored for extended periods of time (from six months to one year in controlled atmosphere cold storage) to provide high quality fruit year-round to consumers. During storage, the most common fungal plant pathogens in the US, *Penicillium expansum* and *Botrytis cinerea*, cause decay and are primarily controlled using postharvest fungicides. However, it is unclear if additional pathogens are causing decay during storage and if resistance has developed to various fungicides in *P. expansum* and *B. cinerea*. The objectives of this study were to identify fungi causing postharvest decay on apples and to determine tolerance to postharvest fungicides in *P. expansum* and *B. cinerea*. Decayed apples were collected from farm stands, packinghouses, and storage rooms in Pennsylvania and Maryland from 2010 to 2013. The causal fungi were isolated, identified, and characterized using morphological and molecular methods. Sensitivity of the isolates to pyrimethanil (the active ingredient [a.i.] in Penbotec™) was determined *in vitro* and *in vivo* for *P. expansum* and *B. cinerea*. Five fungal plant pathogens (*Alternaria alternata*, *Alternaria tenuissima*, *Colletotrichum fiorinae*, *Fusarium avenaceum*, and *Mucor piriformis*) were identified which have not been previously reported to cause postharvest decay on apple in the Mid-Atlantic area. Pyrimethanil-resistant *Penicillium expansum* and *Botrytis cinerea* isolates were detected which grew above discriminatory doses *in vitro* and caused decay on Penbotec™-treated apple fruit. Data from this study revealed that several newly characterized fungal plant pathogens may impact future management practices and pose potential barriers for the apple trade. Resistance to pyrimethanil in *P. expansum* and *B. cinerea* emphasizes the importance of rotating chemicals possessing different modes of action to ensure adequate control and illuminates the need for development of additional control strategies.

P FUNGI 23

Incidence and molecular characterization of fenhexamid-resistant isolates of *Botrytis cinerea* from strawberry and greenhouse grown tomatoes in Greece

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Introduction: *Botrytis cinerea* Pers., the causal agent of gray mold, is a high risk pathogen for fungicide resistance development. In the past resistance was developed to several botryticides, including fenhexamid, an inhibitor of 3-ketoreductase (*Erg27*) in the ergosterol biosynthesis pathway.

Objectives: The study was conducted to: i) determine the frequency of resistance to fenhexamid in isolates obtained from strawberry and tomato, ii) detect mutations in *Erg27* and iii) investigate the effect of the mutations to the level of resistance.

Materials and methods: The isolates were obtained from rotten strawberry (n=213) or tomato (n=200) fruits and were characterized as fenhexamid-resistant or -sensitive using the discriminatory dose of 3 µg ml⁻¹. The *erg27* gene of all the resistant isolates was amplified and sequenced to detect point mutations. Then, isolates were selected on the basis of their genotype and the sensitivity to fenhexamid was measured in terms of EC₅₀ values using a colony growth test at different fenhexamid concentrations ranging from 0.03 to 30 µg ml⁻¹.

Results: A high frequency (30%) of fenhexamid resistance was observed in strawberry isolates, while resistance to fenhexamid was very low (2%) in the tomato isolates. *Erg27* sequencing revealed the presence of 14 different point mutations (F412S, F412I, F412C, ΔP298, T63I, K59R, P250F, W400L, H72D, M218T, E363D, E328G, L438F, M165L). Six of them are reported for first time worldwide (K59R, M218T, P250F, E263D, W400L, L438F), while 4 (ΔP298, T63I, F412I, F412C) are reported for first time in Greece. F412S was found to be the predominant mutation. Measurements of sensitivity to fenhexamid revealed that M165L and F412I conferred the higher levels of resistance, while ΔP298 was associated with the lower levels of resistance.

Conclusions: The results showed that resistance to fenhexamid is widespread in strawberry, but not yet in tomato fields. The increasing frequencies of fenhexamid resistance in strawberry fields and the detection of resistant isolates in tomato greenhouses necessitate continuous monitoring and implementation of antiresistance strategies.

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Antifungal activity of some fungicides to control apple scab (*Venturia inaequalis*) with different pH *in vitro* and field trials

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This study was carried out to determine the effectiveness of 5 fungicides, Cyprodinil, Propineb, Pyraclostrobin+Boscalid, Dodine, Floupyram+Tebuconazole, solved in different pH water against apple scab *in vitro* and *in vivo* between the years 2013-2014 in Eğirdir, Isparta, Turkey. For *in vitro* experiment, fungicides were added in PDA medium at 15, 20 and 25 °C temperatures and 6, 7 and 8 pH values combinations. The colony diameters were measured at the end of experiment for the effects on mycelium development of pathogen.

Field trials were performed using 2 year-old Scarlet Spur apple variety in 2 different fields in Eğirdir, Isparta, Turkey. Fungicides were applied at the recommended dose according to an early warning system times; evaluation of disease severity were performed between April- May 2013-2014. Fungicides were applied at 3 different pH values (6-7-8). Disease severity (%) was calculated according to untreated control plots.

All fungicides inhibited of mycelial growth at 3 different temperatures and 3 different pH values (0.0±0.0) compared with untreated control (P<0.05) *in vitro* experiments. There was no statistically differences between temperatures and pH values combinations (P<0.05).

The most effective fungicide was found Floupyram+Tebuconazole and diseases severity was ranged 20.1 to 31.6 % in experiment 1 (P<0.05). In the other hand, Dodine was the most effective fungicide with 25.3 - 39,8 % diseases severity in experiment 2 (P<0.05). Different pH values did not affect fungicide efficiency for controlling of apple scap in both field trials (p<0.05).

P FUNGI 25

Efficacy of different fungicide application methods to control of *Ascochyta* blight (*Ascochyta rabiei*) in chickpea

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Chickpea (*Cicer arietinum* L.) is an annual grain legume that is used extensively for human consumption and also, it is widely cultivated in Turkey. *Ascochyta* blight caused by *Ascochyta rabiei* (Pass.) is most important disease of chickpea that affects the quantitative and qualitative chickpea yield in Turkey. The fungus can infect all above ground parts of the plant and yield losses increase due to this disease in cool, cloudy and humid weather conditions during the crop season. Fungicides management is essential to control this disease. In this study, three fungicide, Mancozeb, Thiram, Pyraclostrobin + Boscalid, were tested against *Ascochyta rabiei* at different applications including seed-treatment, seed-treatment + foliar spray and foliar spray. This study was conducted on resistant chickpea variety, Koçbaşı and susceptible Sarı 98 under control conditions. Experiments were evaluated after three weeks. Disease severity (%) was calculated according to control plants.

Pyraclostrobin + Boscalid and mancozeb showed significant reduction in disease severity at resistant Koçbaşı and susceptible Sarı 98 varieties in all applications (P<0.05). However, thiram was less effective in disease severity with 44-69 % (P<0.05). Seed-treatment + foliar spray combination was the highest effect against disease on chickpea varieties Kocbaşı (22%) and Sarı 98 (12%) (P<0.05). Seed treatment had higher effect than foliar spray to control disease (P<0.05). The lowest efficiency was determined in foliar spray application. The comparison of means showed that application of fungicide was a suitable strategy for reduction of *ascochyta* blight severity.

P FUNGI 26

***In vitro* effect of five molecules pyridazine *Phytophthora parasitica*, *Phytophthora citrophthora* and *cholletotrichum gleosporioides* isolated citrus**

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The most important fungal diseases of the Moroccan citrus orchard are those that are caused by *Phytophthora spp.* The objective of this study was to evaluate the *in vitro* effect of five Molecules Pyridazine based on mycelial growth and spore production of two species of *Phytophthora spp* (*P.parasitica* and *P.citrophthora*) and a species *cholletotrichum gleosporioides*

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from citrus fruit grown in the Gharh region of Morocco. In addition, this study was complemented by an *in vitro* test of the effect of the five molecules on the development of *phytophthora spp* on branches of a sensitive rootstock. The results showed that both *Phytophthora* species tested are sensitive to different molecules used and in varying degrees; while the species of *C. gloeosporioides* require much higher doses of the five molecules for inhibition of mycelial growth. It seems that the tested molecules inhibit the two stages of the life cycle of *Phytophthora* isolates tested at moderate concentrations. By cons, they act more on the production of *C.gloeosporioides* spores than on their mycelial growth due to the strong IC90 values.

P FUNGI 27

***Cercospora Beticola* Sensitivity to Strobilurins and Triazoles in Italy**

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Introduction: Commercial mixtures of difenoconazole, fenpropidin, tetraconazole, trifloxystrobin and azoxystrobin are the most frequent fungicides used in Italy to control cercospora leaf spot (CLS), caused by the fungus *Cercospora beticola*. The resistance development risk to these fungicides is moderate for triazoles and piperidines, whereas the risk is high for Qols (Brent and Holloman, 2007). Therefore, this study was aimed to evaluate the sensitivity of *C. beticola* isolates collected from Northern Italy to difenoconazole and strobilurin fungicides.

Material and methods: Total 228 isolates were collected from trial plots, commercial fields and one garden in September 2012 and 2013 for fungicide sensitivity studies. Conidial germination tests were examined using trifloxystrobin as technical grade (Sigma) to achieve 0.01, 0.1, 1, 2 mg/L and commercial formulation of 100 mg/L (Flint, 50%, Bayer). Technical grade of difenoconazole (Sigma) (0, 0.01, 0.1, 1, 10 mg/L) was tested for mycelial growth inhibition. Mean EC₅₀ values were calculated by probit analysis and MIC values were determined. Mean percentage of relative germination (RG%) at 2 mg/L and 100 mg/L were calculated per each isolate for trifloxystrobin.

Results: Experimental and commercial field samples showed lower sensitivity to trifloxystrobin (EC₅₀ >2 mg/L, RG > 78% at 2 mg/L and >64% at 100 mg/L) than that obtained on the samples collected in garden (EC₅₀ 0.17 mg/L, RG 10.48% and 2.12% at 2 mg/L and 100 mg/L, respectively). Decrease in sensitivity was observed comparing to the baseline study (Cioni *et al.*, 2013) carried out in Northern Italy by our group in 2009 and in USA (Secor *et al.*, 2010). EC₅₀ values for difenoconazole were from 0.83 to 3.04 mg/L for experimental and commercial field samples and EC₅₀ 0.13 mg/L for isolates sampled from the garden. MIC of all isolates were found >10 mg/L. The data obtained for difenoconazole presented lower sensitivity in experimental and commercial field samples according to the studies in USA and Greece (Secor *et al.*, 2010, Karaoglanidis and Bardas, 2006).

Conclusion: Sensitivity tests indicated that Qol resistance had developed in all *Cercospora beticola* populations. As for difenoconazole, some cases of decreased sensitivity of the pathogen were observed. This decreased sensitivity had lower intensity with respect to strobilurins.

P FUNGI 28

Role of single site-specific Allele replacement into SVHK1 Locus in the study of Stemphylium Vesicarium Dicarboximide and Phenylpyrrole Fungicides Resistance

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Introduction: *Stemphylium vesicarium* is the fungal agent of pear Brown Spot and its resistance to dicarboximide fungicides has been a known concerning phenomenon since the 1990s. Henceforward, pear orchards have been monitored and field strains have been tested by mycelial growth inhibition assays to understand the sensitivity to dicarboximide and phenylpyrrole fungicides. Four phenotype classes were recognized according to *in vitro* responses to procymidone and iprodione: S (sensitive), S+ (low resistance), R1 (moderate resistance), R2 (high resistance). Cross-resistance to fludioxonil was only detected in R2 phenotype. Previous molecular studies correlated dicarboximide resistance class with single aminoacid substitutions observed in a two-component histidine kinase (HK1), corresponding to single nucleotide polymorphism (SNPs) in the nucleotidic sequence of *SvHK1* gene (Alberoni *et al.*, 2010). The goal of this ongoing study is to define the role of known SNPs in *SvHK1* sequence on dicarboximide resistance by the replacement of the S allele with S+, R1 or R2 alleles.

Material and methods, results: A reference sensitive strain was selected through biological and molecular assays and DNA was properly extracted. Fusion PCR technique was used to build the linear disruption vector (KOSvHK1). Fungal protoplast were obtained by enzymatic lysis of cell wall and transformed. KOSvHK1 replacement of *SvHK1* gene produced null mutants which were able to grow up on Hygromycin B. Transformants will be screened for unique and site-specific insertion of KOSvHK1 using

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PCR and Southern Blotting assays. Interesting mutants will be transformed with linear complementation vectors (S+SvHK1 or R1SvHK1 or R2SvHK1) and complemented strains will be tested for the expected acquired resistance level.

Discussion: Assessment of the role of SNP mutations in *SvHK1* sequence in *S. vesicarium* resistant phenotypes to dicarboximides will make available to use a RealTime PCR assay to quickly determine resistant allele-frequency in monitored populations. The final results will be able to increase the possibility of quantify, prevent and manage the iprodione and fludioxonil resistance risk in practice.

P FUNGI 29

G143A Detection in *Venturia Inaequalis* in Turkish Apple Orchards

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Introduction: Strobilurins have been used in Turkey since 1998 and unfortunately, in the late 2000's control failures were observed in apple orchards due to intensive treatment. The G143A substitution in the inhibitor binding site of cytochrome *b* of *Venturia inaequalis* confers a high level of resistance to strobilurins. This study was undertaken to evaluate strobilurin resistance by *in vitro* assays and through molecular assay for detecting the A143 allele position in Turkish *V. inaequalis* field populations.

Material and methods: The study was carried out on ten bulk populations each obtained from 40-50 scabbed leaves collected in western and southern Turkish orchards in 2011. Samples were originated from different scab management (wild type, untreated and poor control by strobilurins). Conidial germination tests were examined using trifloxystrobin as active material (Sigma) to achieve 0, 0.001, 0.01, 0.1, 2 mg/L. Mean EC₅₀ values were calculated by probit analysis. CAPS (Cleaved Amplified Polymorphic Sequence) PCR analysis with two specific primers ANK 10 and ANK 283 which amplify a 413 bp fragment of *V. inaequalis* cytochrome *b* was carried out to evaluate the presence of G143A substitution.

Results: Samples collected from untreated orchards showed low mean EC₅₀ values ranging from 0.00001 to 0.04 mg/L and absence of G143A substitution, including a wild type population. They can be considered sensitive to strobilurins according to the baseline study by Kung Farber *et al.*, 2002. Using CAPS-PCR method, the G143A substitution was found in five field samples originating from Turkish orchards in which apple scab proved difficult to be controlled by strobilurins and they presented very low sensitivity to trifloxystrobin (mean EC₅₀ ranging from 1.46 to >2 mg/L). Therefore, they can be considered resistant (Kung Farber *et al.*, 2002).

Conclusion: The results lead us to conclude that in some Turkish apple growing areas *V. inaequalis* populations resistant to strobilurins are present. The PCR-based method in this study efficiently reveals the presence of the G143A substitution in Turkish *V. inaequalis* field populations. The quantification of mutated alleles through real-time PCR analysis has been in progress.

P FUNGI 30

Transposon mediated DMIs resistance in *Penicillium digitatum*

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Penicillium digitatum, causing green mold, is the most destructive postharvest pathogen of citrus worldwide. The phenotype and genotype of 383 isolates of *P. digitatum*, collected from Zhejiang, China, during 2000 to 2010, were characterized in terms of their imazalil sensitivity. The frequency of IMZ-R isolates increased from 2.1% in 2000 to 84% in 2010. Only 6.5% and 4.5% of the IMZ-R isolates belong to the IMZ-R1 and IMZ-R2 previously described, respectively. To determine the resistance mechanism of the predominant and unknown IMZ-R isolates of *P. digitatum* (termed as IMZ-R3), *PdCYP51B*, homologous to the *PdCYP51A* gene, was cloned from IMZ-R3 and IMZ-S isolates. Sequence alignments revealed that a unique 199bp insertion was present in the promoter region of *PdCYP51B* in all IMZ-R3 isolates examined, but was absent in all tested IMZ-S isolates as well as in IMZ-R1 and IMZ-R2 isolates. Introduction of another copy of *PdCYP51B*^S (from IMZ-S) into a IMZ-S isolate did increase the resistance of *P. digitatum* to DMIs; whereas introduction of a copy of *PdCYP51B*^R (from IMZ-R3) dramatically increased the resistance level of *P. digitatum* to DMIs. Further studies indicated that this 199 bp element was a MITE-like element, designated as PdMLE1. BLAST searching and southern blot showed that PdMLE was unique to *P. digitatum*. *P. digitatum* mutant harboring the PdMLE1 fused GFP gene showed the strong green fluorescence, indicating the powerful promoter activity of PdMLE1. By promoter deletion, we identified a 20 bp core sequence in PdMLE1 which was associated with its promoter activity. Thus, we proposed a model that PdMLE1 acted as a powerful promoter and most likely recruited the transcription factor(s), therefore led to the overexpression of *PdCYP51B* gene and conferred *P. digitatum* with DMI resistance. This is the first regulation model of transposon resulted fungicide resistance proved in plant pathogens.

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P FUNGI 31

Developing a new biopesticide Shenqinmycin using the secondary metabolites phenazine-1-carboxylic acids from the PGPR *Pseudomonas* strain by genetic and metabolic engineering

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The development of green pesticides with high efficiency and low toxicity has aroused wide public interest in recent years. Phenazine-1-carboxylic acid (PCA) produced by pseudomonads has proven effectively against a range of soil-borne fungal phytopathogens and has great potential for development as a new kind of fungicide. The melon rhizosphere-originating *Pseudomonas* sp. M18 produces two different antibiotics, PCA and pyoluteorin. During the last decade, several rounds of genetic modifications, including inactivation of the quorum sensing repressor QscR and the global regulator GacA, and increasing the copy number of PCA biosynthesis cluster, have been conducted and PCA yield in the engineered strain has been significantly increased. Through optimizing the culture medium components, PCA yield was achieved as high as 4,000 mg/L, which is economically applicable for large-scale commercial purposes. PCA has been registered as "Shenqinmycin" in China. The control effects of Shenqinmycin on rice sheath blight disease have been tested in field during 2008-2009 in 13 provinces of China. In 2011, 1% Shenqinmycin suspension was officially approved as a new biopesticide to protect rice and vegetables against diseases caused by *Rhizoctonia solani*, *Fusarium oxysporum* etc. Sales in China have reached over 2.2 million US dollar during the last 2 years. Recently we have sequenced the genome of M18 and we found that seven genomic islands and six biocontrol-related gene clusters probably contribute to its biocontrol activities and living abilities in rhizosphere niches. We are currently investigating the mutual relationship of two PCA biosynthesis cluster, how quorum sensing mechanism controls PCA production, and the global regulatory network of PCA biosynthesis. Our latest results will be presented.

P FUNGI 32

Polymorphisms in fungicide resistance genes of *Venturia inaequalis* from a sanitation trial orchard

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Venturia inaequalis causes large crop losses in South Africa and is currently managed through fungicide application only. Fungicide resistance buildup has been shown to occur in the pathogen populations and it has been hypothesised that this trend can be reverted through primary inoculum reduction.

Therefore, the aims of the study were to (i) isolate *V. inaequalis* from a field trial using orchard sanitation and (ii) analyse three candidate fungicide resistance gene loci for occurring polymorphisms.

Venturia inaequalis samples from the Ceres and Grabouw regions were isolated from single spores and tested for fungicide sensitivity against two classes of fungicides (anilinopyrimidine cyprodinil, and demethylation inhibitor flusilazole) by determining the EC₅₀ using fungicide amended agar plate tests. DNA was extracted from all *V. inaequalis* isolates and PCR-RFLP analyses as well as sequencing was conducted for nucleotide binding site 2 of the ABC2 transporter gene (ABC2) and cystathione gamma ligase and cystathione gamma synthase regions from 40 resistant and 40 sensitive isolates per fungicide.

Most of the isolates (80%) found to be sensitive to flusilazole, whereas only 33% of isolates were sensitive to cyprodinil. Multisite resistance was found in 17% of isolates. Non-synonymous polymorphisms with potential functional consequences were found in the ABC2 gene and the CGL gene, where two main haplotype groups were identified.

Certain CGL haplotypes were predominant in the fungicide exposed population, thus could have been selected for due to resistant phenotypes.

P FUNGI 33

Screening of potato varieties and evaluation of fungicides against late blight of potato, *Phytophthora infestans*

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Late blight of potato caused by *Phytophthora infestans* (Mont.) de Bary, is one of the most important diseases in all potato (*Solanum tuberosum* L.) growing areas of Pakistan. Field experiments were conducted to screen out thirty potato varieties and to evaluate the efficacy of three fungicides namely, Tazoline (mancozeb + metalaxy), Kocide (copper oxychloride) and Ridomil Gold (mefenoxam) on three most susceptible potato varieties against late blight. Isolation, identification, pathogenicity test, fungicidal evaluation and yield data were used to evaluate the efficacy of late blight disease on potato. Most of the potato

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varieties showed susceptibility to *P. infestans*. Fourteen varieties were moderately resistant, eight were moderately susceptible, five were susceptible and three were highly susceptible. Very low disease incidence (Disease rating scale, DRS) was recorded in varieties Desire and SH-297. Ridomil Gold (4.4) showed the best result followed by Tazoline (6.0) and Kocide (7.3) as compared to control (8.2). Variety SH-479 was the best one having mean disease severity (6.0) followed by FD-76-35 with mean disease severity (6.2). Variety SH-479 gave higher potato yield (217.7g) per plant while SH-332 gave 194.3g_yield per plant as compared to control treatment (without fungicidal spray) which gave 124.7g_yield per potato plant. It is recommended that potato varieties Desire and SH-297 are more promising one and fungicide Ridomil Gold is the best way to control late blight of potato.

Figure 1

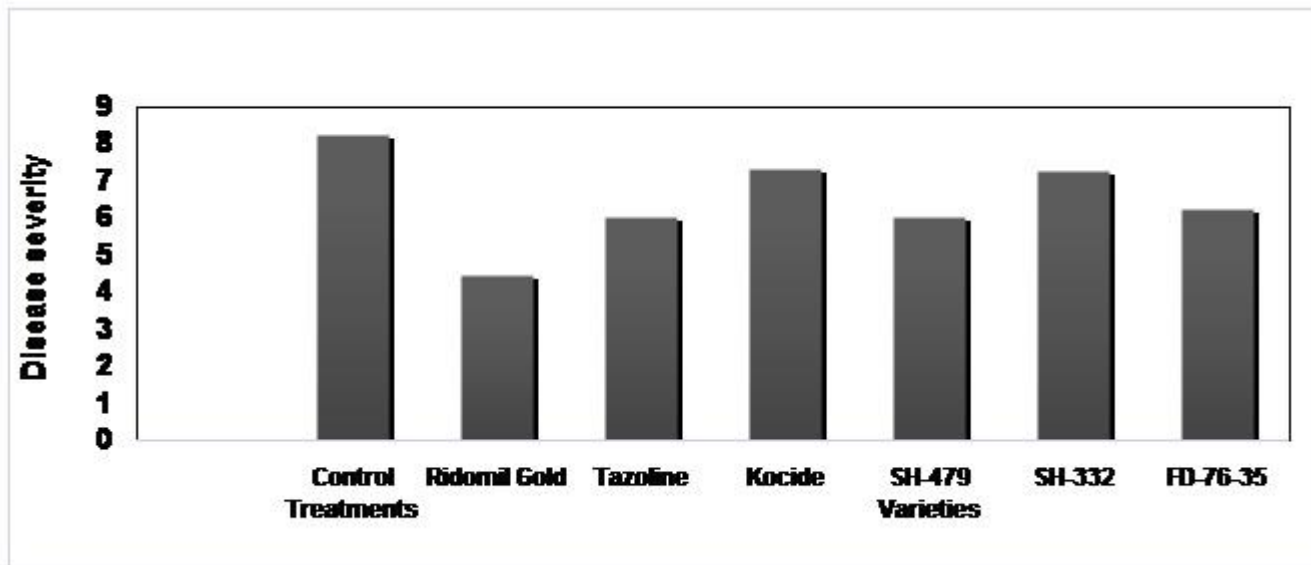
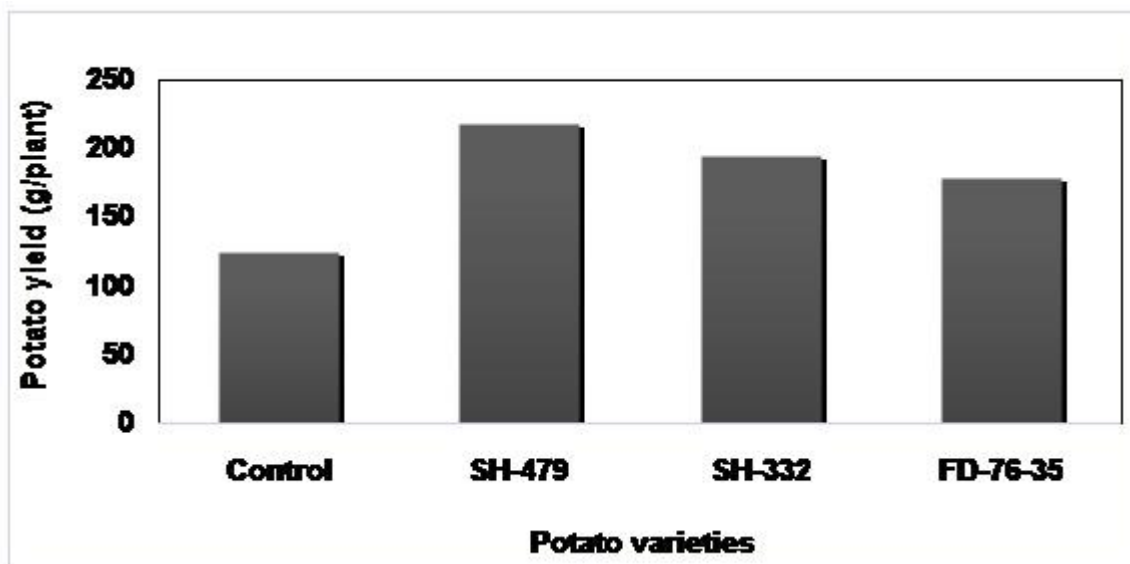


Figure 2



P FUNGI 34

New findings about the sensitivity of *Plasmopara Ticola* to CAA fungicides

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Introduction: Carboxylic Acid Amides (CAA) are widely used to control grapevine downy mildew (*Plasmopara viticola*) worldwide. Dimethomorph, the first CAA fungicide (Albert *et al.*, 1988), has been authorized in Italy since 1994. Other CAA

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compounds as iprovalicarb, bentiavalicarb, valifenalate have been in use since the early 2000s, while mandipropamid was introduced in 2009. In 2010 the single point mutation leading to an amino acid exchange from glycine to serine at codon 1105 (G1105S) in the Cesa3 protein was discovered, conferring CAA resistance in *P. viticola* (Blum *et al*, 2010). During the last years, resistance has increased gradually in Europe although field performances has been good (FRAC). A wide monitoring work has been carrying out to evaluate the sensitivity of Italian *P. viticola* populations to CAA fungicides. In this text some results obtained in biological assays conducted towards mandipropamid and dimethomorph will be reported.

Material and methods: Twenty-five samples were collected in Northern Italy vineyards during 2013-2014 years. Bioassays were carried out on leaf discs and seedlings (grown in greenhouse) applying 5 concentrations (from 3 to 300 mg/L a.i.) of mandipropamid (Pergado SC) and dimethomorph (Forum 50 WP) 24 hours before inoculation. For each concentration a total of 15 leaf discs (22 mm ϕ) were soaked in the fungicide suspensions. Dried leaf discs were then transferred to survival water agar medium (1.5%) in Petri dishes in triplicates. Seven samples out of 25 were processed for bioassays on seedlings (3/concentration) sprayed till run off. The inoculations were done by spraying a sporangial suspensions (5×10^4 spores/ml) onto the adaxial face of each leaf disc or of the 2-3 leaves per seedling. The sporulation was assessed 8-10 days after the treatment evaluating the sporulated leaf surface. The EC₅₀ values (mg/l) were calculated by probits analysis.

Results: Data obtained from both bioassays were consistent. Thirty-two percent of samples were considered sensitive with an EC₅₀ <1 mg/l for both fungicides. The remainder samples showed an EC₅₀ >300 mg/l for mandipropamid while dimetomorph had EC₅₀ ranging from 1.6 to 22.54 mg/l.

Conclusion: The results obtained from bioassays seem to demonstrate the presence in Italy of *P. viticola* strains with different sensitivity to dimethomorph and mandipropamid. We have been studying this phenomenon also by other experimental approaches (molecular analysis and field trials) to better define the possible different resistant mechanism.

P FUNGI 35

Influence of selected preparations on *in vitro* growth of *Fusarium* spp.

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The aim of the study was to compare the fungistatic impact of fungicides containing various biologically active substances with the effect of preparations based on natural active substances and bacteria strains on the colony growth of 10 isolates of *Fusarium* spp. in laboratory conditions. Isolates originating from Polish herbs and medicinal plants were selected. They came from a Bank of Plant Pathogens Institute of Plant Protection - National Research Institute in Poznan.

In the experiments, three fungicides (containing various biologically active substances), and three different preparations based on natural active substances: Biosept 33 SL (extract of grapefruit), Biochikol 020 PC (chitosan) and Polyversum WP (oospores of *Pytium oligandrum*) were used. Evaluation of fungistatic activity of preparations were carried out on Petri dishes with Potato Dextrose Agar medium (PDA). Tested preparations were added to sterile medium at active substances concentrations of 1, 10, 100 and 1000 ppm. The plates were incubated at 24°C. Measurement of cultures growth were taken for 14 days along two perpendicular lines. The results were compared with the control growing on a PDA medium without the addition of formulations. Each experimental combination included 4 replicates.

During the experiment different degrees of growth inhibition of *Fusarium* spp. colonies were observed. The sensibility of the fungi was dependent on the type of formulation and on the dose. Laboratory tests have confirmed the possibility of the use of biological formulations for plant protection against *Fusarium* spp. It is very important in herbs and medicinal plants cultivation.

P FUNGI 36

Efficacy of Fungicides against Downy Mildew of Basil (*Peronospora Belbahrii*) in Italy

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Introduction: Sweet basil (*Ocimum basilicum*) is in Italy an economically important herb crop cultivated both in greenhouse and in field for fresh and mainly processed (pesto sauce) consumption. Downy mildew (*Peronospora belbahrii*) (Belbahri *et al*, 2005; Thines *et al.*, 2009) is the major threat of this crop and it needs many fungicide applications. Over many years, metalaxyl-M was the most utilized active ingredient (a.i.) even if during the last years efficacy reductions were pointed out probably due to pathogen resistance issues (own data not yet published). Mandipropamid, azoxystrobin and fluopicolide+propamocarb a.i.s are also authorized but, because of the risk of resistance development, the availability of other products, possibly with different

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MoA, should be useful. The aim of this study was to evaluate the activity against *P. belbahrii* of some fungicides authorized in Italy on other herb crops but not on basil as dimethomorph, fosetyl-Al, amisulbrom and cyazofamid.

Materials and methods: The study was carried out under greenhouse and in field. Twenty-four hours after the spraying with the above mentioned fungicides, potted plants grown in greenhouse were inoculated with a sporangia suspension (10^5 /ml). The field trial was conducted in late summer 2014 testing fosetyl Al and amisulbrom in randomized blocks design with four repetitions. Four treatments were applied with intervals of 6 days by a Carpi backpack hand-sprayer. The assessments were made as percentage of sporulated leaf surface area in both experimental trials: at the end of an appropriate incubation period in greenhouse, after the appearance of natural infections on untreated plants in field. Data were processed by analysis of the variance and mean values compared by the Duncan test ($p=0.05$).

Results: Greenhouse and field results were consistent, showing a high efficacy degree (from 75 to 100%) for all tested products, especially considering the elevated infection degree (from 75 to 90%) observed on untreated plants.

Conclusions: The results showed that dimethomorph (Forum 50WP, 50 g/100 l), fosetyl-Al (Aliette, 300 g), amisulbrom (Leimay, 60 ml) and cyazofamid (Ranman Top, 50 ml) should be successfully used against *P. belbahrii* on basil also in order to reduce the risk of appearance of less sensitive strains to chemicals currently available.

P FUNGI 37

Copper sensitivity of Italian *Pseudomonas syringae* pv *actinidiae* strains

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Introduction: *Pseudomonas syringae* pv. *actinidiae* (*Psa*) is the agent of the bacterial canker of green and yellow-fleshed kiwifruit (respectively *Actinidia deliciosa* and *A. chinensis*). It is the cause of severe economic losses in Japan (Takikawa *et al.*, 1989), South Korea (Koh *et al.*, 1994) and Iran (Mazarei and Mostofipour, 1994). This pathogen was reported on *A. deliciosa* in Italy for the first time in 1994 (Scortichini, 1994) without yield losses up to 2008 when the disease infected most part of orchards located in Latium region. *Psa* is currently a worldwide pandemic disease, threatening the kiwifruit producing countries, particularly Italy, France, New Zealand and Chile. The current chemical control of *Psa* in the field is reliant on spraying of copper-based compounds (Koh *et al.*, 1996; Nakajima *et al.*, 2002; Vanneste *et al.*, 2011). Unfortunately, copper may lead many bacteria to develop different strategies to overcome its toxicity (Nies, 1999). This study was undertaken to establish the copper sensitivity of many strains of *Psa* coming from North of Italy isolated during 2009-2013 years.

Materials and methods: A total of 53 strains (24 collected during 2009-2011, 29 in 2012-2013) were evaluated for copper sensitivity by their ability to grow on media supplemented with copper sulphate (0-0.64-1.2-2.4-4.8 mM of Cu^{++}). The medium Ceria 132 (Vanneste *et al.* 1992) was chosen because of its limited copper binding capacity. Bacterial suspensions containing 1×10^3 CFU/ml of *Psa* were spotted on plates which were then incubated at 24°C for two days. The assessment was made as Minimal Inhibitory Concentration (MIC).

Results: The fifty-four percent of strains isolated from 2009 to 2011 (namely before the widespread and massive use of copper in field) showed 1.2 < MIC values < 2.4 mM of Cu^{++} while the remainder had a MIC < 1.2 mM. The percentage of strains with 1.2 < MIC values < 2.4 mM of Cu^{++} increased up to 82.7% on isolates collected during 2012-13.

Conclusion: The *Psa* isolates sampled in northern Italy during 2009-2013 years showed a normal sensitivity to copper ion according to other studies (Nakajima *et al.*, 2001). Nevertheless, we have to consider that the number of the strains with 1.2 < MIC values < 2.4 mM of Cu^{++} has increased during the last years (2.4 mM of Cu^{++} corresponds to half of the minimum dose currently used in field during the vegetation period).

P FUNGI 38

Antifungal activity of essential oil from *Ammodaucus leucotrichus* Coss. & Dur. growing wild in South West of Algeria on the fungic growth

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Objectives: This work studies the antifungal capacity of the essential oil of spontaneous aromatic plant with vocation medicinal used in the traditional treatments in the South-West of Algeria: *Ammodaucus leucotrichus* Coss Dur.

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Materials and methods: The essential oil was isolated from seeds of plant material by hydrodistillation, for 4h, using a Clevenger-type apparatus. The physico-chemical analysis [1] of the essential oil of this plant specie has enables to us to even characterize to identify our oil. Antifungal activity of the essential oil was studied witch respect to seven fungal strains with various concentrations.

Results: The local plant tested gives a good essential oil yield (1.55%). The results of direct contact method show that the oil of *Ammodaucus leucotrichus* Coss Dur is proven very effective on the mycelial growth of the moulds. All strains were inhibited at concentration as weak as 1/1000 (V/V). *Fusarium oxysporum f.sp.albedinis*, *Alternaria*, *Cladosporium* and *Pénicillium expansum* were most sensitive, being inhibited as from 1/5000 (V/V).

The evaluation of fungal biomass on liquid medium [2] of the seven fungal strains, showed a clear reduction in the biomass formed until a total inhibition showed. Majority of strains were inhibited at concentration as weak as 1/150 (V/V). Whereas *Cladosporium* and *Alternaria* were most sensitive, being inhibited as from 1/5000 (V/V).

Conclusions: This essential oil has a fungistatic effect.

References:

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P HERBI 1

Changes in photosynthetic efficiency and color in Alfalfa by Mesosulfuron Methyl + Iodosulfuron Methyl

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Introduction: In Mexico, specifically at Mexicali Valley and San Luis Río Colorado, 29,545 ha of alfalfa are sown, who often accidentally sprayed with mesosulfuron methyl + iodosulfuron methyl herbicide for weed control in wheat, leaving without production and commercial value of this forage to the producer. It's known this herbicide inhibits ALS (Acetolactate synthase, E.C. 2.2.1.6) and therefore not valine, leucine and isoleucine amino acids are synthesized by the plant, but the effects in photosynthetic efficiency and color by phytotoxicity in alfalfa are unknown.

Objective: Evaluate changes in photosynthetic efficiency, color and greenness index in alfalfa sprayed with three doses of mesosulfuron methyl + iodosulfuron methyl.

Materials and methods: Three doses of herbicide Sigma Forte[®] (6,25 g + 1,25 g ha⁻¹; 12,5 g + 2,5 g ha⁻¹; 25 g + 5 g ha⁻¹ of active ingredient; mesosulfuron methyl and iodosulfuron methyl, respectively) and control (water) were applied on alfalfa field with a motorized sprayer. Before herbicide doses application and after that, was performed *in situ* sampling every third day to evaluate photosynthetic efficiency (Fv/Fm; Junior Pam, Walz), color (°Hue; X-Rite SP60) and greenness index (Spad units; SPAD 502, Minolta) for 15 days. Random completely design was performed, one way ANOVA and Tukey test (p=0.05) by Statistix 8.0 was run.

Results: In general, a decrease in photosynthetic efficiency (Figure 1), color and greenness index were observed in response to herbicide doses (Figure 2), from the third day after the application of herbicide. These effects were more severe at 25 g + 5 g ha⁻¹ dose.

Conclusion: The herbicide applied doses affect negatively photosynthetic efficiency, color and greenness index in alfalfa although the action mode inhibits ALS enzymatic activity.

Figure 1. Photosynthetic efficiency behavior in alfalfa by mesosulfuron methyl + iodosulfuron methyl doses.

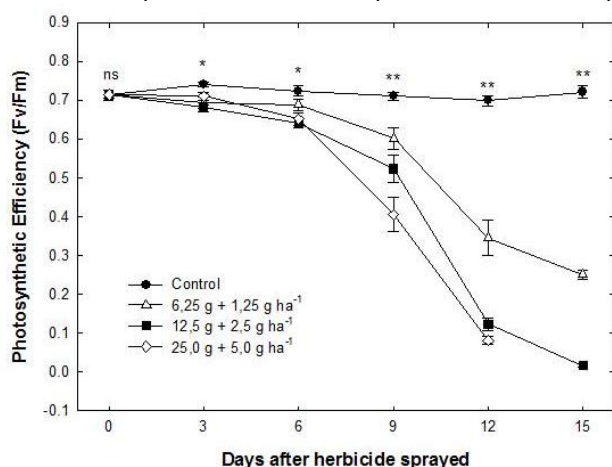
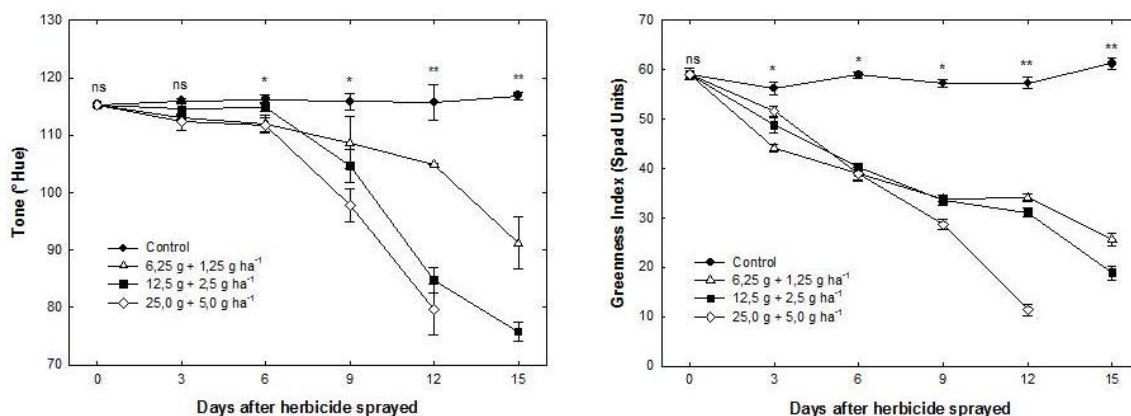


Figure 2. Color and greenness index changes in alfalfa by mesosulfuron methyl + iodosulfuron methyl doses.



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Mechanism of *Ammannia arenaria* resistance to Bensulfuron-Methyl

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Ammannia spp. are popular harmful weeds in paddy rice field worldwide. *A. arenaria* has been becoming one of the most harmful weeds, and its occurrence area was expanding rapidly in China in recent years. The efficacy of mainly used herbicide bensulfuron-methyl (BSM) to the weed was very poor, and it was confirmed that the biotype AH014 (from GuangDe, Anhui) of the weed was resistant to BSM with resistance index of 8.0, but the mechanism of the resistance kept unknown. The object is to clarify the reason of *A. arenaria* resistance to BSM. The gene coding BSM target enzyme acetohydroxyacid synthase (ALS) of the resistant biotype and susceptible biotype HZ001 (from Hangzhou, Zhejiang) were analyzed. The full length of ALS DNA sequence was 2235 bp without intron, coding 667 amino acids and 86% of them were similar to *Descurainia Sophia* and *Conyza canadensis* as a result of comparison by BLAST software. There was mutation in ALS gene in the resistant biotype, and the 197th amino acid proline (Pro) was substituted by serine (Ser). The amino acid substitution in this position has been confirmed as BSM resistance mechanism in several weed. The results showed that the ALS gene mutation and 197th amino acid substitution serine for proline might be the mechanism of *A. arenaria* resistance to bensulfuron-methyl, It was the first report of the molecular mechanism of the weed even the *Ammannia* spp. resistance to BSM in the world, it is the basic knowledge for the resistant weed risk evaluation and management strategies making (The work was funded by National Natural Science Foundation of China (31171863), Special Fund for Agro-scientific Research in the Public Interest (201303031, 201303022). Project supported by Zhejiang Entry-exit Inspection and Quarantine Bureau (ZK201324))

P HERBI 3

Interaction effect between seed treatments and pre-emergence herbicides on maize cultivars in South Africa

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In South Africa maize seed is treated with either a fungicidal or insecticidal product, or with both to protect seedlings from soil-borne fungal diseases and insect pests. The use of registered pre-emergence herbicides for effective control of weeds are commonly practiced by maize producers and questions with regard to interactions between seed treatments and herbicide products were raised. The interaction effect between seed treatments and the use of pre-emergence herbicides on the germination and emergence of maize seedlings was evaluated and quantified. Greenhouse trials were conducted using cultivars from Pannar and Pioneer Hi-Bred. Two sets of seed were tested, i.e. treated and not treated. Three soil applied herbicides (acetochlor 700 g ai SE, acetochlor 840 g ai EC and s-metolachlor 915 g ai EC) were applied at the label rate and double the label rate. The experimental design was a complete randomised design with four replicates per treatment. Mean time to emergence, total number of emerged seedlings and visual symptoms of phytotoxicity were recorded. Plant height was measured weekly and dry mass was determined at 54 days after planting. Data were expressed as a percentage of control treatments and subjected to an ANOVA using Genstat® for Windows Release 14.1. Cultivars treated with the respective seed treatments emerged faster and total number of seedlings emerged was higher when compared to untreated seed. The interaction between seed dressing and herbicides applied was significant for plant height and dry mass of Pannar cultivars. Pannar seedlings were stunted where double the label rate of all herbicides was applied in seed treatments. Stunting was only observed for Pioneer Hi-Bred cultivars that were untreated at double the dosage rate of acetochlor 700 g ai SE. Phytotoxicity symptoms of cultivars was observed as a tight folded coleoptile, usually dark green in colour and twisting or curling of the whirl. Phytotoxicity was more severe where Pannar seed was treated while Pioneer Hi-Bred cultivars showed more phytotoxicity where seed was not treated. The interaction between seed dressings and herbicide applications was cultivar related and was mostly significant for the first 4 weeks after emergence. All seedlings, however, outgrew stunting and visual phytotoxicity symptoms 10 weeks after planting.

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P HERBI 4

Glyphosate resistant biotypes of goosegrass and horseweed in Japan

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Introduction: Glyphosate has been used in agricultural and non-agricultural areas in Japan. Some wild populations of goosegrass (*Eleusine indica* (L.) Gaertn.) and horseweed (*Conyza canadensis* (L.) Cronquist) grow and display high survival rates after the application of glyphosate at 2.3 kg ai ha⁻¹, the recommended dose. This suggests that resistance to glyphosate has evolved in the wild populations of the two species. To clarify the mechanisms conferring glyphosate resistance is important for managing the glyphosate resistant biotypes of the two species.

Objectives: The objectives of the study are to elucidate a mutation in the target enzyme, 5-enolpyruvylshikimate-3-phosphate synthase (EPSPS) gene, amplification of the EPSPS gene and reduced glyphosate translocation.

Materials and methods: Complementary DNA (cDNA) sequencing analysis of the EPSPS gene was conducted to elucidate a mutation in the EPSPS gene and quantitative real-time PCR was conducted. Studies on the translocation of glyphosate in resistant biotypes of goosegrass and horseweed are undergoing.

Results: Complementary DNA sequencing analysis of the EPSPS gene indicated that there were no mutations conferring amino acid substitution at codon 106 in the EPSPS gene in the resistant biotypes of both species. Amplification of the EPSPS gene did not occur in the resistant biotypes of both species. Researches on the translocation of glyphosate in resistant biotypes of both species are in progress.

Conclusion: Any mutation in the EPSPS gene and amplification of the EPSPS gene were not found in the glyphosate-resistant populations of both species.

P HERBI 5

Arylex™ active (halauxifen-methyl): A novel post-emergence herbicide for cereal crops with a broad activity on dicotyledonous weeds

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Introduction: Dow AgroSciences is constantly seeking to develop new herbicides with new or broader weed spectrums to close unmet needs and with alternative modes of action to help manage resistant weed biotypes.

Objectives: Arylex™ active is a novel arylpicolinate herbicide and has been evaluated in all major cereal cultivating regions. Results will be presented that describe the active substance and its key characteristics.

Material and methods: Field trials and numerous lab and greenhouse studies have been conducted to investigate the biological performance of Arylex and to evaluate its toxicological and environmental profile.

Results: Arylex is a new post-emergence herbicide for the control of dicotyledonous weeds in cereal crops. It is the first member of a new class of synthetic auxin herbicides. Arylex is highly active at low doses (5-10 g ae/ha). When applied in post emergence in spring and winter cereals, Arylex herbicide controls a broad range of important weeds in cereal markets such as CENCY, CHEAL, DESSO, GAETE, FUMOF, GALAP, GERSS, LAMSS, AMBEL and PAPRH including weed biotypes resistant to other mode of actions such as ALS inhibitors. But unlike other synthetic auxin herbicides, the activity of Arylex on weeds is not significantly influenced by temperature. When applied with the safener cloquintocet, Arylex is selective in winter and spring cereals. Selectivity in cereals is due a slower rate of Arylex de-esterification to the active and mobile form of Arylex: halauxifen-acid. Cloquintocet enhances the metabolism rate in cereals through demethylation and conjugation prior to the formation of halauxifen-acid. To susceptible weed species, Arylex causes the typical symptoms of synthetic auxins and symptoms can occur within a few hours. Arylex degrades rapidly in soil and plants and does not induce any risk to rotational crops. Arylex exhibits favorable environmental and toxicological profiles. Arylex will be combined with other herbicides from Dow AgroSciences such as florasulam or fluroxypyr and will be offered in a range of dry or liquid formulations.

Conclusion: Arylex™ active is a new herbicide in development by Dow AgroSciences for the control of broadleaf weeds. It offers effective post-emergence control including herbicide resistant species at low dose rates, provides consistent weed control under adverse climatic conditions, and degrades rapidly in soils and crop plant tissues.

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P HERBI 6

GF-2644 & GF-2819: two new herbicides containing Arylex™ Active herbicide (halauxifen-methyl) to control wide range of broadleaved weeds in cereals in Europe

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Introduction: After the recent development of Arylex™ active herbicide, two new post-emergence herbicide mixtures containing this active substance have been developed by Dow AgroSciences: GF-2644 (Arylex + florasulam) and GF-2819 (Arylex + fluroxypyr).

Objectives: Arylex has been developed globally as a broadleaf weed herbicide with several Arylex based mixtures already created for the North America, Asian, and Australian cereal markets. Field data for two first concepts will be presented that show herbicidal efficacy and crop safety.

Material and methods: Field trials with both herbicides have been carried out across Europe to evaluate herbicidal efficacy and crop safety.

Results: GF-2644 and GF-2819 are new post-emergence herbicide mixtures for use in winter and spring cereals, including wheat (durum and spelt), rye, barley, and triticale. GF-2644 contains Arylex™ and florasulam formulated as an oil dispersion (OD), with a maximum use rate of 1 L/ha which corresponds to an Arylex rate of 6 g ae/ha and a florasulam rate of 5 g ai/ha. Depending on the country, GF-2644 can be applied from BBCH 11 to BBCH 45. When applied at the end of the winter or beginning of spring (BBCH 13-32), GF-2644 controls a wide range of broadleaf weeds including key species such as *Galium aparine*, *Matricaria ssp.*, *Papaver rhoeas*, *Stellaria media*, *Lamium ssp.*, *Centaurea cyanus*, *Chenopodium album*, *Cruciferae weeds*, *Fumaria officinalis*, *Geranium ssp.* and others.

GF-2819 contains Arylex and fluroxypyr in the form of an emulsified concentrate (EC), and at the maximum use rate of 0.5 L/ha delivers Arylex at 6 g ae/ha and fluroxypyr at 140 g ae/ha. GF-2819 is a flexible product which can be used from BBCH 13 to BBCH 45. When applied from tillering to BBCH 32, GF-2819 exhibits excellent control of *Galium aparine*, *Stellaria media*, *Lamium ssp.*, *Centaurea cyanus*, *Chenopodium album*, *Fumaria officinalis*, *Geranium ssp.*, *Galeopsis tetrahit*, *Ambrosia artemisiifolia*, and others.

Conclusion: GF-2644 and GF-2819 are two new and unique herbicides mixtures developed by Dow AgroSciences for post-emergence use in winter and spring cereals. Both products offer a wide spectrum of weed control, excellent crop safety, and no limitations for rotational crops. Both GF-2644 and GF-2819 contain Arylex™ active herbicide, a new tool to manage hard to control and ALS resistant broadleaf weed species.

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P HERBI 7

The impact of fungicide and herbicide timing on foliar disease severity, and barley productivity and quality

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Question: Barley producers in Western Canada are interested in mixing herbicides with a half-rate of fungicide at herbicide timings for weed and disease control. However, a one pass herbicide-fungicide application may not provide direct protection from pathogens for the upper cereal canopy leaves. The objective of the current study was to determine the effects of herbicide and fungicide timings on barley leaf disease severity, and the productivity and kernel quality of the malting barley cultivar AC Metcalfe.

Methods: At six sites across the Canadian prairies from 2010 to 2012 combinations of herbicide and the fungicide Tilt® (propiconazole) were applied to barley at the 2-3 leaf stage (herbicide and half-rate fungicide), 5-6 leaf stage (herbicide and half-

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rate fungicide), and/or the flag leaf stage (full or half-rate fungicide only). Each plot area was cross-seeded with tame oat as a model weed prior to seeding. Upper canopy leaf samples were collected for leaf disease assessment at the early dough growth stage. Weed biomass, and grain yield and quality were determined.

Results: Total leaf area diseased, (a combination of scald, both forms of net blotch and spot blotch) was greater for the 2-3 or 5-6 leaf stage herbicide only treatments and the combination herbicide and half-rate fungicide treatments compared with fungicide at the flag leaf stage. Yield, thousand kernel weight, kernel plumpness and test weight were greatest and kernel thins lowest for treatments with a flag leaf stage fungicide application. Split applications of fungicide at the time of herbicide application and at flag leaf emergence did not improve disease management and crop productivity compared to a single full rate fungicide application at the flag leaf stage. Weed biomass was generally not influenced by the treatments because weed control was excellent at all sites. However, yield was lower when herbicide was applied at the 5-6 versus 2-3 leaf stage.

Conclusions: For improved leaf disease management and yield in barley, fungicide applications should include a flag leaf stage timing for adequate protection of upper canopy leaves, which are key contributors to yield and grain filling. Delaying herbicide application to the 5-6 leaf stage in an attempt to accommodate a fungicide application reduces barley yield due to early-season weed interference.

P HERBI 8

Effect of Imazapic residues on photosynthesis traits and chlorophyll fluorescence of maize seedlings

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The influence of various levels of imazapic residues (0~800 µg/kg) on the growth, chlorophyll concentration, photosynthetic characteristics and chlorophyll fluorescence of maize seedlings were studied by adding imazapic to soil in the simulated pot experiment in greenhouse. Upon treatment, plant height, root length and whole plant dry weight of maize decreased with the increase of imazapic residues concentrations. Under stress, the root/shoot ratio initially decreased and then increased, which indicated that effect of imazapic residues on plant height and root length of maize seedling may be different. The decline of chlorophyll content and the net photosynthetic rate in the leaves of maize seedlings were observed for all treatments, and these indexes showed a dose-response relationship to the concentrations of imazapic. Intercellular CO₂ concentration (Ci), transpiration rate (Tr) and stomatal conductance (Gs) were also declined to varying extent, but chlorophyll a/b value increased gradually. Chlorophyll fluorescence analysis showed that imazapic could significantly reduce the primary light energy conversion efficiency (Fv/Fm), PS II actual photochemical efficiency (ΦPS II) and photochemical quenching (qP), but increase non-photochemical quenching (NPQ) as the imazapic dosages were raised. Maize seedling leaves had a decreased fraction of light energy allocated to photochemical reactions, while the fraction of light energy allocated to heat dissipation and nonphotochemical reactions were increased with the rise of the imazapic residues concentrations. These results indicated that imazapic may impair PS II and block the electron transport in light reaction. Together, this study indicated that maize seedling might have its photosynthetic protection mechanism in some degree under imazapic stress.

P HERBI 10

Transcriptomic evaluation of enhanced bioactivity caused by derivatization of allelochemical of Thunberg's meadowsweet (*Spiraea thunbergii*)

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Introduction: Thunberg's meadowsweet (*Spiraea thunbergii*) is a popular garden shrub known to produce *cis*-cinnamoyl glucosides as predominant allelochemicals. The essential chemical structure responsible for the bioactivity of them is *cis*-cinnamic acid (*cis*-CA), which strongly inhibits the growth of several plant species. As we thought *cis*-CA is promising candidates for the development of agrochemicals, chemical derivatization of *cis*-CA has been conducted and some *cis*-CA analogues with intensified plant growth-inhibitory activity were successfully designed.

Objective: While these *cis*-CA analogues might serve as the lead chemicals in developing new herbicides, the reasons for their ability to enhance growth-inhibitory activity need to be clarified through the use of molecular biology.

Materials and methods: To establish a rapid high-throughput evaluation system for the enhanced plant growth-inhibitory activity caused by modifications of *cis*-CA's chemical structure, a DNA microarray assay was used to analyze the changes in early gene responses of *Arabidopsis thaliana* seedlings.

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Results: After a 6-h exposure to (Z)-3-(3-iodophenyl)acrylic acid, we observed an upregulation in three classes of early auxin-responsive genes, *Aux/IAA*, *GH3*, and *SAUR*, which was similar to the transcriptional response to indole-3-acetic acid (IAA), together with an upregulation of the genes related to environmental stress and toxin detoxification responses. The genes belonging to the Gene Ontology (GO) terms “responses to heat”, “toxin catabolic process”, “response to jasmonic acid stimulus” and “UDP-glucosyltransferases”, were overrepresented. Gene responses to 2-(3,4-dihydronaphthalen-1-yl)acetic acid were similar to those to IAA. In contrast, fewer genes were upregulated in response to its double-bond isomer, (Z)-2-[3,4-dihydronaphthalen-1(2H)-ylidene]acetic acid, than to *cis*-CA.

Conclusion: Structurally different *cis*-CA analogues trigger diverse gene responses and a correlative relationship between the strength of the *cis*-CA analogues' bioactivity and the number of responsive genes was observed. Also, our results suggest that DNA microarray analysis is effective for the rapid evaluation of substituent effects on plant growth-inhibitory activity caused by the bioactivities of chemical derivatives.

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The Evaluation of Oxadiargyl dosages at different growth stages for potato (*Solanum tuberosum*) yield

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In order to study the effect of Oxadiargyl doses, on potato yield, field experimental design was conducted at Alaroug Research station in Ardabil 2013. The factorial experiment with control (weedy and weed free) was performed in the base of Complete Randomized Design with three replications, and potato cultivar was Agria (common cultivar in Ardabil). The first factor was Oxadiargyl dosages with six levels (0.05, 0.1, 0.2, 0.4, 0.6 and 0.8 lit a.i /ha), and second factor was Oxadiargyl time of application at different potato growth stages including three levels (Potato emergence, Stolon initiation and Potato tuber bulking). The results showed that higher dosages of Oxadiargyl were controlled maximum percent of weeds. 0.8 lit ai/ha of Oxadiargyl reduced the weed density and biomass to 48.40 and 66.16 percent. Among oxadiargyl application time different potato growth stages, maximum reduction percent of weed density and biomass was at potato emergence, which proves the effectiveness of the Oxadiargyl for potato emergence. Statistical analysis showed that using different dosages had significantly effects on potato plant height, main stem diameter per plant, yield per plant and total tuber yield per hectare. Among oxadiargyl application time at different potato growth stages, potato emergence increased potato plant height, main stem diameter per plant, yield per plant and total tuber yield per hectare maximally.

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Table 1: Mean comparison of control percentage of weed density and biomass at different doses of Oxadiargyl

Dose (lit ai/ha)	Weed density (%)	Weed biomass (%)
0.05	62.4 ^a (3.53)	104.6 ^a (2.46)
0.1	101.1 ^a (3.47)	186.3 ^a (4.53)
0.2	138.1 ^a (3.49)	263.8 ^a (4.31)
0.4	29.8 ^b (3.92)	50.81 ^b (3.65)
0.6	46.7 ^b (3.26)	61.2 ^b (3.03)
0.8	48.4 ^b (2.82)	66.16 ^b (3.22)

Table 2: Mean comparison of number of yield per plant and total tuber yield at different doses of Oxadiargyl

Dose (lit ai/ha)	Yield per plant	Total tuberyield
0 (Weedy)	223.39 ^a (6.21)	9.04 ^a (0.27)
0.05	223.64 ^a (6.68)	9.49 ^a (0.29)
0.1	272.22 ^a (5.82)	11.20 ^a (0.25)
0.2	313.87 ^a (11.51)	13.08 ^a (0.51)
0.4	332.11 ^a (7.17)	13.88 ^a (0.51)
0.6	395.78 ^a (13.92)	16.71 ^a (0.61)
0.8	440.29 ^a (16.38)	18.67 ^a (0.72)

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***Streptomyces* sp. KRA14-329 Producing Herbicidal Metabolites as Potential Biocontrol Agent**

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Introduction: Bioherbicidal metabolites produced by *Streptomyces* sp. are being studied as possible herbicides or herbicidal adjuvants to develop biological agents that are easily degradable and environment friendly. Also, isolation and structural identification of natural herbicidal-active compounds from *Streptomyces* sp. has been proved to be an effective approach for novel lead discovery of the herbicide development.

Objectives: The aim of this work was to screen bioherbicidal isolates, to identify active compound and to evaluate *in vivo* herbicidal activity of selected isolate as bioherbicidal agent.

Materials and method: *In vivo* herbicidal activity of culture broth of the actinomycete isolates was examined against five grass species and four broad leaf species on Plastic pot(superficial 350cm²) at 30/20 ± 5°C, day/night temperature with an about 14h photoperiod in a greenhouse condition. Active metabolites were purified from culture broth by solvent extraction, C₁₈ silica gel, Sephadex LH-20 column chromatography and preparative HPLC. The metabolites were identified by electrospray ionization mass spectra (ESI-MS) and ¹H-, ¹³C- and 2D NMR spectral data analysis.

Result: One of about 600 different soil actinomycete isolates, KRA14-329, showed strong herbicidal activity against grass species, *D. sanguinalis*, *Panicum dichotomiflorum*, *Sorghum bicolor*, *Echinochlia crus-galli*, *Agropyron smithii* and broad leaf species such as *Solanum nigrum*, *Aeschynomene indica*, *Xanthium strumarium*, *Calystegia japonica*. The culture broth of KRA14-329 by foliar application showed phytotoxic symptoms of wilting or burn-down of leaves and stunting and finally plant death. The isolate KRA14-329 was identified as *Streptomyces* sp. based on its 16S rRNA gene sequence and morphological characteristics. Two herbicidal compounds, 329-M1 and 329-M2, were identified as cycloheximide derivatives including glutarimide moiety by NMR and ESI-MS analysis.

Conclusion: These results suggest that *Streptomyces* sp. KRA14-329 producing bioherbicidal metabolites can be developed as a biocontrol agent (BCA) for weed control and/or may provide as a lead molecule for a more efficient herbicide.

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Sugarcane bagasse as support for immobilization of *Bacillus pumilus* HZ-2 and its use in bioremediation of mesotrione-contaminated soils

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The use of herbicides has contributed to over 40% of the pesticide consumption in the world market, which globally ensures crop cultivation and food production. Unfortunately, problems emerged in recent decades. Herbicide resistance and its side effects are the two main concerns while inappropriate use of the herbicides potentially impact non-target organisms in aquatic ecosystem and non-farming areas. With the growing demand for food free of agrochemicals, therefore, the removal of herbicide residues from environment has received increasing attention and an effective strategy is an urgent need. Microorganism-based remediation is a group of promising techniques introducing microbes with specific catabolic potential isolated from *in situ* microbial communities to degrade target contaminants. However, the degrading microorganisms isolated from environment usually fail to degrade pollutants when applied for bioremediation of contaminated soils, thus additional treatments are required to enhance biodegradation. In this study, the potential of sugarcane bagasse as bacteria-immobilizing support was investigated for the first time in herbicide mesotrione biodegradation. A novel bacterial isolate *Bacillus pumilus* HZ-2 (collection number: CCTCC M 2013380), which was capable of degrading over 95% of mesotrione at initial concentration ranging from 25 to 200 mg L⁻¹ within 4 days in liquid cultures, was chosen for immobilization. Scanning Electron Microscope (SEM) images showed the bacterial cells were strongly absorbed and fully dispersed on bagasse surface after immobilization. Significantly, the microbial degrading activity was retained, and 86.5% and 82.9% of mesotrione was eliminated by bacteria immobilized on bagasse. In the sterile soil, approximately 90% of mesotrione was degraded after supplementing 5.0% of molasses in bacteria-bagasse composite, which largely enhanced microbial adaptability and multiplication in the real soil environment. Analysis of the degradation products by high performance liquid chromatography (HPLC) determined 2-amino-4-methylsulfonylbenzoic acid (AMBA) and 4-methylsulfonyl-2-nitrobenzoic acid (MNBA) as the main metabolites in biodegradation pathway of mesotrione. In the field tests, the immobilized preparation demonstrated wide availability of mesotrione degradation in various environments (5.0 to 8.0 of pH, 25 to 35 °C), especially at low concentrations of mesotrione (5 to 20 mg kg⁻¹). Compared with the control,

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75.1% of applied dose of soil mesotrione was continuously eliminated within 14 days. As expected, the potential of sugarcane bagasse as support in microbe immobilization was validated. More importantly, our studies demonstrated a novel approach for better utilization of degrading microorganisms in bioremediation of herbicide-contaminated environment.

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A comparative study between non-linear regression and the probit model for the evaluation of *Echinochloa crusgalli* resistance levels

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The resistance to herbicide butachlor was measured within 24 populations of barnyard grass (*Echinochloa crusgalli*) which were collected from advanced rice cultivating areas in China. The results showed that all the sampled populations have developed resistance to butachlor with relative resistance index (RI) of 1.36 to 10.61 except Ledong (V24) (the relatively sensitive population, $ED_{50}=160.07$ g a.i./ha). Differences in sensitivity were determined using the logit model and the butachlor-resistance level of grass population Jilin (V7) were the highest with RI value of 10.61, following by Dongying (V8), Meizhou (V20), Loudi (V17) and Binzhou (V9) with RI values of 7.29, 6.58, 5.59, 4.46, respectively. Relative RI values in other barnyard grass populations were evaluated below 4. Moreover, the whole-plant bioassay was investigated in terms of the total dry weight, total fresh weight, shoot fresh weight and shoot dry weight, respectively and the logit analysis method was employed to calculate the ED_{50} values, which followed the general order: shoot fresh weight > total fresh weight > shoot dry weight > total dry weight. The RI values based on those four parameters by the probit model were lower than those values calculated by the logistic dose-response model, which may indicate the former method tended to underestimate the weed resistance levels in general. Therefore, it is suggested that the ED_{50} values of herbicides applied in soil should be determined by the logistic model based on the index of the total fresh weight or total dry weight.

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First population of Black Grass (*Alopecurus myosuroides*) resistant to herbicides in northern Spain (Navarre)

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Introduction: In Navarre, the most cultivated crops (regarding surface) are cereals (wheat and barley). The application of herbicides to control grass weeds is one of the most important action to get a proper yields. The resistant populations of wild oat, raygrass and other broadleaf species have been already selected. From the last few years, black grass (*Alopecurus Myosuroides*) became a difficult weed to control due to its density increase. The herbicide mode of action most commonly used are HRAC groups A and B, which are being less and less effective. Therefore, a suspect of resistance is patent.

Objectives: The main objective of the work reported here was to prove the existence of a resistant population of black grass to A and B herbicide mode of action.

Another important objective is to compare the efficacy of A and B groups with other mode of actions.

Materials and methods: The trial was carried out in wheat in Ripodas. A randomized blocs was designed with 4 replications. All treatments were applied in a water volume equivalent to 300 l/ha at a pressure of 3 bar.

Weed assessments were recorded at specific times after herbicide application and were based on four dime-meter quadrant counts per plot (2×10 m²). Weed control results are listed as weed score in this report where 0 indicates no control and 10 indicates total weed control. The cereal herbicides were applied in two times: autumn (1), when the crop had 1 to 3 leaves (BCHH 10-13), and the weed had 1-2,5 leaves; and winter (2), when the crop had 4 tillers detectable (BCHH 24), and the weed had 2-4 tillers detectable.

Results: The results of efficacy on black grass of herbicide applications were:

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Table 1: Efficacy results.

Herbicide	g a.i. x h ⁻¹	Spray timing	HRAC Group*	Weed score**
1 Untreated				0
2 Polarpec+Mohican	2400+125	1	N+F	7
3 Polarpec+Mohican+Protur	2400+125+1500	1	N+F+C	9
4 Herold	(120+240)	1	K+F	8
5 Herold+Protur	(120+240)+1500	1	(K+F)+C	9
6 Herold+Polarpec	(120+240)+2400	1	(K+F)+N	8
7 Atlantis	(15+3)	2	B+B	2
8 Broadway	(188+63)	2	B+B	2
9 Traxos Pro	(300+300)	2	A+A	3

* In bold, mode of action with activity on grass weed.

** 0 = no weed control; 10 = 100% weed control

The herbicides belonging to HRAC groups A and B failed and their weed control were nearly non-existent. The rest of treatments gave almost complete control of black grass. The best results were when Polarpec and Herold were combined with Protur, or rather, two modes of action were combined.

Discussion: The result of this trial indicates that the herbicides commonly used to control black grass were commercially non acceptable. It seems that the replication during the last years of these modes of action (A and B) have finally selected a resistant population.

These control problems occur in lands nearby.

When mode of action is changed, the result of efficacy is successful. Therefore, mode of action on black grass must be changed or alternated in the coming years, even if population are still sensible to HRAC groups A and B.

Figure 1



Figure 2



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Development of an herbicide resistant tomato by mutagenesis techniques

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ALS inhibiting herbicides are characterized by a broad weed control spectrum, low mammalian toxicity, high selectivity and high activity with low application rates. There are now over 30 herbicides belonging to this group of herbicides that are registered for use all over the world. These herbicides act by inhibiting the enzyme acetolactate synthase (ALS), a key enzyme in the branched chain amino acid biosynthesis pathway leading to the formation of leucine, valine and isoleucine. Tomato plants are sensitive to these herbicides, except some herbicides of the sulfonylurea group which are detoxified **by a P450-type oxidase in tomato leaves and therefore are not effective in root parasites management**. Development of a tomato variety resistant to the imidazolinones herbicides may serve as a reasonable approach for broomrape control, the most troublesome tomato pest in the Middle East. EMS (ethyl methane sulfonate) mutagenesis was conducted on 20,000 seeds of the commercial tomato line M82. About 100,000 tomato second generation seedlings were screened for resistance to pulsar (imazamox). As a result, a novel tomato mutant HRT-1 was obtained. The mutant is resistant to high rates of imidazalinone herbicides pulsar, cadre (imazapic) and arsenal (imazapyr) in all stages of its vegetation, tissue culture, germinating seeds and tomato plants grown in the field. Several field experiments demonstrated that even a rate high as 144 g a i. ha⁻¹ did not cause any visual damage or yield loss of HRT1 tomato plants. The resistance is due to a change in the herbicide's target site on the ALS molecule as a result of point mutation in the ALS gene located on chromosome three. The substitution of Alanine to Valine in position 194 which corresponds to Alanine₂₀₅ in Arabidopsis confers HRT1 resistance to the imidazolinones.

P INSECT 1

The Efficacy of Ceranock Attract and Kill System as a Control Methods of Mediterranean fruit fly, *Ceratitis capitata* in Citrus and Apricot Orchards in Central Iraq

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Introduction: The Medfly, *Ceratitis capitata*, is considered as one of the most important economic pest worldwide.

Objectives: A field experiment was conducted using Ceranock bait station, "attract and kill" system to combat Mediterranean fruit fly, *Ceratitis capitata*, in Citrus and apricot orchards, central of Iraq during the 2013-2014 field season.

Materials and methods: Control program was implemented in three orchards of mixed citrus and apricot varieties. Ceranock traps were hung in each tree of two separate orchards(each to citrus and apricot). Delta and McPhail traps were used to monitor Medfly population density in each treated and control orchards.

Results: Results indicated that the total of trapped insects in monitoring traps after one week of Ceranock application in apricot orchards were zero, 1 and 31 adults for the 1st, 2nd and the control orchards respectively. At the end of apricot season and after 45 days of Ceranock trap application, the number of trapped insects in monitoring traps reached 110, 111 and 2349 adults/ week respectively, for the 1st, 2nd and 3rd orchard. The use of Ceranock traps reduced Medfly population density by 86-97%. The percentage of fruit injuries in early maturing varieties were 0.75%, 1%, and 34% for the 1st, 2nd and control orchards, respectively. While, for late varieties were 2% and 3% for 1st and 2nd orchard, and 69% for the control. The percentage of reduction in fruit damages reached to 95% and 97%, respectively in the 1st and 2nd orchard. The total of trapped insects in monitoring traps after two week of Ceranock application in citrus orchards were 205, 277, 765 adults for the 1st, 2nd ,3rd orchards respectively. The percentage of citrus fruit injuries were increased when fruits maturity and yellowish increase dramatically 21 % , 32% in mandarin and orange fruits respectively compared with 4% , 5% (fruit injuries decrease 95%, 96%) in treated respectively. the 1st, 2nd and control orchards, respectively. The percentage of reduction in Medfly population density reached to 56%, 67% respectively in the 1st and 2nd orchard treated.

Conclusions: The results of this study demonstrate clearly the efficacy of Ceranock bait station, "attract and kill" system as a control measure for Medfly in citrus and apricot orchards.

P INSECT 2

First Record of Collembolans Carboxylesterase and Glutathione-S-Transferase Activities Exposed to Several Agrochemicals

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Collembolans (springtails) are a group of small animals that live in dead vegetation and leaf litter and in the soil pores and cavities to a depth of about 15 cm. They are important consumers of dead plant material, and eat saprophytic microbes. Carboxylesterases (CbEs) and glutathione-S-transferase (GST) play a key role in the detoxification of many agrochemicals. These enzymes are involved in the biochemical mechanisms underlying resistance to pesticides in some pest species. They also provide an efficient protective mechanism against toxicity caused by several agrochemicals in invertebrates. To gain knowledge on the role of CarbEs and GST activities in the natural tolerance in collembolan insects, we performed enzyme kinetic analyses to determine whether these insects are able to generate these enzymes in their bodies after exposure to various chemicals. However, there are no biochemical studies on the impact of pesticides upon these soil organisms. Our studies provide a baseline study on the impact of various agrochemicals on the levels of the detoxification enzymes CarbE and GST for collembolan insects. Application of pesticides such as Bordeaux mixture, imidacloprid, methiocarb and glyphosate resulted in significant increases of CarbEs in collembolan insects. Polyacrylamide gel electrophoresis (PAGE) of CarbE levels revealed visible stronger bands in the response of collembolans to all the applied pesticides, compared to control. A significant increase in glutathione S-transferases (GST) activity was recorded only after application of Bordeaux mixture.

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P INSECT 3

Neonicotinoid resistance in the cotton whitefly, *Bemisia tabaci* (Genn.), (Hemiptera: Aleyrodidae), populations from Antalya
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Cotton whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), is one of most damaging insect pest on numerous cultivated crops worldwide. Furthermore, it has the ability to develop resistance to diverse group of insecticides rapidly, hence controlling the pest is problematic. The aim of the current study is to investigate resistance status of different populations collected from Antalya, Turkey. A set of insect populations collected from six different counties of Antalya (seven different locations), and a susceptible lab population (Koçarli) collected earlier and maintained since 11.08.2009 at the Entomology lab of Akdeniz University were used as insect materials. Leaf dip bioassay was employed for resistance screening in the populations. The results of bioassays were shown that resistance ratios ranged from 0.34 to 48.62 for thiamethoxam and 0.10 to 30.41 for acetamiprid. Moreover, *mtCOI* sequencing results indicated that all populations were B biotype. Overall, the results indicated that the use of neonicotinoid insecticides on whitefly may not be effective on the regions sampled. Furthermore, the pest has been exposed to thiamethoxam more frequently in recent years and the resistance level may have reached to uncontrollable levels in three years.

P INSECT 4

Physicochemical and microbiological characterization of the essential oil of *Syzygium aromaticum* and its use in biological control against *Tuta absoluta*

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This study aimed to make the extraction of essential oil of clove (*Syzygium aromaticum* syn *Eugenia caryophyllata*) and to evaluate the physicochemical quality, like studying its antimicrobial effect for some beneficial and the other pathogenic ones. The essential oil of clove was obtained by steam distillation, the results of the physico-chemical study (density, refractive index, acid value, ester index, index of saponification, pH) and sensory (smell appearance and color) revealed essential oil obtained meets the requirements of the European pharmacopeia.

The characterization of the composition by Infrared spectroscopy showed that there was an abundance of specific binding to phenols O-H, = C-O and even aromatic rings which confirms the presence of eugenol and acetate eugényl in our analyzed essential oil.

The results of microbiological analyzes revealed that the essential oil of clove (*Syzygium aromaticum*) presents - *in vitro*- a moderately inhibiting activity opposite all the studied bacteria: *Bacillus thuringiensis*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus vulgaris* *Klebsiella pneumoniae*.

Except *Beauveria bassiana*, the fungi stocks in our study *Candida albicans*, *Penicilium* sp are more sensitive to the essential oil of the clove.

However the study of the minimum inhibitory concentration (MIC) shows that clove essential oil concentrations of 0,06% for *Bacillus thuringiensis* and lower than 0.03% for all other bacteria and even lower than 0.015% for the fungi stocks are sufficient to give a bacteriostatic effect.

The results of the CMB (bactericidal minimal concentrations) and the CMF (fungicidal minimal concentrations) show a strong bactericidal effect for the majority of the studied stocks.

P INSECT 5

Pink Bollworm *Pectinophora gossypiella* (Saunders) Resistance to Dipel 2x and Cross-Resistance to Different Insecticides With Special Reference to Assay Some Enzymatic Parameters for Characterization of the Resistant Strain

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The aim of the present work is to investigate the development of resistance of pink bollworm *Pectinophora gossypiella* to the bio-insecticide Dipel 2x and cross-resistance to different insecticides. Also, to assay some enzymatic parameters for characterization of the resistant strain. Laboratory selection of the resistant strain of *Pectinophora gossypiella* indicated an

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increase in the resistance ratio to Dipel 2x. The resistance ratio reached to 10.57-fold in G25, then increased to 46.29-fold in generation 43. Study the response of Dipel 2x resistant strain to other different insecticides indicated that such strain was low cross resistance to Protecto (local product of *BtK*) but no cross resistance to alpha-cypermethrin, profenofos and diflubenzuron. Biochemical analysis of detoxification enzyme levels indicated that protease activity increased significantly in all of the measured generations, the activities were 119.14 and 121.48 µg casein/min, in G25 and G27, respectively compared with the susceptible strain (91.10 µg casein/min). Regarding, - and β-esterase activity increased significantly between 15.45 to 22.74 µg - naphthyl/min/g larval weight in generation 38 and between 17.88 to 18.17 µg β-naphthyl/min/g larval weight in generation 43. The electrophoretic patterns of the -esterase isozyme of the Dipel 2x resistant strain shows presence of two bands in susceptible, G25, G27 and G28, one band appear in G34 while three bands were present in G38, 41 and 43. The electrophoretic patterns of β-esterase isozyme of Dipel 2x resistant strain shows two bands in susceptible strain and in different generations of Dipel 2x resistant strain with high density in G41 and 43. The occurrence of new bands may be a directory of the changes between the susceptible and resistant strains.

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Figure 1

Table (1): Rate of resistance development in *P. gossypiella* (Saunders) toward Dipel 2x during the selection pressure for 18 generations

Selected generations	LC ₅₀ (g/L) (Fiducial limits)	Slope (±SE)	Resistance Ratio (Fold)
Susceptible(s)	0.07(0.05-0.99)	1.244±0.15	—
Parent(G20)	0.57(0.4-0.77)	1.14±0.13	8.14
G25	0.74(0.3-1.11)	1.28±0.33	10.57
G27	0.94(0.69-1.22)	1.42±0.17	13.43
G28	0.99(0.43-1.59)	0.90±0.22	14.14
G29	1.29(0.84-1.85)	1.34±0.27	18.43
G30	1.53(0.97-2.15)	1.11±0.20	21.86
G31	1.35(0.96-1.84)	1.53±0.22	19.29
G34	1.68(0.94-2.41)	1.53±0.30	24
G38	2.14(1.18-3.10)	1.24±0.24	30.57
G41	2.35(1.39-3.33)	1.29±0.24	33.57
G43	3.24(1.87-4.65)	1.40±0.28	46.29

Table (2): Response of Dipel 2x resistant strain of *P. gossypiella* (Saunders) to the tested insecticides:

Tested insecticides	Susceptible strain		deltamethrin resistance strain		Resistance ratio
	LC ₅₀ (ppm) (Fiducial limits)	Slope (±S.E)	LC ₅₀ (ppm) (Fiducial limits)	Slope (±S.E)	
alpha-cypermethrin (Super-alpha®)	2.07(0.53-3.3)	1.43±0.47	3.71(2.42-5.27)	1.37±0.31	1.79
Profenofos (Curacron®)	0.07(0.02-0.13)	0.94±0.26	0.22(0.12-0.36)	1.51±0.45	3.14
Diflubenzuron (Dimilin®)	183.76(93.76-265.49)	1.93±0.50	214.91(125.61-304.18)	2.02±0.49	1.17
Protecto	320(200-440)	1.20±0.22	1890(710-3020)	1.55±0.41	5.91

Figure 2

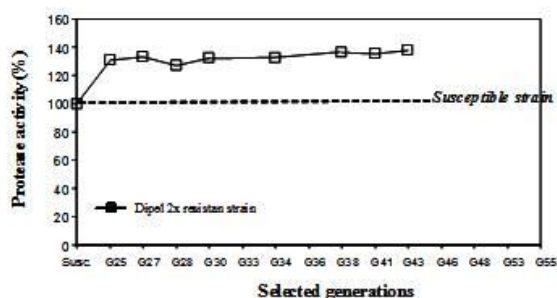


Fig.(1): Activity percentage of protease in full grown larvae of some of the selected generations of *P. gossypiella* (Saunders) during resistance development against Dipel 2x.

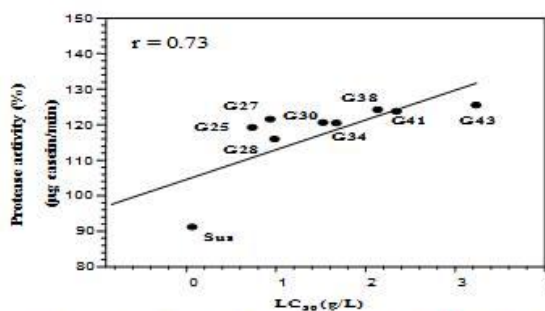


Fig.(2): The relationship between the determined LC_{50} values of Dipel 2x in susceptible strain and the selected generations of Dipel 2x resistant strain of *P. gossypiella* (Saunders) and the determined activity percent of protease.

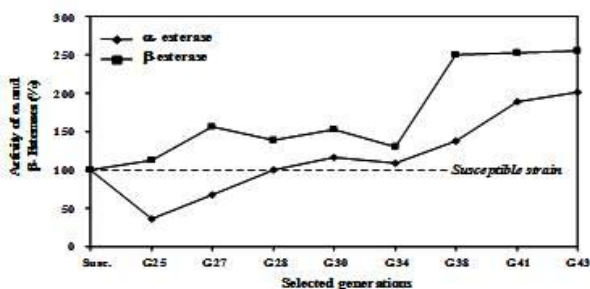


Fig.(3): Activity percentage of α - and β -esterases in full grown larvae of some of the selected generations of *P. gossypiella* (Saund.) during resistance development against Dipel 2X.

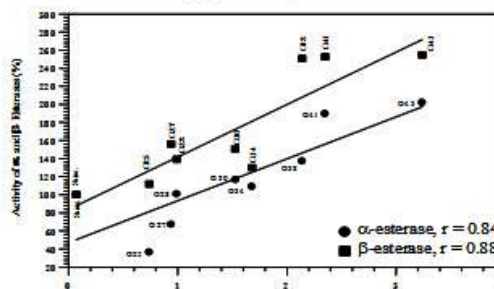


Fig.(4): The relationship between the determined LC_{50} values of Dipel 2x in susceptible strain and the selected generations of Dipel 2x resistant strain of *P. gossypiella* (Saunders) and the determined activity percent of α - and β -esterases.

P INSECT 6

Lethal and Sublethal Effects of Spinosad and Abamectin on *Spodoptera littoralis* (Lepidoptera: Noctuidae)

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The effects of sublethal concentrations of two biorational insecticides, spinosad (Tracer[®]) and abamectin (Vertimec[®]) on the biological characteristics of the cotton leafworm, *Spodoptera littoralis* Boisd. were investigated through 48h-feeding of the fourth instars on treated cotton leaves. According to toxicity data after 72h of treatment, spinosad ($LC_{50} = 63.4$, $LC_{25} = 14.0$ mg L⁻¹) seemed to be more effective than abamectin ($LC_{50} = 248.9$, $LC_{25} = 57.8$ mg L⁻¹). The pupation rate and pupal weight were significantly lower in treated groups in which the fourth instars were treated with either spinosad or abamectin at LC_{25} and LC_{50} compared with those of the controls. Both spinosad and abamectin treatments significantly reduced fecundity (mean cumulative number of eggs laid per female) than in the control groups. On the other hand, the residual activity of spinosad and abamectin, applied on cotton, under field condition, at labeled field- and subfield-rates were examined against the fifth-instar larvae of *S. littoralis*. For spinosad, our results indicated that feeding deterrent effects were significantly demonstrated in larvae that fed on leaves collected from field plots with residual deposits of spinosad at 3 and 7 days old after application (DAA). The residual activity of spinosad on feeding and other metabolic parameters was decreased after 21 DAA indicating that the chemical started to degrade under field conditions. Unlike spinosad, abamectin applied at either selected concentrations, had no significant residual activity on larval feeding efficiency indices through 21 day-old residues, compared to the controls. A histological study on midgut from larvae that fed on leaves treated with selected concentrations of either spinosad or abamectin showed that spinosad had strong histomorphological symptoms to the midgut, fat bodies, and Malpighian tubules. But, the histopathological symptoms in the midgut caused by abamectin were less pronounced than those observed for spinosad. Our results suggest that the sublethal concentrations of spinosad may reduce population growth of the cotton leafworm by affecting its survival, development and reproduction, and it is suitable for integration into an IPM program for this pest.

P INSECT 7

Current status on the use of microbial insecticides in Japan

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The use of insect pathogens to control insect pests dates back to the 19th century when Metchnikoff (1879) of Russia first used a fungus (*Metarhizium anisopliae*) to control sugar-beet weevil (*Cleonus punctiventris*). In Japan, Hidaka (1933) was the first to attempt this task, by using a fungus (*Beauveria bassiana*) to control pine moth (*Dendrolimus spectabilis*). Since then, various attempts have been made to develop a method to control insect pests using insect pathogens. In Japan the cypovirus product, Matsukemin, was the first microbial control product to be registered in 1974, and inactive and live *Bacillus thuringiensis* products were also registered and put on the market as pesticides in 1980 and 1981, respectively. Since then, there is a significant increase in the number of microbial insecticides registered as agricultural chemicals in Japan. Currently, there are 32 microbial insecticides on the market in Japan. However, the shipments in value terms have fallen since the 1996 agricultural chemical fiscal year (period from October to September in the following year) when it peaked at 1.9 billion yen. In the 2013 agricultural chemical year, the value decreased to 0.67 billion yen that constitutes less than 1% of all insecticides used in Japan; given the benefits of using microbial insecticides, a broader use of the products is desired.

P INSECT 8

Monitoring and Minimizing Pesticide Residues in Strawberry

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The present work aimed to monitor and minimize the pesticide residues in and on strawberry fruits in Egypt. A total number of 1537 samples of strawberry were collected from local Egyptian markets during 2008 to 2010. A multi residue method (QUECHERS) sample preparation method and gas chromatography coupled to mass spectrometry (GC-MS/MS) and liquid chromatography coupled to mass spectrometry (LC-MS/MS) were adopted for the analysis of pesticide. The obtained result indicated that 39.9% of the total number of all samples analyzed had no detectable pesticide residues. However 61.1% contained detectable residue, of which 15.6% contained residues that exceeded the maximum residue limits (MRL'S) which could pose adverse effects on health of consumers. The resulting data indicates that, 47 pesticides have been identified as detectable residues in all analyzed samples collecting during 2008. However, the number reached 75 pesticide detected in 2009 and decreased in 2010 to be 57 pesticide. Considering monitoring of heavy metals in strawberry, data showed that 22.7% of all samples analyzed were free from any detectable amount of trace elements. However, 77.3% were contaminated of which 1.7% was violated. Cadmium was the most frequently detected contaminant in all samples where the contamination percentage was 48%. Washing process with tap water for three minutes induced considerable relative reduction in pesticide residues, contaminated the strawberry samples. The boiling/cooking was observed to be more effective in reducing the residue.

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Figure 1

Table (1) Percentage of strawberry samples contained one, two, three or more pesticide residues during 2002, 2009, and 2010 in Egypt.

parameters	2008	2009	2010
Sampling year			
Total on of analyzed samples	499	716	322
None contaminated samples (%)	67.5	44.8	38.5
Samples contained one pesticide residues (%)	23.4	25.2	18.9
Samples contained two pesticide residues (%)	9.8	13.4	19.9
Samples contained 3 or more pesticide residues (%)	6	15.4	22.7

Table (2) The free, contamination and violation percentage of strawberry samples subjected to pesticide residues collected in Egyptian local market, during 2008, 2009 and 2010*

parameters	2008	2009	2010
Total no. of analyzed sample	(499)	(716)	(322)
Free contaminated (%)	40.9 (204)	39.5 (283)	34.5 (111)
Contaminated (%)	59.1 (295)	60.5 (433)	65.5 (211)
Contaminated not Violated (%)	42.1 (201)	48.6 (348)	43.8 (136)
Violated (%)	17.0 (83)	11.9 (83)	21.7 (73)

* Number between brackets represent the number of analyzed samples

Figure 2

Table (3) the effects of some household processes on pesticides residues ($\mu\text{g g}^{-1}$) in strawberry samples collected from Egyptian local markets during 2009.

Sr no	Pesticide	Raw					Percentages of loss in washing					Percentages of loss in Cooking				
		1	Carbendazim	5.5	5.2	4.3	1.9	1.7	1.82	1.92	2.326	5.263	5.88	54.5	53.85	58.14
2	Chlorpyrifos	1.2	0.8	0.66	0.38	0.3	49.2	50	53.03	52.63	46.7	73.3	73.75	71.21	63.16	66.67
3	Ethion	0.92	0.47	0.23	0.21	0.11	57.6	59.6	65.22	61.9	45.5	72.8	65.96	56.52	57.14	90.91
4	Fenpropathrin	0.38	0.33	0.1	0.09	0.08	2.63	3.03	10	11.11	12.5	60.5	63.64	100	100	100
5	Methomyl	1.3	1.2	0.5	0.47	0.42	0	8.33	2	2.128	2.38	76.9	76.67	70	63.83	69.05

Table (4) The free, contamination and violation percentage of heavy metals in strawberry samples collected from Egyptian local markets during 2008-2010.

parameters	2008	2009	2010
Total no. of analyzed sample	(155)	(84)	(74)
Free contaminated (%)	19.35 (30)	35.71 (30)	14.86 (11)
Contaminated (%)	80.64 (125)	64.28 (54)	85.14 (63)
Contaminated not Violated (%)	78.71 (122)	64.28 (54)	83.78 (62)
Violated (%)	1.93 (3)	0.00 (0)	1.36 (1)

* Number between brackets represent the number of analyzed samples

P INSECT 9

A sequential testing program to evaluate the efficacy of seed-treatment insecticides on cotton flea beetles as indicators of early-season pests in Sudan

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Introduction: Seed treatments promote seedling establishment, help ensure yield and reduce quality losses due to many pests and diseases. Protecting cotton plant from the attack of early-season insect pests and diseases is of prime importance to ensure a healthy and strong establishment of this strategic crop.

Poster Presentations

Insecticides

Objectives: The present study tried to measure the susceptibility of cotton flea beetles (*Podagrica* spp.) to the neonicotinoid imidacloprid as a single seed treatment or in a mixture with two antimicrobial pesticides.

Materials and methods: The efficacy of some single pesticides or mixtures at different dosage rates on cotton flea beetles was measured using three different kinds of experiment: visual counts in the field, no-choice semi-field laboratory tests, and no-choice laboratory tests. Flea beetle damage was assessed by counting shot-holes resulting from adult feeding. The data were subjected to appropriate transformation (square root for counts). Statgraf software was used for data analysis (ANOVA). Using the antimicrobial bronopol alone did not prevent flea beetle damage

Results: Treatments containing imidacloprid significantly reduced damage in the three experiments, but not 10 weeks after sowing in field experiments.

Conclusion: This insecticide can be used successfully in integrated pest management programmes to combat early-season pests

P INSECT 10

Lethality and Repellency Effects of Imidacloprid, Thiacloprid and Insecticidal Soap on *Aphelinus mali*, the Parasitoid of Woolly Apple Aphid

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Introduction: The parasitoid *Aphelinus mali* controls woolly apple aphid (WAA, *Eriosoma lanigerum*) in apple orchards of Iran.

Materials and methods: The effects of three pesticides (Imidacloprid, Thiacloprid and insecticidal soap) were evaluated on *A. mali*. To investigate the contact effect, adults were caged for 16 hours in Petri-dishes containing apple leaves sprayed with each insecticide. To determine the oral toxicity in the same period of time, some other Petri-dishes containing a filter paper soaked in a poisoned solution of 10% sugar were used. To determine the repellency effect of the insecticides, we used Busvine Y shaped tube and host choice experiment between treated and untreated leaf disks was carried out.

Results and conclusion: In the contact effect, the results indicated that Thiacloprid caused more mortality rate (77.85%) on *A. mali* compared to other insecticides. In the oral test, the results demonstrated a higher mortality caused by Thiacloprid and Imidacloprid (68.42 and 68.39%, respectively). The repellent effects of test compounds on *A. mali* showed significant differences among treatments. The Imidacloprid and insecticidal soap treatments, had the most and lowest, repellency effects on *A. mali* in comparison to control.

P INSECT 11

Effects of pirimicarb and thiamethoxam on survivorship and detoxification enzymes activity in *Aphis fabae* Scopoli (Hemiptera: Aphididae)

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Aphis fabae Scopoli (Hemiptera: Aphididae), is one of the most important pests of Chenopodiaceae and Fabaceae. We evaluated the effects of thiamethoxam and pirimicarb in sublethal concentrations on the detoxification enzymes. For detection of lethal effects, the bioassay was done by contact method with dry residual pesticides on the *Faba vulgaris* leaf. The median lethal concentration for the aphid exposed to the thiamethoxam and pirimicarb was 113.85 and 2.94 mg(ai)L⁻¹, respectively. For the biochemical tests, the female adults were exposed to the LC₁₀, LC₂₀, LC₃₀, LC₄₀ and LC₅₀ of both insecticides. After 24 hours the survival individuals detected for the enzyme assays. The effects of both insecticides on *A. fabae* caused significant increase in P450 (P<0.0001). Using CDNB as substrate, GST induced in *A. fabae* parallel by increasing the sublethal concentrations of pirimicarb (df=5, 12, F= 18.17, P= 0.0001). Also thiamethoxam in higher concentrations (48.07, 75.06 and 113.85 mg(ai)/L) induced this enzyme (df=5, 12, F= 9.15, P= 0.0009). On the other hand, DCNB as substrate didn't show any observed data in the aphid. Pirimicarb inhibited the aphid AChE (df=5, 12, F=9.88, P=0.0006) and general esterases when alpha-naphthyl acetate was used as a substrate (df=5, 12, F=6.60, P=0.0036). However esterase activity didn't show any significant changes in pirimicarb treatments using beta-naphthyl acetate. In addition to, thiamethoxam didn't have any significant effect on AChE and esterase activity. The results illustrated that thiamethoxam and pirimicarb are two important aphicides that in sublethal concentrations, can be detected by detoxification enzymes as biochemical markers. Consequently, prediction of poisonous effects on the aphid populations in the field will be possible.

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Insecticides

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P INSECT 12

Resistance comparison of different populations of the diamondback moth, *Plutella xylostella* (L.) (Lep.: Plutellidae) to conventional insecticides in central regions of Iran

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The diamondback moth, *Plutella xylostella* (L.), is the most important pest of cruciferous plants in Isfahan, Alborz and Tehran provinces of Iran. The misuse of insecticides against *P. xylostella* has led to several problems such as resistance in many field populations of the pest. The present study aimed to evaluate the efficacy of currently-in-use insecticides against *P. xylostella* in cabbage fields of south Tehran, Mohammad shahre Karaj and Mobarake Isfahan province. The vulnerability of *P. xylostella* larvae to four insecticides, including Indoxacarb, Hexaflumuron, Chlorpyrifos ethyl and Thiodicarb was tested. The leaf-dip method was used for conducting bioassays. Bioassay tests were performed using the insecticides with five different concentrations, six replications for each concentration, and ten third larval instar of *P. xylostella* for each replication under standard environmental conditions (25±2°C, 70±5% RH and 16L:8D h photoperiods). The LC₅₀ value of Indoxacarb, Hexaflumuron, Thiodicarb and Chlorpyrifos ethyl for Mobarake population were 7.72, 7.32, 39.14, and 22.26, ppm. For south of Tehran were 1.02, 5.18, 26.50, and 64.90, ppm and for Mohammad shahre Karaj were 2.35, 11.79, 16.44, and 25.38, ppm, respectively. Pest populations of Mohammad shahre Karaj, Mobarake Isfahan, Mohammad shahre Karaj and Mobarake Isfahan are more resistant to Indoxacarb, Hexaflumuron, Chlorpyrifos ethyl and Thiodicarb insecticides, respectively, compare to other populations.

P INSECT 13

Imidacloprid sublethal effects on the ovarian development of the Neotropical brown stink bugs *Euschistus heros*

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Introduction: Much of the economic losses occurring in soybean plantations in Brazil are related to the action of insect pests, especially the stink bugs. The Neotropical brown stink bugs *Euschistus heros* (Hemiptera: Pentatomidae) is the major pest in most Brazilian soybean fields. The management of this pest is heavily dependent on insecticide applications, especially the neonicotinoids. Sublethal exposure to the neonicotinoids has been associated with enhanced reproductive outputs observed for the *E. heros* in the Brazil, although no scientific proofs were provided so far.

Objectives: This investigation was carried out aiming to evaluate the sublethal effects of the neonicotinoid imidacloprid on the morphology and development of *E. heros* ovaries.

Material and methods: Newly emerged (≤ 24 h) adult females were exposed for 48 h to dry imidacloprid residues (0.042 mg/cm², equivalent to 1% of the field rate dose [375 mg a.i./L]) and their ovarian morphology were assessed over the time (6, 8, 10, 12 and 14 days of adulthood). The control treatment consisted of exposure to distilled water. Adult insects at 2, 4, 6, 8, 10, 12 and 14 days after emergence were used, and the following morphometric parameters were determined on the median ovariole: length (mm), number of follicles, and the area of the most developed follicle (mm²).

Results: Although the exposure to imidacloprid did not alter the number of follicles per ovariole, imidacloprid-exposed females anticipated their ovarian development exhibiting greater ovariole length and a larger area of the most developed follicle in their ovaries up to the 6th day of adulthood.

Conclusion: The ovarian development anticipation might indicate the potential involvement of the imidacloprid sublethal exposures in the recent outbreaks of the Neotropical brown stink bug *E. heros* observed in Brazilian soybean regions.

P INSECT 14

Bio-effectiveness and safety evaluation of an effective IPM compatible juvenile hormone mimic insecticide, pyriproxyfen 10% EC (Daita) [4-phenoxyphenyl (rs)-2-(2-pyridyloxy) propyl ether] against *Myzus persicae* Sulz. infesting chilli in Gangetic alluvial plains of West Bengal, India

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Introduction: *Myzus persicae* is one of the most important pest of chilli responsible for transmission of *chilli vein banding virus* and huge loss in yield. To protect the crop farmers apply several rounds of insecticides which lead to disruption in ecological balance *vis-a-vis* resurgence, resistance and residue problems. In recent past, attempts are being made to develop new IPM compatible molecules of different modes of action. The present study was conducted under field conditions to derive the most effective dose of pyriproxyfen 10% EC as well as its effect on non-target fauna.

Objective: *Myzus persicae* culminate huge economic loss in chilli. The objective of the study was to evaluate the bio-effectiveness of pyriproxyfen 10%EC for management of the pest.

Materials and methods: The experiment was carried out on chilli (cv Canning Bullet) in the growing seasons of 2012 and 2013 in RBD with seven treatments and three replications. Three rounds of sprays were imposed at an interval of 15 days coinciding ETL. Aphid population were recorded at weekly intervals from five randomly selected plants a day before and 1, 2, 7, and 10 days after each application. Among the non target species coccinellids, pollinators and neutrals were recorded. Green fruits were picked at an interval of 14 days. Data thus generated were subjected to ANOVA after suitable transformation wherever necessary.

Results:

Effect on target pest (*Myzus persicae*): In terms of reduction of mean population of aphids at 7 days interval and highest yield with pyriproxyfen 10%EC @500 & 1000 ml⁻¹ ha were statistically on par. At 10 days, the efficacy of the test insecticide reduces to the tune of 60.34 and 64.23 %, respectively.

Effect on Predators, pollinators and neutrals: Seven days after application, the plots treated with pyriproxyfen 500 ml⁻¹ ha showed no mortality of predators (*Coccinella septempunctata* and *Coccinella transversalis*), pollinators (*Apis cerana indica* and *Apis mellifera*) and neutrals. But in case of 1000 ml⁻¹ ha only 2.25 % reduction of their population of were recorded.

Conclusion: Considering the cost effectiveness, pyriproxyfen 10% EC an IPM compatible insecticide at 500 ml⁻¹ ha could be recommended in the sustainable and bio-rational management of *Myzus persicae* in chilli under West Bengal agro-climatic conditions.

Figure 1

Table. Relative efficacy of different treatment schedules of Pyriproxyfen 10% EC against aphid infesting Chilli under Gangetic alluvial plains of West Bengal,India (Mean of three sprayings)

Serial No.	Treatment	Formulation (ml/g/ha)	Pre-treatment count (Mean population / leaf)	% Reduction(-) / increase (+) in population after different days of treatment				Mean % reduction in aphid population over untreated control	Yield (q/ha)	Percent increase in yield over control
				1	2	7	10			
1	Pyriproxyfen 10% EC	300	6.29	49.62 (44.79)*	52.70 (46.55)	60.60 (51.14)	48.37 (44.06)	52.82	11.80	54.88
2	Pyriproxyfen 10% EC	500	7.04	59.12 (50.29)	69.77 (56.69)	85.17 (67.55)	65.37 (54.00)	69.85	13.72	61.02
3	Pyriproxyfen 10% EC	1000	7.58	72.87 (58.50)	76.82 (61.28)	87.12 (69.47)	67.25 (55.13)	76.01	14.92	63.70
4	Pyriproxyfen 5%+ Fenpropathrin 15%EC (Sumigremp)	500	6.33	57.00 (49.06)	56.50 (48.74)	65.37 (53.97)	56.50 (48.75)	58.84	12.22	56.35
5	Pyriproxyfen 5%+ Fenpropathrin 15% EC(Sumigremp)	750	7.54	63.37 (52.81)	71.50 (57.77)	81.50 (64.77)	62.35 (52.17)	69.68	14.57	63.04
6	Thiamethoxam 25WG	200	8.08	74.37 (59.64)	79.12 (62.88)	71.00 (57.43)	59.92 (54.99)	71.10	13.52	60.55
7	UTC		7.17	+9.70 (0.00)	+11.00 (0.00)	+10.75 (0.00)	+17.12 (0.00)	0.00	5.37	-
	CD (p=0.05)			(4.37)	(4.72)	(4.51)	(4.81)		1.15	

* Data in parentheses are angular transformed values. Data were arc-sin transformed and then analyzed by one-way ANOVA. UTC – Untreated control.

Figure 2

Table:II Relative efficacy of different treatment schedules of Pyriproxyfen 10% EC against prevailing natural enemies in Chilli eco-system (Mean of three sprayings) during August, 2011-March, 2012 in Gangetic alluvial plains of West Bengal,India

Serial No.	Treatment	Formulation (ml/g)	Pre-treatment count (Mean population / plant)	Number of Coccinellids after different days of treatment/ five plants			Mean population of pollinators and neutrals/ five plants at different days after treatment			Over all mean population of coccinellids	Over all mean population of pollinators and neutrals*
				1	7	14	1	7	14		
1	Pyriproxyfen 10% EC	300	2.92	1.33 (1.35)	2.33 (1.68)	3.33 (1.95)	0.16 (0.81)	1.00 (1.22)	2.33 (1.68)	2.33	1.16
2	Pyriproxyfen 10% EC	500	3.08	2.33 (1.68)	2.50 (1.73)	3.00 (1.87)	0.00 (0.71)	0.50 (1.00)	1.33 (1.35)	2.61	0.61
3	Pyriproxyfen 10% EC	1000	3.00	1.00 (1.22)	1.33 (1.35)	2.33 (1.68)	0.33 (0.91)	0.33 (0.91)	1.00 (1.22)	1.55	0.55
4	Pyriproxyfen 5%+ Fenprophatin 15% EC (Sumiprospt)	500	2.33	2.00 (1.58)	2.33 (1.68)	2.67 (1.78)	0.33 (0.91)	0.83 (1.35)	1.66 (1.47)	2.33	0.94
5	Pyriproxyfen 5%+ Fenprophatin 15% EC (Sumiprospt)	750	3.33	1.67 (1.47)	2.00 (1.58)	2.33 (1.68)	0.33 (0.91)	0.67 (1.08)	0.67 (1.08)	2.00	0.56
6	Thiamethoxam 25%WG	200	3.04	1.00 (1.22)	1.33 (1.35)	1.67 (1.47)	0.00 (0.71)	0.67 (1.08)	2.67 (1.78)	1.33	0.83
7	UTC	-	2.5	2.00(1.58)	6.00(2.55)	10.00(3.24)	4.00(2.12)	6.00(2.55)	12.00(3.54)	6	7.33
CD (p<0.05)			(NS)	(0.82)	(1.68)	(1.06)	8.00	7.00	7.00		

Data in parentheses are arcsine transformed and then analysed by one-way ANOVA.
 UTC - Un-treated control, NM : No mortality **Predatory complex consisted of: *Coccinella septempunctata* and *Coccinella prasinivaria*.
 *Pollinator fauna consisted with *Apis cerana indica* and *Apis mellifera*

P INSECT 15

The mode of action of novel meta-diamide insecticide, broflanilide, on insect RDL GABA receptor

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Introduction: Broflanilide is a novel meta-diamide insecticide developed by Mitsui Chemicals Agro. Broflanilide and its desmethyl analogue exhibit high insecticidal activity against *Spodoptera litura* (SL) larvae. Desmethyl analogues of meta-diamides acted as antagonist on insect RDL GABA receptor when evaluated by membrane potential assay¹. Radio ligand binding assay also demonstrated that desmethyl analogues bound RDL GABA receptor with higher affinity than N-methyl analogues including broflanilide².

Objectives: To better understand the mode of action of meta-diamide insecticides on insect GABA RDL receptor, electrophysiological experiments were conducted.

Materials and methods: Two-electrode voltage-clamp (TEVC) electrophysiology was performed using *Xenopus laevis* oocytes expressing the SL-RDL GABA receptor (GeneBank: DD171257).

Results: TEVC studies showed that the IC₅₀ value of desmethyl-broflanilide for SL-RDL GABA receptor was 463 pM. In contrast, the IC₅₀ value of broflanilide was greater than 3 μM, the activity being about 1000 times lower than that of desmethyl-broflanilide.

Desmethyl-broflanilide showed the same inhibition activity level against wild type and S285N (S2'N) mutant located in channel pore, although the mutation abolished the inhibition activity of fipronil. In contrast, the G319M mutation located in inter subunit cavity abolished the inhibition activity of desmethyl-broflanilide, although the effect of G319M mutation on that of fipronil was small.

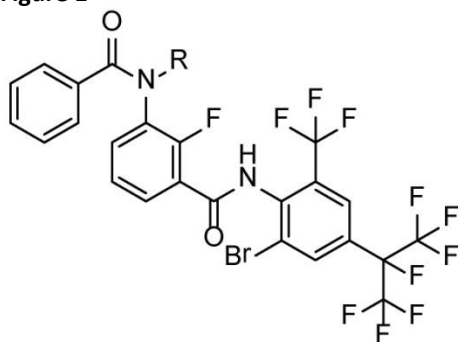
Conclusion: Desmethyl-broflanilide showed high inhibitory activity against SL-RDL GABA receptor, whereas broflanilide did about 1000 times lower than that of desmethyl-broflanilide, suggesting that broflanilide is metabolized to desmethyl-broflanilide to act as an insecticide. Overall, broflanilide is expected to be a novel insecticide that is effective on pests that are resistant to conventional noncompetitive antagonists of the RDL GABA receptors.

References:

- 1) Nakao T, Banba S, Nomura M, Hirase K. *Insect Biochem. Mol Biol.* 2013; **43**: 366-75
- 2) Ozoe Y, Kita T, Ozoe F, Nakao T, Sato K, Hirase K. *Pestic. Biochem. Physiol.* 2013; **107**: 285-92

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Figure 1



R = Me : broflanilide
R = H : desmethyl-broflanilide

Fig. 1 Structure of meta-diamide insecticides

P INSECT 16

A new NeemAzal® technical slow release formulation shows high control levels against larvae of the tomato leaf miner *Tuta absoluta* (Lep., Gelechiidae) and other pests

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Introduction: Plant derived botanical NeemAzal®-T/S is commonly used as foliar application. Because the active ingredient NeemAzal® technical degrades during a few days, a further treatment within 10-12 days is usually necessary. In this regard a new way for NeemAzal® technical application was developed. The new formulation - in the form of sticks applied to the soil - slowly released the a.i. into the soil. The a.i. got absorbed by the plant roots and was translocated into the foliar parts where it was consumed by respective pest insects sucking or biting on the plant's foliage.

Objectives: The overall objective was to demonstrate the slow release of the a.i. NeemAzal® technical into the soil and the subsequent uptake and transport into the leaves. The goal was to show that this type of formulation is suitable for the control of insect pests like leaf miners.

Materials and methods: Mortality of larvae of the tomato leaf miner *Tuta absoluta* was assessed in tomato plants, treated with the new NeemAzal® technical formulation, 7 and 14 days before infestation with first instar *T. absoluta* larvae, respectively.

Results: Emergence of adult *T. absoluta* was significantly lower in treated than in non-treated tomato plants, indicating the good systemic translocation of the a.i. into tomato leaves and the high efficiency against leafmining larvae.

Conclusion: The slowly dissolving granular formulation (the stick) containing NeemAzal® technical showed very good efficacy to control *T. absoluta* larvae and has therefore a high potential against a variety of insect pests. Further studies will thus be conducted against a variety of target insects. Its systemic potential to control leaf and stem-eating insects could be proven.

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P INSECT 17

Key Biological Properties Of a Novel Insecticide Cyclaniliprole

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Introduction: Cyclaniliprole is a new insecticide that is now being globally developed by Ishihara Sangyo Kaisha, Ltd. This compound has a low toxicity to mammals and other non-target organisms such as birds and fishes. The mode of action of cyclaniliprole is categorized into the diamide class (IRAC 28); it activates the ryanodine receptor modulator, accelerating the release of calcium from the sarcoplasmic reticulum and causing continuous muscle contraction in insects.

Objectives: In this study, we describe the key biological properties of cyclaniliprole.

Materials and methods: The key biological properties (target spectrum, rapid action, rainfastness, residual activity, and adulticidal activity) of cyclaniliprole and its activity against strains of diamondback moth (DBM) collected in Japan with low susceptibility to diamides were evaluated in laboratory and pot tests.

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Results: In comparison with other insecticides of the diamide class, cyclaniliprole exhibited a high level of insecticidal activity against a broad spectrum of Lepidoptera, Hemiptera, Thysanoptera, Diptera, and Coleoptera. The larvae of Lepidoptera exposed to this compound through oral ingestion or even direct skin contact rapidly stop feeding, become paralyzed, and finally die within several days. In the residual activity and rainfastness test, cyclaniliprole exhibited long-lasting protection in the form of larvicidal activity against common worm on cabbage. Compared with other diamides, cyclaniliprole exhibited high adulticidal activity and consequently reduced the number of eggs laid by peach fruit moth. In addition, in comparison with other diamides, cyclaniliprole exhibited high larvicidal activity and thereby prevented invasion of the fruit. This compound also exhibited good performance against DBM with low susceptibility to other existent diamides.

Conclusion: Cyclaniliprole had a wide target spectrum and rapid action and exhibited inhibition of feeding behavior, long residual activity, and high adulticidal activity. Although cyclaniliprole is categorized into the diamide class, it exhibited high activity against strains with low susceptibility to other existent diamides. Cyclaniliprole can therefore become an excellent tool for use in insect pest control programs and resistance management strategies in crop protection.

P INSECT 18

Study of effects of some entomopathogenic bacteria isolated from Algerian soil against *Locusta migratoria*

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Soil bacteria are of particular importance for crop production. Various bacteria are able to fix atmospheric nitrogen, while others play major roles in the cycles of certain fertilising elements (Fuchs, 1999; Kowalchuk and Stephen, 2001). Other bacteria protect plants against pests. Most of these bacteria produce antibiotics or toxins that seem to play a significant role in the mechanisms of plant protection (Davet, 1996; Alvarez *et al.*, 2002; Van Loon, 2007).

It is in this context that our work focuses on the isolation and identification of some bacterial strains from Land to valorize into the best their entomopathogenic effectiveness towards the locust migratory *Locusta migratoria* in order to broaden their spectrum of action.

A total of 17 bacterial strains were isolated from in the Algerian desert soil. They are mobile aerobic, with a positive catalase.

From these bacterial strains, four were selected for their entomopathogenic power and identified based on their genetic traits. The rDNA16S sequences of these named strains (B3, B4, B5 and B6) were recorded in the EMBL / EBI data bank and their phylogenetic analysis revealed that they belong to the genera *Pseudomonas*, *Bacillus* and *Enterobacter*.

This work is achieved by highlighting the selected entomopathogenic activity against larvae of the fifth stage of the migratory locust *Locusta migratoria*. The results obtained one week after treatment show that the treated larvae were highly sensitive to our isolated bacteria, with mortality rates of 100 %, 98 %, 71 % and 65 % respectively for the obtained *Pseudomonas sp.* Strain B3 (HF911369), *Pseudomonas sp.* Strain B4 (HF911366) *Enterobacter sp.* Strain B6 (HF911368) and *Bacillus sp.* Strain B5 (HF911367), in comparison with the controls (3.33%).

These isolates could find then their places in biotechnological applications aiming at improving yields and preserving the environment for sustainable development

P INSECT 19

Genetic Characterization of cry Gene Diversity in *Bacillus thuringiensis* Isolates from Kyrgyzstan

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Question: The Gram-positive bacterium *Bacillus thuringiensis* (currently referred to as "Bt") is the most important entomopathogen for insect biocontrol. Bt kills its host through the action of highly specific, crystal-forming Cry protein toxins (in addition to further toxin types). A large number of cry toxin encoding genes have been analyzed and organized into several groups that in part reflect host group adaptation. In particular, proteins encoded by cry1, cry3, and cry4 genes are generally toxic for Lepidopteran, Coleopteran, and Dipteran insects, respectively.

The present study addresses the characterization of cry genes present in a set of Bt strains that have previously been isolated from insect and soil samples from different environments in Kyrgyzstan.

Methods: A PCR-based diagnostic approach using cry1, cry3, and cry4 gene specific primer sets was employed in a first assessment of cry gene diversity in Kyrgyz *B. thuringiensis* isolates.

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Results: Experiments have revealed pronounced differences in the frequency of cry genes in Kyrgyz Bt isolates. Whereas cry1 and cry4 genes were regularly detected, cry3 genes were found present in only a small number of strains investigated. Several copies of cry1 and cry4 genes appeared to be present simultaneously in numerous isolates. Interestingly, at least single copies of cry1 and cry4 genes appeared combined frequently, whereas the combination of cry1 and cry3 occurred only in a single Bt strain.

Conclusions: A high degree of cry gene diversity is present within the set of *B. thuringiensis* isolates from Kyrgyzstan. The rather regular presence of several cry gene copies, potentially combining protein toxins of different specificities, within a single strain is of high interest with respect to the possible application of these strains for biocontrol purposes.

Experiments aiming towards the further exploration of the biodiversity of entomotoxins - including further cry as well as vip or cyt genes - in *Bacillus thuringiensis* strains isolated in Kyrgyzstan are currently under way.

P INSECT 20

Monitoring of insecticide resistance in pollen beetle (*Meligethes aeneus* F.) populations in Denmark

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Oilseed rape (*Brassica napus* L.) is one of the most important oil crops in the world after soybean. In the northern countries, the pollen beetle (*Meligethes aeneus* F.) is a major pest in winter oilseed rape. Insecticides from the group of pyrethroids were for more than two decades the only available control method. A metabolic resistance developed where the first case was reported in France in 1999 (Zimmer and Nauen, 2011). Since then metabolic resistance of pollen beetles spread across Europe, which causes serious problems in the control of the pollen beetle. The monitoring of the current susceptibility level against λ -cyhalothrin and thiacloprid of 52 Danish pollen beetle populations was conducted in 2014. Furthermore experiments were designed to evaluate fitness parameters of pollen beetles with different levels of susceptibility towards λ -cyhalothrin. Monitoring results from 2014 and primary results from fitness studies will be presented and discussed.

Reference:

Zimmer, C. T., and Nauen, R. (2011). Pyrethroid resistance and thiacloprid baseline susceptibility of European populations of *Meligethes aeneus* (Coleoptera: Nitidulidae) collected in winter oilseed rape. *Pest Manag Sci* **67**, 599-608.

P INSECT 21

Dropleg - an innovative application method for oilseed rape

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The dropleg application method was originally invented for specific vegetable uses. A dropleg sprayer consists of tubes extending vertically downwards from the boom, on which are mounted one or more horizontally-aligned spray nozzels. This allows application to the leaves from below the level of the flowers. It has been tested by Bayer CropScience over the past years with the aim to optimize the spray coverage on lower leaf layers, to minimize spray drift and to reduce product residues. Small plot and large scale field studies with Biscaya for the control of *Meligethes aeneus* and *Dasineura brassicae* were carried out at nine locations in Germany in 2014. The average efficacy values obtained were slightly lower as compared to overhead application, whereas rapeseed yield benefit compared to untreated could be demonstrated. Few data points from fungicide trials performed under low infection situations revealed no significant differences between overhead and dropleg application. The focus of the 2015 field trials program will be to confirm efficacy against key pests and diseases. Results will be part of the presentation to be given during the congress.

P INSECT 22

Monitoring of insecticide resistance levels and investigation of toxicodynamic resistance mechanism to carbamate insecticide in *Nilaparvata lugens*

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Nilaparvata lugens Stål is one of the important migratory pests of rice paddy fields in Korea. Resistance levels to nine insecticides were monitored in 12 local strains. The local strains revealed 1.3- to 28.0-, 1.6- to 6.0-, 2.8- to 237.0-, 0.6- to 0.9-, and 0.7- to 1.3-fold resistance to carbamates, organophosphates, neonicotinoids, fipronil and etofenprox, respectively. Molecular mechanisms of carbamate insecticide resistance were investigated by cloning of the type-1 AChE. The open reading frame of Nlace1 is composed of 1,989 -bp (approximately 74 kD) and revealed 52.5% amino acid sequence identities to those of *Nephotettix cincticeps*. Screening of point mutations identified four amino acid substitutions (G119A, F/Y330S, F331H and I332L) in the resistant strain that likely contribute to AChE insensitivity. The frequencies of these mutations were well correlated with resistance levels, confirming that they are associated with reduced sensitivity to carbofuran. For the rapid detection of resistance levels, a quantitative sequencing (QS) protocol was established to determine the allele frequency of point mutations. The allele frequencies of the four mutations (G119A, F/Y330S, F331H and I332L) in field strains ranged from ca. 0.0~51.7%, 0.0~88.9%, 2.5~47.7%, 5.1~56.0% and 6.7~57.3%, respectively. The F331H and I332L were tightly linked each other, suggesting these mutations may occur simultaneously. A QS protocol would be employed as a supportive tool for rapid monitoring of CB insecticide resistance levels in *N. lugens*.

P INSECT 23

Fungicide susceptibility, genetic characterization, and strain-specific diagnosis of *Lecanicillium* fungi, potential aphid biocontrol agents

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Question: Mitosporic fungi of the genus *Lecanicillium* are of particular interest as biological control agents for phloem-sucking plant pests including aphids. Bioprospection for these fungi in Argentina has given rise to a set of single-spore derived *Lecanicillium* strains isolated from a wide range of original hosts.

Reliable molecular taxonomic markers and diagnostic tools are highly solicited for these fungi. Fungicide susceptibility of *Lecanicillium* strains is of particular interest in view of both co-applicability with agro-fungicides and the development of selection markers for basic research.

Species-level characterization, diagnostic tool development and fungicide susceptibility assessment for *Lecanicillium* isolates from Argentina were aims of the present study.

Methods: A set of five genetic markers comprising one mitochondrial (NMS) and two nuclear (ITS, IGS) ribosomal RNA operon together with one mitochondrial (*nad1*) and one nuclear (*ef1a*) protein-encoding sequences, has been employed for species-level characterization.

The development of strain-specific diagnostics has been based on self-splicing group-I introns located within the rRNA encoding genes.

Susceptibilities to several fungicides were determined using a semi-quantitative test on solid media containing the respective compound.

Results: A subset of markers permitting the unequivocal distinction of the three *Lecanicillium* core species, *L. lecanii*, *L. muscarium*, and *L. longisporum*, was defined. Most, but not all of the Argentine isolates were shown to belong to these species.

Feasibility of strain-specific diagnosis for *Lecanicillium* fungi was demonstrated for one potential biocontrol isolate.

Between strain differences in fungicide susceptibilities were found important and not necessarily in line with systematics. However, the fungicidal polyketide compound soraphen shows outstanding activity against a wide variety of isolates from all species.

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Conclusions: The results obtained fully confirm the view that for *Lecanicillium* fungi sound molecular taxonomic characterization and fungicide sensitivity determination are both feasible and inalienable prerequisites and important criteria of biocontrol agent selection.

P INSECT 24

Investigating the mechanisms involved in diamide resistance in tomato borer *Tuta absoluta*

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Introduction: *Tuta absoluta* (Lepidoptera, Gelechiidae) is a major pest of tomato that was initially endemic in South America. Within a few years, after invading Europe, it rapidly expanded to Africa and Asia becoming a global threat for tomato production. Pest control is largely based on chemical insecticides. The diamides flubendiamide and chlorantraniliprole are amongst the novel and effective chemistries registered for *T. absoluta* control however resistance development to these chemistries was recently reported in Europe. High resistance levels (RR>1000-fold) were detected in Italy while moderate resistance levels were detected in Greece (RR>10-fold).

Objectives: The aim of this study was to investigate the mechanisms involved in diamide resistance.

Materials and methods: To investigate the involvement of metabolic resistance the effect of synergists (i.e. PBO and DEF) on insecticide toxicity in standard bioassays was estimated. We also investigated the differences between resistant and susceptible strains regarding the activity of common detoxification enzymes such as carboxylesterases, glutathione S-transferases and cytochrome P450 monooxygenases. Target site resistance was investigated by sequencing the putative diamide C-terminal binding site region of the ryanodine receptor (RyR). Finally we examined the stability of resistance in absence of selection pressure.

Results: Use of synergists exhibited no effects on toxicity levels in both moderately or highly resistant strains. No difference in the activity of detoxification enzymes was detected, except for P450s that investigation is still ongoing. Sequence analysis revealed a novel amino acid substitution at the binding site region of the RyR. In the absence of selection pressure the resistance remains stable.

Conclusion: Initial results indicated that metabolic resistance may not be the major resistance mechanism. Resistance stability suggests limited fitness cost conferred by the mechanisms involved in diamide resistance in *T. absoluta*. The association of this mutation with diamide resistance is currently investigated. In addition investigations on the genetics of resistance are also conducted in order to check the underlying mode of inheritance.

P INSECT 25

Imidacloprid sub-lethal effects on mating and reproductive performances of the Neotropical brown stink bugs *Euschistus heros*

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Introduction: The Neotropical Brown stink bug *Euschistus heros* (F.) is one of the most important pest species in Brazilian soybean. Its control is achieved mainly by applications of neonicotinoid insecticide imidacloprid. Increases on the density of naturally occurring populations of *E. heros* has been registered over last few years in Brazil and it has been related, without no scientific proof so far, to increases on the reproductive output induced by sublethal exposure to imidacloprid.

Objectives: This investigation was carried out aiming to evaluate whether sublethal exposure to imidacloprid results in changes on the mating and reproductive performances of *E. heros*.

Material and methods: Groups of newly emerged (< 24 h) adults (males and females) were exposed for 48 h to dry imidacloprid residues (0.042 mg/cm², equivalent to 1% of the field rate dose [375 mg a.i./L]). The insects were kept separately for 13 days to reach the sexual maturity. Then, virgin male and female were coupled in four different combinations (untreated female and male, treated female and untreated male, treated female and treated male and finally untreated female and treated male) and filmed for 13h. After that, the males were removed. The film analysis allowed evaluating the duration of courting; number of times mating and the duration of mating for each couple. Twenty mated females and 20 mated males per combination were

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then separately monitored for 60 days and number of laid eggs per female, number of egg- masses per female, egg hatching and survival rates were recorded daily.

Results: Mating and survival rates were not affected by the imidacloprid exposure but fecundity and fertility parameters differed among the treatments. The time to the first viable egg laying was shorter for the treated male and untreated female couples. A higher number of eggs per day per female was registered for the couples where only males were treated. The fertility rate was higher for the couples where only males were treated and its pick was delayed 6-7 days for couple combinations where the females are treated.

Conclusions: Our results showed that the sublethal exposure to imidacloprid induces alterations on the reproduction parameters of *E. heros* and that the effects are sex dependent, which might contribute to the recent outbreaks of *E. heros* observed in Brazilian soybean fields.

P INSECT 26

Characterization of thiamethoxam resistance in *Liriomyza sativae* Blanchard (Dip.: Agromyzidae)

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Introduction: Vegetable leafminer (VLM) is a key pest of vegetable crops and ornamental plants in greenhouses and fields worldwide. Biological characteristics of this pest and reliance on chemical control may lead to development of resistance against insecticides in this pest.

Objectives: The study was conducted to determine some characteristics of thiamethoxam resistance in VLM to be used in integrated management of this pest.

Materials and methods: Two Iranian strains of VLM with proven resistance to thiamethoxam were used in this study. Enzyme assays for general esterases (EST), glutathione S-transferases (GST) and monooxygenases (MFO) were performed on adults and larvae of *L. sativae*. These strains were tested for cross-resistance to azadirachtin, cyromazine, chlorantraniliprole and spinosad. The resistant strains were also reared in absence of pesticide pressure and tested periodically to check for stability of resistance.

Results: The activity of GST in the resistant strains was not significantly different from that of the susceptible strain. The activity of EST and contents of MFO in resistant strains were higher than the susceptible strain. The results of biochemical assays revealed that EST and MFO played a role in resistance of *L. sativae* to thiamethoxam. These strains did not show cross-resistance to azadirachtin, chlorantraniliprole and cyromazine. Only a slight cross resistance to spinosad was observed in these strains. Resistance ratios decreased in the strains after 10 generations of rearing in pesticide free conditions.

Conclusion: Biochemical and cross-resistance assays in this study and those of other researchers suggest that increased activity of metabolic enzymes and reduction in target site sensitivity are joint factors involved in *L. sativae* resistance to thiamethoxam. Resistance to thiamethoxam in *L. sativae* was unstable in absence of pesticide pressure. Therefore, thiamethoxam can be used in rotation with other effective insecticides such as azadirachtin, chlorantraniliprole, cyromazine and spinosad in order to manage insecticide resistance in leafminer control programs.

P INSECT 27

The effect of neonicotinoid Actara 25 WG on the feeding activity of the large pine weevil *Hylobius abietis* L.

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Introduction: The large pine weevil, *Hylobius abietis* L. (Coleoptera: Curculionidae) is an important pest of conifer seedlings and transplants in commercial forests of Europe. The adults feed on the bark of young seedlings often killing or damaging them severely. Various chemical insecticides have been used against *H. abietis*. In Estonia pyrethroids have been recently replaced with neonicotinoid Actara 25 WG (a.i. thiametoxam) to protect young conifers from damage caused by pine weevils.

Objectives: The aim of the study was to test the influence of Actara 25 WG on the feeding behaviour of the adult *H. abietis*, and to assess the antifeedant effect of thiametoxam on weevils.

Materials and methods: In the experiment the effect of Actara 25 WG lethal (0.2%) and sub-lethal (0.05%) concentrations were tested on *H. abietis* feeding activity in choice-feeding tests under laboratory conditions. The ends of the Scots pine twigs were dipped in melted bees wax and the twigs were then dipped in a solution. The weevils' feeding area was estimated from the removed (consumed) outer cortex bark and phloem after 24 h, 48 h and 72 h for each twig. The laboratory results were compared using antifeedant index (AFI).

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Results: Higher concentration (0.2%) had very strong lethal effect to *H. abietis*, insects died during 24 hours (Table 1). Sub-lethal concentration reduced significantly the feeding activity of weevils in comparisons with control. 72 hours later 50% of females and 75% of males were dead due to lower dose. The males were more sensitive to thiametoxam compared with the females.

Conclusions: Neonicotinoid Actara 25 WG had a strong antifeedant effect on the large pine weevil *H. abietis* in laboratory conditions.

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Table 1: Mean feeding bark and phloem area ($\text{mm}^2 \pm \text{SD}$) of *H. abietis* females (♀♀) and males (♂♂) depending on the Actara 25 WG treatment of Scots pine twigs and feeding time. Antifeedant index (AFI) indicates the antifeedant effect of different concentrations. AFI < 0 - feeding stimulant, AFI = 0 - no effect, AFI > 0 - antifeedant.

Feeding time, h	Weevil sex	Treatments					
		Actara 25 WG 0.2%	Control	AFI	Actara 25 WG 0.05%	Control	AFI
24	♀♀	11.8±8.9	40.4±31.1	0.55	12.0±11.4*	48.4±45.0	0.60
	♂♂	11.6±5.0*	9.3±12.8	-0.11	11.0±7.3*	27.4±25.3	0.43
48	♀♀	dead	dead	-	17.6±7.7*	59.1±59.8	0.54
	♂♂	dead	dead	-	11.1±7.2*	38.4±43.6	0.55
72	♀♀	dead	dead	-	18.0±7.6*	59.1±59.8	0.53
	♂♂	dead	dead	-	11.6±7.0*	62.6±107.3	0.69

*Significant difference between treated and untreated control twigs ($p \leq 0.05$; Wilcoxon test) (n = 40)

P INSECT 28

This Study was carried out to evaluate the effectiveness of bacterial insecticide (Vertimic) of the fourth star larve of *Trogoderma granarium* (Everts)

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This Study was carried out to evaluate the effectiveness of bacterial insecticide (Vertimic) of the fourth star larve of *Trogoderma granarium* (Everts) by four treatments (A, B, C, D) at seven concentrations 100, 10, 1, 0.1, 0.01, 0.001 PPM in the treatment A, B and at four concentrations 1, 0.1, 0.01, 0.001 PPM in the treatment C, D. Mortality rate of larve was 100% at concentration 1000, 100 PPM in the treatment A and B after 24 hours, and after 48 hours in the treatment D at 1 PPM. The efficiency of the treatment A was greater as compared to the treatment B at all concentrations and all exposure times. For example at 0.01, 0.1, 1, 10, 100 PPM and after 216 hours the Mortality rate of larve was 3.33, 13.33, 43.33, 100.00% in the treatment A respectively and 0.00, 0.00, 3.33, 23.33, 96.67% in the treatment B respectively. The efficiency of treatment D was greater as compared to the treatment C in all concentrations and all exposure times for example at 0.001, 0.01, 0.1, 1 PPM and after 120 hours the Mortality rate of larve was 6.67, 13.33, 43.33, 100.00% respectively in the treatment D and 0.001, 0.01, 0.1, 1 PPM respectively in the treatment C.

P INSECT 29

Identification and characterization of a novel mu class GST from citrus red mite, Panonychus citri (McGregor)

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Background: The citrus red mite (*Panonychus citri*) is a worldwide citrus pest and has developed severe resistance to different types of acaricides. However, the molecular mechanisms of resistance to acaricides in this mite remains unknown. In this study, one full length of cDNA encoding glutathione S-transferases (GSTs) was identified and characterized in *P. citri*. The effect of abamectin and citral exposure on this gene expression was also investigated.

Results: Phylogenetic analysis revealed that this GST gene belongs to mu class. After both the exposure of abamectin and citral, the relative expression of *PcGSTm5* were up-regulated. When treated with the LC₁₀ of abamectin, after the treatment of 12h, 24h and 36h, the expression of *PcGSTm5* was increased to 19.5, 19.8 and 5.9-fold, respectively. Similarly, the fold change of relative expression of *PcGSTm5* after the treatment of citral LC₁₀, was also increased to 14.6, 2.3 and 7.6-fold.

Conclusion: This is a novel GST gene characterized in *P. citri* and its molecular characteristics was investigated. The elevated transcripts of *PcGSTm5* after exposure to abamectin and citral in *P. citri* might be one of the mechanisms of detoxification to this acaricide and secondary compounds of citrus.

P INSECT 30

The effect of neonicotinoid Actara 25 WG and botanical insecticide NeemAzal-T/S on the mortality of the carabid beetle, *Pterostichus aethiops* Panz

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Introduction: Carabid beetles (Coleoptera: Carabidae) are important polyphagous natural pest control agents in agricultural fields. Pesticides threaten the survival of non-target carabid beetles living in both treated and untreated areas, because carabids move relatively fast. They may contact pesticides directly or feed on pesticide-treated seeds and pests.

Objectives: The aim of the study was to test the effect of widely used neonicotinoid Actara 25 WG (a.i. thiametoxam) and botanical insecticide NeemAzal-T/S (a.i. azadirachtin) on the predatory adult carabid beetle *Pterostichus aethiops* Panz.

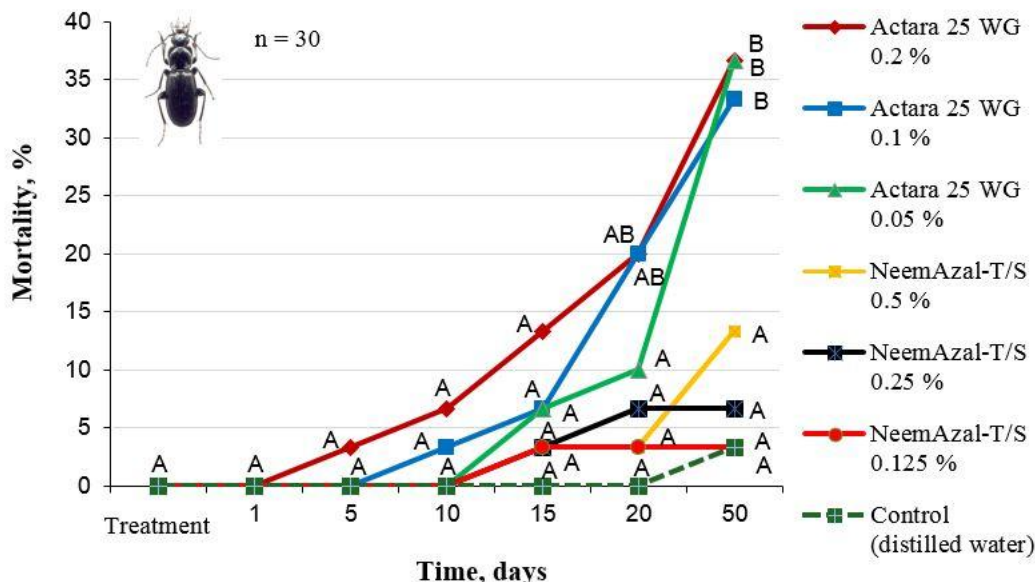
Materials and methods: In the experiment the effect of neonicotinoid Actara 25 WG lethal (0.2%) and sub-lethal (0.1%, 0.05%) concentrations were tested on *P. aethiops* mortality in laboratory conditions. The results were compared with NeemAzal-T/S lethal (0.5%) and sub-lethal (0.25%, 0.125%) concentrations. 5 µL of solution was applied between the beetles' thorax and abdomen using distilled water as the solvent. Beetles were fed with moistened cat food and provided clean water daily. The mortality rate was registered within 50 days after treatment.

Results: Botanical insecticide NeemAzal-T/S did not have the influence on *P. aethiops* mortality compared to control (distilled water). The faster impact on insect mortality caused the Actara 25 WG recommended concentration (0.2%) (Figure 1). Both tested insecticides caused paralysis in beetles, whereas neonicotinoid had stronger impact.

Conclusions: Carabid beetle *P. aethiops* is vulnerable to neonicotinoid Actara 25 WG. When neonicotinoids are applied to agricultural land their toxic effect on predaceous beetles should not be ignored.

The study was supported by the projects: IUT36-2, 8-2/T12115MIMK, ETF9449.

Figure 1: The dynamics of Actara 25 WG and NeemAzal-T/S treatments effects on the mortality of the adult carabid beetle *Pterostichus aethiops*. Means followed by the same letter are not significantly different ($p \leq 0.05$) (ANOVA, Tukey HSD test).



P INSECT 31

Morphological measurements and chemical control of tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae)

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Leaves infested by *T. absoluta* were collected from a tomato field in Beni-Swaif Governorate, and transferred to the laboratory. They were examined under a binocular in order to differentiate the different immature stages by measuring length and diameter of eggs, head capsules and bodies of the four larval instars, and bodies of pupae and adults using a micrometer lens. Egg was 0.28 - 0.38 mm in length and 0.18 - 0.28 mm in diameter. The length of head capsules were 0.11-0.14, 0.175-0.245, 0.28-0.35 and 0.46-0.56 mm for the 1st, 2nd, 3rd and 4th larval instars, respectively. On the other hand the main of the body's length, were 0.99, 2.33, 4.17 and 7.54 mm for the 1st, 2nd, 3rd and 4th the larval instars, respectively. The pupal body length was 4.03 - 4.66 mm. and adult was 5-6 mm. Efficacy of emamectin benzoate, chlorfenapyr, indoxacarb, spinetoram and chlorantraniliprole was conducted against *T. absoluta* adults (one day old) in laboratory. Samples of tomato leaves contains different larval instars of *T. absoluta* were collected in April, 2012 from commercial fields in Fayoum Governorate, and transferred to a holding room in bio-control Lab, and the values of LC50 for each insecticide was 7, 18, 20, 22.5 and 24 ppm, respectively, and LC90 was 1000, 265, 500, 500 and 170 ppm, respectively. Field trials were carried out in 2012 and 2013 growing seasons to evaluate the efficacy of tested insecticides against larvae of *T. absoluta* infesting tomato plants in Fayoum and Giza Governorates. The candidate insecticides; Coragen 20% SC, Evisect S 50% SP, Challenger 36% SC, Avaunt 15% SC, Proclaim 5% SG, Radiant 12% SC, and Tracer 24% SC were applied at their recommended rates of application 60 ml, 125 g, 45 ml, 25 ml, 120 g, 100 ml and 30 ml, respectively. The tested insecticides can be arranged descendingly as Coragen (77.56%), Evisect (67.79%), Proclaim (57.9%), Challenger (52.07%), Avaunt (44.6%), Radiant (41.83%) and Tracer (10.91%) according to their efficacy against *Tuta absoluta* larvae after ten days of application. Second season 2013 showed the same results for the first one except Evisect S became more effective than Coragen with a percentage (89.3%).

Figure 1



Fig. (1): General view of different stages and larval instars of *T. absoluta*.



Fig. (2): The head capsules of different larval instars of *T. absoluta*.



Fig. (3): The head capsules of different larval instars of *T. absoluta* separately.

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Figure 2

TABLE (IV)
Percent infestation of the first, fourth and seventh leaflets of tomato leaves by *T. absoluta* larvae during the experimental period of season 2012

No	Insecticides	Rate/ 100L	Before application				After 5 days of application				After 7 days of application				After 10 days of application				General mean
			1 st	4 th	7 th	Mean	1 st	4 th	7 th	Mean	1 st	4 th	7 th	Mean	1 st	4 th	7 th	Mean	
1	Coragen	60 ml	71.25	77.55	76.6	75.13	26.04	39.81	31.40	32.42	21.43	20.00	23.29	21.57	0.00	0.00	0.00	0.00	18.00
2	Evisect S	125g	68.75	89.29	69.74	75.93	17.10	26.39	23.45	22.31	15.38	30.19	23.40	22.99	31.51	27.78	40.80	33.36	26.22
3	Challenger	45 ml	70.00	72.0	73.08	71.69	28.46	40.43	34.91	34.60	29.89	40.37	34.50	34.92	41.84	44.21	40.26	42.10	37.21
4	Avaunt	25 ml	68.75	67.24	67.00	67.66	32.43	41.18	40.34	37.98	23.08	30.09	27.72	26.96	61.90	54.56	55.83	57.43	40.79
5	Proclaim	120 g	67.50	68.83	66.67	67.67	24.04	34.21	36.44	31.56	31.46	30.38	32.26	31.37	37.00	31.67	20.00	29.56	30.84
6	Radiant	100 ml	66.25	61.39	60.46	62.70	30.0	28.97	32.70	30.56	21.15	22.30	33.06	25.50	71.00	55.83	64.08	63.64	39.90
7	Tracer	30 ml	72.50	69.37	92.00	77.96	62.67	58.51	62.5	61.23	71.0	63.64	73.30	69.31	88.78	72.30	88.89	83.32	71.29
8	Control	---	78.75	88.89	77.46	81.70	85.05	92.03	82.69	86.59	86.26	91.24	84.87	87.46	94.35	90.62	74.76	86.58	86.88

TABLE (V)

Percent reduction in alive larvae of *T. absoluta* in the first, fourth and seventh leaflets of tomato plants

No	Insecticides	Rate/ 100L	After 5 days of application				After 7 days of application				After 10 days of application				Mean			General mean
			1 st	4 th	7 th	Mean	1 st	4 th	7 th	Mean	1 st	4 th	7 th	Mean	1 st	4 th	7 th	
1	Coragen	60 ml	66.16	50.41	61.59	59.39	72.73	74.88	72.24	73.28	100	100	100	100	79.63	75.10	77.94	77.56
2	Evisect S	125g	77.00	68.29	68.49	71.26	79.58	67.07	69.37	72.01	61.73	69.48	49.10	60.10	72.77	68.28	62.32	67.79
3	Challenger	45 ml	62.35	45.76	55.24	54.45	61.02	45.39	56.90	54.44	50.09	39.76	52.07	47.31	57.82	43.64	54.74	52.07
4	Avaunt	25 ml	56.32	40.84	43.58	46.91	69.34	56.41	62.23	62.66	24.82	20.40	27.50	24.24	50.16	39.22	44.44	44.60
5	Proclaim	120 g	67.02	51.99	48.79	55.93	57.45	57.01	55.82	56.76	54.23	54.86	73.90	61.00	59.57	54.62	59.50	57.90
6	Radiant	100 ml	58.07	54.41	49.32	53.93	70.84	64.62	50.08	61.85	10.51	10.79	7.79	9.70	46.47	43.27	35.73	41.83
7	Tracer	30 ml	20.00	18.52	23.55	20.69	10.60	10.65	12.63	11.29	0.0	2.24	0.0	0.747	10.2	10.47	12.06	10.91

P INSECT 32

Bioinformatics analysis of amino acid sequence of acetylcholinesterase from *Bemisia tabaci* Genn

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Methods: Sequence analysis was aligned using ClustalX 1.83 package. Sequence homology was analyzed by DNASTar software. We modeled three-dimensional model of acetylcholinesterase structure using the SWISSMODEL web server, which can search for similar templates of available structures from the PDB, and SWISSpdb viewer 4.01 was used to generate a three-dimensional image. In addition, phylogenetic trees of amino acid sequences were created using MEGA3.1 program by neighbor-joining method and the bootstrap test was carried out with 1000 iterations.

Results: We compared the amino acid sequences of AChEs from the organisms tested and found that amino sequence of AChEs showed biggish evolution and conservation. The amino acid sequence of AChEs from *Bemisia tabaci* was obtained and analyzed. Phylogenetic tree was created based on the amino acid sequence of AChEs in which can exhibited the phylogenetic relationship of the organisms tested. Homology modeling was completed and the AChEs structure and key amino acid points from the aphids tested was viewed. This paper also discussed the *B. tabaci* AChEs structure characteristics and functional sites.

Discussion: Acetylcholinesterase is a serine hydrolase that serves principally to terminate signal transmission at cholinergic synapses by rapid hydrolysis of the neurotransmitter acetylcholine (ACh) in the synaptic gap. The alignment of amino acid sequences revealed that AchEs from *B. tabaci* and AchEs from the other pests had sequence and functional similarities, especially in the key substrate binding sites. Previous studies found that fourteen aromatic amino acid residues lining the catalytic gorge played an important role in the combination of AChE with the substrate, which may accelerate the substrate to the active sites of AChE. The result of multiple alignment showed that eight aromatic residues lining the catalytic gorge were different in tested materials. Some of these alternatives may be more beneficial or detrimental to identify the substrate. Studies have found that the replaced aromatic amino acid residues played the important role in the insect resistance to insecticides.

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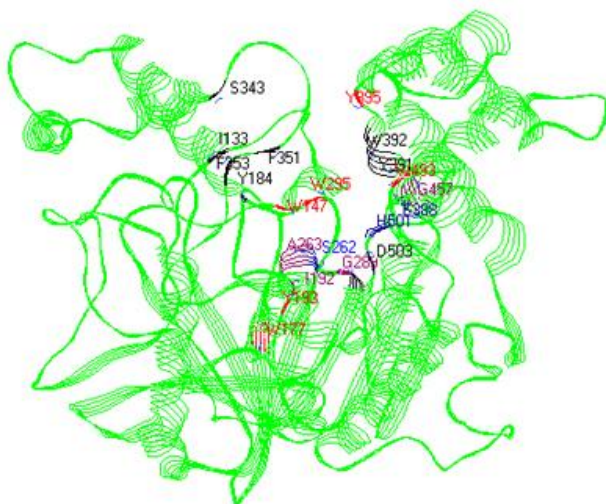
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Figure 1

		Percent Identity																
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15		
Divergence	1	■	81.1	64.4	64.4	68.4	68.3	60.2	59.7	60.1	60.2	61.6	44.9	34.5	34.5	34.8	1	JF343436
	2	25.6	■	67.1	67.3	69.4	69.2	61.6	61.4	61.9	62.0	63.6	45.5	33.9	33.9	34.4	2	HQ260968
	3	50.8	44.1	■	83.9	62.7	62.6	59.9	59.6	59.7	60.2	61.7	42.8	32.9	32.9	31.1	3	AY970293
	4	49.2	42.3	17.8	■	62.2	62.1	60.5	60.1	60.3	60.9	62.3	42.1	33.4	33.4	30.0	4	DQ186605
	5	46.4	43.7	50.4	50.0	■	99.9	60.7	60.3	60.6	61.0	63.6	45.0	33.7	33.7	35.8	5	EF675188
	6	46.6	43.9	50.7	50.3	0.2	■	60.7	60.3	60.6	60.9	63.6	44.9	33.6	33.6	35.7	6	EF675187
	7	60.3	56.3	65.4	61.7	55.2	55.2	■	98.6	97.1	97.4	96.7	41.5	32.5	32.5	30.7	7	XP-001948653
	8	61.6	56.9	66.4	62.7	56.1	56.1	1.6	■	96.7	96.9	96.4	41.4	32.5	32.5	30.7	8	AAV68493
	9	60.6	55.7	66.0	62.0	55.5	55.5	3.3	3.8	■	97.9	95.7	41.4	32.3	32.3	30.4	9	AAT76530
	10	60.3	55.4	64.7	60.7	54.5	54.8	3.0	3.6	2.4	■	95.4	41.6	33.0	33.0	30.8	10	BAD51408
	11	56.2	51.0	59.5	55.7	49.8	49.8	3.3	3.6	4.5	4.8	■	42.8	33.7	33.7	31.9	11	AAN71600
	12	112.1	110.3	107.7	106.6	106.9	107.5	112.2	112.9	112.9	111.6	102.4	■	33.9	33.9	40.3	12	CAA27169
	13	126.9	128.4	140.6	139.6	128.7	129.4	140.9	140.9	141.7	137.8	136.4	115.6	■	100.0	47.6	13	EF675190
	14	126.9	128.4	140.6	139.6	128.7	129.4	140.9	140.9	141.7	137.8	136.4	115.6	0.0	■	47.6	14	EF675189
	15	128.2	130.2	128.9	130.0	120.2	120.9	128.9	128.9	130.5	128.2	121.1	126.1	66.5	66.5	■	15	CAA29323
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15			

Figure 2



P INSECT 33

Resistance to Imidacloprid in Different populations of *Aphis gossypii* Glover (Hem.: Aphididae) in Fars Province, Iran

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The cotton aphid, *Aphis gossypii* (Glover) (Homoptera: Aphididae), is a key cucurbits pest in Iran and Fars Province. In Iran in addition to cotton, it is the major pest of Cucurbitaceae, especially on cucumber. *A. gossypii* causes direct damage through sucking nutrients from the plant and indirect damage through contamination with honeydew and by vectoring viral pathogens. It is managed with repeated insecticide applications. Due to its short life cycle and high reproductive capability, *A. gossypii* has a high potential for resistance development to insecticides and reports of insecticide control failures have recently increased, particularly with imidacloprid.

To quantify resistance of cotton aphid populatoins to imidacloprid, seven populations were collected from different places in Fars province (Shiraz, Jahrom, Saadatshahr, Marvdasht, Kavar, Sadra 1 and Sadra2). To estimate the response of 5 days old A.

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gossypii populations to imidacloprid, leaf dip bioassays was performed in the laboratory. LC₅₀ values were estimated by probit analysis and used to calculate the resistance ratios (RR). The bioassay results showed significant discrepancy in susceptibility to imidacloprid among the populations. The LC₅₀ values for populations of Sadra1, Jahrom, Kavar, Marvdasht, Saadat Shahr, Sadra2 and Shiraz was estimated as 632.88, 263.76, 203.23, 183.26, 142.94, 54.69 and 37.09 µg ml⁻¹ respectively.

The highest levels of resistance to imidacloprid was detected for Sadra1 (RR = 17.06 fold). Also in other populations some levels of resistance were detected. In Jahrom, Kavar, Marvdasht, SaadatShahr, Shiraz and Sadra2 populations the RRs were 7.11, 5.48, 4.94, 3.85, 1.47 and 1, respectively. Because of the slopes and comparison of RRs with other studies we thought resistant population is in first stages of resistance and has the ability to be more resistant.

P INSECT 34

A new age in development of insecticides

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In recent agricultural process, farmers are largely depended on chemicals for pest management. However the intensive use of the chemicals has led to many serious health problems not only for the applicators but also the consumers. It also has caused to the pest resistance against insecticides and the environmental pollution. It has now become essential to search for efficient and environmentally friendly ways for sustainable pest management.

Thanks to developing technology, there are some new ways to solve problems that are caused by insecticides. One of them is: to formulise insecticides which are developed by nanotechnology.

There are two main aims to develop the nanopesticides: to increase solubility of poor soluble insecticides, to release the active ingredient slowly, to get better efficacy on target pest and to protect the active ingredient against premature degradation.

In this study we have focused to explain the importance to develop new nanopestices for environmentally friendly and more efficient pest management. Also, some advantages and disadvanges of nanopesticides are discussed.

Poster Presentations

Integrated Pest Management

P IPM 1

***In- vivo* Control of tomatoes *Fusarium* spp. wilt using plant extracts**

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Fungal suspensions were prepared from *Fusarium solani*, *Fusarium oxysporum*, and *Fusarium solani* isolated from the roots, stems, and rhizosphere of tomatoes' wilt plants. We used methanol extracts of the following plants in pots: fig's (*Ficus carica* L.) leaf powder extract, myrtle leaf extract (*Myrtus communis* L.), and marigold's (*Tagetes patula* Linn.) stems, leaves, flowers, and roots extracts separately at our experiments in pots. The results show that both 6% marigold's stems and 6% marigold's leaves' extract separately infected with *F. solani* isolated from tomatoes' roots significantly reduced the tomatoes diseased plants, and activated the growth of the tomatoes' roots and stems comparing with the control. 6% marigold's stems and 6% marigold's leaves' extract separately infected with *F. oxysporum* isolated from tomatoes' stems significantly reduced the tomatoes diseased plants comparing with control, and activated the growth of the tomatoes' stems, moreover only 6% marigold's stems extract infected with *F. oxysporum* activated the growth of the tomatoes' roots. 6% marigold's stems and 6% marigold's leaves' extract separately infected with *F. solani* isolated from rhizosphere reduced the tomatoes diseased plants comparing with control and activated the growth of the tomatoes' roots and stems comparing with the control.

P IPM 2

Compatibility studies among selected entomopathogenic fungi, insecticides and fungicides in chili

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Among the test insecticides, spinosad was found to be highly compatible with the *Beauveria bassiana* (Bals.) Vuillemin and *Metarhizium anisopliae* (Metsh.) Sorokin by recording no inhibition of growth, sporulation and viability of spore germination followed by indoxacarb, novaluron and cartap hydrochloride. The propiconazole could not be used in combination with *B. bassiana* and *M. anisopliae* due to its detrimental effect as no radial growth, zero conidial concentration/cm and conidial viability was recorded, whereas tebuconazole, azoxystrobin and chlorothalonil showed little inhibition on above fungi. The LC₅₀ of the insecticides viz., indoxacarb, spinosad, novaluron and cartap hydrochloride against *H. armigera* was determined as 110.34, 99.83, 383.30 and 224.32 ppm, respectively, whereas the fungicides viz., propiconazole, chlorothalonil, and azoxystrobin and tebuconazole recorded LC₅₀ values of 0.76, 11.85, 1.92 and 8.26 ppm, respectively. The tested insecticides were non toxic to *C. capsici* at LC₅₀, field recommended and other concentrations, whereas fungicides were also non toxic to *H. armigera*. Synergism was displayed by the combinations of indoxacarb 110.34 ppm with chlorothalonil 11.85 ppm and tebuconazole 8.26 ppm, whereas antagonism was noticed in the combinations with propiconazole 0.76 ppm and azoxystrobin 1.92 ppm against *H. armigera*. Spinosad in combination with chlorothalonil, azoxystrobin and tebuconazole at their LC₅₀ values displayed synergism against *H. armigera* and they significantly differing among themselves in this regard. The effect of fungicides in combination with insecticides on the spore inhibition of *C. capsici* at their LC₅₀ values revealed that all the combinations were antagonistic to each other.

P IPM 3

Plant protection in ecocycle-based agricultural systems: aquaponics as an example

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Aquaponics is a new, rapidly emerging, ecofriendly agricultural technology that integrates recirculating aquaculture with hydroponics (plant production in water without soil) (Kiraly et al., 2013). The technology is highly efficient, because it uses the waste fish produce to feed the plants with nutrients, providing a symbiotic environment for producing fish and plants in a closed system. In such systems, however, health issues related to plants and fish deserve serious consideration. Thus, a large number of infectious agents may cause diseases to fishes, and some of these agents are known plant pathogens. In addition, the recycling of water within the system strongly limits the available methods of pest and disease control. This paper calls the attention to a particularly important issue in aquaponics often neglected until it is too late: the management of plant pests and diseases. The complexity of an aquaponic system (that is based on the stable coexistence of fish, bacteria and plants) makes this task extremely difficult, because fish toxicity strongly reduces the number of the applicable chemical pesticides. Therefore, the

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design and the successful operation of aquaponic systems should be based on a careful evaluation of all the tools of integrated pest management.

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P IPM 4

Faunistic composition, population trends and resistance status of certain sap feeding pests inhabiting selected cucurbit cultivars

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Faunistic composition and the population trends of certain sap feeding pests and their associated natural enemies inhabiting cucurbit plantations have been studied in Assiut Upper Egypt. Twenty one arthropod species belonging to 18 genera, 11 families and 4 orders were encountered by using the sweeping net method. Amongst the identified species nine piercing and sucking pests and five predators belonging to order Hemiptera-Heteroptera were recorded. Population trends of the main sap feeding pests, namely, *Campylomma impicta* (Wagner); *Creontiades pallidus* Ramb; *Empoasca* sp. and *Bemisia tabaci* (Gennadius) were estimated on the selected cucurbit cultivars. Resistance status of three sweet melon, three cucumber and two squash cultivars to the whitefly *B. tabaci* (nymphs), the leaf hopper *Empoasca* sp. (nymphs and adults) and the two spotted spider mite *Tetranychus urticae* Koch (mobile stages) was determined. The tested cucurbit cultivars showed different susceptibility degrees to the aforementioned pests. Al-Wafeer cucumber cultivar and Cabili squash cultivar showed some sort of resistance against the three selected pests. The remaining cucurbit cultivars showed variable degrees of resistance to these pests. Correlation coefficient values between selected plant phenomena and the incidence of the selected pests was calculated.

P IPM 5

The evaluation of eggplant (*Solanum Melongena* L.) germplasm against jassid (*Amrasca Amrasca Biguttula Biguttula* (Ishida) resistance

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The eggplant is the most popular vegetable and jassid is the harmful and major pest in Pakistan. The present study was carried out for evaluating relative plant resistance and susceptibility of various germplasm of eggplant against the jassid. The randomized complete block design was laid for this experiment and nine eggplant germplasm viz. Rubi, Vrib-01, Virb-02-F1, Vrib-0401, Vrib-04, Bemissal, Vrib-9901, Nirala and Cluster king were screened against jassid. The population of jassid present at eggplant genotypes were Rubi (1.42), Vrib-01 (1.67), Virb-02-F1 (1.78), Vrib-0401 (2.02), Vrib-04 (3.03), Bemissal (3.36), Vrib-9901 (1.92), Nirala (3.03) and Cluster king (1.67). The parameters were pest preference and host plant susceptibility indices (HPSI). The Rubi, Vrib-01 and Virb-02-F1 showed comparatively high resistance of jassid/leaf while Vrib-9901, Vrib-0401 and Bemissal appeared as intermediate resistance and Vrib-9901, Nirala and Cluster king were comparatively susceptible of jassid/leaf. The germplasm Bemissal showed the most susceptible between the experimented materials. The maximum level of HPSI in Bemissal was 18% and while in Rubi it was very less at 7%. The selected genotypes showed significant difference ($P \leq 0.05$).

P IPM 6

Evaluate the performance of several types protein hydrolyzate in monitoring and control of olive fruit fly in Guilan province

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Olive Fruit Fly *Bactrocera oleae* Gmelin. is most important pest in olive groves in Iran and Guilan province that causes heavy losses production to farmers each year. In some of years with increasing population has been decreased production of table olives and quality of olive oil. Lack of information about the efficiency of the best of protein hydrolyzate composition available in markets for farmers, cause they have been confused that which of compound is appropriate for monitoring and control of Olive

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Fruit Fly. In this study, comparison of efficiency of several of protein hydrolyzate such as : Biocebo from Bioiberica company of Spain , Dacus bait 100 from EVYP company of Greece and Fly cap from Green universe company of Spain has been evaluated. The study was conducted in Randomized Complete Block Design (RCBD) in olive orchard in Roudbar olive research station and data were collected weekly. Sampling continued until the end of the harvest season. During each sampling, male and female olive flies and non-target insects were counted and recorded in tables. Results showed although that the protein Fly cap in the first months of sampling performed better than other traps. But with decreasing temperature, the Dacus bait 100 is better than from others that mean of Fly cap in 1 th month is 14.00 ± 3.25 and for Dacus bait is 1.40 ± 0.50 for attraction of female olive fly and 15.20 ± 0.29 to Fly cap and 3.60 ± 0.13 to Dacus bait for attraction of male olive fly. But in total attraction but in total of duration of study , Dacus bait 100 with of mean 48.23 ± 2.58 is the best protein hydrolyzate for attraction of female olive fly and Biocebo and Fly cap with means 30.13 ± 4.42 and 15.66 ± 4.92 were after Dacus bait. In attraction of male olive fly , mean of Dacus bait 100 was 41.7 ± 2.64 the best protein hydrolyzate and means of Biocebo and Fly cap were 28.05 ± 4.21 and 14.88 ± 4.97 after Dacus bait 100. and Dacus bait 100 from EVYP company of Greece is the best protein hydrolyzate for monitoring and mass trapping .

P IPM 7

Biological control of chestnut blight: persistence of biocontrol agent *Cryphonectria hypovirus 1* in healed chestnut cankers

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Chestnut blight is a disease caused by the plant pathogenic fungus, *Cryphonectria parasitica*, introduced to North America and Europe from Asia. The pathogen causes cankers on infected trees. Biocontrol of this fungus is mediated via *Cryphonectria hypovirus 1* (CHV1), which reduces virulence and reproductive capacities of the fungus. This phenomenon is called hypovirulence. If the hypovirus is introduced naturally or artificially into active canker (Fig 1a) caused by virulent *C. parasitica* strain(s), canker expansion ceases and 'healing' canker - callus (Fig 1b) is formed. Besides active and healed cankers (Fig 1c), also occurring in nature are superficial cankers caused by hypovirulent *C. parasitica* isolates, as well as non-expanding, inactive cankers. High prevalence of CHV1 in *C. parasitica* populations can induce healing cankers and recovery of chestnut forests. Therefore, it is important that hypovirulent CHV1-infected fungal strains persist in populations. The aim of this research was to investigate the persistence of hypovirulent *C. parasitica* strains in completely healed chestnut blight cankers. Bark samples were collected from seven chestnut populations (six in Europe and one in North America) from more than 100 healed cankers. Samples were taken both from callus tissue and from the callus-wood interface. Neither hypovirulent nor virulent *C. parasitica* strains comprised more than half of the samples isolated from healed cankers. In different populations, the frequency of hypovirulent *C. parasitica* strains was different between callus and interface. Generally, virulent *C. parasitica* strains were found more frequently in healed cankers, which is interesting because hypovirulent strains are thought to promote healing. The relationship between virulent and hypovirulent isolates in healed cankers appears to be highly dynamic. We suspect that the loss of vigor by hypovirulent *C. parasitica* allows virulent strains or other fungi to invade healed cankers.

Figure 1



P IPM 8

Reaction of two *Pisum sativum* genotypes to *Didymella pinodes* infection

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Didymella pinodes is the major pathogen of the ascochyta blight disease complex of field pea (Khan et al., 2013). It reduces both yield and quality of peas (*Pisum sativum* L.). The present work aimed to investigate the reaction of two genotypes of peas (i.e. cv. Messire and Model) to *D. pinodes* isolate infection at their early (4-leaf) growth stage. In a factorial experiment, their response to this pathogen was evaluated with a complete plant nutrient and unfertilized control group. Disease severity was scored on stipules and leaflets at the first, second and third nodes using a 0-6 scale adapted from the 0-5 scale previously described by Roger and Tivoli (1996). The results indicated that there were significant differences ($p < 0.05$) between the two genotypes in disease severity, plant height, estimated leaf chlorophyll content or “greenness” and dry matter yield. The lowest infection rate and the slowest progress of the disease expressed as relative infection rate were noted in cv. Model as compared to Messire (Fig. 1A). On the other hand, the plant height (data not shown) and dry matter production (Fig. 1B) were lowest in the infected and unfertilized cv. Messire. The mean values of leaf greenness were similar in both infected and healthy plants in cv. Model (data not presented). We observed no upward diseases progress on the infected pea plants. Finally, we suggest a time series infection test to understand completely the effects of *D. pinodes* on the growth, development, final yield and quality of pea genotypes.

Figure 1: Effects of *Didymella pinodes* infection on (A) disease severity and (B) total dry matter production of two pea genotypes. Abbreviations are: C (un-fertilized control); F (mineral fertilizer); H (healthy plant); I (infected plant). Error bars indicate standard error (n = 4).

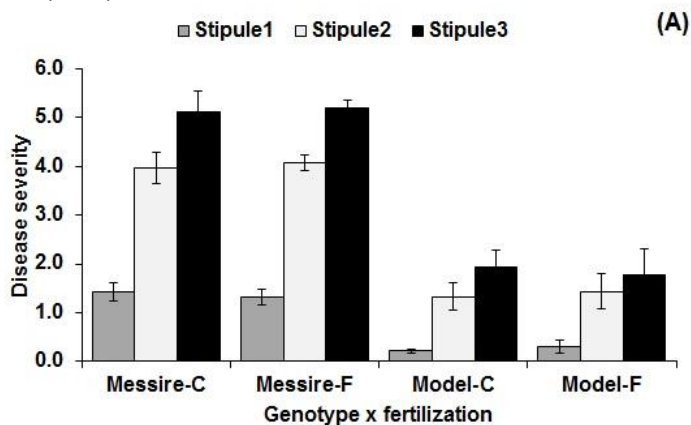
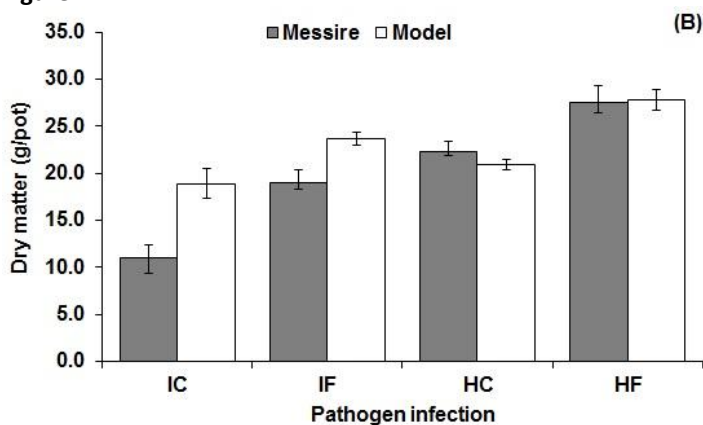


Figure 2



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P IPM 9

Ecology and Management of brown spot of rice (*Oryza sativa* L.) under the undulating red and lateritic zone of West Bengal, India

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Rice (*Oryza sativa* L.) has been regarded as one of the most important cereal crops and a major food grain contributor to the total world food grain basket. Brown spot of rice (*Drechslera oryzae*, *Helminthosporium oryzae*) is a chronic disease that affects millions of hectares of rice every growing season, grown by some of the most resource-poor farmers. Attempts were made to know the present severity level of the disease, their ecology and to manage the disease under field condition. The severity of brown spot was varied greatly with the location, variety and season. A terminal disease severity ranging from 14.65 % to 34.14% was obtained from different locations in *Kharif* rice. While, the severity was little bit lesser in *Boro* rice (19.4 % to 30.6%). Maximum severity of disease (37.35 % PDI) was recorded from the field transplanted 30 days later than the schedule time followed by 32.85% from 45 days. Maximum yield (4921 kg/ha) was obtained from the field transplanted on 25.07.2013 (schedule time of transplanting) followed by (4785 kg/ha) from 15 days early transplanted rice (10.07.2013). Among the phyto-extracts, minimum severity of brown spot disease (15.3% PDI) and maximum yield 49.23 qt/ha were noticed in the field treated with leaf extract of *Derris indica* (karanj) followed by *Lantana camera* (19.3 % PDI and yield 48.75 qt/ha). Fungicide Azoxystrobin 23 SC (Amistar 25 SC) was found to be very effective against brown spot which reduced the severity up to the level of 86.07%. The disease was increased initially at a faster rate (5.18 % periodical increment in PDI) during September 18th to 24th 2013 and finally reached at a lower level of 0.35% PDI during 30th October-5th November'2013. When max. temperature ranged from 32.6 to 34.21^oC, min. temperature maintained between 23.61^o C to 26.18^oC, average RH varied between 80.71 to 85.81%, having moderate rainfall, average sunshine of 4.63 to 6.16 h/ day and moderate wind speed from 1.0 to 1.57 km/h persist in rice field maximum periodical increment in PDI might be expected. From the R² value it can be stated that, these six meteorological factors responsible up to the extent of 85.2 percent for the development of the disease. The study provide the information about the present status of disease severity, the trends of disease progression and environmental relationship. It also offers a complete package of controlling this disease in the region.

P IPM 10

Current Technologies in Locusts Plague Management Program in China

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Locust (*Locusta migratoria*) plague was one of three biggest disasters with flooding, drought in China. For a long time, due to there were several key problems unsolved, such as excessively using chemical pesticides, intensive labor monitoring and control, the outbreak of locust and grasshoppers occurred frequently. To solve these problems, since 1990s, we have researched and developed biological control, ecological control, information technology based locust control supporting information platform.

1. Biological and ecological control. 1.1. Higher virulent strains of *Nosema locustae*, a protozoan, have been obtained through traditional screening. It has been demonstrated that epizootics of *Nosema* disease could be caused by application of *Nosema* spores in China both in crop field and grassland. 1.2. The mass production technology for *Nosema* spores has been developed. The yield is more than 21×10⁹ spores/locust. A novel formulation of *Nosema* spores, water suspense formation has been developed, which can keep spores alive longer than for one year at room temperature and be applied by airplane ULV. *Metarhizium* has been commercialized and played an important role. The field application methods of both *Nosema* and *Metarhizium* have been developed. 1.3. It has been demonstrated that one bird, Rosy Starlings (*Stumus roseu*) can predate 120-160 individuals of grasshoppers per day, and can eat 50 thousands grasshoppers for its whole life. Since 1990s, artificial bird nests with stones or bricks were built totally 50,000m³. 1.4. Ecological control methods have been improved. In Hebei, Shandong, and Henan provinces some non-host plants of locust, such as alfalfa, cotton, jujube etc. have been planted to replace reeds which is host plant of locust in wild locust breeding regions, reduced effectively locust densities more than 50%.

2. Efficient systems for management. To monitor and control locusts efficiently, an information platform for locust control based on the GPS, RS, and GIS was developed. The platform can provide accurate information about locust occurrence and control strategies, consists of three systems, a mobile GPS pad, a processing system for locust information based on GIS and RS,

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and a WebGIS-based real-time monitoring system. And all of these technologies have been applied 3.36 million ha in 20 provinces.

P IPM 11

Evaluation of Damage Induced by the Shedder bug, *Creontiades pallidus* Rambur (Hemiptera: Miridae) on different cotton cultivars

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The Shedder bug, *Creontiades pallidus* Rambur (Hemiptera: Miridae) is one of the most important pests of cotton in Khorasan Razavi province of Iran. This insect as a serious pest of cotton in recent years has been considering in the eastern areas of Iran (Khorasan) and particularly Sabzevar region. Damage caused by *C. pallidus* on the different commercial cultivars of cotton (including Bakhtegan, Khordad, Sahel, Sepid, Mehr, Varamin) was evaluated in Sabzevar region during 2013-2014 in Agricultural Research Station. The experimental design was a split plots design with 4 replications with 6 cultivars. Ten plants per plot were considered to be constant until the end of the project and on each sampling date, the number of black spots on the all bolls per plant was counted and mean of ten plants was recorded. During the season, 14 sampling intervals were carried out from 14th August to 14th October. Results of analysis variance showed that effect of cultivar on damage rate was significant ($P < 0.01$). Bakhtegan and Mehr cultivars had the highest and lowest rate of infestation, respectively. Also interaction between cultivar and time was significant ($P < 0.01$). Analysis variance of results indicated that the highest number of bugs was observed in 16th August on Varamin cultivar. Also the lowest number of bugs was seen in 4th October on Mehr cultivar. Totally, Mehr is the most resistant cultivar to *C. pallidus* while Bakhtegan and Varamin are the most susceptible cultivars to *C. pallidus*.

P IPM 12

Evaluation of infestation percentage of cotton fields to the spiny bollworm, *Earias insulana* Boisduval. (Lep.: Noctuidae) and its relationship with pheromone traps

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The spiny bollworm, *Earias insulana* Boisduval. (Lep.: Noctuidae), is one of the important pest of malvaceous plants throughout the world except America. In recent years, this insect has been serious pest for cotton fields in southern regions of Iran, especially in Darab region of Fars province. In order to evaluate the performance of sex pheromone for reduction of infestation percentage to *E. insulana* by mass trapping method, an experiment was carried out during 2012 in Darab agricultural research station in randomized completely block design with 5 treatments and 4 replications. The treatments were as application of the sex pheromone trap at the rates of 16, 20, 24 and 30 traps/h, application of Larvin insecticide at the rate of 1 Lit/h, and control. Rate of infestation percentage in the field was evaluated with counting 100 bolls and flowers per week. In Larvin insecticide treatment, after reaching to control index, spraying was carried out. Analysis variance of results showed that there are significant differences between time, trap number and time x trap number on infestation percentage per hectare. During sampling time, the highest infestation percentage was in control treatment and the lowest one was observed in 24 and 30 traps/h treatments. The peak of infestation percentage was seen in 28th of November. The best efficiency among treatments was observed in pheromone trap.

P IPM 13

Cropping systems with maize and oilseed rape may reduce the risk of soilborne diseases of wheat

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Introduction: Cropping systems (CS) with crops cultivated in short cycles on the same land provoke phytosanitary problems which may force more chemical inputs. However, the potential effects of CS with maize and oilseed rape on the incidence and severity of stem base and root diseases of wheat have not been investigated thoroughly.

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Objectives: The aim of this study was to conduct field experiments in order to explore how CS including maize and oilseed rape affect the risk of root and stem base diseases in winter wheat. Further, our aim was to determine the microbial community composition in different CS, which may explain the varying occurrence of soilborne diseases of wheat.

Methods: We therefore analyzed the effects of varied percentages of maize and oilseed rape on stem base and root pathogens of winter wheat grown in four different CS and rotations in two locations in Central and Northeastern Germany for three years. Additionally, we determined the fungal and bacterial community with barcoded amplicon pyrosequencing.

Results: Our results demonstrate that short and intensive crop rotations with wheat combined with crops do not necessarily enhance the risk by soil and straw borne diseases. Moreover, a suitable combination of wheat, oilseed rape and maize with adapted cropping methods (late sowing after maize, mouldboard ploughing) can significantly mitigate the threat of stem base diseases in wheat. While disease incidence of sharp eyespot was always <5%, CS had significant effects on the incidence and severity of eyespot, fusarium foot rot and take-all (in Northeastern Germany). Incidence of fusarium foot rot and take-all was significantly reduced by 70% and incidence of eyespot nearly to 0%, when wheat was planted after maize in a system with late sowing and ploughing, compared to wheat after oilseed rape with reduced tillage and early sowing. Further, these CS with maize showed a low level of fusarium head blight. DON levels in grains were always low.

Conclusions: The present study demonstrates that current shifts in crop rotations to a higher prevalence of maize due to novel market developments do not necessarily enhance the phytosanitary risks in the main crop wheat, if a suitable system of agronomic measures is applied, enabling highly productive and sustainable energy crop production systems.

P IPM 14

EU project BIOCOTES develops new biological control products for Integrated Pest Management in agriculture and forestry

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The objective of BIOCOTES (www.biocotes.eu) is to develop 11 new biological control agents (BCAs) and 2 production technologies for key markets in European agriculture and forestry. BCAs were identified through market analysis by six manufacturers of biological control products. BCAs will primarily be for use in open field crops of vegetables (3), of which 2 are also for use in protected crops, arable crops (3), fruit crops (3), and three different types of forests (2). Primary targeted pests are: gypsy moth (*Lymantria dispar*), pine weevil (*Hylobius abietis*), tomato pinworm (*Tuta absoluta*), white flies, aphids of fruit tree crops and *Mamestra brassicae*. Primary targeted pathogens are: damping-off diseases in forest nurseries, soilborne pathogens of oilseed rape and cereals, brown rot (*Monilinia* spp.) of stone fruit, and powdery mildew of cereals (*Blumeria graminis*). The economic sustainability during the entire development process will be assessed by the responsible industrial partners. The environmental sustainability will be quantified for each BCA by means of the Sustainable Process Index method. The entire developmental process for each of the 11 BCA products is guided by a consultancy partner specialized and leading in (bio) pesticide registration including risk assessments for European (bio) pesticide industries. *In vitro* production of entomopathogenic viruses as new innovative technique will be developed aimed at a breakthrough in economic production. Downstream-technology and shelf life for entomopathogenic nematodes will be improved. BIOCOTES will communicate project results with all stakeholders with special attention to European IPM networks throughout the whole project duration. BIOCOTES combines the expertise of 10 industrial SME partners, 3 larger industrial partners and 14 research partners with 38% of the requested EU contribution supporting SMEs. All 11 BCA solutions will be novel IPM tools and new alternatives to replace major pesticide applications in European agriculture and forestry.

This project has received funding from the European Union's Seventh Framework Programme (Grant Agreement 612713).

P IPM 15

Microbes contamination controle by using ginger juice

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The study was conducted at College of Agriculture / University of Baghdad cooperation with Biotechnology and Research Center / Univ. of Al-Nahrain to study the effect of ginger juice on some microbes contaminated cow milk such, Escherichia coli , salmonella, Pseudomonas fluorescents that have pathogen and spoiled effect on cow milk were studied . Isolated bacteria have been brought from college of Agriculture. Microbes were cultured on media that has been prepared and then media were sterilized by two methods, first by using autoclave with 1210 c for 15 minute, and second by using microbes filter. Concentration 1,2,3,4,5,10,20,30,40,50 % ginger juice were prepared . Result showed positively an inhibition that correlated with concentration , it was noticed that halo diameter of inhibition from 10 to 50 %concentration were 11.19, 12.13 ,12.62 ,13.31 ,14.11 mm

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respectively for salmonella whereas its effect on *Escherichia coli* were 8.96 , 10.90 , 11.37 , 12.12 , 12.56 mm for the same above concentration . On other hand its effect on *Pseudomonas*. Were had the trend but with lower value .That concentration 10 to 50 % were 7.11, 8.42, 9.15, 10.83, 11.21 mm respectively. It can be concluded that increasing ginger juice concentration cussed increasing bacteria inhibition in milk.

P IPM 16

Monitoring and control of the olive moth *Prays oleae* Bern. (Lepidoptera: Yponomeutidae) in the West of Algeria

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Prays oleae commonly called the olive moth is a lepidopteran insect of the family Yponomeutidae. This pest preferentially attacks the olive tree (*Olea europaea*), which it is a major pest, the economic importance of the damage caused by this insect depends on the size of pest population, and also the generation in question. *P. oleae* developing three generations per year each generation is associated with a part of the host plant.

At the beginning of this research we developed a pest monitoring plan at 6 plots distributed in western Algeria. 3 stations in Relizane, 2 stations in the province of Mascara and a station at Oran.

Delta traps pheromone, provided by Russell IPM, was installed in each olive orchard.

Samplings were carried out weekly to explore the pest *P.oleae*. The results obtained from the pest samples show the presence of the insect in all orchards prospected with differences in the rate of infestation of trees. The period of the summer of 2014 was a very warm period that has not allowed the insect to do major damage in the period from July to September 2014.

P IPM 17

Geostatistical analysis of spatial distribution of alfalfa spotted aphid *Therioaphis maculata* and coccinellid lady beetles

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Introduction: Understanding the spatial dynamics of insect distributions provides useful information about ecological requirements of insects and can also be used in site-specific pest management programs. Interactions between preys and predators are spatially and temporally dynamic and can be affected by several factors.

Objectives: The objectives of this study were to determine and map the spatial distribution pattern of alfalfa spotted aphid, *Therioaphis maculata* and coccinellid lady beetles and their spatial interactions using geostatistical tools.

Materials and methods: The study was conducted in three (0.5, 3.1 and 7.3 ha) alfalfa fields and two growing seasons, 2013 and 2014. The 0.5 ha field was divided into 10×10 m grids and 3.1 and 7.3 ha fields were divided into 30×30 m grids. Weekly sampling began when height of alfalfa plants reached about 15 cm and was continued until the cutting of alfalfa hay. Forty and 10 stems were chosen randomly in 30×30 m and 10×10 m grids, respectively and shaken into a white pan three times. The aphids and coccinellids fallen in the pan were counted and recorded. Semivariance analysis and variograms were used to determine the spatial autocorrelations between samples. Data analysis was conducted using GS+5.1. The results of semivariance analysis were used to generate distribution maps of the insects using ArcGIS 9.3.

Results: The results of spatial analysis indicated that distribution of *T. maculata* was aggregated in the fields and spatial autocorrelation was strong in 20, moderate in 16 and weak only in four out of 40 data sets. Semivariance analysis of the coccinellids showed moderate to weak spatial dependency. Comparison of the distribution maps of aphid and ladybeetles showed that there was overlap between the maps, but they did not coincide completely. Several factors including prey availability, inter and intra-specific interactions and environmental factors can influence the spatial synchrony of prey and predator. In this study low population density of lady beetles in the alfalfa fields can be one of the reasons for lack of spatial correlation between the spotted alfalfa aphid and lady beetles.

Conclusion: The aggregated spatial pattern of *T. maculate* indicated that site-specific management of this aphid is possible and distribution maps can be used for this purpose.

P IPM 18

An assessment of indirect energy used for pesticide applications for field crop production in Southeastern Anatolia region of Turkey

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The manufacture of pesticides is a highly complex process, resulting in high-energy inputs per kilogram produced. Physical, chemical, and thermodynamic characteristics of the manufacturing process determine the energy cost of a pesticide. Most pesticides are derived from ethylene and propylene, which are obtained by catalytic cracking of crude petroleum oils, or from methane from natural gas. The total energy cost is the sum of the energy sequestered in the material itself and that required to apply it to the crops. This energy is directly utilized in the manufacturing process and indirectly utilized in formulation, packaging, and transportation.

Energy use pattern and contribution of energy inputs vary depending on farming systems, crop season and farming conditions. Considerable work has been conducted on the use of energy in agriculture with respect to efficient and economic use of energy for sustainable production. The aim of this study was to assess indirect energy used for pesticide applications for wheat, lentil and cotton production in the Southeastern Anatolia region of Turkey. Data were collected from growers by using a face to face survey in the production years. Taking actual farm size as the variable, the total 132 farms was randomly selected by using stratified random sampling. The total indirect energy used for pesticide applications were 161.1 MJ, 195.7 MJ and 385 MJ per hectares for wheat, lentil and cotton production, respectively. The indirect energy for herbicides represents 85.1%, 61.8% and 59% of the total indirect energy of pesticides used for lentil, cotton and wheat production, respectively.

P IPM 19

Exploitation of bioactive metabolites from new or rare fimicolous fungi against plant pathogenic fungi

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Introduction: Fimicolous fungi play an important ecological role in decomposing and recycling nutrients from animal, especially herbivorous, dungs. To win the struggle for life, these fungi produce a plethora of bioactive secondary metabolites to compete with other fungi, whose growth can be inhibited, thus resulting in an enhancement of the ecological fitness of producer strains. Actually these antifungal metabolites are of interest especially in medicine while very few information are available concerning a possible use in agriculture against plant pathogenic fungi.

Objectives: Aims of the present work was to investigate the effect of secondary metabolites from fimicolous fungi as bioactive molecules against plant pathogenic fungi.

Materials and methods: An isolate of the rare *Cleistothelobolus nipigonensis* and isolates of the recently described *Neogymnomyces virgineus* and *Rodentomyces reticulatus*, collected from different herbivorous dungs, were investigated. The organic extracts and the aqueous residues from Solid State Fermentation (SSF) on rye flour were tested for their antifungal activity against some important plant pathogenic fungi. Organic extracts showing the most interesting antifungal activity have been further purified resulting in different fractions and some pure metabolites, whose chemical and biological characterization has been performed.

Results: Antifungal bioassays indicated a significant activity against almost all tested pathogenic fungi by the organic extracts from *N. virgineus* and *C. nipigonensis* SSF. Further, both *n*-hexane and dichloromethane extracts were purified by several chromatographic steps obtaining different fractions, some of them particularly effective against *A. brassicicola*, *F. graminearum* and *B. cinerea*. In addition, pure metabolites were also obtained. These last showed interesting antifungal activity and have been chemically and spectroscopically characterized.

Conclusion: The use of natural derived ingredients of pesticides is in line with the EC Dir. 128/2009, establishing a framework to achieve sustainable use of pesticides. Results here reported demonstrate that fimicolous fungi are an underexplored reservoir of bioactive metabolites that could be exploited as active ingredients of plant protection products.

P IPM 20

A jump into ATP Binding Cassette (ABC) transporters of the biocontrol agent *Trichoderma gamsii*

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Introduction: Fusarium Head Blight (FHB) causes yield losses and accumulation of mycotoxins (such as Deoxynivalenol - DON) in cereals. The biocontrol agent *Trichoderma gamsii* 6085 (T6085), an antagonist of mycotoxigenic *Fusarium graminearum* and *Fusarium culmorum*, can grow in presence of high concentration of DON, without degrading or modifying it. ATP binding Cassette (ABC) transporters, by moving natural toxic compounds, such as mycotoxins, across biological membranes, could be involved in this resistance.

Objectives: Aims of the present work was to use the recently sequenced and annotated genome of T6085 as a tool to deeply investigate genes potentially involved in the interaction of this isolate against FHB causal agents. Particularly, a phylogenetic analysis focused on ABC-B, ABC-C and ABC-G subfamilies is here reported in order to investigate the potential role of this system in the resistance to DON.

Material and methods: T6085 ABC-proteins have been manually annotated using reference proteins. Phylogeny was made by Neighbour-Joining method in MEGA 6.06 software. The JTT amino acid substitution model was used with uniform rates among sites and pairwise deletion of gaps. Statistical support for phylogenetic grouping was assessed by 1000 bootstrap re-samplings.

Results: T6085 has a repertoire of 48 predicted ABC-proteins. The phylogenetic analysis shows 36 out of 48 proteins clustered within the ABC-B (10), ABC-C (15) and ABC-G (11) subfamilies. The remaining 12 ABC-proteins have a role in the viability of fungal cell and are not closely related to transport. TGAM01_01326 and TGAM_02332 (ABC-B) clustered along with *S. pombe* pmd1, a transporter of leptomycin, whereas TGAM01_08025 (ABC-C) clustered with *S. cerevisiae* Yor1, a transporter of oligomycin, and *S. cerevisiae* Bpt1, a heavy metals transporter. TGAM01_07870 (ABC-G), the homologous of *Trichoderma atroviride* Taabc2, was also found.

Conclusion: Results here reported represent a preliminary step to analyse the role of these proteins in the interaction between T6085 and mycotoxigenic causal agents of FHB. The availability of the genome of this fungus represents a platform for further investigations, such as expression and functional analyses and comparative genomics of other fungi with similar ecological fitness.

P IPM 21

Crop protection, biotic stress, biology of canola pathogens and insect pests: Blackleg (*Leptosphaeria maculans*)

Comparing efficacies of major blackleg resistance genes in winter oilseed rape against different regional populations of *Plenodomus lingam* in Germany

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Background: Blackleg disease, caused by *Plenodomus maculans* (PM) is one of the most important fungal diseases in oilseed rape (OSR) production world-wide. Genetic resistance is an important tool to control this disease. Seedling resistance is conferred by single major genes. Due to its sexual propagation, PM isolates evolving rapidly from avirulent to virulent strains on cultivars harboring major resistance genes. Therefore, resistance of OSR against PM conferred only by major resistance genes was often overcome and led to severe yield losses in the past.

Objectives: The aim of this study was to determine the efficiency of major resistance genes to PM in different OSR growing regions in Germany by identifying the frequency of virulent isolates and determine the race spectra of PM.

Methods: We cultivated two OSR cultivars in fields throughout Germany from 2011 to 2014: i) 'NK Bravour' harboring no known major genes against PM (serving as trap crop) and ii) 'Exocet' harboring the efficient major gene *Rlm7* to observe resistance breakage in the field. In autumn and spring we collected true leaves with typical Phoma lesions to gain isolates of PM. Single pycnidia isolates were tested with a French and Canadian differential set through cotyledon inoculation for their virulence to different major genes. The differential set consisted of 10 OSR genotypes harboring the major genes *Rlm1*, *Rlm2*, *Rlm3*, *Rlm4*, *Rlm7*, *Rlm9* and *LepR1*, *LepR2* and *LepR3*. Thereby, the frequency of virulent isolates in a region was determined. Isolates showing the same complement with virulence alleles were grouped to the same race.

Results: The frequency of isolates being virulent to *Rlm1*, *Rlm2*, *Rlm3*, *Rlm4* and *Rlm9*, respectively, was above 85%. Conversely, the frequency of virulent isolates to *Rlm7* was very low (< 5%). Interestingly, the frequencies of isolates being virulent to the major genes *LepR2* and *LepR3* showed a high variability between different regions, ranging from 35% to 100%. There was no isolate showing virulence to *LepR1*. Most isolates belonged to two races with a high virulence complexity.

Conclusions: Most tested major genes lost efficiency to PM. Only *Rlm7* and *LepR3* are still mediating resistance in OSR to PM in Germany. We assume that *Rlm7* may lose its efficiency with increasing deployment of this major gene in OSR in Germany.

P IPM 22

Influence of *Metarhizium anisopliae* on age-specific survivorship of *Habrobracon hebetor* as a numeric variable

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Question: The ectoparasitoid wasp, *Habrobracon hebetor* Say (Hymenoptera: Braconidae) and the entomopathogenic fungus, *Metarhizium anisopliae* (Metsch.) Sorokin (Hypocreales: Clavicipitaceae) are valuable biocontrol agents attacking *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae) larvae (Fathipour and Sedaratian 2013). Since, the age-specific survivorship (I_x) of a parasitoid is very important for the success of a biological control program, sub-lethal effect of *M. anisopliae* was studied on survivorship of immature stages of *H. hebetor* as a numeric variable.

Methods: Based on mortality data from bioassays, third instar larvae were first infected with LC₃₀ (2×10^6 conidia.ml⁻¹) of *M. anisopliae* isolate M14 which were then exposed to one pair of male and female parasitoids (< 24h) at different time intervals (0, 24, 48 and 72 h). A χ^2 goodness-of-fit was computed to determine if there was any deviation from the expected survivorship.

Results: Our results indicated that the highest and lowest survivorships of immature stages were 0.76 % and 0.41 % for control and 72 h treatment, respectively. The LC₃₀ of *M. anisopliae* had no adverse effect on survivorship of *H. hebetor* when the exposure to the parasitoid wasp was immediately after fungus application ($\chi^2 = 0.107$; $df = 1$; $P = 0.744$). Furthermore, statistical analysis of the survival curve for *H. hebetor* revealed that there were significant differences among the treatments tested ($\chi^2 = 36.657$; $df = 4$; $P < 0.0001$) (Table 1). Similar to our results, survival of *Spalangia cameroni* (Hymenoptera: Pteromalidae) was significantly reduced by the highest concentration of *M. anisopliae* (Nielsen et al. 2005).

Conclusions: Our findings will be useful to prevent negative intraguild interaction of these two biocontrol agents during integrated management of *H. armigera* by development of proper timing strategies for their combination.

Table 1 Survivorship for *Habrobracon hebetor* parasitizing *Metarhizium anisopliae*-treated larvae of *Helicoverpa armigera* larvae at different time intervals

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Figure 1

Treatments	Control	0 h	24 h	48h	72 h
Control		$\chi^2 = 0.107$ $df = 1$ $P = 0.744$	$\chi^2 = 6.587$ $df = 1$ $P = 0.010$	$\chi^2 = 12.500$ $df = 1$ $P = 0.000$	$\chi^2 = 25.229$ $df = 1$ $P = 0.000$
0 h	—	—	$\chi^2 = 5.050$ $df = 1$ $P = 0.025$	$\chi^2 = 10.382$ $df = 1$ $P = 0.001$	$\chi^2 = 22.281$ $df = 1$ $P = 0.000$
24 h	—	—	—	$\chi^2 = 0.992$ $df = 1$ $P = 0.319$	$\chi^2 = 6.480$ $df = 1$ $P = 0.011$
48 h	—	—	—	—	$\chi^2 = 2.432$ $df = 1$ $P = 0.119$

P IPM 23

Sub-lethal effect of *Metarhizium anisopliae* on reproduction of *Habrobracon hebetor* parasitizing *Helicoverpa armigera*

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Question: The ectoparasitoid wasp, *Habrobracon hebetor* Say (Hymenoptera: Braconidae) and the entomopathogenic fungus, *Metarhizium anisopliae* (Metsch.) Sorokin (Hypocreales: Clavicipitaceae) are valuable biocontrol agents attacking larval stages of *Helicoverpa armigera* (Hübner) (Lepidoptera: Noctuidae). There is no detailed information on compatible application of these

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two bicontrol agents. Accordingly, fecundity of *H. hebetor*, parasitizing *M. anisopliae*-treated larvae of *H. armigera* were studied under laboratory conditions.

Methods: Third instar larvae were first infected with LC₃₀ (2×10^6 conidia.ml⁻¹) of *M. anisopliae* isolate M14 by immersion method which were then exposed to one pair of male and female parasitoids (< 24h) at different time intervals (0, 24, 48 and 72 h).

Results: Results revealed that the LC₃₀ of *M. anisopliae* adversely affected age-specific fecundity (m_x) of *H. hebetor* as a function of time interval between fungus application and exposure to the parasitoid. The highest daily and total fecundity values were 131.75 and 7.01 (egg) for control and 72 h treatments, respectively whereas the lowest were 2.90 and 45.67 (egg).

Conclusions: Our findings will be useful in development of proper timing strategies for combination of these two biocontrol agents during integrated management of *H. armigera*.

P IPM 24

Effects of different plant protections strategies on cereal yield in a long term field trial in Germany (2002-2014)

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Introduction: Politics and community have a critical look at plant protection in agriculture. In accordance to the strategy of integrated plant protection the pesticide application should be reduced to the required necessary pesticide dosage.

Objectives: The presented long term field trial provides opportunities for the identification of the saving potentials for pesticide usage and the determination of the necessary minimum to avoid economically relevant losses and therefore ensure high yields and high resource efficiency.

Materials and methods: In 2002 a long term field trial was established at the Julius Kühn research field in Dahnsdorf, state of Brandenburg in Germany. The crop rotation consists of maize - winter wheat - winter barley - potato - winter wheat and winter rye. Four different plant protection strategies were investigated: (1) non chemical plant protection (2) application of pesticides according to the integrated plant protection (3) reducing the treatment frequency index of pesticides by 25 % compared to strategy 2 and (4) reducing the treatment frequency index of pesticides by 25 % compared to strategy 2. Weed emergence before treatment and occurrence of fungal diseases were periodically determined in all cereals.

Results: Plant protection strategy 1 (non chemical) shows drastic losses of 30% in yield for all cereals. Strategy 4 shows also losses in yields especially in winter barley and generally in years with a high infection pressure for fungal diseases. The highest yields could be achieved in strategies 2 and 3. These two strategies show no significant differences in yield over the years, which could be explained through the good crop rotation with the changing of winter and spring sown crops. Also the weed infestation rises in the strategies with no or lower herbicide input.

Conclusion: In our field trials a slight reduction of pesticide amount is possible, but the risk of yield losses increases with this reduction. Non chemical plant protection gives lower yields and the risk of dramatic losses increases.

P IPM 25

Monitoring of Resistance and Baseline Sensitivity of *Setosphaeria turcica* to Azoxystrobin in Gansu

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The sensitivity of *Setosphaeria turcica* isolates to azoxystrobin in Gansu was tested by Petri plate in order to master their baseline sensitivity and resistance factor. The results showed that the sensitivity of *Setosphaeria turcica* isolates to azoxystrobin in Gansu is very significant from southeast to northwest, EC₅₀ range is 0.0349~5.2132 µg/ml, mean EC₅₀ is 0.5409 µg/ml. Normality test of EC₅₀ in thirty-two isolates is continuously normal distribution, baseline sensitivity of *Setosphaeria turcica* to azoxystrobin in Gansu is 0.1044 µg/ml from mean EC₅₀ in 32 isolates. The highest and mean resistance factor as well as resistance frequency of *Setosphaeria turcica* isolates to azoxystrobin is respectively 49.9348 and 5.1808 as well as 21.05% on this base in Gansu. Seven moderately and highly resistant isolates with 12.28 percentage appeared in southern humid area and in east semihumid and semiarid area. Five lowly resistant isolates with 8.77 percentage appeared in east semihumid and semiarid area and in central rainfed dryland. Sensitive strains appeared in Hexi corridor, mean EC₅₀ is 0.1631 µg/ml, mean resistance factor is 1.5617, the highest resistance factor is 3.8914, and there is temporarily no resistance.

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P IPM 26

The effects of rice varieties and nitrogen fertilization rates on nymphal performance of Malayan black bug, *Scotinophara coarctata*

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Question: Malayan black bug (*Scotinophara coarctata*) was a major insect pest in the island of Palawan, the Philippines. Northward migration of the insect has been documented and Malayan black bug is now routinely found in Luzon, the northernmost island of the Philippines. Monitoring of research fields at the International Rice Research Institute showed relatively higher numbers of black bug on rice with high nitrogen fertilization rates. We conducted a controlled experiment study to investigate the effects of rice varieties and nitrogen fertilization rates on nymphal performance of Malayan black bug.

Methods: The experiment was conducted in a greenhouse with black bug colonies originated in the Philippines as the insect source. The treatments consisted of a combination of three N rates and three rice varieties selected on the base of their popularities among Philippino farmers. There is very little information available on the susceptibilities of rice varieties to black bug. Each combination of treatment was replicated nine times. Fifteen 1-2 day old eggs of *S. coarctata* were used to invest each replicate. After six weeks, the numbers of surviving black bug were counted and their developmental stages recorded.

Results: We found that the survivorship, development rate and adult biomass of *S. coarctata* is lower on plants treated with high N rate compared to the ones treated with low N rate. This relationship between black bug's ecological fitness parameters and nitrogen fertilization rate is in direct opposition of that found in brown planthopper, another crucial pest of rice, in which higher nitrogen fertilization rate resulted in higher survivorship, performance and fecundity.

Conclusion: Reduction in nitrogen fertilization rate acts as a component in the management brown planthopper populations. The same practice may have a very different effect on Malayan black bug, as indicated in our study. Studies on the effects of nitrogen fertilization rate on black bug reproduction and adult performance is missing. These studies, together with the results reported in this poster, will provide better information for rice farmers to manage risks associated with Malayan black bug.

P IPM 27

Effects of Dazomet and *Purpureocillium lilacinum* on root-knot nematode *in vitro*

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Root-knot nematode (*Meloidogyne* spp.) is one of the most serious soilborne diseases and causes enormous losses in protected vegetable production in China. In this paper, the integrated effects of fumigant Dazomet and biocontrol agent *Purpureocillium lilacinum* YES-2-14 on root-knot nematode were investigated *in vitro*. The results demonstrated that nematode eggs and juveniles were extremely sensitive to Dazomet. When exposed to Dazomet for simulation of field application, the hatching rate of eggs and vitality of second-stage juveniles decreased significantly with the increasing concentration of fumigant ($P < 0.05$). The hatched juveniles were less than 5% compared to the control under the fumigation dosage of 5 mg/kg soil. When 10 and 20 mg/kg soil Dazomet were used, the corrective mortality of the juveniles reached 41.9% and 100%, respectively. The isolate YES-2-14 showed strong parasitic capacity to the eggs of root-knot nematode. When encountered with 6×10^4 spores/ml fungal suspension, the percentage of eggs colonized by *P. lilacinum* was 55.5% in 132 h. However, when Dazomet was exerted with the dosages of 5-25 mg/kg soil, the parasitic rate reached 77.9%-85.6%, though there was a delay at early parasitic process under higher fumigation concentration. Fermentation filtrate of YES-2-14 suppressed second-stage juveniles intensively. However, no significant difference was detected between the treatments with and without Dazomet fumigation before application of the nematophagous fungus. In greenhouse, the infection of tomato roots by second-stage juveniles treated by both practices decreased significantly, and the numbers of knots per root reduced by 99.7% compared to the separate use of Dazomet (71.9%) and *P. lilacinum* (23.9%), indicating that combined application of Dazomet and *P. lilacinum* could improve the control efficiency against root-knot nematodes. The research is of great theoretical and practical meaning in efficient control of plant nematodes.

P IPM 28

Conservation and Classical Biological Control of Citrus Pests in Eastern Mediterranean Region of Turkey

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Eastern Mediterranean region is the largest producer of citrus in Turkey for fresh consumption and export. Citrus is the host for many pests, mainly sucking insects belonging to order Hemiptera. Scale insects, mites, whiteflies, aphids and Lepidopteran species are controlled by either native and/or introduced natural enemies, successfully established by several classical biological control studies over the last few decades. Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae), Citrus mealybug, *Planococcus citri* (Risso) (Hemiptera: Pseudococcidae) and California red scale, *Aonidiella aurantii* (Maskell) (Hemiptera: Diaspididae) are considered as major pests in the region. *Planococcus citri* and *A. aurantii* are controlled by either native or introduced natural enemies. Only *C. capitata* cannot be suppressed without chemical control. The status of biological control, including classical and conservation strategies, against citrus pests in eastern Mediterranean region of Turkey is reviewed. Conservation of natural enemies, by proper cultural practices and the application of specific acaricides and insecticides in combination with summer oil applications seems to be an essential component of the management of pests in citrus in the region.

P IPM 29

First results of monitoring local migration dynamics of cabbage whitefly (*Aleyrodes proletella*) in winter oilseed rape fields

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From the early 2000s onwards, the cabbage whitefly *Aleyrodes proletella* (Hemiptera: Aleyrodidae) has become an important pest in Brassica vegetables throughout Germany. Although this whitefly species has been reported as pest already in the 1930s, there exist substantial knowledge gaps on biology, hibernation and short-distance migration of *A. proletella*. Within the scope of a 3-year research project funded by the German Federal Office for Agriculture and Food (BLE), migration behavior and flight activity of *A. proletella* are investigated in the light of temporal dynamics. Based on the data collected, this knowledge may especially be used for scheduling agronomic and plant protection measures. It can be assumed that *A. proletella* adults overwinter in oilseed rape fields and that take-off in early summer is most frequent when the host plant is senescing. Therefore, we examined short-distance migration of *A. proletella* under field conditions from April to October 2014. For this purpose four oilseed rape fields about 50 km to the south of Rostock were selected. Population development and emigration behavior of *A. proletella* were monitored during the entire growing season. Bait plants (Kale, *Brassica oleracea* convar. *acephala* var. *sabellica*) were used as passive traps and were placed along two equally spaced transects radiating from the fields of at least 250 m in prevailing wind direction. Furthermore, changes in oilseed rape maturity and quality were assessed in order to identify potential factors, which may trigger whitefly emigration. Preliminary results show that *A. proletella* started to emigrate from oilseed rape fields in early June. Consistent flight activity was observed until oilseed rape harvest at the end of July. However, it has not yet been finally determined, whether or not whitefly take-off is triggered by host maturity. Shortly after harvest the abundance of *A. proletella* on bait plants increased again, while highest colonisation was reached in September. However, the consistent flight activity of *A. proletella* also after rapeseed harvest suggests that on the landscape level other alternative hosts offer additional temporary reproduction sites. In this regard we often noticed that after harvest it is common practice of regional farmers to use re-growing oilseed rape stubbles or volunteer growth rapeseed for green manure, aiming at soil coverage and N catch crop effects. Consequently, under these circumstances, whitefly immigration into Brassica vegetable crops is probably not limited to a defined timeframe.

P IPM 30

Two-year race monitoring for *Exserohilum turcicum* in European maize growing regions

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Introduction: Northern corn leaf blight (NCLB) caused by *Exserohilum turcicum* is one of the most important leaf diseases in maize growing regions in Europe. Qualitative resistance genes are effectively used to control this disease. However the usefulness is limited by the evolution of virulent races.

Objectives: The aim of this study was a race monitoring for *E. turcicum* in central Europe to answer the question for the regional efficacy of monogenic resistance genes against NCLB.

Material and methods: Isolates were collected over two years (2011 & 2012) in ten different countries and from 165 different locations. A total number of 548 single spore isolates have been characterized 302 from 2011 and 246 from 2012. To classify the races we assessed the disease reaction on a differential set of near isogenic inbred lines based on the nomenclature proposed by Leonard et al. 1989. We used the lines: B37 (no *Ht* gene), B37Ht1, B37Ht2, B37Ht3 and B37HtN.

Results: For each tested resistance gene we found at least one virulent isolate. The four mainly occurring races over both years are race 0 (avirulent to all tested R genes) with 45%, race 1 (22%), race 3 (15%) and race 3N (13%). The races 13, 123, 23, 2, 23N, 12, 1N and 13N were present at very low levels. Race 0 isolates had the highest frequency in both years with 50% in 2011 and 40% in 2012. The three virulent races 1, 3 and 3N together covered 44% in 2011 and 57% in 2012. In the northern and coastal regions with high percentages of maize race 0 is predominant with 70%. In the Upper Rhine Valley with higher temperatures and long tradition in maize cultivation 45% of the isolates are virulent to *Ht1* and 26% to *Ht3*. Isolates collected in south west France and the Inn valley in southern Germany and Austria showed frequencies for *Ht3* of 43%. A unique characteristic of the south west region of France in contrast to other regions in Europe is the high virulence frequency of 35% for *HtN*. Race 3N is predominant in this region. In the Po valley in northern Italy the R gene *Ht3* has lost its effectiveness against 60% of the tested isolates.

Conclusions: The efficacy of single tested R genes is regionally decreased. To ensure the efficacy it is important to take additional sources of qualitative resistances into account and beyond that quantitative resistances can help to avoid a fast development and wide spread of virulent races.

P IPM 31

Phytochemical-based management of phytoparasitic nematodes

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Secondary metabolites occurring in plants as constituents of defence mechanisms against biotic stresses may be a potential source of biorational biocidal compounds to apply in the management of plant pests, among which also phytoparasitic nematodes. Role of phytochemical-based nematicidal formulations is furtherly enhanced by the dramatic lack of commercial synthetic nematicidal products following to the drastic EU pesticide revision. A huge number of plant-derived pure compounds or commercial formulations has been investigated, both in laboratory and field conditions, by our workgroup throughout the past decade. Based on this experimental work, glucosinolates, triterpenoidic saponins and essential oils and their main constituents seem to be the groups of plant secondary metabolites most promising for the development of new effective but safe nematicidal products. Glucosinolates are thioglucosidic secondary metabolites occurring mainly in the Brassicaceae and in Capparidaceae families. Products (isothiocyanates, thiocyanates, indoles etc.) of their hydrolytic degradation are highly toxic to soilborne pest, pathogens and weeds and then can be considered an ecological alternative to fumigants previously used for the control of nematode pests. Plant-derived saponins are triterpene glycosides present in top and root tissues of plant species of botanical families as Leguminosae, Alliaceae and Asteraceae. Due to their chemical, physical and physiological properties, naturally occurring saponins display a broad spectrum of biological effects, among which also nematicidal properties. Essential oils are mixtures of heterogeneous volatile substances, mainly terpenes, terpenoids and other aromatic and aliphatic constituents, formed as secondary metabolites by aromatic plants belonging to a number of botanical families, like Lamiaceae, Myrtaceae, Lauraceae, Asteraceae. Due to their great number of constituents, essential oils are able to affect several targets at the same time, thus decreasing the target organisms' resistance or adaptation. Biological activities of EOs and their components are extended to a broad spectrum of phytonematode species through different mechanisms of action, such as behaviour and feeding deterrence effects, fumigant and contact toxicity. The *in vitro* and *in vivo* studies undertaken by the author's workgroup are reviewed and discussed in this communication.

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P IPM 32

Toxicity of indoxacarb to diamondback moth (*Plutella xylostella* L.) and their hyperparasite *Dedegma fenestralis* Holmgr. in white cabbage

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White cabbage *Brassica oleracea* L. var. 'Capitata' is widely cultivated vegetable in the Europe as well in Lithuania. One of the most harmful pests of cabbages is Diamondback moth (DBM) (*Plutella xylostella* L.). DBM larvae cause economic damage in cabbage in the fields, especially in sustainable growing system. In order to save infested plants pest control is necessary in most cases. A lot of insecticides are now ineffective in the control of DBM. The objectives of this research were to determine the toxicity of new insecticide Steward 30 WG to DBM and their hyperparasite *Diadegma fenestrale* Holmgr. The investigation was conducted in the experimental fields at the Institute of Horticulture (55°08' N, 23°80' E) in 2013. Insecticides Steward 30 WG (a.i. indoxacarb 300 g kg⁻¹) at rates 0.085, 0.10, 0.20 kg ha⁻¹ and Decis Mega EC (a. i. deltamethrin 50 g l⁻¹) at rate 0.15 l ha⁻¹ (standard) were tested. The experiment was designed by randomized blocks at four replications. The number of pests was compared among treatments using a single factor analysis of variance (ANOVA). Mortality (according Abbot) of larvae after two applications of Steward (all rates) varied from 61% to 90% and was higher compared with plots treated by Decis Mega. There were no significant differences found in abundance of DBM between all treated insecticides. The parasitism was highest in untreated plots, lowest - in plots treated with Steward 30 WG 0.10 kg ha⁻¹. In plots treated with Steward 0.20 kg ha⁻¹ any parasitized pupas was not found. All reared parasitoids belonged to Ichneumonidae family. As a percentage of the parasites recovered from the pupae of DBM and their hyperparasite *Diadegma fenestrale* was 75% in untreated plots, in plots treated with Steward (0.085 and 0.10 kg ha⁻¹) reached 50% and in plots treated with Decis Mega was 66.7%. This research was funded by a grand ("Horticulture: agro-biological basics and technologies" implemented by Lithuanian Research Centre for Agriculture and Forestry") from the Research Council of Lithuania.

P IPM 33

Enhancement of eggplant capacity to cope with pest-caused stress through BABA treatment

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Background: BABA (β -aminobutyric acid) is a non-protein amino acid, acting as a priming agent, retaining plant fitness. BABA priming mitigates adverse effects of a range of biotic and abiotic stressors. The objective of this study was to evaluate potential protective effects of BABA treatment against piercing-sucking pests (thrips and mites), the key pests of eggplants grown under cover.

Materials and methods: The eggplant (*Solanum melongena* L. Scorpio F₁) was grown on soilless media in greenhouse, closely resembling standard production conditions. The plants were treated either with water (control) or with BABA at concentrations of 25 mM or 50 mM applied as seed imbibition (SI) or soil drench (SD). Experiment was conducted in a randomized complete block design with 3 replications, 4 plants each. All the experimental plants were exposed to natural infestation by the resident population of thrips (*Frankliniella occidentalis* Pergande) and/or the two-spotted spider mites (*Tetranychus urticae* Koch). Following pest infestation, eggplant growth, yield and leaf chlorophyll fluorescence (Fv/Fm - maximum quantum efficiency of PSII, PI - performance index, Φ PSII - quantum efficiency of PSII) were assessed.

Results and conclusions: BABA applications had negligible effect on the density of the two-spotted spider mite but hampered development of thrips on the thrips-infested eggplants. BABA application had no negative impact on eggplant fitness measured as plant growth and productivity. It is worth mentioning the increase of leaf Fv/Fm in BABA-treated (SD) and mite-infested plants compared to the control. The study provides evidence that BABA may modulate fitness of eggplant, however it seems to have very limited potential in mite and thrips management.

P IPM 34

Bioinsecticidal effect of the crude ethanolic extract of the plant *Artemisia judaica* against *Aphis fabae*

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The crude ethanol extract of the plant *Artemisia Judaica* was tested on the black bean aphid *Aphis fabae*. Four doses (12.5, 6.25, 3.12 and 1.56 mg/mL) were tested on contact wingless adults. The results have showed that the tested extract has been very powerful to aphids. At the highest dose 12.5 mg/mL, the 100% of mortality were recorded 2 hours after treatment, and after 96 hours for the lowest dose (1.56 mg/mL). The LD50 calculated 2 hours after treatment from the regression lines Probit = f (doses) shows that it is 2.75 mg/mL. This powerful insecticidal activity of the tested crude extract could be due to the richness of the plant on terpene compounds known for their bio-insecticide action.

P IPM 35

Effect of the flavonoid rutin on the biology of *Spodoptera frugiperda* (Lepidoptera: Noctuidae)

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The fall armyworm *Spodoptera frugiperda* is a major pest species of maize crops. In Brazil, one of the factors that contributes to failures in the control of *S. frugiperda* is the large number of hosts caused by the succession of crops with different phenologies. The activity of plant chemical substances has been promising, and new components with insecticidal potential have been discovered with potential use in pest management. The effects of plant metabolites on the biology and behavior of insects is rarely studied. Therefore, the current study aimed to evaluate diet containing the flavonoid rutin and your affect on the biology of *S. frugiperda*. The study was conducted in the Laboratory of Agricultural Entomology of the Goiano Federal Institute - Campus Uruaí, in completely randomized design with 25 replicates. The study evaluated four treatments: regular diet (control) and diets containing 1.0, 2.0 and 3.0 mg/g of rutin. The following biological parameters were evaluated: larvae and pupae development time and viability, weight of 10 day old larvae, weight of 24 hour old pupae, longevity and adult total life cycle. The flavonoid rutin negatively affected the biology of *S. frugiperda* by prolonging the larval development time, reducing the larval and pupal weight, decreasing the pupal viability and prolonged the life cycle.

Figure 1

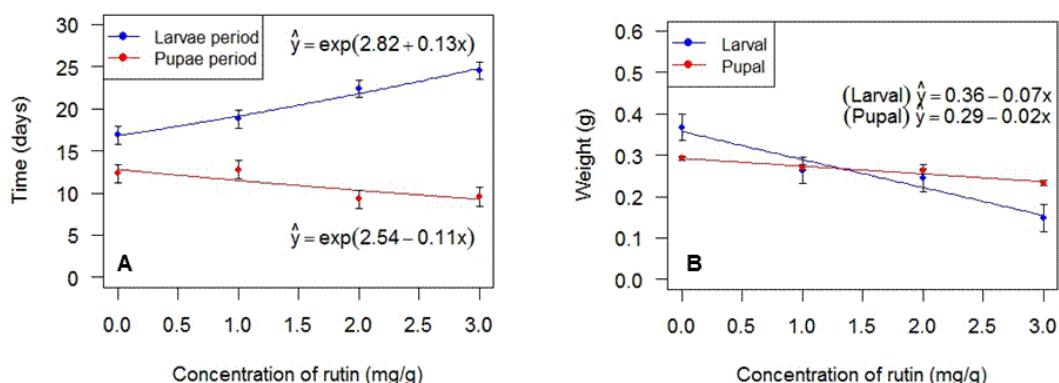


Figure 1. Development time (A) and weight (B) of larvae and pupae of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) fed an artificial diet containing different concentrations of rutin. Uruaí, Goiás state, Brazil. 2014.

Figure 2

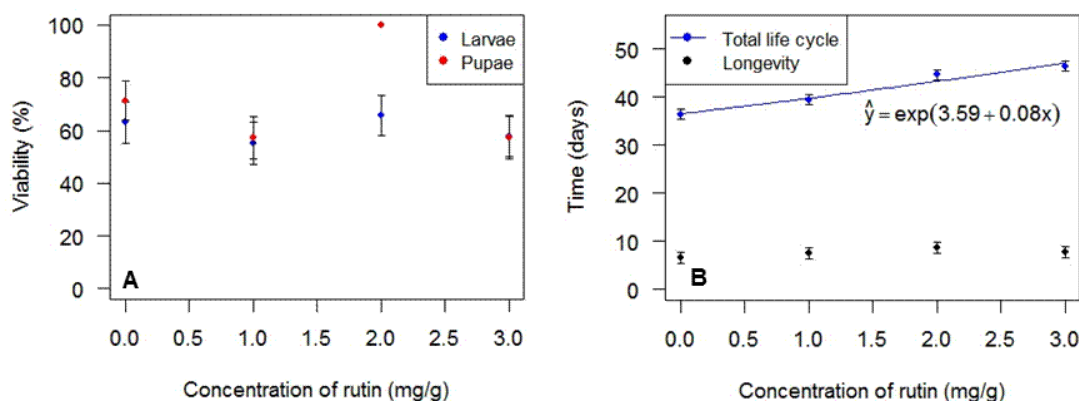


Figure 2. Viability (A) and total life cycle and longevity (B) of *Spodoptera frugiperda* (Lepidoptera: Noctuidae) fed an artificial diet containing different concentrations of rutin. Urutaí, Goiás state, Brazil, 2014.

P IPM 36

Resistant soybean varieties and silicon in the biology of *Euschistus heros* (Hemiptera: Pentatomidae)

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Seed-sucking insects are economically relevant pests impacting the soybean crop in Brazil. The aim of the present study was to evaluate the effect of inducers of soybean resistance on the neotropical brown stink bug *Euschistus heros* (Hemiptera: Pentatomidae). Seeds of the soybean varieties IAC 100 and IAC 17 (antibiosis-type resistance and non-preference varieties in terms of insect feeding preference), Conquista (moderately resistant) and Jataí (susceptible) were planted in polyethylene pots filled with a mixture of soil and organic compost. Green pods of soybean varieties treated with inducers or a control solution were offered individually to *E. heros* nymphs in Petri dishes (6 cm in diameter) to monitor the biology of the insect. Each Petri dish containing one insect represented one replicate, and 20 replicates were performed for each treatment using a completely randomized design. The daily evaluations of the varieties with or without inducer application were performed in the morning after the confinement of nymphs. The variables assessed were stage duration (N2, N3, N4 and N5) and the egg-to-adult period. The antibiosis resistance of varieties IAC 17 and IAC 100 to *E. heros* were evidenced by the negative effects observed when plants of these varieties treated with resistance inducers were fed to *E. heros*. The potassium silicate, calcium magnesium silicate and acibenzolar-S-methyl (ASM) inducers were found to have a synergistic effect with IAC 17 and IAC 100 on insect resistance.

Figure 1

Tabela 1. Mean duration of the nymphal and adult stages of *Euschistus heros* (Hemiptera: Pentatomidae) on different soybean varieties treated with different resistance inducers.

Cultivars (C)	Stages of insects ^{1,2}				
	2 ^o instar	3 ^o instar	4 ^o instar	5 ^o instar	Adult ¹
IAC 17	3.82 a	5.78 ab	5.77 a	5.05 a	2.65 a
IAC 100	3.68 a	4.82 a	4.60 ab	2.05 c	2.15 a
BRS Conquista	2.89 b	4.68 b	3.00 b	3.20 bc	1.20 b
BRS Jataí	2.46 b	4.72 ab	5.17 a	3.70 ab	1.70 b
F (C)	9.24**	2.70*	6.64**	11.13**	17.92**
Inductors (I)					
ASM	3.13	4.22 b	5.79 a	2.44 bc	.2
K Silicate	3.50	4.90 ab	4.29 ab	6.06 a	3.75 a
Na Silicate	3.04	4.62 ab	3.83 ab	1.94 c	1.50 ab
Ca+Mg Silicate	2.87	5.87 a	3.62 b	3.44 bc	2.69 a
Untreated	3.52	5.37 ab	5.62 ab	3.62 b	0.81 b
F (I)	1.48 ^{ns}	3.26*	3.95**	14.64**	34.26**
F (CxI)	1.83*	0.52 ^{ns}	6.24**	22.17**	67.24**
C.V. (%)	23.62	22.39	24.06	20.53	16.52

¹Data transformed into $(x + 0.5)^{1/2}$ for analysis. ²Means followed by the same letter within a column do not differ according to Tukey's test at 5% probability. C.V. coefficient of variance. ^{ns}non-significant. * Significant at 1% probability. ** Significant at 5% probability.

Figure 2

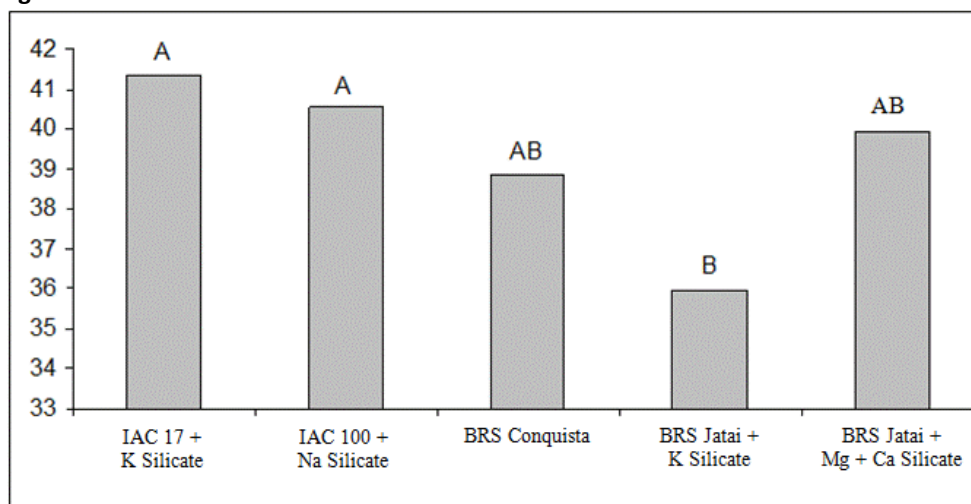


Figure 1. Egg-to-adult development (days) of *Euschistus heros* on different soybean varieties treated with different inducers. Urutaí, Goiás State, Brazil. Means followed by the same letter in the column do not differ according to Tukey's test ($P>0,05$).

P IPM 37

Microbial control of Phyllophaga (Coleoptera: Scarabaeidae) by small farmers in México

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Soil dwelling larvae of insects from the family Scarabaeidae are pests of many crops in Mexico. The Laboratory of Pathology and Microbial Control of Insects (INIFAP, Uruapan Experimental Station, Michoacan) has focussed on the use of entomopathogenic fungi for control of these pests. Investigations have been carried out to find, evaluate and produce native strains of *Beauveria bassiana* and *Metarhizium anisopliae*. A bank of isolates from diverse regions has been formed and strains tested for efficacy against soil pests. *Phyllophaga* spp. are the main pests of maize and many other crops. Promising strains for control of these insects have been identified and application in farmers' fields has produced up to 80% larval control and increases of >1000kg/ha in yield. An IPM system for pest scarabs in maize has been developed combining application of entomopathogenic fungi and use of native varieties of maize with strong root systems. The biocontrol programme has been extended to small fruit production on the initiative of the local agricultural producers. A biofactory for the production of entomopathogenic fungi has been constructed in the municipality of Los Reyes with the support of the Federal and State Governments and is staffed by local personnel trained for mass production of fungi through solid state fermentation. Entomopathogenic fungi are now produced for applications in regional programmes of biological control against grasshoppers, locusts, and whitefly, promoted by local authorities and producers' organisations in cultures of blackberry, peach, maize and vegetables. The biofactory is owned and administered by the organisation "Productores Agropecuarios por la calidad" (PROCAL). INIFAP advises on the process of production, develops research and implements transfer of technology.

P IPM 38

On farm management of sudden mango death disease in Pakistan

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Pakistan is the worlds fourth for the production of mango (*Mangier indica* L.) crop with 167.5 hectares with production of 1732 tones. Sudden mango death has caused a serious threat to this economically important mango crop. Strategies to manage this disease were developed and demonstrated at farmer's field. For dissemination of these management strategies an extension approach "On-Farm Demonstrations" was adopted whereby disease was managed on selected sites for adaptation by rest of the farmers. The selected sites were two villages of tehsil Mianchannu district Khanewal, Punjab, Pakistan. Two garden sites

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comprising of five and two acres in villages such as 44/15-L and 124/15-L, respectively were selected in the East and Southern sides of Mianchannu city. Ten mango trees from each garden suffering with 70-80 % disease were randomly selected for treatment of disease. Mixture of copper sulphate (grinded) 350 gm, lime 2 kg, rottened wheat straw 10 kg, furadon 350 gm and in these ingredients 20 kg compost was added. This mixture was mixed in the soil with hoeing 2 feet around and irrigation was applied. This practice controlled 80-85 per cent of disease. This practice then adopted in five other districts of Punjab and yielded similar results. These results are discussed in the light of economic impact of sudden death disease control for mango industry.

P IPM 39

Effects of nutritional supplements on seed germination, plant growth and resistance to *Ralstonia solanacearum* causing bacterial wilt disease in tomato

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The cultivated tomato (*Solanum lycopersicum*) is economically important and the second most consumed vegetable crop in the world. To produce high-quality tomato is very important to both breeders and farmers. For this, tomato plants should be healthy during growing season. In addition, diverse diseases in tomato should be well-managed to minimize impacts of diseases in tomato fruit production and quality. The bacterial wilt disease caused by *Ralstonia solanacearum*, a soil-borne pathogen, is one of the most destructive diseases in tomato. Although some tomato varieties have been shown to be resistant to this disease, resistance was easily broken particularly when they were placed in the high temperature and humidity. In this study, we analyzed effects of nutritional supplements on seed germination, growth of tomato roots and shoots, and the degree of resistance to bacterial wilt disease. As nutritional supplements, two commercial products of Cytozyme company, Seed+Extra (liquid) and Soil Max were tested. To examine their efficacy in germination and plant growth, Seed+Extra was applied to tomato seeds, as manufacturers described in the products. Seed+Extra treatment increased both germination rate and plant growth. Next, to examine the effect of Seed+Extra or Soil Max on bacterial wilt disease, 4-week-old tomato seedlings pre-treated with each of two products were transplanted and inoculated with *R. solanacearum* strain SL341 (race1, biovar3) in the controlled growth chamber. Disease severity was measured from one week after inoculation until plants died with rating from 0 (no symptom) to 5 (completely died). As results, Seed+Extra treatment significantly reduced bacterial wilt diseases in tomato, while Soil Max did not. These results indicate that Seed+Extra could make tomato plants healthier and more resistant to certain diseases.

P IPM 40

Antimicrobial activity of *Rosmarinus officinalis* extract on *Magnaporthe oryzae*

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The control of rice blast is mainly accomplished with the application of fungicides, but the indiscriminate use of these products cause environmental and food residues and therefore risks to human health. This leads to the need to develop new control strategies for rice blast, such as the use of plant extracts. The aim of this research was to evaluate the antimicrobial activity of *Rosmarinus officinalis* extract on the germination and apresorium formation of *Magnaporthe oryzae* and its effect in suppressing rice blast severity, in greenhouse conditions. The bioassays were conducted in a completely randomized design. We tested the efficiency of the *R. officinalis* extract at the concentrations (50, 40, 30, 20, 10, 5, 2.5 and 0 mg/mL) for inhibition of conidial germination and apresorium formation; and in the concentrations (150, 100, 50 and 0 mg/mL) for the suppression of rice blast. The concentrations of 40 and 50 mg/mL of the *R. officinalis* extract inhibited conidial germination and apresorium formation at 4, 6 and 24 hours. All tested concentrations of *R. officinalis* extract reduced rice blast severity, especially 150 mg/mL, which suppressed over 90% of the affected leaf area. The *R. officinalis* extract proved to be a potential antimicrobial for *M. oryzae* *in vitro*, and an alternative to control rice blast *in vivo*.

P IPM 41

Tomato growers' application for the correct methods used in tomato leaf miner pest control in plastic tunnels in the (Aljazeera / 61) desert region / Karbala province

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Tomato, *Lycopersicon esculentum* Miller (Solanaceae), is considered to be one of the most economically important crops all over the world. Between 100 and 200 pest species are reported to attack tomatoes worldwide. Some of the pest species are known to be of great economic importance such as; the tomato leaf miner, *Tuta absoluta* (Meyrick) (Lepidoptera: Gelechiidae). This research aimed to determine the tomato growers' application level for correct methods implemented in controlling *T. absoluta* through three controlling areas and as a follow: using chemical control, using agricultural control, using pheromones and light traps control. And yet, to determine the reasons prevented those growers' of application the correct methods. The research community included all tomato growers in the area (Aljazeera / 61) / desert region in Karbala province where those number was (504) growers. The sample was (50) growers that means 10% of the total tomato growers' number. The results showed that the application level was low for the using chemicals and agricultural control, and was none for the using pheromone and light control, This research is highly recommended the following trends: i) The adoption of research results by the department of extension and extension centers and agricultural offices in all tomato producing provinces all over the country, ii) Providing all the control necessary requirements for the pest control, iii) Getting the growers satisfaction through the application of new techniques of insect control in their farms, Awareness of growers about the dangerous uses of pesticides on human health .

Table 1: interviewee's distribution based on the implemented level

D.S		Implemented Level Mean	Percentage	No	Implemented Level Degree	Implemented Level
1.33	6,52	5 ,18	54	27	6 - 4	Low
		7 ,61	42	21	9 - 7	Medium
		11	4	2	12 - 10	High
				100	50	Total

The percentage of the interviewee's distribution based the preventing reasons criteria

The Percentage	No	Preventing Reasons	N
90	45	The interviewees whom persist to implemented and applying the tradition control methods and	1
42	21	Lack of the possibilities	2
74	37	The extension weakness	3
52	26	government support weakness The	4
84	42	The undesirable environmental factors	5

P IPM 42

The impact of seed treatment, foliar fungicide timing, and plant growth regulator on leaf-disease severity and productivity of barley

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Question: Over the past decade Western Canadian barley producers have moved to a canola/cereal rotation due mainly to economic considerations. As a consequence the risk and impact of plant diseases has increased. The objectives of the current

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study are to determine the effects of seed treatment and plant growth regulator (PGR) application, with flag leaf emergence or head emergence fungicide application on disease development, and barley quality and yield.

Methods: At Lacombe and Lethbridge, Alberta, Melfort and Indian Head, Saskatchewan, Brandon, Manitoba, and Charlottetown, Prince Edward Island, the impact of seed treatment, foliar fungicide timing (flag leaf versus head emergence), and plant growth regulator (PGR) on leaf disease severity and crop productivity of barley was assessed in 2013. Insure™ (triticonazole + pyraclostrobin + metalaxyl) seed treatment was used at two times the recommended rate, while Twinline™ (metconazole + pyraclostrobin) and Prosaro™ (tebuconazole + prothioconazole) fungicides were applied at recommended rates at flag leaf and head emergence, respectively. The PGR Ethrel™ (Ethepon) was applied between flag leaf emergence and just prior to head emergence.

Results: Preliminary results suggest a negative effect of seed treatment on emergence at most sites. Reduced emergence due to seed treatment may have also led to slightly later maturity at some locations. Final disease severity on flag -1 and flag -2 leaves collected at late milk/early dough was significantly affected by some of the treatments in 2013. Leaf disease severity was lowest following in-crop fungicide application, although at some sites seed treatment also resulted in slightly lower leaf disease levels. Yields tended to be highest at most sites when an in-crop fungicide was applied, especially at the head emergence stage. Yield increases were observed with seed treatment, especially at Lethbridge, Melfort, and Indian Head. The application of PGR also increased yields, especially at sites where significant lodging occurred.

Conclusions: When the risk of leaf disease and lodging are increased the use of in-crop fungicides and PGR's should be used to improve crop productivity.

P IPM 43

Economical evaluation of different methods for controlling fig longihorne beetle, *Hesperophanes griseus* (Coleoptera: Cerambycidae) on fig trees.

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The long-horned beetle (L.H.B.), *Hesperophanes griseus* (Fabricius) (Coleoptera: Cerambycidae) is one of the most destructive pests of fig trees (*Ficus carica* L.) in Egypt. Recommended chemical insecticide Anthio 33% (250 cc /100 L. water), Anthio 33% plus the plant extract of *Antholyza* (*Antholyza ringens* Andr.) at 5% concentration, Anthio 33% plus entomopathogenic nematode, *Steinernema carpocapsae* and *A. ringens* plus the entomopathogenic nematode, *S. carpocapsae* were experimented for controlling L.H.B. infested fig orchards at El- Twayle village, Arish city, North Sinai Governorate, Egypt. All above mentioned treatments significantly increased average yield of fig. Anthio 33% and *A. ringens* was the most effective treatment caused increased in average production /fig feddan. While, *Antholyza ringens* and Nematode showed the least increased average yield of fig. All treatments are considered good recommendation for reducing the population of *H. griseus* larvae to minimum level in fig orchards, and increased the monetary value.

P IPM 44

PBPs enhance the sensitivity of PRs to sex pheromone components in *Chilo suppressalis*

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Insect monitor the chemical environment with specialised chemosensilla localised on dedicated sensory organs. Previous studies showed multiple olfactory proteins in chemosensilla involved in different steps in peripheral signal transduction pathway. It is generally accepted that the OBPs and ORs are both required for a correct detection of the stimulus. Therefore, a functional study of ORs should better be performed in the presence of OBPs, in order to reproduce in vitro a system as similar as possible to the physiological conditions. This is far from easy task, given the high numbers of both ORs and OBPs in insect, producing a number of theoretical combinations in the order of several hundreds to few thousands. The sub-system present in Lepidoptera, constituted by PRs and PBPs and dedicated to the perception of sex pheromones, offers a simplified scenario to model and investigate interactions between ORs and OBPs. In this studies, we have measured the binding affinities of the four recombinant PBPs to pheromone components and analogs. We have also expressed the six PRs in heterologous expression system and monitored their responses to the same ligands in the presence/absence of each PBP. The results suggested PBPs and PRs produce a system endowed with improved sensitivity and in some cases more narrowly tuned. These studies may suggest focused strategies to disrupt semiochemical detection and recognition in agricultural pests.

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Evaluation of nutrient indices, digestive enzymes and hemolymph components of the Colorado potato beetle (CPB), *Leptinotarsa decemlineata* (Say) (Col.: Chrysomelidae) on six potato cultivars

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The Colorado potato beetle (CPB) is a serious and economically important pest of potatoes throughout the world and Iran. In this study, the effects of six commercial potato cultivars including: Arinda, Sprit, Markiez, Lotta, Santae and Agria were studied on nutritional indices and enzymatic activities in midgut and hemolymph. There was a significant difference in the nutritional indices and enzymatic activities of larvae and adults reared on different potato cultivars. The lowest efficiency of conversion of ingested food (ECI) and efficiency of conversion of digested food (ECD) was observed on Lotta and the highest was on Agria. The index of plant quality (IPQ) and larval growth index (LGI) were significantly low on Lotta and high in Agria. The lowest and highest activity of chymotrypsin in both stages was observed on Lotta and Agria, respectively. The activities of α - and β -glucosidases in both stages were significantly lower on Lotta and higher on Santae. The activities of aspartate amino transferases (AST) and alanine amino transferase (ALT) were significantly higher on Agria in both stages. Amount of high-density lipoprotein (HDL) in the larval stage was highest on Agria and no significant difference was observed among other cultivars. Also, the highest amount of triacyl-glyceride (TAG) and protein was found in the larvae fed on Agria. These results revealed that Lotta is the most unsuitable cultivar for the larvae and adults of CPB as compared to other evidenced by the lower nutrient and intermediary metabolism.

P IPM 46

Spatial distribution pattern of alfalfa leaf weevil *Hypera postica* and root weevils *Sitona* spp. (Coleoptera: Curculionidae) in alfalfa fields

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Introduction: Alfalfa is an important forage crop in Iran and many parts of the world. Leaf weevil *Hypera postica* (Gyllenhal) and root weevils *Sitona* spp. (Coleoptera: Curculionidae) are important pests which cause considerable loss of alfalfa yield. Spatial heterogeneity is an important source of variation in populations and is affected by many biotic and abiotic factors.

Objectives: This study was done to determine spatial distribution pattern of alfalfa leaf and root weevils using geostatistics and generate distribution maps.

Materials and methods: This study was conducted in East Azabaijan province of Iran in two growing seasons, 2012 and 2013. Two alfalfa fields (0.5 and 1 ha) were chosen in Ilkhchi region in 2012 and four alfalfa fields (0.4, 0.5, 3.1 and 7.3 ha) were located in karkaj in 2013. The 3.1 and 7.3 ha fields were divided into 30×30 m grids and 0.4, 0.5 and 1 ha fields were divided into 10×10 m grids. Weekly sampling were started at the end of winter and continued until the yield harvest. Depending on the height of alfalfa plants, a 1×1 m quadrat and a standard sweep net were used for sampling the weevils. Spatial analysis of data was done using GS+5.1. Components of variogram models were used for determining the degree of spatial dependency of alfalfa weevils. Degree of dependence (DD) was calculated for detecting the strength of spatial dependency.

Results and conclusion: In semivariance analysis, spherical, exponential and Gaussian models were best fitted for the data with spatial autocorrelation. DD values were >26% for 97 out of 116 data sets indicating that the weevils were aggregately distributed in the alfalfa fields and had medium to strong spatial dependency. These results provide useful information about ecology of leaf and root weevils in the alfalfa fields and can be used in site-specific management of these pests.

P IPM 47

Predicting severity of bacterial canker and wilt caused by *Clavibacter michiganensis* subsp. *michiganensis*

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Introduction: *Clavibacter michiganensis* subsp. *michiganensis* is the causal agent of bacterial canker and wilt of tomato. In 2000 there was an increase in the number of infected greenhouses and in the severity of the disease in Israel.

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Objectives: To characterize the spatial pattern of the disease spread and to identify the relative significance of management practices used.

Methods: A comprehensive survey was conducted in tomato greenhouses and net-houses during the years 2009 - 2012 in Southwest Israel. Scouts visited the surveyed plots (681 in total) monthly and assessed the severity of the disease and recorded the relevant details about the crop and the cultural practices employed. These variables were divided into three groups: (1) variables affecting primary inoculum inside and (2) outside the plots, and (3) variables affecting disease severity during the season. We tested these three groups of variables including or excluding three hierarchical scales: grower, village and county, as variables. We characterized the spatial pattern of the disease spread and used semivariance analysis to examine the degree of anisotropy at directions of 0°, 45°, 90° and 135°. Next, we investigated the predictive power of these variables using generalized linear models.

Results: The results suggested an absence of anisotropy pattern. Global Moran's *I* analysis showed that disease severity had significant spatial autocorrelation revealing the existence of potential spatial patterns for the severity index. Next, we found that the strongest spatial autocorrelation occurred within a 1900 m neighborhood. The contribution of the three hierarchical scales decreased when ascending from small (grower) to larger (county) scales. When the grower was included in each group of variables, the explained variation increased from 0.09, 0.04 and 0.35 to 0.49, 0.44 and 0.65 for the first, second and third groups of variables, respectively.

Conclusions: Overall, the results demonstrate that the most influential factor on bacterial canker severity was the grower. The spatial autocorrelation at small scale (1900 m) can potentially also be attributed to the grower, as growth structures located at these distances commonly belong to the same farm and are cultivated by the same grower. This variable probably encompasses variation in experience and differences in agricultural practices between growers.

P IPM 48

Effect of Farm yard Manure on Fusarium wilt of Tomato in Copper polluted soil

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Current research work was carried out to check influence of farm yard manure (FYM) in *Lycopersicon esculentum* L. against *Fusarium oxysporum* f. sp. *lycopersici* (FO) in copper polluted soil. Silt-loam soil naturally enriched with 70 ppm of Cu was inoculated with FO and incorporated with 0%, 1%, 1.5% or 2% FYM. The multilateral interaction of host-pathogen-metal-organic amendment was assessed in terms of morphology, growth, yield, physiology, and metal uptake in tomato plant after 30 and 60 days of sowing. When soil was inoculated with FO, plant growth and biomass was significantly increased during vegetative stage, while declined during flowering stage with substantial increase in productivity over control. Infected plants exhibited late wilting and disease severity was found on 26-50% of plant during reproductive stage. Incorporation of up to 1% FYM suppressed disease severity, improved plant growth and biomass, while decreased yield. Rest of manure doses was found ineffective in suppressing disease. Content of total chlorophyll, sugar and protein were significantly declined in FO inoculated plants and incorporation of FYM caused significant reduction or no influence on sugar and chlorophyll content, and no pronounced difference among different FYM doses were observed. Whereas, proline, peroxidase, catalase and nitrate reductase activity were found to be increased in infected plants and incorporation of 1-2% FYM further enhanced the activity of these enzymes. Tomato plant uptake 30-40% of copper naturally present in the soil and incorporation of 1-2% FYM markedly decreased plant uptake of metal by 15-30%, while increased Cu retention in soil. Present study concludes that lower dose (1%) of FYM could be used to manage disease, increase growth and biomass, while ineffective for yield and productivity in Cu-polluted soil. Altered physiology/biochemistry of plant in response to any treatment could be served as basis for resistant against pathogen and metal homeostasis in plants.

P IPM 49

Digestion profiles of olive fruit fly (*Bactrocera oleae*) by predators using qPCR

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Olive tree has a great economic, social, environmental and landscape importance mainly in the Mediterranean region. The Mediterranean countries dominate the world olive market by producing 80% of the world's olive oil. One of the major concerns around the olive crop is the different pests and diseases that can limit olive production. *Bactrocera oleae* (Rossi) (Diptera: Tephritidae) is considered as one of the major pests and can be responsible for a loss of nearly 600 million euros per year. The

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control of this pest has relied mainly on the use of pesticides, but this strategy can harm human health and cause environmental contamination, besides plants might be able to acquire resistance. For this reason, the use of alternative pest management has been promoted to find a more sustainable production system that gives priority to non-chemical methods. Taking into account this situation a trophic interaction study between *B. oleae* and their natural predators (arthropods) was performed, in order to conceive a biocontrol strategy for restricting this pest. Specific primers designed for the mitochondrial cytochrome oxidase subunit I (COX1) gene regions from *B. oleae* were used for analysing the gut content of field-caught arthropods. The identification of arthropods was done using taxonomic keys and by sequencing their barcode region of COX1 gene. Results revealed *Calathus granantensis* and *Forficula auricularia* as potential predators of *B. oleae*. To select the more suitable predator for *B. oleae*, a feeding assay was performed, in which the potential predators were fed with *B. oleae* pupae and their gut content evaluated after different periods. The amount of *B. oleae* DNA in the intestinal content of predators was analysed by qPCR. Results are discussed taking into account their possible use as biocontrol agents of olive fruit fly.

Acknowledgments: This work is funded by FEDER funds through COMPETE (*Programa Operacional Factores de Competitividade*) and by national funds by FCT (*Fundação para a Ciência e a Tecnologia*) in the framework of the project EXCL/AGR-PRO/0591/2012.

P IPM 50

Successful use of honeybees for grey mould (*Botrytis cinerea*) biocontrol on strawberries in Turkey

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Entomovector Technology is a novel application strategy using bees for the delivery of microbial control agents. This technology is an environmentally friendly control strategy against plant pathogens and pests that makes use of pollinators to vector powder formulations of plant protection products from the hive to the flowers and leaves of pest-infested crops and improves crop pollination resulting in increased yield and crop quality. In practice, a dispenser is attached to the hive and loaded with a powder formulation of the desired biological control agent. Biological control in organic agricultural systems is more friendly to the environment and more sustainable than the other farming systems. The study was performed to determine the efficacy of entomovector technology in suppression of grey mold (*Botrytis cinerea*) was tested in field conditions using Prestop Mix biopreparation (*Clonostachys rosea* f. *catenulate* J1446). The experiments were conducted on experimental fields of Erzincan Horticultural Research Station in Erzincan, Turkey in 2014. The experimental design was completely randomized with 4 repetitions. The frigo plants of 'Aromas' strawberry cultivars were used. Each plot (no cage and cage) consisted of 70 strawberry plants planted in an area of 3.30 m × 4 m. The number of diseased fruit of each plot was recorded. The number of diseased fruit was averagely 139 in the treatment of *Clonostachys rosea* f. *catenulate* J1446 treatment disseminated with *Apis mellifera*. On the other hand, the untreated plots were calculated 432 in the diseased fruit number. There were significant differences number of diseased fruit between treatments. BICO POLL is the first application of the use of honey bees in entomovector technology in Turkey. Results have shown that *Clonostachys rosea* f. *catenulate* J1446 can be effectively delivered by honey bees for prevention of *Botrytis cinerea* infection in strawberry. This method has important significance in organic farming, biocontrol is an environmentally friendly approach to control the development of grey mold. The project BICO POLL was founded by transnational CORE Organic II Funding Body within the FP7 ERA-Network.

P IPM 51

Effects of *Trichoderma harzianum* and nitrogen treatments on brown rust of wheat

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Introduction: The economic loss of wheat crop due to the brown rust (*Puccinia triticina*) depends on many factors including cultivar sensitivity, stage of infection and also such nutritional conditions as the nitrogen content of soil.

Objectives: Effects of treatment with the antagonistic fungus *Trichoderma harzianum* and different amounts of nitrogen fertilizer (urea), on the response of two wheat cultivars, Boolani (sensitive) and Kavir (moderately sensitive), to the brown rust disease of wheat was studied.

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Materials and methods: The average recommended amount of urea fertilizer for wheat fields (350 kg ha^{-1}) was estimated as equal to 1 g kg^{-1} soil. Treatments were performed under greenhouse conditions and consisted of four nitrogen levels: 0.5, 1, 1.25 and 1.5 g kg^{-1} soil, applied at the time of planting. Wheat seeds were surface sterilized with 70% ethanol, washed with sterile water and soaked into different concentrations (10^5 , 10^6 and 10^7 conidia ml^{-1}) of *T. harzianum* conidia for three hours. Di-carboxymethyl cellulose was used to help adhesion of conidia to the surface of seeds. After ten days, seedlings were inoculated with urediospores of *P. triticina*.

Results: Assessment of average disease severity after three weeks showed significant differences among treatments. Up to 17.18 % and 59.25% reduction of disease severity was observed for Boolani and Kavir cultivars, respectively.

Conclusion: This study reveals the induction of resistance against brown rust in wheat plants treated by *T. harzianum*. Nitrogen concentration plays a key role in disease suppression or increase and should be applied cautiously.

P IPM 52

PGPR as a Bio control agent against *xanthomonas oryzae* pv. *Oryza* Bacterial leaf Blight in rice

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Bacterial leaf Blight (BLB) caused by *Xanthomonas oryzae* pv. *oryzae* (Xoo) is main menace to rice crop and cause substantial economic losses to farmers in all over the world. PSA medium was used to isolate Xoo from diseased leaves and later was confirmed through PCR by using specific primers TXT4R. The virulence of Xoo strains was also confirmed by in vivo pathogenicity test. Plant growth promoting rhizobacteria (PGPR) were isolated from the rhizosphere of rice plants grown in different regions of Punjab, Pakistan. Three hundred bacterial isolates were screened in vitro for their antagonistic activity against Xoo using hole plate diffusion method. SA33, Tk229, Tk179, SP12 and SA37 showed positive isolates against Xoo. In greenhouse experiment, isolate SP12, Tk229 and SA37 were effective in reducing BLB incidence with disease suppression up to 79%, 72% and 68%, respectively. These three antagonistic isolates were further studied for growth promotion mechanism from which strain TK229 showed significant role in indole acetic acid (IAA) activity and phosphorous solubilization. Rice plants sprayed with fresh suspension of isolate SP12 provided 75% protection from Xoo, followed by TK229 and SA37 with 66 % and 62%, respectively. Kinetics of these bacterial strains also studied in order for efficient fresh spray. These strains can also be used along with other strategies to achieve greater role of crop protection, formulation of biopesticides and to sustain rice yield.

P IPM 53

Use of Integrated Pest Management in Scottish soft fruit production

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Cultivation of Soft fruit, particularly of strawberries, is a growing and economically important component of Scottish horticulture. The aim of this study is to present temporal trends in pesticide use and IPM uptake in the Scottish soft fruit industry over the last decade.

The Scottish Government currently conducts statutory monitoring of pesticide use, and aspects of horticultural practice, on a sample of soft fruit growers every two years. Using this sample, statistical estimates are made of total pesticide use in Scottish soft fruit production.

Chemical pesticide input to soft fruit crops is higher than in any other type of Scottish agricultural or horticultural system. Soft fruit crops receive on average almost 10 kg/ha of pesticides, of which around three quarters are fungicides. In contrast, Scottish vegetable crops receive around 3 kg of pesticides per hectare.

However, over the last decade the use of alternative pest control methods, such as polythene mulches to suppress weeds and biological control of pests, has increased. In addition, the type of biological control agents used has changed over time, from predominately those controlling insect pests to a combination of organisms used for insect and disease control.

Adoption of IPM in the soft fruit industry is likely to become increasingly important in light of the threat to several major use pesticides resulting from the implementation of the EU pesticides approval regulation (EC 1107/2009) and water framework directive (2000/60/EC).

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P IPM 54

Molecular Identification of the Biotype of Whitefly Inhabiting the in Saudi Arabia

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The whitefly *Bemisia tabaci* (Genn.) is a worldwide important insect pest and vector of plant viruses. Recently, the whitefly populations upsurges dramatically in Al-Ahsa region of Saudi Arabia due to the expansion in greenhouse cropping system and it causes severe economic damage to many vegetable crops in greenhouses. Proper classification of the whitefly that prevails in the area is a crucial step towards efficient management of the pest. The whitefly is highly polymorphic with extreme plasticity in key morphological characters that vary according to the host which makes the taxonomic identity of existing biotype is difficult and sometimes ambiguous. The specimens for this study were collected from nine different locations of the whitefly populations in Al-Ahsa region. The study was done on the basis of three molecular techniques namely: (1) PCR amplification and polymorphic analysis of restriction enzyme digestion of mitochondrial cytochrome oxidase I gene (mtCOI), (2) Cloning, sequencing and phylogenetic analysis of mitochondrial 16S rRNA gene and (3) Random Amplified Polymorphic DNA Polymerase Chain Reaction (RAPD PCR) analysis. The results of these molecular techniques clearly indicated the dominance of B biotype of the whitefly on crops grown in greenhouses in Al-Ahsa region of Saudi Arabia.

P IPM 55

Risk management tools help to implement Best Management Practices (BMPs) to reduce losses of Plant Protection Products (PPP) to water from runoff

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Introduction: PPPs should be applied in a sustainable way to reduce impacts to the environment. Research indicate that point sources contribute > 50% of the PPP - findings in surface water.

Point sources originate from the farmyard.

Diffuse source contamination originate from applications in the field, due to runoff / erosion and spray drift. Runoff is the major diffuse entry route for PPP into surface water. TOPPS projects work in various EU countries with local experts to develop BMPs, risk diagnosis tools and materials for training and dissemination (www.TOPPS-life.org). Mitigation measures address the correct use of the PPP, techniques, infrastructure and for runoff the water pathways, soil, landscape factors and agronomic practices.

Materials and methods: Based on research from France risk management tools were developed and adapted to the local situation in 7 EU- countries. The team of experts developed three risk management tools (Dashboards). These start with an analysis of the water movement in a catchment and then focus on the situation in a specific field.

a) Runoff risk due to infiltration restrictions (spring /early summer with high intensity rains)

Proximity to water, permeability of soil, and slope are the main factors.

b) Runoff risk due to saturation access (winter with high volume of rain)

Proximity to water, drainage system, topography of the field, permeability of the soil and the field capacity are the main factors.

c) Concentrated runoff

Concentrated runoff shows signs of erosion.

Advisers in 7 countries have been trained to use the dashboards and to make test audits in pilot catchment areas. The risk classifications of the fields were discussed with the farmers in the catchments and the selection of appropriate mitigation measures were discussed. Mitigation measures were implemented for demonstrations.

Results and discussion: Intensive research done in a long term project in Fontaine du Theil, France demonstrated that applying risk analysis and implementation of mitigation measures strongly reduced PPP loads in the surface water.

Feedback from advisers who worked with the TOPPS risk management tools experienced fast learning effects related to the time required. The risk management tools are practical and reflect to a large extend the real farmers' expertise. The transparency of results support acceptance by the farmers and supports implementation of measures.

Conclusion: The proposed tools offer to link risk analysis with BMP measures to an efficient risk management system protecting surface water from contaminations due to runoff not only for PPP.

Acknowledgement: Contributions from TOPPS partners and ECPA are acknowledged.

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P IPM 56

RNAi-based integrated management of citrus insect pests

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Citrus fruits accounted for the highest share of fruits market in terms of international trade. The economic loss caused by citrus diseases transmitted by insect vectors, as well as insect pests is inestimable. However, the present strategies for citrus insect pests control mainly rely on spraying chemical insecticides, which brings up many environmental and ecological problems, such as insecticides residue and resistance. RNA interference (RNAi) has been proven to work in selectively controlling insect pests, and even in resistance management, which circumvents the problems brought by insecticides usage. Hence, we are interested in exploring the potential application of RNAi-based strategies in integrated citrus pests management. *Bactrocera dorsalis* (Hendel), as one of the most damaging citrus pests, was used as a model in this study. We first use the genes involved in the ovary development as the targets of RNAi. The results indicated that knock-down of those genes by dsRNA could significantly affect delay the normal development and affect the normal morphology of the ovary, suggesting RNAi might possess great potential in controlling the population of *B. dorsalis*. In addition, the knock-down of esterase genes could also significantly increase the susceptibility of *B. dorsalis* to insecticides, implicating that dsRNA could also work together with the insecticides as synergists. Our study may open the avenue for RNAi application in citrus pests management.

P IPM 57

Integrated pest management strategy to minimize stripe rust of wheat, *Puccinia striiformis* f.sp. *tritici*, in cereal crops

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Introduction: Yellow (stripe) rust (Yr) was formerly widespread in cooler areas of Northern Germany, but now also occurs on wheat and triticale in warmer areas of Southern Germany. Long-term studies by the JKI show that the Yr race composition may drastically change from year to year. The limited durability of race-specific resistances urgently affords a novel approach of integrated pest management that integrates intelligent fungicide strategies and durable resistant cultivars.

Objectives: The research project aims to

1. monitor virulences, pathotypes and diversity of German Yr populations,
2. test the sensitivity of Yr populations to the most common fungicides,
3. identify race-specific resistance genes in new wheat germplasm
4. select new, durable adult plant resistances by biotechnological methods.

Materials and methods:

1. Test seedlings of a differential set with Yr isolates from the main wheat growing areas.
2. Investigate fungicide sensitivity of Yr populations using a miniaturized test system.
3. Phenotype 4 wheat populations in field trails with artificial inoculation at 3 locations for 2 years.
4. Genotype 4 wheat populations by 15K Infinium wheat chip.

Results: The new Warrior race first detected in 2010 was present in 69% of the samples tested in 2014. The race is characterized by a complex virulence profile, increased aggressiveness and extended adaptation to previously unfavourable environments. In Germany, only a few, monogenic resistances remain effective (Yr 5, 8, 10, 15, 24) to this race. In contrast, adult-plant resistances (APR) have proven durable despite dissemination in widely grown cultivars. Field tests of the 4 selected wheat populations suggested a wide range of genetic variation.

Conclusion: With the occurrence of the new Warrior race, Yr became a major biotic threat for wheat production in Germany. Genetic control of Yr by effective APR will offer a cost effective and environmental-friendly strategy to reduce losses in wheat production.

P IPM 58

***In vitro* control of *Mycosphaerella Arachidis* Deighton the Early Leaf Spot Disease Pathogen of Groundnut by the extracts from six medicinal plants.**

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Ground nut (*Arachis hypogaea*) is one of the most popular commercial crops in Nigeria. Its successful production has been drastically affected by early leaf spot disease caused by *Mycosphaerella arachidis* Deighton. *In vitro* control of the pathogen by six medicinal plants (*Entada africana*, *Vitex doniana*, *Lawsonia inermis*, *Azadirachta indica*, *Acalypha hispida* and *Nuclea latifolia*) was assessed in this study. The extracts of the plants were prepared using cold and hot water and alcohol. The pathogen was isolated from ground nut infected with early leaf spot disease. The results revealed a great significant difference ($P < 0.05$) in yield of extracts between cold water, hot water and alcohol extracts. A significant difference ($P < 0.05$) was observed in percentage concentrations of the various phytochemical constituents present in the extracts. Flavonoids percentage concentration was the highest (0.68 - 1.95%) followed by saponnin (0.09 - 1.53%) in *N. latifolia* extracts. Steroids had the least percentage concentrations (0.00 - 0.09%) followed by terpenoids (0.02 - 0.71%) and proanthocyanin (0.05 - 0.86%). *N. latifolia* extracts produced the highest percentage concentrations (0.07 - 1.95%) of all the phytochemicals followed by *A. indica* (0.05 - 1.64%) and least concentrations were obtained in *A. hispida* (0.09 - 0.87%) and *V. doniana* (0.00 - 0.88%). The extracts inhibited spore germination and growth of *M. arachidis*. The inhibition by alcohol extracts was high and significantly different ($P > 0.05$) from cold and hot water extracts. Alcohol extract of *L. inermis* gave 100% spore germination inhibition followed by *N. latifolia* and *A. indica* with 97.75% and 85.60% inhibition respectively. Therefore, field trials of these six medicinal plants on the control of early leaf spot disease of ground nut are recommended.

P IPM 59

Evaluation of zeolite and Agri-fos 600® in control of Verticillium and Fusarium wilt diseases and *Pseudomonas syringae* pv. *tomato*

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Plants, during their evolutionary path, formed complex resistance mechanisms against their invading pathogens (fungi, bacteria and viruses). The phenomenon of Induced Resistance is part of the innate immune system of plants and is expressed after the biochemical or chemical stimulation of latent resistance mechanisms that are activated using non-pathogenic microorganisms or chemical inducers. The objective of this study was the evaluation of zeolite and Agri-fos 600® in control of Verticillium and Fusarium wilt diseases and bacterial speck caused by *Pseudomonas syringae* pv. *tomato*. Management of these diseases is mainly based on prevention, thus the discovery of alternative means for their control is essential. Zeolite is a microporous, aluminosilicate mineral with specific physicochemical properties commonly used as a commercial adsorbent and catalyst. Zeolite is commonly used as a soil improvement substance but its role in disease management has not been studied. Agri-fos 600® is a special formulation consisting of potassium phosphonate anions that except their role as nutrients, have the ability to induce the defense mechanism of plants. Pathogenicity experiments were performed in *Arabidopsis thaliana*, eggplants and tomato plants infected with *Verticillium dahliae* (10 ml suspension of 10^6 conidia/ml) or *Pseudomonas syringae* pv. *tomato* (spray with 10^8 cfu/ml) where zeolite (5gr/lt) and Agri-fos 600® (1ml/lt) applied in the form of root drenching in three booster doses of 10 ml. Virulence assays showed that zeolite and Agri-fos 600® have the ability to reduce the rate of Verticillium wilt mainly in tomato and *A. thaliana* between 5%-20%. Repeated applications of zeolite and Agri-fos 600® are necessary for the effective response of Verticillium wilt. Finally, it was found that Agri-fos 600® confers faster plant growth and greater leaf mass. Future experiments will be extended to the role of these substances in the control of other important plant pathogens.

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Egg deposition mediates defence of *Ulmus minor* against a major pest insect, the elm leaf beetle

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Plants may take egg deposition by insect pests as a warning signal for future feeding damage by hatching larvae. The warning improves the feeding-inducible defence mechanisms of plants, resulting in pest reduction. Responsible agents for this phenomenon are, however, largely unknown, particularly in perennial plants such as trees. The Collaborative Research Centre (CRC) 973 elucidates the mechanisms of 'priming and memory of organismic responses to stress'. The here presented project as part of the CRC 973 investigates if and how prior egg deposition affects defence of field elm against the multivoltine elm leaf beetle, a pest insect of elm especially in Southern Europe, the USA and Australia. Indeed, we found evidence that egg deposition by the elm leaf beetle improves the elm's defence against the feeding stages of this herbivorous species. Chemical analyses suggest that a phenolic compound in egg-deposited, feeding damaged leaves contributes to the worse performance of the elm leaf beetle on these leaves when compared to egg-free ones. Hence, a small but significant metabolic change can provoke a striking effect on herbivory. This knowledge may be valuable for future integrated plant management concepts.

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Research Progress on Plant Protection in China during 2010-2013

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A series research progress has been made in the basic research, high-tech development, and key forecast and control technique improvement of crop diseases and insect pests by a team work. The advancements in plant protection have played an important role in the disaster prevention and mitigation in agriculture, and made a tremendous contribution to ensure the grain output increased for the tenth consecutive year.

Progress in the basic research. Focusing on the functional genomics for pathogens and insect pests, insect chemical ecology, invasive biology, biosafety on GMO etc, a significant improvement has been made in basic research of plant protection. For example, southern rice black-streaked dwarf virus (SRBSDV) was proved to be a new specie in Feijin viral family by genomic sequencing. And the ecological efficiency of wide plantation of Bt-transgenic cotton, and mechanisms of resistance development, genetics and evolution of the cotton bollworm to Bt-transgenic cotton have been verified.

Development of high-tech. Detection technique systems of plant pathogen based on immunological technique, and PCR technology have been developed rapidly. For example, twenty viruses detection technique systems based on monoclonal antibody have been widely used in many regions of China, which promote the pathogen rapid detection. DNA barcoding technique, and remote comparative analysis system for important invasive insects have been created, which greatly shortened the invasive species monitoring time. Nearly 100 kinds of insect pheromones of agricultural and forestry insect were identified.

IPM strategy and technology. A great breakthrough has been made in IPM strategies and technologies aimed at major diseases and insect pests on crops in China. For example, the inoculum source areas of *Puccinia striiformis* f. sp. *tritici* (*Pst*) in China have been found, and the technology for long-term monitoring and forecasting of regional epidemics of wheat stripe rust in remote area has been developed. The IPM strategy and technique system of wheat stripe rust has been put forward, and widely applied in the areas of wheat plantation at a scale of more than 25 million hectares during 2009-2013, resulting in the sustainable control of wheat stripe rust epidemics and remarkably economic retrieve.

P IPM 62

Studies on shelf life of *Pseudomonas Fluorescens* and *Bacillus Subtilis* in two different carrier materials

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Inoculation of biopesticide in crop cultivation plays very important role in sustainable farming. It is well known that the carrier-based bioinoculants are being very effective as carrier determines the shelf life of the inoculant. Hence the selection of better carrier is very essential for maintaining shelf life of the inoculant during storage and for better performance in the field use. In the present study, the effect of Vermiculite and Farm Yard Manure (FYM) in maintaining the shelf life of bioinoculant such as *Bacillus subtilis* and *Pseudomonas fluorescens* was studied up to 6 months from the date of preparation of inoculant.

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Comparatively, FYM based bioinoculants showed longer shelf life than vermiculite based bioinoculants. Among FYM based bioinoculants *B. subtilis* showed maximum population of 7.60×10^8 cfu/g of dry wt on 180th day followed by *Pseudomonas* 10^8 cfu/g of dry wt respectively

P IPM 63

Possibilities of mass trapping with *Agriotes sordidus* and *Agriotes lineatus* with pheromone traps in south west French conditions

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Click beetle larvae (wireworms Coleoptera: Elateridae) are significant crop pest in France with more and more damage, mainly on corn, potatoes and different vegetables crops (tomatoes, melon, lettuces, carrot, asparagus...). The augmentation of wireworm problems en culture seem to be the combination between the reduction of active ingredients available for growers and the development of a click beetle specie with a cycle shorter : *Agriotes sordidus*. The development of pheromone traps give us new possibility of control, especially mass trapping. The technique could be used to reduce population of click beetle, mainly male, in a habitat. We have conduct a mass trapping trial at Lanxade center, in south west of France from 2003 to 2013 in the same field. On Lanxade station we have two click beetle species more present: *Agriotes lineatus* with a long life cycle (5 years), and *Agriotes sordidus* with short life cycle (2-3 years). The level of catching is very dependent of the weather of the year and also of the position of the trap in the field. The relation between the levels of click beetle catch, the population of wireworms presents in the field and finally the crop damage is not clear. After ten years of mass trapping, we observe a reduction of number of *Agriotes sordidus* catch; on the other hand, we note an increase of *Agriotes lineatus*. We diminution of damaged are observed in the two crop used for controlling the level of damage : potatoes and carrot.

P IPM 64

New Symptoms and Management Of Vascular Streak Dieback On Cocoa

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Vascular Streak Dieback (VSD) is a serious disease that has contributed to declines in the production of cocoa in the entire region, particularly Indonesia. It is very detrimental to cocoa farmers, some of whom are changing to other crops as a result of disease losses. Since 2004, symptoms have changed to become predominantly necrotic, with leaves remaining attached for longer periods. Formerly, the predominant symptom was leaf chlorosis and rapid abscission. It is unknown whether the new symptoms of VSD are related to changes in the resistance of cocoa genotypes. The newer symptoms generally involve a greater degree of dark necrosis of the leaf lamina and vascular tissue than originally observed associated with the disease. Examination of hyphae in infected twigs and sporocarps on leaf lamina and leaf scars on stems showed that the fungus associated with new symptoms is identical in all aspects to *Oncobasidium theobromae* (syn. *Ceratobasidium theobromae*) as described by Talbot and Keane (1971). Isolation of the fungus from infected xylem that the fungus emerging from the vascular tissue is a slow-growing - like fungus with an average hyphal width of 5 mm. Mycelium with similar characteristics was observed in sporocarps on leaf and petiole cracks of infected, attached leaves. Basidiospores were asymmetrical and approximately 17×7.5 mm, consistent with earlier descriptions of the fungus by Talbot and Keane. While a previous report suggested *C. ramicola* was associated with VSD-infected cocoa, spores consistent with dimensions of this species in previous reports have not been detected in Sulawesi to date. Examination of longitudinal sections of infected twigs by high-power microscopy indicated hyphae with *Rhizoctonia*-like characteristics in xylem vessels, including perpendicular branching and narrowing at the junctions, occurs in the xylem tissue, consistent with previous descriptions. It is possible that the new symptoms of vascular streak dieback are caused by a change that affects the host response to the fungus. Since the newer symptoms are region-wide it is likely that an environmental factor, such as changes in climate or soil fertility, triggered a change in host response. However, a genetic change in the pathogen cannot be ruled out. Recommendations for management of the disease remain unchanged: this includes pruning branches 30-40 cm below the extent of infection, raising seedlings in covered nurseries and the propagation of resistant cocoa genotypes.

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Strategies to manage Plum Pox Virus transmission in peach orchards in the Niagara region of Canada

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Plum pox virus (PPV) is the most devastating viral disease of stone fruit (*Prunus* spp.) worldwide. The disease is a member of the genus Potyvirus and family Potyviridae. PPV is spread both vegetatively and transmitted in a non-persistent manner by aphids. The Dideron strain (PPV-D), first detected in Canada in Ontario and Nova Scotia, led to the implementation of an eradication program in 2000 by the Canadian Food Inspection Agency (CFIA). Laboratory and field studies of the transmission of PPV by the green peach aphid, *Myzus persicae* (Sulzer), were carried out using foliar applications of horticultural mineral oils (HMO) and other novel insecticides. The residual activity of the HMO's on green peach aphid ability to transmit PPV was measured. Changes in aphids probing and feeding behaviors induced by HMO and antifeedant insecticide applications were also monitored using an electrical penetration graph (EPG) system.

P IPM 66

Using *Bacillus mycoides* isolate J induced resistance in IPM programs

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Bacillus mycoides isolate J (BmJ) induced resistance provides control of bacterial, fungal, oomycete and viral pathogens on a wide range of crop plants. BmJ induced resistance is signaled through the NPR-1 gene and involves several PR proteins and ethylene. Optimal systemic resistance induction occurs 3-5 days after application of live cells and provides disease control for 14-21 days depending on the plant induced. BmJ is compatible with a wide range of pesticides including; triazole, EDBC, and QoI class fungicides and a wide range of insecticides. Disease control equal to commercial standards has been achieved with BmJ alone or in combination with low rates of fungicides or in alternating programs where BmJ replaces half the fungicide used in fungicide alone programs. BmJ has also been demonstrated to be of benefit to triazole and QoI fungicide resistance management programs in *Cercospora* leaf spot of sugarbeet control programs. BmJ used in combination with insecticides and roquing has produced >50% control of potato PVY in multiple years in the field. Data will be presented for disease control of cucurbit crops, tomato, pepper, potato, pecans, spinach, lettuce and sugarbeet with comparisons to commercial pesticide standards. Control of postharvest diseases using preharvest application will be discussed. BmJ is licensed to CERTIS USA by Montana State University and is grown in liquid fermentation will be sold as a WDG formulation. Application rates are based on 1×10^7 cfu/ml (60-240 gm/A depending on spray volume). Registration materials have been submitted to Canada's Pest Management Registration Authority (PMRA), the USEPA and OMRI listing is expected in 2015.

P IPM 67

Effect of nitrogen and potassium fertilizer on yield and fruit quality of tomato in greenhouse conditions

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In order to investigate the effects of nitrogen and potassium on yield, quality and resistant for insects to tomato trial factorial arrangement in a randomized complete block design with four replications in a pot in a greenhouse located in the Emamzadeh city of Urmia was conducted The first factor consisted of four levels of nitrogen (N0 = 0, N1 = 0/20, N2 = 0/41, N3 = 0/64) grams and the second factor consisted of four levels of potassium (K0 = 0, K1 = 0/54, K2 = 1/01, K3 = 1/61) grams recommended by the soil and Water Research Institute. The results showed that nitrogen and potassium treatments on morphological traits affecting tomato and lead to a decrease or increase the amount of macro nutrients absorbed by plant leaves and fruits. The results showed that with increasing levels of nitrogen and potassium, nitrogen and potassium increased leaf and fruit, but with increasing nitrogen uptake of potassium in the leaves decreases. Increase the amount of nitrogen in increasing chlorophyll and thus lead to increased fruit yield increased nitrogen soluble solids in the plant fruit quality is a major risk factor for both treatments led to a decrease in titratable acidity due to the restoring effect of ammonium nitrogen molecules and alkali element was potassium.

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P IPM 68

Disease incidence and effects of fungicides on the control of stalk rot of maize cause by *Fusarium moniliforme* and *Macrophomina phaseolina* in vitro

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A survey of the stalk rot caused by *Fusarium moniliforme* and *Macrophomina phaseolina* was carried out in Remo, Ijebu, Yewa and Egba zones of Ogun state, Nigeria to assert on the incidence and effects of fungicides (Apron star, Benlate and Dithane M-45) on the control of associated fungi. The results showed that the incidence and severity of the disease with respect to causal agents vary from one zone to another and from one farm to another. *F. moniliforme* whose average incidence of 72.1 % was significantly higher ($p < 0.05$) than that of *M. phaseolina* - 52.5 % was most prevalent in all the areas sampled. On average, there was significantly high ($p < 0.05$) disease index for *F. moniliforme* (41.95) and *M. phaseolina* (27.2) at Ijebu zone compared to other zones sampled. Effects of fungicides on the growth of *F. moniliforme* and *M. phaseolina*, revealed that there were significant differences on the response of the pathogens to these fungicides. Results obtained showed significantly great reduction on the radial growth of *F. moniliforme* at low concentration level of 25 ppm to varying degrees. Apron star, Dithane M-45 and Benlate appeared to be most effective in controlling *F. moniliforme*. On *M. phaseolina*, Apron star appeared to be effective in controlling the pathogen at lower concentration level of 50ppm. The result of this survey suggests that *F. moniliforme* is the predominant causal organism of stalk rot of maize in the state. And it is hereby recommended that the research institute should endeavour to develop resistant varieties to this stalk rot pathogens, since over the years, attention has always been on foliar diseases in contrast with soil born diseases. Observation on the fungicide treatments revealed that Apron star was effective in inhibiting growth of both organisms in the laboratory. Therefore, it is recommended to farmers that instead of purchasing two chemicals for their control, Apron star could be used on the on the field as seed dresser.

P IPM 69

Characterization of *Erwinia carotovora* subsp. *carotovora* and subsp. *atroseptica* by PCR-based methods

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Nine isolates of *Erwinia carotovora* subsp. *atroseptica* (*Eca*) and *E. carotovora* subsp. *carotovora* (*Ecc*), were obtained taken from potato tubers collected from different localities of El-Minia, Assiut and Sohag Governorates were subjected to this investigation. Three different molecular techniques were employed to differentiate the two subsp. of *Erwinia carotovora*. The primers used in RAPD-PCR technique generated an unique distinct bands which could be used as genetic markers to distinguish the isolates in respect of their sub species (*carotovora* or *atroseptica*) or their virulence (high or low virulence). Also, the 16s rRNA gene sequences was obtained from the bacterial isolates of *Ecc* and *Eca*. The phylogenetic tree couldn't distinguish between the two subsp. of *E. carotovora*, where some isolates of *Ecc* connected in the same cluster with *Eca*. Moreover, SDS-PAGE analysis technique was used. The application of protein (SDS-PAGE) analysis may aid to detect the differences between the isolates of *E. c.* subsp. *carotovora* or *atroseptica* in the base of the region of collection. Where, protein analysis shown polypeptide fractions could be used as a genetic marker to distinguish isolates collected from same area.

P IPM 70

Antifungal activity of aqueous extract of Egyptian *Citrullus colocynthis* against Botrytis onion umbel blight disease

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The antifungal activity of aqueous extract of bitter apple (*Citrullus colocynthis*) against botrytis onion umbel blight (BOUB) pathogens was studied *in vitro* using dual culture assay on potato dextrose agar (PDA) medium. The activity of aqueous extract was measured by the diameter of inhibition zone. Three different concentrations (1, 5 and 10 %) of bitter apple were tested against *Botrytis allii*, *B. cinerea*, *B. aclada*, and *B. squamosa*. All tested concentrations of aqueous plant extract showed antifungal and mycelial growth inhibition of pathogenic fungi. The highest pathogen inhibition of *B. allii* was (70 %) *B. squamosa* (60%), *B. acalda* (50 %), and *B. cinerea* (44 %) caused by *C. colocynthis* at concentration 10 %. While, the lowest pathogen inhibition of *B. aclada* (28 %) was observed at concentration 1 % of bitter apple. Application of aqueous extract of *C. colocynthis* to control

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BOUB disease *in vivo* under greenhouse conditions caused disease reduction up to 72.2 and 56.7 % before and after 48 h of pathogen inoculation, respectively. The aqueous extract of *C. colocynthis* was analyzed by gas chromatography-mass spectrometry (GC-MS). The most intensive components in aqueous extract of *C. colocynthis* were: 9,12-Octadecadienoic acid; linoleic acid, squalene, linolein, tocopherol, which have antimicrobial activity. This study proved that aqueous extract of *C. colocynthis* could be used as a biocontrol agent of botrytis onion umbel blight disease.

P IPM 71

DMI and QoI fungicides for the control of coffee leaf rust

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This study investigated the ability of different triazole (tz) to the soil and tz + strobilurin (st) fungicides sprayed to the leaves and tz + st sprayed to control Coffee Leaf Rust (CLR) using a susceptible variety Catuai in the greenhouse (sprayed) and in the field (soil and sprayed). The effects of tz (triadimenol (T), cyproconazole (C) and epoxiconazole (E)) and st (azoxystrobin (A), trifloxystrobin (Tri), pyraclostrobin (P)) were first studied in a greenhouse to evaluate the translaminar effect on CLR control. The field experiment was performed to evaluate the effect of tz applied to the soil and tz + st sprayed on CLR control. The different fungicides applied in the greenhouse reduced CLR severity by 8.9 % (Tri), 79.4 % (A), 82.0 % (T), 93.2 % (C), 96.6 % (E) and 98.5% (P). In the field experiment tz applied to the soil and tz + st sprayed and tz + st only sprayed were effective in CLR control. C applied to the soil in Nov and sprayed twice with C + A and the application of E + P (twice) or C + A sprayed three times were the most effective control of CLR and to maintain the highest coffee berry yield (CBY). The CLR incidence was 72 % (average of three seasons) considered very high for the control treatment and 15 % (very low) for the best treatment. The average CBY increased by 33.9% (lowest value) when applying T to the soil in Nov followed by C + Tri sprayed in Dec and Feb, and increased by 99.1% (highest value) in the treatment with E + P sprayed in Dec and Mar compared with plants that did not receive any fungicide application. The control treatment and that of T applied to the soil in Nov followed by C + Tri sprayed in Dec and Feb yielded less than 30 (22 to 30) bags of processed coffee/ ha, whereas the other treatments more than 35 (35.7 to 44.6). Treatments with C applied to the soil in Nov followed by C + A sprayed on the leaves in Dec and Feb, E + P sprayed on the leaves in Dec and Mar, and C + A sprayed on leaves in Dec, Feb and Apr yielded more than 39 (39.0 to 44.6) bags of processed coffee/ ha. This study reports for the first time that a tz fungicide applied to the soil and tz + st sprayed, efficiently controlled CLR and increased significantly CBY for three seasons.

P IPM 72

Microbial Products for Agriculture in Uruguay

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Biotechnologies based on microbial resources have made possible the development of biological products to improve plant protection and plant nutrition, without compromising the environment. These technologies also contribute to the achievement of high end market requirements for low agrochemical use. The Platform of Microbial Products for Agriculture at INIA-Uruguay (National Institute for Agricultural Research) has emphasized the role of microorganisms in biocontrol of plant pests and diseases, and in nitrogen and phosphorus plant nutrition. The long term strategy lies on strengthening research facilities and team work, recruitment and continuous training of human resources, research and technology ventures with the public and private sectors, fostering legal framework for registration and intellectual property of products, and promoting education and extension for farmer adoption. Research areas include: (1) biological control, (2) biological nitrogen fixation, (3) microbial mediated phosphorus availability to plants, and (4) soil health indicators. Current biocontrol projects focus on identifying strains for product development, to be incorporated in Integrated Pest Management (IPM) for targeted crops and pests. Biological nitrogen fixation is being efficiently exploited by inoculating legumes with suitable rhizobial inoculants, supported by appropriate legislation, which sets requirements for product registration, mandatory strain recommendation, and quality surveillance of commercial inoculants. Supporting research addresses bioproduction and formulation, inoculation and seed coating technologies, fungal endophytes, as well as metagenomics for assessment of microbial diversity. The joint development of Lecafo[®] (INIA-Lage&Cia), based on a selected strain of the entomopathogenic fungal species *Lecanicillium lecanii*, will be presented as the first biopesticide nationally produced in Uruguay.

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P IPM 73

Effect of certain antioxidant compounds on incidence of root and pod rot diseases of peanut

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The effect of certain antioxidant compounds (salicylic acid, ascorbic acid, sodium benzoates, thiouria and catechol) on incidence of root and pod rot diseases of peanut caused by *F. moniliforme* (No. III), *F. oxysporum* (No. I) and *F. solani* (No. II) was investigated *in vitro* and *in vivo*. *In vitro* test, all tested antioxidants at different concentrations (2, 4, 6, 8 and 10 mM) reduced the mycelial growth of the tested pathogenic fungi compared with control. Each tested antioxidant at concentration 8 mM and 10 mM gave the greatest reduction of mycelial growth of the pathogens. Under greenhouse conditions treated seeds with each antioxidant (8 mM) were able to reduce disease severity percentage of root and pod rot diseases at concentration of 8 mM. Salicylic acid gave the greatest reduction of both diseases of peanut. Spraying of peanut plants with each of antioxidant (8 mM) significantly reduced root and pod rot diseases severity percentages compared with control. Spraying plants with salicylic exhibited the highest reduction in both of root and pod rot diseases of peanut

P IPM 74

Evaluation of Mosquitocidal Activity of *Bacillus amyloliquefaciens*, *Lysinibacillus* spp. and *Cellulosimicrobium cellulans* Isolated from *Culex* spp larvae

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Biological control alone or as a part of integrated vector management stands to be a better alternative to chemical controls aimed against pest mosquitoes. Here, we screened for microorganisms that can be utilized as new host cells as mosquito larvicides. As persistence in the environment is required of host cells, we examined bacterial populations in mosquitoes collected from natural breeding ponds around Mansoura University, Egypt. Bacterial sequences were isolated using standard 16S rRNA gene sequencing. The isolates were *Bacillus amyloliquefaciens* from isolate 3, *Lysinibacillus* spp from isolate 2 and *Cellulosimicrobium cellulans* from isolate 1. *Lysinibacillus* spp was found to be the dominant species and toxic to *Culex* larvae (Population mortality was 55%, 65%, 25% against 2nd, 3rd, 4th instar larvae, respectively after 36 hours at 10⁶ CFU/ml). The entomopathogenic bacteria, *B. amyloliquefaciens* was isolated from *Culex* larvae for the first time in Egypt and it was also effective against culex larvae (Population mortality was 30%, 67%, 32% against 2nd, 3rd, 4th instar larvae after 72 hours using 10⁶ CFU/ml), respectively. *C. cellulans* was also isolated and had the lowest effect.

P IPM 75

Effect of nutritional attractant (Lurem-tr®) and yellow sticky cards on decreasing *Thrips tabaci* (Thysa: Thripidae) population in cucumber greenhouses

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Thrips tabaci is the world-wide pests in greenhouse. To control this pest more than 10 times spraying should be applied in Iran. Non chemical methods like attractant are useful method to decrease thrips populations. In this study comparing between yellow sticky cards (20×40 cm) and yellow sticky cards associated with (Lurem-tr®) was done. The experiments were done in two commercial cucumber's greenhouses (2000 m²). Thrips population was monitored every week with 8 sticky cards (10×20 cm) in each greenhouse. When the average of thrips on cards had been 20, hung up yellow sticky cards increased at each greenhouses. Additionally at treatment greenhouse Lurem-tr® was attached on each sticky cards. Yellow cards replaced every two weeks. In 8th weeks after transplanting (average adult thrips on all cards was more than 20) Lurem-tr® and sticky cards were hung up. The results showed that sticky cards with (Lurem-tr®) was trapped 15% more than sticky cards without them (table 1). Furthermore in treatment the population of thrips received to peak three weeks later than control (table 2). These results showed (Lurem-tr®) is the suitable mass trapping, and can be applied in IPM.

P IPM 79

The evaluation of black and white Plastic efficacy mixed with Chemical and Physical treatment for weeds control and increasing Potato (*Solanum tuberosum*) yield

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In order to evaluate different management treatments on weed structure and potato yield, an experiment was conducted in Ardabil (North west of Iran) as randomized complete blocks with four replications in 2009. Treatments included weedy (check), weed free (check), Metribuzin as a preplant (standard), black plastic (polyethylene), white plastic (polyethylene) Trifluralin, Trifluralin + black plastic , Trifluralin + white plastic, cultivation, Treflan + cultivation. Statistical analysis showed that different treatments had significant effect on weed density, weed dry weight and potato yield. The results showed that weed densities were reduced to lowest level in black polyethylene included treatments. In this treatment weed density were less than 4 per/m² and 93.57% less than weedy treatment. The most weed densities were in treatments with weed controlling at earlier potato growing stage. Metribuzin preplant application was reduced weed dry weight comparing to weedy check significantly. In this treatment weed dry weights was less than 2 times comparing to weedy treatment. Different treatments had significant effect on tubers yield and the highest yield production was 39.04 t/ha in black polyethylene included treatments.

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Table 1: Total weed density in different IWM treatments

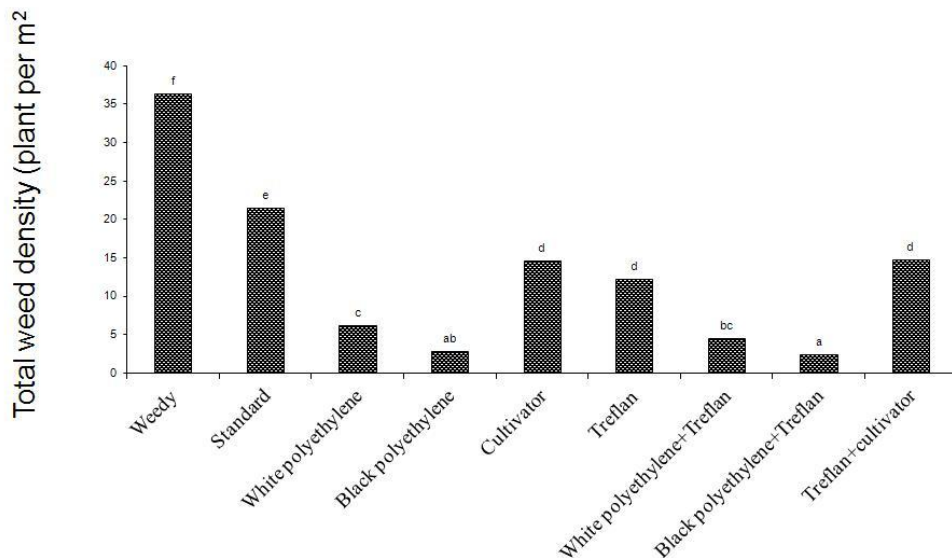
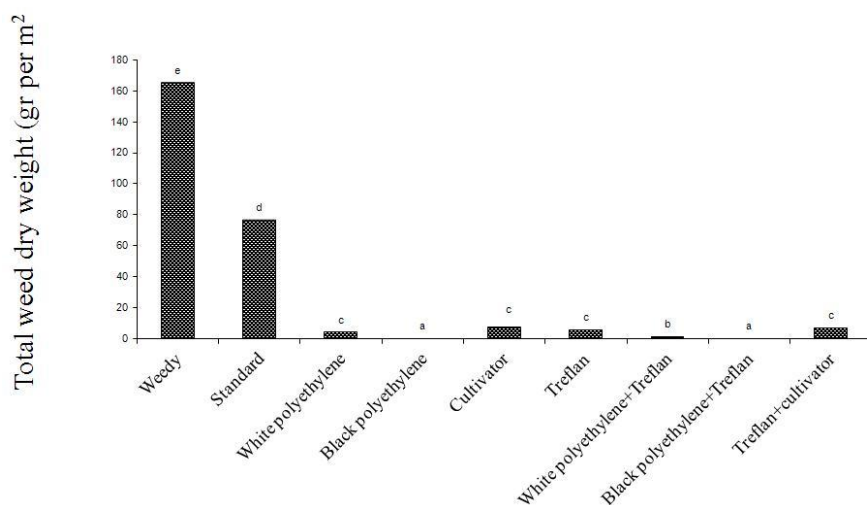


Table 2: Total weed dry weight in different IWM treatments



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Effect of different diets on development of *Amblyseius swirskii* (Acari: Phytoseiidae)

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The predacious mite *Amblyseius swirskii* Athias-Henriot is used as a biological control agent against various pests in greenhouses. In many phytoseiid species, pollen grains not only provide important food elements such as proteins, carbohydrates and lipids, but also play an important role in providing the energy source of migration and aid reproduction process, especially if being consumed along with main preys. The objective of this study is to appraise and compare the development, mortality and reproduction of *A. swirskii* on five different foods, bee pollen alone, date palm pollen alone, *Tetranychusurticae* Koch (Acari: Tetranychidae) immatures in the presence and absence of bee pollen and date palm pollen. This study was investigated in laboratory by using the bean excised leaf method at 25 ±1 °C, 16L: 8D hours photoperiod and 65 ±5 % RH. The predator eggs were individually placed on bean squares and were supplied with the sufficient amount of food. Experimental units were checked daily and the raw data were recorded for both female and male individuals separately. Developmental time (from egg to adult emergence) of the predator on different diets varied from 8.17 to 13.71 days for females and from 7.75 to 12.50 days for males. Survival rate was highest (95%) when the predator fed on immatures of *T. urticae*, while the lowest rate (52.9%) was recorded on bee pollen grains. *Amblyseius swirskii* had significantly longer oviposition period, along with the highest total fecundity on *T. urticae* immatures with date palm pollen. The shortest female and male longevity was found when the predator fed on bee pollen grains and the highest was on date palm pollen and the mixture of *T. urticae* immature with date palm pollen. Overall, use of date palm pollen with the main prey increased the adult survival, as well as egg production rate and finally caused optimizing experimental mass rearing of *A. swirskii*.

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The Effect of Seed Proteinaceous Extracts from Two Wheat Cultivars against Digestive α-amylase and Protease Activities of *Phthorimaea operculella* Zeller (Lepidoptera; Gelechiidae)

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Introduction: The potato tuberworm, is a worldwide pest of solanaceous crops especially damaging to potatoes. Since insect pests rely on their digestive enzymes to feed on plants, so the enzyme inhibitors which are encoded in transgenic plants could be an advisable strategy to control of phytophagous insect-pests.

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Objectives: The goal of current study, was to investigate the effect of seed proteinaceous extracts from wheat (*Triticum aestivum* L. cv. MV17 and cv. Azar) against digestive α -amylase and protease activities of potato tuberworm and also determination of the optimal pH of these enzymes activities was done.

Material and methods: Fifth instar larvae of the pest was used for enzyme extraction. The optimal pH of α -amylase and protease activities were determined using different pH values of universal buffer. The extraction of seeds proteins, was done by NaCl, ammonium sulfate, Tris-HCl buffer and dialyzing the extract against the same buffer. Electrophoretic detection of amylolytic and proteolytic activity using 10% (w/v) polyacrylamide gel was done.

Results: The optimum pH of α -amylase and protease activities was found to be highly alkaline. Amylase activity was significantly affected by extracts from MV17 and Azar by pH ($p=0.05$; maximum effect at pH 9) and influencing of protease activity by extracts did not highly vary by pH 8 and 11 and pH 8, 9 and 11, respectively. Inhibition manner of various concentrations of extracts were dose-dependent. In polyacrylamide gel assay, both enzymes, without inhibitors showed two isozymes, which at highest concentration of extracts, both bands disappeared or their intensity decreased.

Conclusion: In conclusion, potato tuberworm α -amylases are more sensitive to be inhibited by tested proteinaceous seed extracts in comparison with protease. Finally, it should be said that since MV17 and Azar could affect the insect digestive enzymes specially α -amylase, these seed extracts can be tested to produce resistant potato crops against potato tuberworm.

P IPM 83

Host-plant odours for *Bruchus rufimanus* monitoring

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Question: *Bruchus rufimanus* is a serious pest of Broad Bean in Europe damaging the beans and decreasing the commercial value. The objective of the study was to develop a host-plant odour based attractant for pest monitoring.

Methods: Headspace samples of volatiles were collected from broad bean plants at different development stages (leaf, flower, pod). The volatile organic compounds (VOCs) collected on SPME fibers were analyzed and identified with GC-MS. The perception of identified molecules was tested in electroantennography (EAG). Formulations of the semiochemicals identified by GC-MS and detected by the antennae were tested in field trapping experiments.

Results: Broad bean plants emit about 30 VOCs. The three development stages are characterised by a specific chemical signature: the leaf stage release a large amount of green leaf volatiles (cis-3-hexenol, cis-3-hexenyl acetate) and monoterpenes whereas benzoic compounds and sesquiterpenes dominate the flowering stage. The pod stage is characterized by abundance of monoterpenes (limonene, ocimene, linalool) and of cis-3-hexenyl acetate. Electroantennography revealed that the female *B. rufimanus* are more sensitive to host-plant VOCs than males. They detect alcohols (phenyl ethanol, benzyl alcohol, trans-2-hexenol) and other flower scent compounds such as benzaldehyde and terpenes. We formulated semiochemical lures based on the pod odours evaluated in field trapping experiment. Glued traps baited with a five-component blend caught a large number of *B. rufimanus*, significantly more than unbaited control traps.

Conclusion: The host-plant blend was attractive with traps under field conditions. The prototype lure and trapping system developed could be used as a monitoring tool to determine infestation levels of *B. rufimanus* in bean fields or to develop new control methods.

P IPM 84

Exploration of alternative fire blight management strategies

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Few methods exist for fire blight disease control in apple. The main available controls are removal of infected portions of trees, antibiotic sprays at bloom time, and use of less susceptible varieties and rootstocks. In addition, antibiotic use in agriculture is coming under increasing scrutiny out of concerns about the development of antibiotic-resistance among bacteria, and the use of streptomycin in U.S. organic apple production was phased out in 2014. In this study, we explored possible novel avenues for fire blight control: induced immunity to fire blight in apple trees; and the use copper-bacterial product spray mixtures applied post-dormancy to manage shoot blight. Most studies of systemic acquired resistance (SAR) have focused on annual plants and SAR responses that last a few weeks or months. The area of multi-year SAR in woody perennials has not yet been explored in detail by plant biologists, and this could represent a promising approach in tree fruit crops. From 2013 - 2015, we evaluated year-to-

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year SAR induced by natural fire blight infection and through previous season applications of acibenzolar-S-methyl and prohexadione-Ca. Using an apple block with a history of fire blight, we monitored at the level of individual trees throughout the season for natural disease incidence from late May through July. In 2013, 8% of the total number of trees in the apple block had 6 or more shoot strikes; 44% in 2014. Since the disease pressure was so severe in 2014, we are continuing to monitor this apple block for disease incidence at the level of individual trees for the 2015 season from two weeks after bloom until mid-July. We also evaluated during the 2015 season blossom and shoot blight incidence of 12 year old 'Gala' trees treated during the 2014 season with acibenzolar-S-methyl and prohexadione-Ca. Acibenzolar-S-methyl is well known to support protection against blossom infection as a result of SAR induction; however, protection beyond postbloom applications has not been evaluated. Prohexadione-Ca is a plant growth regulator often used to prevent shoot blight infection during the season, but the lasting effect beyond the current season has not been evaluated. Since few methods exist for controlling the shoot blight phase of the disease, we also wanted to determine during the 2015 season how weekly topical sprays, which included copper octanoate mixed with different strains of *Bacillus sp.*, mitigated the progression of the disease throughout the course of the season. Previous research using a copper octanoate and *Bacillus amyloliquifaciens* mixture was promising (1). Results are pending for the 2015 season.

(1) Yoder, K., Cochran, A. 2013. Shoot blight suppression, fruit finish, and summer disease control by Cueva and Double Nickel on Gala apple, 2013. Plant Dis. Manag. Rep. 8:PF023 doi:10.1094/PDMR08.

P IPM 85

Effect of cultural practices and seed-treatment with insecticides on the spread of persistently aphid-transmitted viruses affecting food legume and cereal crops

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Food Legume and cereal crops are subject to infection with a wide range of viruses, many of which cause serious diseases and major yield losses. In this paper, examples of successful deployment of cultural practices (planting date, plant densities, zero tillage) and chemical control (seed-dressing, foliar spray) on the spread of persistently aphid-transmitted viruses affecting legume and cereal crops are presented. In Syria, Egypt and Tunisia, faba bean crops planted early were often severely attacked by *Faba bean necrotic yellows virus* (FBNYV), leading to 100% infection. In such circumstances, farmers plough the crop under and replant with another crop. Delaying sowing led to reduced incidence of infection and consequently less crop losses, mainly due to fewer viruliferous aphid vectors arriving from neighboring virus sources. Changing in planting date from mid-December to late January increased the rate of *Barley yellow dwarf virus* (BYDV) infection on barley and reduced grain yield and biomass (10.04% and 33.95%, respectively). The rate of BYDV incidence was reduced to 25.28% when plant density was increased from 200 seeds/m² to 300 seeds/m², which led to an increased grain yield and biomass of 3.39% and 12.55%, respectively. In Syria, we found that the incidence with Luteoviruses on chickpea crops was decreased (40%) when zero tillage was applied, while chickpea yield was increased by 61.5%, compared with traditional tillage. Field experiments conducted in Syria, showed that treating barley seeds before planting with Imidacloprid insecticide (Gaucho) (1.8 g a.i./kg seeds) reduced BYDV incidence by 84% and increased the grain yield and biomass by 9.2% and 46.45%, respectively. In Tunisia, seeds treated before sowing with Celest top (25 g/L difenoconazole + 25 g/L fludioxonil + 262.5 g/L thiamethoxam) and with Apron Star 45 WS (200 g/kg thiamethoxam, 200 g/kg mefenoxam, 20 g/kg difenoconazole) showed that spread of FBNYV and BYDV and yield loss caused by them were significantly reduced in treated plots compared with untreated plots. Based on the results obtained, it can be concluded that seed treatment and use of cultural practices can effectively reduce the incidence of persistently transmitted aphid-borne viruses affecting cereal and legume crops.

P IPM 86

Biological activity of aromatic plant's aqueous extracts against plant-pathogenic fungi and aflatoxin biosynthesis

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The aim of this work was to evaluate the biological activity of five Lamiaceae species (*Melissa officinalis*, *Hyssopus officinalis*, *Origanum dictamnus*, *Origanum vulgare*, *Salvia officinalis*), and *Crocus sativus* aqueous extracts against three plant pathogenic

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fungal species: *Fusarium oxysporum*, *Alternaria alternata* and *Aspergillus flavus*. The concentration of the aqueous extracts was 1 g/10 mL for the *Lamiaceae* species and 1 g/10 mL as well as 0.01 g/mL for *Crocus sativus*. Each fungal species was inoculated in PDA (Potato Dextrose Agar) medium, which contained the aqueous extracts of these 6 plants. The mycelium growth and the production of the conidia were measured. In the case of *Fusarium oxysporum*, *Origanum vulgare* extract appeared more active as the conidia production quadrupled compared to the control. A similar activity was demonstrated on the effects of *Salvia officinalis* extract on *Alternaria alternata* and *Origanum dictamnus* extract on *Aspergillus flavus*. In contrast, *Origanum vulgare*, *Crocus sativus* (0.01g/mL) and *Hyssopus officinalis* extracts reduced conidia production in *Alternaria alternata*. In further experiments, a study was conducted on the effects of the aqueous extracts of the aromatic plants in aflatoxin production of *Aspergillus flavus* in CCA (Coconut Cream Agar) media. The toxin was extracted by the TLC (Thin Layer Chromatography) method. Furthermore, the effects of the different extracts on the expression of the transcription factor *afIR* and the biosynthetic gene *nor-1*, involved in the aflatoxin biosynthesis pathway, were examined by Real-Time PCR. The results of the TLC analysis showed a notable reduction of the aflatoxin production after the application of the aqueous extracts of *Origanum vulgare*, *Origanum dictamnus*, *Melissa officinalis* and *Crocus sativus* (1g/10ml). Real-Time PCR showed that the extract of several species significantly reduced the expression of the examined genes. Further investigation is currently in progress to fully understand the effect of these aqueous extracts on the three fungal species.

P IPM 87

Characterization of resistance genes against actual races of *Pyricularia oryzae* in Uruguay

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Rice blast caused by the fungal pathogen *Pyricularia oryzae* is the most devastating disease affecting crop production in Uruguay and the world. In the last seasons the indica type cultivars El Paso 144 and INIA Olimar and temperate japonica type cultivars INIA Tacuarí and Parao were grown in more than 80% of the rice area of Uruguay. These cultivars are susceptible in leaf and/or neck to the actual blast races. A common disease management practice is the application of fungicides which pose a significant concern for human health and environmental safety. Deployment of *R* genes in new cultivars is the most powerful strategy for managing blast disease. The objective of the present study was to characterize blast resistance genes against actual Uruguayan blast races in order to incorporate these genes to new breeding materials.

Thirty two international monogenic differentials obtained from IRRI-Jircas carrying 24 major blast resistance genes and the susceptible parental Lijiangxintuanheigu (Telebanco-Yanoria et al., 2010) were studied during three seasons in a blast nursery in UE Paso de la Laguna of INIA Treinta y Tres, Uruguay. Leaf and neck blast was scored at vegetative (early and late leaf blast) and milk grain (panicle blast) stages based on a 0-9 scale relative to infection percentage (IRRI 2010) and classified as susceptible or resistant.

Fourteen lines carrying twelve different genes were identified possessing resistance to the actual blast races present in Uruguay. Resistance lines for leaf and neck blast have the *R* genes *Piz-5(Pi2)*, *Pi3*, *Pi5(t)*, *Pi12(t)* and *Pii*. Lines carrying genes *Pi9*, *Pish* and *Pi-z* showed resistance to leaf blast and *Pia* for neck blast. Lines with the genes *Pik-s*, *Pita* and *Pita-2* showed different responses depending on the parental where the gen was obtained indicating that these lines carry more than one *R* gene. Eighteen lines possessing twelve different *R* genes were susceptible to leaf and neck blast and unsuitable for deployment in new cultivars (Table 1).

The results suggested that *Pi3*, *Pi5(t)*, *Pi9*, *Pi12(t)*, *Pia*, *Pii*, *Pish* and *Pi-z* may be interesting *R* genes for preventing actual blast races in Uruguay. *R* genes *Pii*, *Pi3* and *Pi5(t)* provided broad spectrum resistance for leaf and neck blast of rice. These *R* genes can be added to current breeding programs focused in the incorporation of previously proposed major resistance genes like *Piz-5(Pi2)* and *Pi33*. Monogenic lines carrying *Pia*, *Pik-s* and *Pi12(t)* were resistant to neck blast and these genes can be incorporated in Uruguayan japonica type cultivars that are very susceptible to neck blast. Alternatively, these genes can be pyramided with genes with broad spectrum of resistance to leaf blast like *Pish*, *Pi-z*, *Pi9* and *Pi12(t)*.

IRRI. 2010. Standard Evaluation System for rice. IRRI, Los Baños, Phillipines.

Telebanco-Yanoria, M.J., Koide, Y., Fukuta, Y., Imbe, T., Kato, H., Tsunematsu, H., Kobayashi, N., 2010. Breeding Science 60: 629-638.

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Figure 1

Table 1. Disease reaction of 32 monogenic lines to leaf and neck blast.

Rice Line	Gene	Leaf blast	Neck Blast
IRBLA-A	Pia	S	R
IRBLA-C	Pia	S	nd
IRBLI-F5	Pii	R	R
IRBLKS-F5	Piks	S	S
IRBLKS-S	Piks	R	R
IRBLK-KA	Pik	S	nd
IRBLKP-K60	Pikp	S	S
IRBLKH-K3	Pikh	S	S
IRBLZ-FU	Piz	R	S
IRBLZ5-CA	Pi2	R	R
IRBLZT-T	Pizt	S	S
IRBLTA-K1	Pita	R	R
IRBLTA-CT2	Pita	S	S
IRBLB-B	Pib	S	S
IRBLT-K59	Pita	S	S
IRBLSH-S	Pish	R	S
IRBLSH-B	Pish	R	S
IRBL1-CL	Pi1	S	nd
IRBL3-CP4	Pi3	R	R
IRBL5-M	Pi5	R	R
IRBL7-M	Pi7	S	nd
IRBL9-W	Pi9	R	S
IRBL12-M	Pi12	R	R
IRBL19-A	Pi19	S	S
IRBLKM-TS	Pikm	S	S
IRBL20-IR24	Pi20	S	S
IRBLTA2-PI	Pita2	R	S
IRBLTA2-RE	Pita2	R	R
IRBLTA-CP1	Pita	S	S
IRBL11-ZH	Pi11	S	nd
IRBLZE-CA	Pi2	R	R
LTH	0	S	S

R= resistant, S= susceptible, nd= no data.

P LIEE 1

Pesticides use in cocoa sector in Cameroon: characterization of supply source, nature of actives ingredients, fashion and reasons for their utilization²

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² Reference to chemicals in this article should not be taken as recommendations of particular products.

The objective of this study was to provide insight in current pesticide use in the cocoa sector in Cameroon. A stratified sampling scheme was used. A total of 251 cocoa farmers, 20 post-harvest cocoa traders and 37 chemical retailers were randomly selected and interviewed. The study showed that 35 different chemicals were marketed in Cameroon for use in cocoa: four herbicides, 11 fungicides and 20 insecticides. Of 251 farmers consulted, 96.8% said that they used pesticides on their farms while 3.2% did not. Fungicides were used most often by farmers (61.8%) followed by insecticides (38.2%). Eight active ingredients although officially banned are still being used on cocoa farms. Over 77% of farmers do not respect the official spray recommendations for chemicals. Moreover, and 64% do not respect recommended doses. Two main pesticide-supply-pathways exist in Cameroon: a legal and illegal supply chain, which provides 51% of pesticides to cocoa farmers, resulting in an estimated loss of VAT for the Cameroonian government of 550 thousand to around 2.4 million Euros per year. These results suggest that improved control by the state and raising farmer awareness about pesticide use in cocoa could greatly attribute to a more sustainable cocoa economy in Cameroon.

P LIEE 2

Australia's Grains Farm Biosecurity Program - a national initiative in plant biosecurity awareness, education and training

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Introduction: Sound biosecurity systems contribute to achieving resilient and sustainable agricultural and environmental systems, reducing the threat of introducing unwanted pests and diseases. Within Australia, the Grains Farm Biosecurity Program (GFBP) is a national initiative to assist in the development and implementation of improved biosecurity practice within Australia's grain industry. Initiated in 2007, the extension focused program contributes to the grain industry's risk mitigation activities, and promotes a shared responsibility involving governments, industry and community.

Objectives: The GFBP aims to promote and improve the management of, and preparedness for, biosecurity risks in the Australian grains industry at the farm and industry level.

Materials and methods: The GFBP has appointed specialized State Grains Biosecurity Officers in the five key grain growing states of Australia. The program is funded by Grain Producers Australia through grower levies in partnership with state government agencies and Plant Health Australia.

Yearly, over 100 activities are undertaken using a variety of community engagement strategies aimed at increasing biosecurity awareness and adoption throughout the supply chain. Core activities include: identification of priority pests, risk pathways and mitigation strategies; developing education materials; and establishing networks for surveillance and training.

Results: Surveys undertaken throughout the project indicate an increased awareness of biosecurity risks and voluntary adoption of biosecurity best practices throughout the sector.

Key outcomes include: development of a range of practical resources including awareness material for researchers, growers and extension specialists; farm level recommendations to identify and mitigate risks; media articles; forming industry alliances; providing biosecurity training and encouraging surveillance on key pests.

Conclusion: The GFBP is Australia's flagship program for promoting farm biosecurity, with its success encouraging other industries to implement similar extension programs. The focus on adoption of biosecurity best practice through industry

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engagement has seen the GFBP contribute to the safeguarding of production, market access and preserving Australia's grain export reputation.

P LIEE 3

Australia's On-Farm Grain Storage Extension Project - a national initiative improving stored grain pest management and maintaining phosphine fumigation efficacy on-farm for the Australian grains industry.

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Introduction: Phosphine is a cost effective and widely used fumigant to control grain insect pests in on-farm and central storage systems in Australia. Phosphine's continued use is threatened through increased resistance in both frequency and strength in target insect pests. Effective fumigation combined with best practice integrated pest management is essential to the sustainability of grain biosecurity and market access for Australian post-harvest grain systems. The National Stored Grain Extension Program (NSGEP) is the industry funded initiative developed to facilitate best practice grain storage practices, involving growers, agribusiness and industry.

Objectives: The NSGEP aims to improve grain storage management practices and the efficacy of phosphine fumigation within Australia's grains industry.

Materials and methods: The NSGEP uses a multi approach engagement strategy aimed at increasing awareness and knowledge to build capacity and support to enable farmers and industry to manage their grain storage systems and meet best practice requirements. These include: workshops, field days, industry forums, information, multi-media and website development and building networks with grower groups, government agencies and agribusiness.

In southern Australia, training workshops for farmers, advisors and agribusiness on best practice fumigation and grain storage principles that use a variety of adult learning principles and training techniques (e.g. practical demonstrations) is a key element with over 50 delivered yearly.

Results: Various evaluation methods have shown that awareness and adoption of best practice in on-farm grain storage management has increased. Key outcomes include increased knowledge in stored grain insect identification and skills development and practice change primarily around improvements in hygiene, aeration, phosphine application, silo testing and planning of storage systems.

Conclusion: The NSGEP contributes to the positive on-going changes observed in Australia's on-farm grain storage systems, primarily through the specialized extension network of information, support and training provided that is highly regarded and in demand. It plays an instrumental role in building capacity and maintaining phosphine fumigation efficacy in the Australian grains industry.

P LIEE 4

National Invertebrate Pest Initiative (NIPI): Engagement and adoption program to improve pest management for the Australian grain industry

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Introduction: Within Australia, practice change in pest management leading to the wider adoption of more sustainable pest management for broad-acre grain crops has been driven by the National Invertebrate Pest initiative (NIPI). NIPI is a collaborative network of researcher and extension specialists funded by grower levies through the Grains Research and Development Cooperation in partnership with governmental agencies, private organisations and universities. Initiated in 2006, NIPI extension services have contributed to sustainable grain production through education, training and support services.

Objectives: NIPI extension activities aimed to increase the uptake of sustainable pest-management practices within Australia's grain sector by building capacity in invertebrate identification and biology, and creating a real-time pest alert network.

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Materials and methods: Key communication engagement approaches used across Australia's southern and western regions included: development and delivery of invertebrate training workshops; establishment of an expert network to provide communication of real-time information (email and diagnostic services); best practice decision support tools and extension materials.

Results: Over 8 years: Approximately 1000 participants undertook invertebrate identification and management training; a comprehensive training manual was developed and distributed widely; an entomologist network provided over 700 real-time pest alerts yearly; and diagnostic support services delivered annually.

A formal review of NIPI was undertaken which demonstrated an increased capacity in invertebrate identification and improved management strategies throughout the industry, as well as highlighting IPM extension needs for further adoption.

Conclusion: A hallmark of the NIPI extension program is the extensive network of diagnostic support, real-time insect alerts, and support for pest control decision making. This network allows for continued delivery of regular, topical and useful information and alert services to stakeholders. This strong engagement strategy is highly valued within industry. Its success in up-skilling industry and providing a solid foundation to build upon has, and will continue to, reduce Australian grains reliance on broad-spectrum insecticides.

P LIEE 5

Improving the availability of plant protection products in minor uses - A joint project of DBV, ZVG and JKI

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Introduction: Minor uses in plant protection are areas of usage of minor economical impact. For some areas of minor uses no appropriate control methods (chemical, biological or mechanical) are available for practical usage. In order to face the future challenges and also in respect to the new European registration legislation, the German grower associations Deutscher Bauernverband (DBV) and Zentralverband Gartenbau (ZVG) have established a joint project in cooperation with the Julius-Kühn Institut (JKI). The work is financially supported by the German Federal Ministry of Food and Agriculture (BMEL) through the Federal Office for Agriculture and Food (BLE, grant number 2810MD005 and 2810MD006) and runs from 2013 to 2017.

The objective of the joint project is to support the activities in Germany concerning minor uses in order to improve the availability of practical plant protection methods by investigations in international databases and cooperation with European member states and other international partners.

Results: The joint project includes three parts with different focus:

Part1: Investigations in international databases (e.g. HOMOLOGA) for pesticides, which are registered in other countries for minor uses in arable farming, ornamental horticulture, tree nurseries, fruits and vegetables. In 2014 the focus was set on the investigation for pesticides to control *Drosophila suzukii* in fruit growing.

Part 2: Development of procedures for communication and data transfer between extension services, grower associations, plant protection industry as well as national and European working groups for minor uses. The present focus is on the control of *Poa annua* and *Senecio vulgaris* in vegetable crops by selective herbicides.

Part 3: Searching international literature databases for state of the art research on plant diseases and pests, and conducting field trials with new plant protection products or new strategies. In 2014 field trials were conducted to control *Delia radicum* and other flies in radishes, Chinese cabbage, carrots and leek.

Conclusion: The project is incorporated into the national network of minor uses (<http://lueckenindikationen.jki.bund.de>). The results of the project are provided to the grower associations, extension services and to the working groups for minor uses for further actions.

P LIEE 6

Mapping stakeholders' and farmers' views on herbicides use

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This work attempts to identify farmers' and stakeholders' reaction to herbicides and especially to pendimethalin use on specific crops. This is achieved by interviewing farmers and stakeholders and analyzing the responses. The primary objective of the survey on weed control use (herbicides and particular pendimethalin) is to gather detailed data on current knowledge, attitudes and beliefs related to herbicide use. In addition, pendimethalin's application is examined in three crops (onion, cotton, processing tomato) investigating the impacts of pendimethalin on the crop production, product quality and farmers' revenues. Local stakeholders (crop consultants, industry experts and agronomists) were engaged in informal conversations about on-farm practices related to the use of pendimethalin in the three selected crops (cotton, onion, processing tomato), to develop essential information for its contribution on the plant viability, crop production and production expenses. Moreover, experts in cotton processing were interviewed to cast the industry's perspective. Finally, in order to identify farmers' reflection and perceptions towards herbicides and specially pendimethalin, 140 farmers were interviewed, filling in the appropriate questionnaires.

Analyzing the responses, useful insights were derived. Thus, it can be stated that pendimethalin is valuable and irreplaceable herbicide, especially in cotton and onion crops; whereas in processing tomato crops its use is moderate. All the stakeholders unanimously stated that pendimethalin cannot be replaced or substituted in those crops by any known herbicide without devastated consequences on the production cost and total production.

According to farmers' answers, herbicides are believed to be more effective compared to other weed control techniques and, pendimethalin as a herbicide is very effective, used extensively and is irreplaceable. Also, pendimethalin use affects farmers' incomes positively. Cotton farmers would increase the use of several less effective substitute herbicides in case of pendimethalin withdrawal, affecting negatively their product quality and income.

P LIEE 7

Farmers' Knowledge and Perceptions of Potato Pests and their Management in Uganda

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As we initiate entomological research on potato in Uganda, there is need to understand farmers' knowledge of existing insect pest problems and their management practices. Such information is important for designing a suitable intervention and successful integrated pest management (IPM) strategy. A farm household survey using a structured questionnaire was conducted among 204 potato farmers in six districts of Uganda (i.e. (Kabale, Kisoro, Mbale, Kapchorwa, Mubende and Kyegegwa) during the dry season of 2013. Diseases, insect pests, price fluctuations and low market prices in order of decreasing importance were the top four highly ranked constraints in potato production. Among insect pests, cutworms (*Agrotis* spp.), aphids (Hemiptera: Aphididae) and potato tuber moth (*Phthorimaea operculella* Zeller) were three most severe insect pests. Ants (Hymenoptera: Formicidae), whiteflies (*Bemisia tabaci* Gennadius) and leafminer flies (Diptera: Agromyzidae) were the other insect pests of moderate importance. Yield losses of pests and diseases if farmers don't apply chemical pesticides reached 100% in Kabale, Kisoro, Mbale and Kapchorwa. On average, farmers had low to medium knowledge about pest characteristics. The predominant control methods were use of fungicides (72% of respondents) and insecticides (62% of respondents). On average, only 5% of the 204 farmers knew about natural enemies. The low to medium knowledge of insect pests recorded in this study calls for training of both farmers and extension workers in insect pest identification. Empowering farmers with knowledge on insect pests is essential for the reduction of pesticide misuse and uptake of more environmentally friendly approaches like IPM. Field sampling is also needed to assess the actual field infestation rates and intensities of each insect pest, confirm the species names and compare the results with the responses received from farmers.

P LIEE 8

CO-FREE: four crops, three years - where are we now?

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Introduction: The project CO-FREE is working on innovative strategies for copper-free low-input and organic farming systems (www.co-free.eu; funded by the European Commission, 7th framework program). In CO-FREE, 11 scientific institutions plus 9 small and medium enterprises are working together over a period of 54 months.

Objectives: The project's aim is to develop potent strategies to replace copper in organic, integrated and conventional farming. The project is constructed as a modular system. Alternative plant protection products (CTPs), decision support systems (DSS), susceptible and disease-tolerant varieties and innovative breeding goals (ideotypes) as well as cropping systems are integrated into management strategies. The project focuses on four crops: apple / *Venturia inaequalis*, grape / *Plasmopara viticola*, and, tomato and potato / *Phytophthora infestans*.

Material and methods: The following CO-FREE alternative test products (CTPs) are under investigation: *Trichoderma atroviride* SC1 and protein extract SCNB, *Lysobacter* spp., yeast-based derivatives, *Cladosporium cladosporioides* H39, oligosaccharidic complex COS-OGA, *Aneurinibacillus migulanus* and *Xenorhabdus bovienii*, sage extract, liquorice extract, PLEX and seaweed extract. Field trials were performed in different European countries in 2012-2014 following EPPO standards.

Results: In summary, stand-alone applications of CTPs in field trials showed:

In apple / *V. inaequalis*

- CTPs were effective as stop treatments (leaves).

- In agroforestry production with high cultivar diversity, more than 50 % disease reduction was found (fruit).

In grape / *P. viticola*

- Disease was reduced up to 40 % (leaves and fruit).

In tomato / *P. infestans*

- Up to 70 % disease reduction was achieved (leaves).

In potato / *P. infestans*

- Disease development was retarded (leaves and stem).

- Influence of cultivar was very important.

- Yield increase reached up to 40 %.

Besides this, further positive side-effects were observed with respect to yield quality (potato), support of beneficial mites (grape) and on reduced leaf fall caused by *Marssonina coronaria* (apple).

Conclusion: Field trials from the three years with stand-alone application of CTPs showed promising results. In 2015, strategies combining CTPs with optimized DSS, cultivars and cropping systems are under way.

P LIEE 9

2014 survey of yellow dent corn diseases in North Dakota, United States

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Yellow dent corn (*Zea mays*) acres in the state of North Dakota (ND) in north central United States has doubled in the past ten years. With the rise in corn acreage, concerns with disease related problems have arisen. Prior to 2014, the prevalence of diseases found in corn in ND was relatively unknown. Therefore, a survey was needed to document the prevalence of corn diseases in ND that will in turn help direct research and Extension efforts. Two separate surveys were organized at different points in the growing season. The first survey was conducted between vegetative growth stages V4 (fourth leaf) to V6 (sixth leaf) and observed the prevalence of root rots. This survey encompassed forty fields that were randomly selected in areas of historically high corn production. To document root rot prevalence, ten plants were extracted from each field and roots were examined for lesions. Semi-selective media was used to isolate pathogens from root lesions. The genus of the isolate was identified morphologically and species identification will be done molecularly. The second survey was conducted after VT (tasselling) to examine the prevalence of foliar diseases. Sixty-two fields were randomly surveyed for foliar diseases, which were identified by visual symptom. For the root rot survey, *Fusarium*, *Pythium*, and *Rhizoctonia* species were isolated from 83%, 30%, and 8% of the root samples respectively. Of the corn fields visited during the foliar disease survey, four diseases of corn were identified. Namely, Goss's leaf blight, common corn rust, northern corn leaf blight, and holcus leaf spot were identified in 39%, 82%, 34% and 2% of the fields respectively. Even though several genus were isolated from corn roots, pathogenicity and species quantification is needed for future disease management recommendations. Common corn rust was the most common foliar disease in ND, however most fields did not achieve economic injury levels as most ND corn hybrids have an acceptable level of rust resistance. Leaf samples exhibiting Goss's leaf blight were collected and will be used for future research as this disease continues to be problematic in US corn production. Continued survey efforts will help direct research priorities, strengthen disease management recommendations and provide training for Extension personnel.

P LIEE 10

PestinfoWiki - an interactive searching tool for publications and other information in the field of pest management

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The "Pest Information Wiki" (<http://wiki.pestinfo.org/wiki/>) has been developed as an open internet site for scientists, students and other professionals to support their search for information in the field of pest management. The site contains currently around 130,000 abstracts of publications and other additional information about pests, plant diseases and weeds.

Apart from agricultural and storage pests, medical and veterinary arthropod pests are also covered. The pests included are insects, other arthropods and vertebrates. The plant diseases include fungi, bacteria, viruses, plant parasitic nematodes and other agents like phytoplasmas or viroids providing short summaries about their biology, including photo galleries. Currently, the site has pages for around 5,000 pests, diseases and weeds with the aims to include additional species and to permanently update the sites already established. In addition, about 1,500 pages covering beneficials have been established. These include agents like parasitoids, predators, antagonists of plant diseases, diseases of arthropod pests, or weed biological control agents.

The Pest Information Wiki also contains a database structure based on the Semantic MediaWiki system. This allows the display of lists of literature, pests or beneficials. The literature lists cover for example a specific pest, disease or weed and can then be filtered for specific research topics, like host plants, countries, or beneficials. Another example would be publication lists for a given author. Pages for more than 10,000 authors have been established together with their publication lists and links to address information.

The Wiki is organized by the International Society for Pest Information (ISPI) and ISPI invites all scientists active in the field of pest management to contribute publication abstracts and other information on pests, diseases and weeds.

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P LIEE 11

Assessing indirect costs of pesticide use

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For many years, farmers have used pesticides to protect plants from pathogens, insects, nematodes and weeds. The initial aim was to ensure a viable yield and to improve the quality of the food produced. However, excessive use of pesticides has led to pest resistance and has had a negative impact on the environment and the health of farmers and consumers. Theoretical developments and empirical measurement of the external costs of pesticides' (TEAMPEST, an EU funded FP7 project) investigated this worrying issue and aimed to provide sustainable solutions. The project team used a combination of traditional theoretical methodologies and recent advancements. They then tested the developed theoretical models and policy tools in a selection of EU countries. The results obtained in the project were used to generate a policy framework. This may serve as a benchmark for future EU policy schemes aimed at achieving sustainable pesticide use and management. In addition, the results may guide future decisions on the level of taxation required. In this paper the main policy oriented results presented and an overview of the issue is offered.

P LIEE 12

Legal base for German recollection systems PAMIRA and PRE

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The PAMIRA disposal system was developed on a voluntary basis by the German plant protection products industry together with the wholesale agricultural trade at the beginning of the 1990's.

PAMIRA, the Packaging Recovery Agriculture system, is a simple system for the safe and environmentally-friendly disposal of empty pesticides packaging. Farmers collect the accrued packaging and hand over this - completely emptied, rinsed and dry - at specified times once yearly free of charge to one of the around 300 collection points in Germany. After checking that the acceptance conditions are satisfied, the packaging is accepted, pressed and recycled for their energy or material values.

The legal base for PAMIRA is the Packaging Ordinance and the Law on Closed Cycle Management. The lecture will deal with the particular legal aspects of the PAMIRA disposal system.

PRE disposal system is as well as PAMIRA a simple system for the safe environmentally-friendly disposal of worn-out plant protection products. German plant protection products industry aim for a permanent recollection and waste disposal of worn-out plant protection products and other chemicals from the German agriculture. With PRE, agriculture and agricultural trade reduce potential risks due to improper storage, application and disposal of plant protection products and ensures a safe, environmentally sound collection and disposal.

PRE is part of the product stewardship established by Law on Closed Cycle Management. By regulating the volunteer recollection of used products by manufacturer, sec. 26 shapes the causative principle and establishes opportunities for volunteer measurements by manufacturers and retailers to avoid further legal regulation. Among others the lecture deals with the legal framework of PRE and product stewardship as part of volunteer recollection measurements.

P LIEE 13

Implementing the National Action Plan in North Rhine-Westphalia on minimizing risks in the use of plant protection agents as well as intensifying Integrated Plant Protection

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The Poster presents the National Action Plan of the Federal Republic of Germany which is based on the EU Directive 2009/128/EG on the framework for action for sustainable use of pesticides (Plant Protection framework directive).

What is new about this Directive?

Up to now integrated plant protection was defined in more general terms. Now the Directive specifies goals, goal quota and time targets. It also gives us recommendations for a broad set of indications that incorporate all ecological aspects. This is a significant improvement of integrated plant protection.

In this way, the National Action Plan focusses our action on risk reduction and integrated plant protection.

Poster Presentations

Legal Issues, Extension, Education

The public plant protection agency of North Rhine-Westphalia is actively applying this program. It has intensified work in the following areas:

- developing, improving and introducing of plant protection measures with less pesticide use or even complete non-use of chemical pesticides
- selecting pilot farms to identify best practice of integrated plant protection
- developing best practice guidelines for each plant culture (e.g. selection of cultivar, tillage, use of pesticides)
- incorporate preventive measures in planning and establishing of non-agrarian areas to minimize plant protection measures (biotope networking)
- certification of competence in applying pesticides in line with the German plant protection law and the regulation for competence
- controlling maximum residue levels
- protection water bodies and doing hot spot analyses
- developing risk reduction measures
- improvement of the public advisory service for example by improving technical advisory support
- monitoring introduction and spread of harmful organisms and quarantine organisms
- applying comprehensive measures to eradicate harmful organisms
- promoting research in the field of plant protection

P LIEE 14

ESENIAS-TOOLS: A project as a result of regional networking

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Question and Methods: Many important pest species have alien origin and their introductions and expansions have been increasing due to human activities and global change. Invasive alien species (IAS) threaten not only biodiversity and unmanaged areas but also socio-economy and managed areas. Most of alien species are not covered by the plant and animal health regimes and EU IAS regulations what might facilitate their spread. Networking on IAS and pests in different scales can be helpful to halt new introductions. ESENIAS (East and South European Network for Invasive Alien Species) was established in 2011 to facilitate solving IAS problems, in the regional level. A new regional project has just been launched: "East and South European Network for Invasive Alien Species - a tool to support the management of alien species in Bulgaria (ESENIAS-TOOLS)". The aim of this presentation is to introduce the project and show possible benefits to halt new pests.

Results: This project, funded under the Programme BG03 "Biodiversity and Ecosystem Services" within the EEA FM (2009-2014), will result in networking and development of IAS tools within the framework of ESENIAS to support the management of alien species in Bulgaria and in the overall ESENIAS region. Eleven institutions from Bulgaria, Croatia, Iceland, Greece, R. Macedonia, Romania, Serbia, and Turkey will take part in the project. Workshops, study visits and meetings are planned within the project. Current databases in the region will be renewed, fact sheets will be produced. The project will facilitate prevention of IAS introduction in the region developing tools for early detection and awareness rising. One of the aims of the project is to establish connections and collaborations with regional and international groups/organisations, what could contribute for integration of regional data and activities in NOBANIS, EEA, EWRS, IAPPS, and EPPO.

Conclusion: Introduction of new pest species in agricultural areas can be minimized applying preventive techniques and early detection systems for IAS, which are the most efficient and cost-effective methods. Networking through governmental and non-governmental organisations can be a useful tool for further studies/projects.

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P LIEE 15

The German Scientific Society for Plant Protection and Plant Health (DPG) - organizer of IPPC 2015

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The **German Scientific Society for Plant Protection and Plant Health (DPG)** is the largest scientific association in plant production in Germany. The Society is membership-based, and its members are professionals within the entire field of phytomedicine. DPG is a scientific association with the purpose of promoting research in the entire field of phytomedicine and the application of the results gained thereby, primarily to advance education within plant health and to support extension services. The Society pursues its goals through: a) organising or supporting scientific meetings and conferences; b) the joint organisation of national and international congresses, symposia etc. (e.g. the German Plant Protection Congress in co-operation with the Federal Research Centre for Agriculture and Forestry and the German Plant Protection Services; the International Symposium *Plant Protection and Plant Health in Europe* and the *International Urban Plant Conferences* in co-operation with European scientific societies); c) offering scientists opportunities to join 24 working groups; d) establishment and development of relationships not only with other organizations that have similar aims and objectives but also with professional colleagues abroad; e) co-operation with universities and other training establishments, with the aim of providing advice in the establishment of study plans and education curricula; f) promotion of young scientists; g) provision of information to the general public on the aims and objectives of phytomedicine; h) publication of research results from the entire area of phytomedicine and the promotion of such publications (e.g. *Journal of Plant Diseases and Protection*, the DPG in-house journal *Phytomedizin*, and *Spectrum Phytomedicine* for specific proceedings of conferences and symposia); i) awarding of prizes and medals; j) promotion of the career, legal and social interests of its members, in collaboration with other (mainly national) organisations.

With its wide membership, DPG includes a huge reservoir of scientific potential, not only for the benefit of the Society and its members but also for the public in general. DPG is able to support and mould the development of phytomedicine on a inter- and a trans-disciplinary level, within scientific circles and in the public arena. As a partner, DPG can offer like-minded organisations a long-term partnership to further all aspects of phytomedicine, whether academic or applied. To this end, we welcome contacts from all organisations that are seeking to establish such collaborative ventures in phytomedicine, as well as from individuals who wish to become DPG members. The most important event in 2015 certainly is the International Plant Protection Congress (IPPC) in Berlin, Germany. You are welcomed to visit the symposium website www.ippc2015.de and, of course, the websites of DPG www.phytomedizin.org, www.plant-protection.net, and others.

Poster Presentations

New and emerging pests and diseases

P NEPD 1

Introduction of beetle *Lilioceris faldermanni* (Guerin) (Col.: Chrysomelidae) as a pest *Lilium ledeburii* (Baker) in Damash forest Guilan Province, Iran and Investigation on the biology its in laboratory conditions

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Lilioceris faldermanni (Guerin) is major pest plant Chelcheragh lily *Lilium ledeburii* (Baker) that emerges with growing of Chelcheragh lily in early spring in forest of Damash, Guilan Province, Iran and damages to aerial parts of plant. Since this plant is a protected species and very important, therefore the study of biology is important for pest control management. Some biological parameters beetle *L. faldermanni* were investigated in 2007-2010 in laboratory conditions. To determine the duration of different developmental stages in different temperatures, 20 specimens from the beginning of each stage were separately selected and kept in petri dishes (10 cm) and fed with lily leaves and appearance time of stages development were recorded. In order to test the survival rate of growth stages, 20 number of new eggs were separately selected and kept in petri dishes (10cm). Percent survival rate was determined by deaths registered in each stage, and determining the difference of total population in each stage. In order to study the pest egg lying at different temperatures, six pairs of adults for each temperature were selected to determine amount of egg lying in plastic dishes (12x8cm). All the experiments were performed under temperatures of 14, 18, 22 & 26 °C, with 65±5% RH, 14L: 10 D. The results of experiments showed that mean of developmental period from egg to adult, were 63/4±0/07, 46/15±0/09, 32/8±0/08 & 27/25±0/1 day and data analysis of variance and mean comparisons using Duncan showed that there are significant differences in the level of 1% among the mean of all the different growth stages. The results of survival rate showed that the maximum percent survival of different developmental stages was at 22 °C. Thermal constant from egg to adult was determined 539/9±4/87 DD. Data analysis of variance and mean comparisons using Duncan showed there are significant differences at 5% level among means daily egg laying, total eggs laid, duration of egg laying and adults longevity. The results revealed that with increasing test temperature from 14 to 16 °C, the mean daily egg laying increased, and the means total eggs laid, duration of egg laying and adult longevity decreased.

P NEPD 2

Isolation and identification of the fungus causing leaf spot of *Eucalyptus Stricklandii* in Sirt, Libya

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Laboratory results showed insulation and field to cause disease spotted eucalyptus trees verity *E.stricklandii* leaves and seeds , which were imported from Australia and the cultivation nurse Alkardabia productive , which is located in the city of Sirte, Libya during the agricultural season 2014 to the presence of fungus *Alternaria .spp* tested in all the papers . As explained field survey of the disease to the results of a high sensitivity of the disease , where the severity of the injury recorded a rate of 12.2% at the upper limbs of trees and 17.5 % at the lower extremities , while the prevalence of the disease record 75% of the trees tested in the study area .

P NEPD 3

Biological protection of pine forests by the control of the pine processionary caterpillar *Thaumetopoea pityocampa*. An important trees parasite in the north-Est of Algeria

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The pine recessionary caterpillar: *Thaumetopoea pityocampa* Schiff is one of the biggest pest of pine forests. Feeding on pine needles, it causes a slowdown in growth, and decreased photosynthetic capacity, the tree is weakened is more vulnerable to attack by other pests. A subsequent attack can lead to total death of the tree.

To deal with this problem and as part of the struggle, many techniques have been the most important are the sex pheromone trappings. This technique requires a daily presence in forests in order to recovered adults. The simplest technique is still the biological treatment in large infested areas. Preliminary tests were conducted in laboratory conditions, then switch to the application in the case of good results.

Poster Presentations

New and emerging pests and diseases

Our work is studied the effect of certain plant extracted on the mortality of caterpillars. We studied the effect of *Eucalyptus camaldulensis*, *Matricaria recutita*, *Zingiber officinale*, The doses used have taken between of 0, 5 and 4 g / l.

The leaves of plants were washed, dried, placed in an oven heated at 40 ° C for 3 days and then ground using an electric blender to obtain a powder in the past using the Soxhlet extraction technique.

The results obtained show that the mortality rate reached 100% for the higher doses. The remarkable doses as LD50 and LD 90 were calculated using software developed at each stage and for each stage.

P NEPD 4

Formation of the number of *Rhopalosiphum padi* (L.) (Homoptera: Aphidoidea) feeding on two bird cherry types in Northwestern Russia

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Aphids are major pests. They generally have a low development temperature threshold (4°C), short generation time and show variation in their life-cycle traits. It is clear that environment changes are likely to affect their pest status.

Objectives: The bird cherry-oat aphid *Rhopalosiphum padi* (L.). The bird cherry: (1) - *Prunus padus* Mill. x *P. virginiana* L., a late-ripening, with buds prone on the branch and the leaf area of $155.7 \pm 7.3 \text{ cm}^2$. (2) - *P. padus*, a mid-ripening, with buds erect on the branch and the leaf area of $114.9 \pm 5.5 \text{ cm}^2$.

Methods: All aphid data used come from the counts on two model plants in 2012-2014. Bird cherry (1) and (2) had the same pattern of insect settlement.

It has been proved that the *Rh. padi* population structure has about 70% clones which are strictly holocyclic whereas 30% are able to lay winter eggs and overwintering in active stage in Northwestern Russia, too. Warmer winters without strong frosts increases the numbers of spring generations of the aphid on cereals during the most vulnerable stage of cereal development.

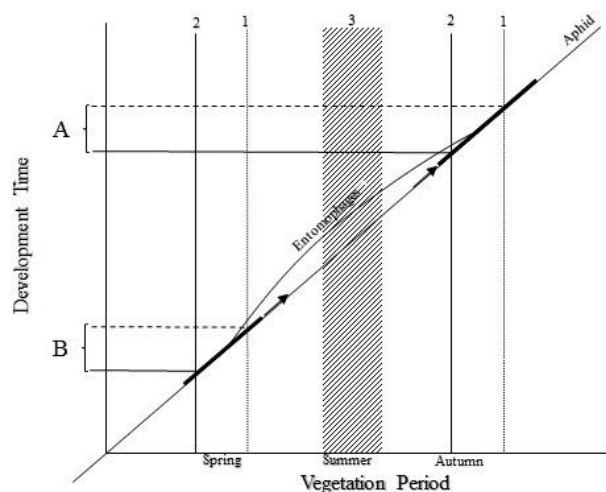
It has been proved that large leaves, prone buds and a later falling of leaves which are characteristic of bird cherry (1) are more attractive for the remigrants and favourable for eggs laying and overwintering. However, the weather conditions of each year influenced the amount of aphid eggs ($F = 87.9^{***}$) more than the angle of bud attachment to the branch ($F = 39.6^{***}$).

The higher temperatures in March and the absence of subzero temperatures in April have caused an earlier buds bursting in bird cherry (2) in spring of 2014. It was very suitable for the 1-st stage larvae survival on bird cherry (2). At the same time, buds burst in bird cherry (1) almost two weeks later than the aphid larvae appeared from winter eggs. As a result, their number decreased even more in 2014 (by 49.2%) than in 2012 (24.5%) or in 2013 (36.3%). As a rule, entomophags cause a reduction in the number of the older and overwintered fundatrices larvae until the completion of their migration onto grasses. The importance of synchronized interaction between the *Rh. padi* development and its winter host (1) and (2) phenology in spring and in autumn is demonstrated (Fig. 1).

The remigrants and aphid eggs abundance is dependent on the parameters of sexual and virginoparae morphs development during the entire life cycle. Their mean values may have variations defined by weather conditions, host-plant suitability, individual and clonal peculiarities.

The obtained results may be used for predicting pest numbers under conditions of the changing climate.

Figure 1



- A-the difference in terms of the beginning of buds burst
 B-the difference in terms of the bird-cherry (1) & (2) leaf fall
 — (spring) the time of eggs hatch
 — (autumn) the time of oviposition
 (1) -bird cherry with buds prone on the branch
 (2) -bird cherry with buds erect on the branch
 3 -grasses (summer host-plant)

Fig. 1 A model showing how the development rate of the winter host-plant – bird cherry (1) and (2) – can influence the numbers of *Rh. padi* at different stages of the vegetative period. The aphid development and host-plant phenology are strongly synchronized. The later bursting of buds and early fall cause a reduction in aphids numbers.

P NEPD 5

Western Corn Rootworm *Diabrotica virgifera virgifera* LeConte - Examinations of control under small scale farming conditions in Austria

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Since the Western Corn Rootworm *Diabrotica virgifera virgifera* LeConte has invaded most areas of Austria only some few incidences of plant lodging occurred. In 2014 however the lodging in monomaize areas reached desasterous dimensions. Moreover concerns grew on yield losses by silk clipping paired with eating damages in milky- to dough ripeness of maize grains late July.

Reduced efficacy by crop rotation in small scaled field structures: small field sizes and alternating crop rotation of maize and oil pumpkin is very common. Carry over of egg deposits by femails beetles into oil pumpkin plots which are neighbouring maize have been observed frequently. This phenomena has been examined 3 years by use of hatching cages:

- when maize is planted after maize hatching numbers are taken as basement 100%
- about 35% of *Diabrotica* hatch in maize after pumpkin

A suppression of the population 90 to 99% was achieved in maize after winter wheat.

Flowering pumkins pollen is a strong attractant to beetles..

Furthermore any fresh green leaves from all kind of plant species are more attractive to beetles after maize flowering and its hard leaf surface from August on.

Similar result like in pumpkins could be obtained in experiments when maize alternates with soybeans.

Calciumcyanamid: hatching rates could not be reduced by even high rates of CaCN₂ in hatching cages.

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Soil preparation: investigations showed that shallow soil preparation methods can lead into higher frequency of goose neck symptoms comparing to plough or deeper (> 20 cm) working cultivators.

Genetic of maize hybrids: the objective was to develop stable a robust root system. Recreation abilities of roots after loss due to larval damages have been tested on several maize varieties. Particular the disastrous maize season 2014 showed lodging symptoms depending on maize varieties. It concludes that breeding have probably a significant influence to achieve consistent yields in heavy *Diabrotica* infested maize growing areas.

P NEPD 6

Bionomics of two lepidopterous pests on *Caragana korshinskii* in arid and semi-arid regions of China

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Question: According to the references, several pests have been found on *Caragana korshinskii* Kom, such as, *Apocheima cinerarius*, *Orgyia antiqua* and so on. Are there any newly pests on the host in recent years?

Methods: To explore the newly pests and make clear their biology, the study was used by field investigation and under laboratory conditions from March, 2013 to September, 2014.

Results: *Neolycaena tengstroemi* (Erschoff) and *Chiasmia saburraria richardsi* (Prout) were newly found in northwestern China. *N. tengstroemi*, an anthophagy pest, belongs to Lycaenidae. The target pest has only one generation per year in Ningxia. These butterflies overwinter as eggs which are dispersed on the foliar buds of the newly sprouted branches of the host plant. The duration of larval stage development begins from late March to mid-May. In early May, the last instar larvae begin to crawl down to litter layer or the surface soil around the host plant root to pupae. The prepupal period is four days, and the pupal stage is about 15 days. The adults emerge in late May with the peak of emergence at the beginning of June. The adults lay eggs in early June, and the eggs stage is about 295 days.

C. saburraria richardsi, a defoliator pest, belongs to Geometridae. The pest has two generations per year in Ningxia and the generation overlapping is very severe. It overwinters as a pupa which can be found at the depth of 10 cm and 30 cm down to surface of the soil from late August. Overwintering adults emerge in mid-May with the peak of emergence in mid-June. The eggs and larvae of the first generation emerge in late May, the last instar larvae of the first generation begin to pupate in late July, the pupal stage is about 10 days. Likewise, the eggs and larvae of the second generation emerge in late July, and the last instar larvae of second generation begin to pupate in late August.

Conclusion: *Neolycaena tengstroemi* and *Chiasmia saburraria richardsi* are newly found on *Caragana korshinskii* Kom.

Figure 1-3: *Chiasmia saburraria richardsi* (Prout)

Fig. 1 Habitue, dorsal view of male; Fig. 2 Habitue, ventral view of male; Fig. 3 The last instar larva

Figure 4-6: *Neolycaena tengstroemi* (Erschoff)

Fig. 4. Habitue, dorsal view of female; Fig. 5 Habitue, dorsal view of female; Fig. 6 The 3rd instar larva



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P NEPD 7

Impact of birds, especially tits as predators of the horse chestnut leaf miner (*Cameraria ohridella*)

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The horse chestnut leaf miner (*Cameraria ohridella*) is an issue in urban green since many years. The larval feeding leads to browning and premature fall of leaves. It is known that birds, in particular blue tits but also great tits, have discovered the chestnut leaf miner as an additional food source and developed the ability to open the mines to pick up the larvae and pupae. How big this influence is, has not yet been quantified.

The objective of this experiment is to determine quantitatively the influence of birds on the population density of the horse chestnut leaf miner.

A total of 34 tree groups à 4 chestnuts on 4 different sites were included in this attempt. Nest boxes were installed at those sites. 17 groups of trees were covered with bird nets in 2014. In mid-September 12 leaves per tree group were collected. These leaves were inspected visually for large / small, opened / closed mines, as well as larvae and pupae each parasitized / not parasitized.

At locations where tits occurred frequently, the number of stages (larvae and pupae) found at groups covered with net was higher than without net. At a part of those sites about 50% less larvae and pupae were found in groups without nets. The proportion of opened mines was higher at groups without net than with net.

The experiment shows initial dimensions of the influence of tits as predators on the population density of the horse chestnut leaf miner. Thus does the promotion of tits, eg. by hanging nest boxes, an important contribution to pest management. The experiment will be repeated in 2015 in order to check the reproducibility of the results.

P NEPD 8

The big challenge in agriculture: Avoidance of bird feeding by plant extractions

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Birds consume seeds and seedlings and cause substantial harvest and income losses in agriculture. And, they consume toxic baits, which are applied for managing other species, leading to unintentional intoxications of birds. A repellent consisting of plant extractions could be a sustainable method to prevent undesirable bird feeding. Our projects' aim is to develop such a bird repellent with focus on the use of this product as a seed treatment. A systematical screening of various plant extractions was the first step. In food- and seedlings-choice tests with pigeons and pheasants in aviaries clearly repellent plant extracts were identified. The same applies for a field test with crows. The experimental results will be presented and consequences will be discussed.

The project is funded by the Federal Ministry of food, agriculture and consumer protection decided by the German Bundestag.

P NEPD 9

The Aphid (Homoptera:Aphidoidea) Species on Vegetable Fields in Tokat Province, Turkey

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In this study, the superfamily Aphidoidea (Homoptera) on vegetables tomato (*Solanum lycopersicum* (L.)), eggplant (*Solanum melongena* , (L.)) pepper (*Capsicum annum* (L.)), okra (*Abelmoschus esculentus* L.), bean (*Phaseolus vulgaris* L.) and lettuce *Lactuca sativa* (L.) was investigated in Tokat province during 2007-2010. The aphid specimens were collected from green parts of the plants and water traps which were placed in vegetable fields. Total of 8 species were determined. These species are

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Macrosiphum euphorbiae (Thomas), *Myzus (Nectarosiphon) persicae* (Sulzer), *Aphis fabae* Scopoli, *Aphis gossypii* Glover, *Aphis craccivora* Koch, *Hyperomyzus lactucae* (L.), *Chromaphis juglanticola* (Kaltenbach) and *Aphis nasturtii* Kaltenbach. The most common species were found to be *M. euphorbiae*, *M. persicae*, *A. gossypii*, and *A. craccivora*.

P NEPD 10

Genome of the whitefly, Q *Bemisia tabaci*, a global invasive pest and vector of hundreds of plant diseases

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The whitefly, *Bemisia tabaci* (Gennadius) (Hemiptera: Aleyrodidae), is a global widespread pest that causes serious damage in protected crops and field crops, through direct feeding and indirect vectoring several hundreds of plant viruses. More than 31 morphologically indistinguishable cryptic species have been identified, among which two members, referred to as B (Middle East - Asia Minor 1) and Q (Mediterranean), are the most invasive and destructive in many parts of the world. To better understand how an invasive and polyphagous sap-sucking arthropod herbivore has adapted to its complex and volatile environment and to provide insights to improve pest control, we analyzed the genome of Q *B. tabaci* as its representative in whitefly.

We have finished Q *B. tabaci* de novo assembly and annotation through BAC-to-BAC pooling combined with Illumina sequencing despite the high levels of polymorphism present in whitefly. The sequenced genome is 658 Mb and identified 20,786 protein coding genes have been obtained. In this paper, we introduce this project status so far, based on which guide for several interested whitefly biology and community questions including virus transmission, resistance, symbiotic bacteria, feeding range and so on.

Our study reveals a series of complex adaptations of the whitefly involving a variety of biological processes, and all these findings highlight potential directions for effective pest control of the whitefly.

P NEPD 11

The home garden: adaptation in response to new and emerging plant diseases

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Introduction: The threat to garden plants from diseases has never been greater due a surge of new and unrecognised diseases emerging as significant risks to cultivated plants and the wider environment. The extreme weather events associated with climate change, along with the reduction in availability of chemicals, amplify the impacts of some diseases already established here. Gardens and garden management need to adapt to these changes.

Objectives: This paper discusses Royal Horticultural Society (RHS) research into new and emerging diseases of UK gardens and the ways in which gardeners can adapt.

Materials and methods: Over 60,000 samples and queries are received by the RHS Advisory Service each year. Potential disease samples that require identification are inspected by the RHS plant pathologists. New records are confirmed using standard techniques and published in peer-reviewed journals. Where appropriate, research projects are instigated to develop strategies to manage these diseases in gardens.

Results: Several emerging diseases have required adaptations in garden design, planting and disease management strategies in the gardens of the UK. Box blight, caused by *Cylindrocladium buxicola*, and to a lesser extent *Pseudonectria buxi*, has proved difficult to manage in gardens and strategies involve avoidance where possible and garden design and cultural methods once present. Box is traditionally pruned to a dense canopy, which encourages disease development and so the position and use of box in gardens may need to change. Methods being developed for the control of heuchera rust, caused by *Puccinia heucherae* involve detection during the lengthy latent period, with a focus on clean planting material. Research on the management of *Armillaria* spp. on ornamental and fruit trees involves choice of replacement planting, cultural and biological measures to enhance the rapid breakdown of fungal rhizomorphs once infected trees are removed, and a focus on good overall plant health.

Conclusion: Gardens must adapt to new and emerging diseases. Management strategies increasingly encompass garden design, planting choice and cultural management.

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P NEPD 12

Soybean rust (*Phakopsora pachyrhizi*) and Witches broom (16SrII *Candidatus* phytoplasma); the two emerging and devastating diseases of soybean in Tanzania

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Soybean production is steadily increasing in Tanzania driven by the growing demand for livestock feed and human consumption. Coupled with this production trend is the emergence of soybean rust (*Phakopsora pachyrhizi*) and soybean witches broom (SWB) caused by 16SrII *Candidatus* phytoplasma that are causing massive yield losses. From fields surveyed in 2012, 2013, and 2014, soybean rust was observed in 5 of 14, 7 of 11, and 14 of 31 fields, respectively. Disease severity ranged from 10-80% on all varieties grown. About 50% of the 20 plants assessed for SWB from one plot in a farmer field, were affected. Accurate diagnosis is a prerequisite to understanding disease etiology and formulating effective control strategies. We used conventional and quantitative Polymerase Chain Reaction (PCR) methods to identify and confirm the presence of soybean rust and SWB in soybean production fields in Tanzania. *P. pachyrhizi* was detected using qPCR with Taqman assays specific for *P. pachyrhizi* (Ppm1 & Ppa2) and a multiplexed exogenous internal control reaction to validate negative results (1). To detect SWB, genomic DNA from SWB suspect samples was PCR amplified using *C. phytoplasma* universal primer pair P1 and P7 targeting the 16S-23S ribosomal RNA encoding region (3). The resulting fragments were directly sequenced for specific identification of phytoplasma agent associated with the disease. *P. pachyrhizi* DNA was detected in excess of 66,000 genome equivalents/cm² in all symptomatic samples. PCR amplicons of expected size (~1700 bp) resulted from the templates of SWB samples. A BLASTn search revealed that the phytoplasma sequences had a nucleotide sequence identity of 99% with those of 16SrII group phytoplasma associated with phyllody and witches'-broom disease of soybean in Malawi and Mozambique (2). These findings reveal the presence of the two diseases in Tanzania and underscore the need for studies to better understand their epidemiology and etiology, especially the presence of natural hosts and vectors associated with the diseases, and formulate management strategies to prevent adverse impacts on soybean production in eastern and southern Africa .

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P NEPD 13

Susceptibility of invasive populations of red palm weevils against exotic isolates of entomopathogenic fungi *Metarhizium anisopliae*

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The red palm weevil, *Rhynchophorus ferrugineus* (Olivier) (Coleoptera, Curculionidae), is the most destructive invasive pest of palms especially date palm in the Gulf regions. Different exotic isolates of *Metarhizium anisopliae* were procured from USDA-ARS to investigate their virulence against red palm weevil larvae. The impact of different isolates of *M. anisopliae* conidia on the growth, susceptibility and antioxidant defense was determined by immersing red palm weevil larvae into each isolate suspension at the concentration of 1×10^7 conidia/ml. Nutritional indices assays results revealed a significant reduction in the relative growth rate (RGR), efficiency of conversion of ingested food (ECI) and efficiency of conversion of digested food (ECD) after infection with different isolates of *M. anisopliae*. Conidial infection of M9374 caused 66.20 %, 36.45 % and 50.72 % reduction in RGR, ECI and ECD compared to uninfected control larvae. The least virulent isolate of *M. anisopliae* M7234 with highest LT₅₀ values could only cause 20.55 %, 9.92 % and 16.51 % reduction in RGR, ECI and ECD, respectively. On the other hand, infectivity of different isolates of *M. anisopliae* induced different levels of studied antioxidant defense-related genes, such as *catalase* and *peroxidase*. The infection of isolate M9374 greatly enhanced the expression of *catalase* and *peroxidase* in hemolymph, gut and fat body of red palm weevil larvae. Significant reduction in ECI and ECD index from infected larvae compared to uninfected larvae revealed that most of the ingested food is being used for energy to combat the invading pathogen, and less food is being utilized for larval growth as evident from the quantification of antioxidant genes by qRT-PCR. It can be concluded that *M. anisopliae* isolate M9374 is good candidate for the development of mycoinsecticides for invasive populations of red palm weevils.

P NEPD 14

Impact of refuge areas on common vole field infestations

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Population outbreaks of common voles can lead to population densities of >2,000 Ind./ha and to severe damage in agriculture. Usually, vole distribution is managed by the use of rodenticides. This often occurs after expansion has already created reasonable crop damage. Also, baiting large agricultural fields is time-consuming and may create empty habitats which can be re-colonized by voles easily and quickly. Refuges like set-aside-land are potential sources for common vole dispersal to adjacent fields. Appropriate population management should take into account the key environmental factors triggering dispersion pressure in these areas. To apply timely and spatially targeted management methods, sound knowledge about the distribution patterns of voles at field-refuge-boundaries is required.

In this study, vole population dynamics and dispersal patterns were investigated as a basis for the development of suitable and sustainable management-methods. The study site was located in Saxony-Anhalt, Germany. Grassland areas distributed in a matrix of arable fields were used as experimental refuges. To measure dispersal pressure, barrier fences that allowed immigration but prevented emigration were installed at 10 of 16 refuges. Capture-mark-release was applied in a two-year-study to compare population development in fenced and unfenced refuges. Although there was no indication from live trapping that voles moved frequently among refuge areas or from refuges to fields, fast re-colonisation repopulated refuges after local extinctions. Population size increased more in unfenced refuges than in fenced ones. Extrapolated densities in refuges reached up to 1,000 Ind./ha whereas densities in adjacent fields remained low. At higher population density and appropriate crop condition voles are expected to invade these fields.

Inhibited dispersal in fenced refuges may lead to lower maturation rates of young voles and to a higher proportion of agonistic behavior between males. Therefore, preventing dispersal from vole source populations may be an effective management method to reduce population numbers and to minimise large-scale rodenticide application across fields. Radio-telemetry, aerial pictures, and DNA analyses are applied to gain further insight in vole dispersal dynamics from refuge areas to fields.

P NEPD 15

Analysis of population genetic structure of *Puccinia striiformis* f. sp. *tritici* from central Gansu and its surrounding areas

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Wheat stripe rust is the most important devastating disease in China. The Longnan region, Gansu province, China, is the main area where the mutation of the pathogen of wheat stripe rust occurred. In order to reveal the population genetic structure and relationship of the *Puccinia. striiformis* f. sp. *tritici* (Pst) from central Gansu and its surrounding areas, 369 Pst isolates were collected from 7 areas including Gansu, South Shaanxi, Qinghai and Xinjiang, and their population genetic diversity was investigated by SSR technique. The statistical analysis showed that Nei's gene diversity (H) and Shannon's information index (I) of Pst population were 0.39 and 0.57, respectively, which suggested that their population genetic diversity was rich in 7 areas. Furthermore, the genetic diversity of Pst populations from different areas represented significant difference. The genetic diversity of Pst population from Tianshui of Gansu was much richer than the other Pst populations, with which its Nei's gene diversity (H) and Shannon's information index (I) were 0.42 and 0.61. In addition, analysis of AMOVA showed that 2.24% of the total genetic variation existed among the populations, while 97.76% of the total variation presented within the population. These results suggested that the populations of Pst possessed relatively high levels of genetic diversity but a lower genetic differentiation in central Gansu and its surrounding areas, and the main genetic variation presented within the population. The genetic similarity of Pst populations from the central area and Longnan of Gansu and Ningqiang of Shaanxi were much high, and the Pst strains intercommunicated closely in the 3 areas.

Figure 1

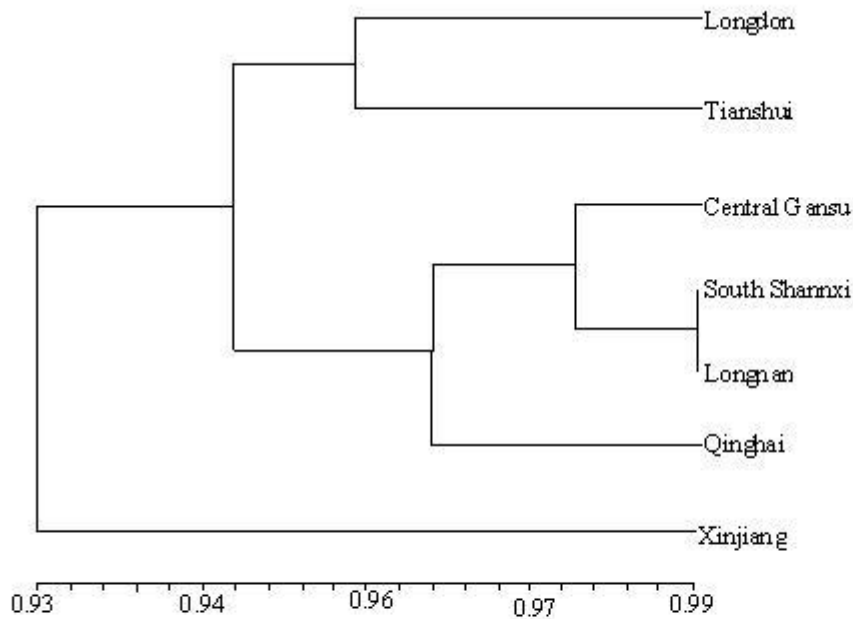


Fig 1 UPGMA dendrogram based on SSR data of *Puccinia striiformis* f. sp. *tritici* including 7 populations from central Gansu and its surrounding areas

P NEPD 16

Carrot psyllid (*Trioza apicalis*) feeding behavior on carrot and potato: an EPG study

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Question: The objectives of this study were to: 1. Determine whether carrot psyllids are capable of phloem feeding on potato. 2. Determine whether there are any differences in feeding behaviour between female and male *Trioza apicalis* and on carrot and potato plants.

Methods: EPG was used to measure the time spent by male and female *T. apicalis* probing each plant tissue type in potatoes and carrots. Ratios of feeding behaviour with time spent not probing the plant were compared.

Results: More time was spent by both males and females in the start of penetration, stylet tip in parenchyma, transition to phloem ingestion and phloem ingestion/salivation on carrots than on potatoes. Both plant and sex of the insect significantly affected the duration of the non-probing phase. Only one male psyllid was recorded phloem feeding from a potato plant.

Conclusions: It may be possible for *T. apicalis* to transmit CLso to potato plants. However, the time spent in phloem ingestion/salivation phase in potato is very short compared the time spent in this phase the on carrot, which suggests that the probability of CLso transmission on potato is low.

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P NEPD 17

Metagenomics sequencing identified for the first time citrus bark cracking viroid (CBCVd) as an aggressive and harmful pathogen of hop (*Humulus lupulus* L.)

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Recent technical advances in next generation sequencing (NGS) technologies and a steady reduction of the associated costs per base have enabled their use for prompt and accurate identification of disease agents. Several recent studies in human, animal or plant pathology have shown that classical identification methods of pathogen detection can be successfully supplemented or even replaced by NGS. In 2007, hop growers in Slovenia observed the appearance of severely stunted hop plants. The disease started to spread rapidly within hop gardens and among farms, with a suggested mechanical and rootstock method of transmission. Classical and selected molecular diagnostic methods were unable to reveal a new pathogen; an NGS approach of total RNA and small RNAs from symptomatic and non-symptomatic plants was therefore applied to find a possible novel pathogen. Non-symptomatic (NSYM) and symptomatic (SYM) hop plant tissues were sampled throughout the growing season. Total bulked RNA samples and bulked small RNA (sRNA) samples (NSYM and SYM) were sequenced-by-synthesis, using the Illumina HiSeq2000 sequencing system. A total of 21 M and 12M sRNA reads and 108 M and 102 M paired-end (PE) total RNA-seq reads were obtained for NSYM and SYM samples, respectively. *De-novo* assembly and reference mapping approaches were applied to discover pathogenic sequences. The results showed the presence of Hop latent viroid (HLVd) and Hop latent virus (HLV) sequences in NSYM and SYM samples, which are considered not-harmful or latent hop pathogens. However, both identification approaches confirmed the presence of Citrus bark cracking viroid species (CBCVd) in the SYM sample, which has never previously been reported in hops. The presence of this novel pathogen on hop was confirmed the following year by RT-PCR analysis and small RNA Illumina sequencing of plants with symptoms from infected hop gardens identified by systematic disease monitoring. The high infectivity of the newly identified CBCVd was also confirmed by biolistic experiment on two hop cultivars, which developed characteristic symptoms in a controlled environment. The presented work shows the feasibility of NGS for prompt and accurate identification of the causative agent of new diseases in hop.

P NEPD 18

Eradicating *Bemisia tabaci* Mediterranean species in the UK

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Introduction: The whitefly, *Bemisia tabaci*, continues to be a major pest of economically important crops worldwide. It is a vector of many plant viruses; several of which do not occur in the UK. In the UK *B. tabaci* remains a notifiable pest subject to a policy of eradication if found on propagators premises, plants moving in trade, and containment/eradication if outbreaks occur at nurseries.

Objective: To screen a range of both chemical products and entomopathogenic fungi for their efficacy against *B. tabaci* Mediterranean species. To determine the compatibility of the fungi with chemical products for the potential of direct tank-mixing.

Materials and methods: The chemical products and entomopathogenic fungi were screened for their efficacy using a standard leaf dip technique against three life stages of *B. tabaci* Mediterranean species; eggs, second instar larvae and adults. Direct compatibility of the fungus with chemicals was determined by suspending conidia in standard dose rates of individual products. Following 24h at 20°C the percentage viability of conidia (germinated spores) from a total of 200 randomly chosen conidia was assessed under the microscope.

Results: There was a significant difference in the mortality of eggs after leaf dipping with the different active ingredients. Exposure to TriTek, SB-Plant Invigorator, Gazelle, Dynamec and Certis Spraying Oil was followed by egg mortalities of 100, 96.6, 88.8, 84.1 and 67.8% respectively. Efficacy of the products against the second larval instar stage also produced promising results. The fungus *Beauveria bassiana* produced the highest mortality of all the products against *B. tabaci* instars (73%). The control given by Agri 50-E, TriTek and SB-Plant Invigorator (all physically acting products) was also over 70%. *Beauveria bassiana* and TriTek gave excellent control of adult *B. tabaci* with total mortality being obtained. Several of the products, including TriTek and Gazelle, offered excellent compatibility with *B. bassiana*, with 100 and 70% spore germination respectively.

Conclusion: Several of the products offer excellent control of *B. tabaci* Mediterranean species. Certain chemicals have been shown to offer better efficacy against individual whitefly life stages. *Beauveria bassiana* also offers great potential to be incorporated into eradication strategies; showing great efficacy when used individually and also offering excellent direct

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compatibility with various chemical products. The integration of the control products into existing eradication strategies for *B. tabaci* is discussed.

P NEPD 19

Insect pests and diseases associated with *Jatropha curcas* L. in Burkina Faso

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We conducted a survey in 2010 through 2014 in 223 randomly selected jatropha plantations in all 4 agro ecological zones of Burkina Faso. We assessed the presence and the incidence of insect pests and diseases associated with this biofuel tree. Insect pests were observed and photographed; some of them were captured and reared under natural conditions. Fungal diseases symptoms were sampled and investigated later in the laboratory. Twenty two insect pests species belonging to 5 orders have been found in the plantations. The most important families included Alticinae observed in 69.05% of the plantations, Scutellaridae, Pyralidae, Pentatomidae and Coreidae found respectively in 56.5%; 49.32%; 30.94% et 28.69% of the plantations. While the Alticinae was the most frequent family, it was not the most widespread. Eighty fungal diseases symptoms were observed in the jatropha plantations. Leaf diseases were the most frequent and were widespread in all agro climatic zones of Burkina Faso. Other symptoms included the colt rot, the withered plant, and the chancre. The analysis of the samples in the laboratory showed that *Pestalotia guepini* was present on the necrosis leaves and *Fusarium moniliforme* on the root colt. This study has revealed the existence of several insect pests and fungal diseases that can cause harm to jatropha plantations in Burkina Faso. Investigations should continue in order to improve the knowledge on these enemies and to develop control methods.

P NEPD 20

EMPHASIS, an European-funded project to provide Integrated Solutions for the Effective Management of Pests and Harmful Alien Species

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Considering the global importance of plant health for sustainable and competitive agriculture, horticulture and forestry sectors, the European Commission recently funded the EMPHASIS project, started on March 2015, addressing native and alien pests threats (insect pests, pathogens, weeds) for a range of both natural ecosystems and farming systems.

The international participatory project is taken over by 22 partners from 10 countries, bringing cross-sectorial and complementary expertise, and including research institutes, enterprises, SME's and international organizations.

The project has the aim to provide advanced practical solutions on the three internationally agreed key pillars to tackle outstanding plant pest challenges:

1. Predict, Prioritize and Planning: pest management challenges and opportunities will be evaluated according to stakeholder-focused criteria and through pathway analysis;
2. Prevent: practical solutions for surveillance in different pathways to enhance preparedness will be provided to end-users, and monitoring tools following outbreaks and eradication will be developed;
3. Protect: practical solutions for managing native and alien pests in agriculture, horticulture and forestry will be developed, their technical and economic feasibility will be demonstrated and their market uptake will be enhanced.

A cross-cutting approach to participatory research and technology transfer is adopted, in order to strengthen the connectivity between agricultural research and other system actors. Thus, on-farm testing and participatory learning activities are being developed since the beginning of the project, in order to facilitate co-design, co-development and co-implementation.

The project is not focused on a single management systems but the plant/pest ecosystems dealt with are treated with a multi-method approach to design true IPM methodology that will be developed for key systems with portability to other similar systems, thereby having a large impact.

Figure 1
EMPHASIS Consortium Partners

N°	Participant organisation name	Type	Country
1	Università degli Studi di Torino (UNITO)	Research	Italy
2	AgraCEAS Consulting (AgraCEAS)	Entreprise	Belgium
3	AgriNewTech (ANT)	SME	Italy
4	Agrobio S.L. (AGROBIO)	SME	Spain
5	Chatim BV (CHATIM)	SME	Netherlands
6	Confederazione Generale dell'Agricoltura Italiana (CONFAGRICOLTURA)	Other	Italy
7	European and Mediterranean Plant Protection Organisation (EPPO)	Intergovernmental Organisation	France
8	Food and Environment Research Agency (FERA)	Other	United Kingdom
9	Imperial College London (IMPERIAL)	Research	United Kingdom
10	Institut National de la Recherche Agronomique (INRA)	Research	France
11	Integrētās Audzēšanas Skola Ltd. (IAS)	SME	Latvia
12	Mendel University in Brno (MENDELU)	Research	Czech Republic
13	Metec Innovation Consulting Srl (METEC)	SME	Italy
14	Moverim Consulting (MOVERIM)	SME	Belgium
15	National Institute of Agricultural Botany (NIAB)	Research	United Kingdom
16	OPTISENSE Limited (OPTISENSE)	SME	United Kingdom
17	Plant Biocontrol International (PBI)	SME	Netherlands
18	The Regional Environmental Center for Central and Eastern Europe (REC)	International Organisation	Hungary
19	Semios BIO Technologies Inc (SEMIOS)	SME	Canada
20	SPIN-TO Srl (SPINTO)	SME	Italy
21	Stichting Dienst Landbouwkundig Onderzoek (DLO)	Research	Netherlands
22	Universidad de Lleida (UdL)	Research	Spain

P NEPD 21

The entomological problems encountering the sweet sorghum [*Sorghum bicolor* (L.) Moench] cultivation in Sanliurfa Province

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Sweet sorghum [*Sorghum bicolor* (L.) Moench] is mainly cultivated with a total acreage of 40 million hectares in Africa, Asia and the America, in approximately 105 countries as a major plant with C4 photosynthesis. About 242 varieties of sweet sorghum that have been determined, will be one of the few plants that can flexibly adapt better for the future climate change conditions especially for drought increasing, soil salinity and high temperature. Sweet sorghum can be used in many fields including food, animal feed and fuel as a multi-purpose promising product. Sweet sorghum is a second product in Günduş and Talat Demirören stations which belonging to the GAPTAEM project in the Sanliurfa province and in 2014 a preliminary study was conducted on sweet sorghum adaptation project. All the pests that create problems and need to be controlled during the sorghum production season were determined. These pests are: *Atherigona* spp (Diptera: Muscidae), *Empoasca decipiens* (Hemiptera: Cicadellidae), *Sesamia cretica* (Lepidoptera: Noctuidae) and *Melanaphis sacchari* (Hemiptera: Aphididae).

P NEPD 22

Lupine leaf weevils (*Sitona gressorius*) in Germany, Belarus, Poland and Switzerland and the potential impact on the European lupine production

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Introduction: The lupine leaf weevils, *Sitona gressorius* and *S. griseus* (Coleoptera: Curculionidae: Entiminae: Sitonini, *syn. Charagmus gressorius*, *C. griseus*) are two important pests affecting European lupine crops.

The weevils cause two different types of damage: the adult weevils feed on lupine leaves chewing notches in leaf margins, whereas the larvae cause severe damage by nibbling on the roots, especially the root nodules. Certainly the primary effects of nodule damage are the decrease of nitrogen fixation and water loss. But even worse, these lesions are entrances for soil born plant pathogens.

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Knowledge about the life cycles and behavior of these emerging pests are scarce.

Objectives: The first objective of this work is to understand the variability of *S. gressorius* populations in Central Europe using a molecular genetic approach.

The second objective is to understand the origin and the migration of these populations in this region.

Materials and methods: During the summer 2012 and 2013, lupine leaf weevils were collected from 60 different sites in Germany, Poland, Switzerland and Belarus. Genomic DNA isolation (innuPREP Forensic Kit, AnalytikJena, Germany) was performed on three legs of each individual weevil. We sequenced two regions: the mitochondrial cytochrome oxidase unit 1 (COX1) gene region and the internal transcribed spacer 2 ribosomal RNA (ITS2).

Different alignment algorithms (MUSCLE, MAFFT, CLUSTAL and TCOFFE) were applied to the sequence data using the Analyses of Phylogenetics and Evolution (APE) library (Paradis et al., Bioinformatics 20, 289-290, 2004) in R. To determine which molecular evolution model best fits our data we used the algorithm PhyML (Guindon & Gascuel, Systematic Biology 52, 696-704 2003).

Results: This study for the first time shows the genetic variability of *S. gressorius*, and furthermore illustrates the migration from south to north in Central Europe.

Conclusion: These results show us how this emerging pest *S. gressorius* adapts and migrates in Central Europe, furthermore this biological information we verified that the statistical methods available in R produce useful results.

P NEPD 23

Morphological, molecular and biological characterization of citrus-associated alternaria species

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Alternaria brown spot is one of the most important diseases of tangerines and their hybrids worldwide. Recently, a disease outbreak in Southern Italy refocused the attention on the disease. Twenty representative cultures of *Alternaria* were selected from a collection of more than 100 isolates from leaves and fruits of cvs Fortune, Nova, Valencia, and Tangerine. Then, they were characterized along with specimen strains of *A. tenuissima*, *A. alternata*, *A. arborescens*, *A. citri*, *A. toxicogenica*, and *A. limoniasperae* ('small-spored' *Alternaria* species) to determine the etiology of the disease and evaluate the virulence of different isolates/species. Morphological characteristics and sporulation patterns separated most *Alternaria* isolates into three main groups corresponding to *A. alternata*, *A. arborescens*, and *A. tenuissima*, of which the first was the most abundant one. Phylogenetic analyses based on endopolygalacturonase (endoPG) and beta-tubulin genes, two anonymous genomics regions (OPA 1-3 and OPA 2-1), and the internal transcribed spacer (ITS) region produced a clustering of isolates largely confirming morphological results. The OPA 1-3 region was more suitable than other tested regions for separating closely related 'small-spored' *Alternaria* species and revealed the existence of intra-species molecular variability. Investigated isolates showed different levels of virulence on leaves and fruits but it was not possible to identify a direct correlation between virulence and genetic/morphological groupings of isolates.

P NEPD 24

The most common species of aphids in different wheat varieties (Homoptera: Aphidoidea) on research

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Aim of this study is to determine the aphids in wheat varieties of Aldane, Selimiye, Saraybosna, Tekirdağ, Bereket and Saban. According to results, in all examined wheat species, *Sitobion avenae* was the most observed aphid species than other species. Aphid population were determined less in Tekirdağ and Saban varieties than others. According to presence ratios of aphids in wheat species, most aphid population was detected in Selimiye, Aldane, Saraybosna and Bereket varieties.

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Cultural control in Switzerland continues to be a sustainable strategy for *Diabrotica v. virgifera* containment

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Ever since 2000 Switzerland belongs to the 22 European countries where the quarantine pest *Diabrotica virgifera virgifera* LeConte, Western corn rootworm (WCR), has been detected. WCR is reported to be the most important maize pest worldwide with annual economic damages reaching 1.5 billion US\$. In Switzerland it is constantly present in the Canton Ticino, south of the Alps, while only few beetles are sporadically found in the north. Observations from 2000 up to 2014 support the hypothesis that populations in the southern part of the Alps are generated by yearly migrations from principal pest foci situated in neighbouring Italian areas of Lombardy. Neither the tight correlation between travel distance and time of first arrival at various points from South to North, nor the steady decline of population along the route can be explained otherwise.

Control measures enacted by Swiss authorities were principally based on a tightly enforced crop rotation scheme without chemical inputs which are usually applied in parts of the European Union.

The effectiveness of crop rotation has been tested in a 7 year field trial comparing a continuous maize cropping system with a crop rotation system and with a maximum of one year of maize within any two year period. Population density was measured using synthetic pheromone baited traps and observations of root damage. Results showed that no economically relevant population built up during this period in the *crop rotation* treatment, whereas in the statistical evaluation of *continuous maize cropping* root damages could be detected after 4 years already.

One to one (1:1) year crop rotations are a common practice, are mandatory since 2004 in Southern Switzerland, and are well accepted by farmers. Consequently, not a trace of pesticide has been employed against WCR in Switzerland up to now. The low level population density also helped to avoid the introduction of WCR populations into Swiss Cantons north of the Alps and thus prevented further spreading towards the state territories of northern neighbour states.

P NEPD 26

Species of the Superfamily Coccoidea (Hemiptera) on Citrus Trees in Eastern Mediterranean Region of Turkey

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This study was carried out to determine the Coccoidea species in citrus groving areas of Adana, Osmaniye, Hatay and Mersin provinces of Turkey. The survey was conducted according to 0.01% of tree number rules at unsteady during April-December in the years 2012-2013. Sixteen coccoid species belonging to four families; Diaspididae: *Aonidiella aurantii* (Maskell), *A. citrina* (Coquillett), *Chrysomphalus aonidum* (Linnaeus), *C. dictyospermi* (Morgan), *Lepidosaphes beckii* (Newman), *L. gloverii* (Packard), *Parlatoria pergandii* (Comstock), *Aspidiotus nerii* Bouché; Coccidae: *Ceroplastes floridensis* Comst., *Ceroplastes rusci* (Linnaeus), *Coccus hesperidum* Linnaeus, *C. pseudomagnoliarum* (Kuwana), *Saissetia oleae* (Olivier); Pseudococcidae: *Planococcus citri* (Risso), *Pseudococcus cryptus* (Hempel); Margarodidae: *Icerya purchasi* Maskell. It was determined that *A. aurantii* and *P. citri* were determined as the widespread species in the citrus orchards at all four provinces.

P NEPD 27

Occurrence of Mediterranean Fruit Fly, *Ceratitis capitata* (Wiedemann) (Diptera: Tephritidae) in Fruit Orchards in Southern Turkey

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The population dynamic of the Mediterranean fruit fly, *Ceratitis capitata* (Wiedemann), (Diptera: Tephritidae) was studied during 2012-2014 in Tarsus/Mersin/Turkey. Jackson traps baited with male-targeted attractant Trimedlure and periodic fruit sampling were used to monitor the population dynamics in peach, pomegranate, apricot, nectarin and plum orchards. The first captures were recorded from the mid of April to July, depending on the year and orchard, and capture rates peaked in June each year. Significant differences were observed in adult population density and in the initiation of fly activity between the orchards that differed in host fruit abundance and availability. The results of fruit sampling showed that apricots were the first fruits infested every year among the other fruit species, in the study site. Though infested at low rates, apricots were very important for breeding the first summer generation. Peaches and nectarins were more infested by the pest and they were important hosts

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for the increase in *C. capitata* population later in the summer. Pomegranates were important for breeding the late summer and early autumn generations and acted as refuges for the transfer of the pest to other hosts. Theoretical and practical implications concerning the population dynamics of this fly are discussed.

P NEPD 28

Hosts of *Parlatoria pergandii* (Comstock) (Hemiptera: Diaspididae) in Eastern Mediterranean Region of Turkey

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The chaff scale, *Parlatoria pergandii* (Comstock) (Hemiptera: Diaspididae) is a cosmopolitan and polyphagous pest. It is a pest of mature citrus groves in Turkey. This study was carried out to determine the hosts of *P. pergandii* in Adana, Mersin, Hatay and Osmaniye provinces of the eastern Mediterranean region of Turkey in 2012-2013. Non-periodic random samplings in the agricultural areas were applied. Fifteen hosts of *P. pergandii* were determined: *Citrus sinensis* (L.), *Citrus reticulata* L. Blanco, *Citrus paradisi* Macfad, *Citrus limon* (L. Burm. f.), *Citrus aurantium* L., *Malus communis* L., *Prunus domestica* L., *Pyrus communis* L., *Prunus persica* L., *Diospyros kaki* L., *Morus alba* L., *Nerium oleander* L., *Ficus carica* L., *Melia azedarach* L. and *Prunus avium* L..

P NEPD 30

New emerging pests within plant-feeding eriophyid mites

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The wheat curl mite (WCM), *Aceria tosichella*, and the cereal rust mite (CRM), *Abacarus hystrix*, are grass-associated eriophyid mites distributed worldwide. The WCM is one of the most important pests known to infest cereals and is able to transmit plant viruses, e.g. Wheat Streak Mosaic Virus (WSMV). Recently DNA barcoding has revealed that WCM and CRM consist of several genetically distinct lineages, possibly cryptic species, with divergent invasiveness and potential to infest cereals [1,2]. It has been shown that some genotypes can colonize many grass species including cereals and have high dispersal potential spreading to North and South America and Australia from its presumed origins in Eurasia [3]. In this project we attempt to resolve core ecological questions concerning distribution of CRM and WCM *sensu lato* as well as its particular genotypes. The general concept of the proposed study was to provide information on habitat use and spatial distribution of these mite pests by using a combined DNA barcoding and species distribution modelling approach. The study was conducted in agricultural landscape in Poland (>300,000 km²) and included: random sampling, quantitative data collecting, DNA barcoding, environmental data analysis and ecological modelling. The results allow to ascertain realized niches and understand factors determining the spatial and host-related distribution of mite pests. Intensive sampling and DNA barcoding enabled to discover twice as many WCM genotypes than currently known and to recognize new relationships between mite pests and their cereal hosts. In general, the project contributed to understanding the role of CRM and WCM as plant pests and should help in designing applied research and management strategies.

[1] Skoracka A, Dabert M. 2010. The cereal rust mite *Abacarus hystrix* (Acari: Eriophyoidea) is a complex of species: evidence from mitochondrial and nuclear DNA sequences. Bull Entomol Res 100: 263-272.

[2] Skoracka A, Kuczyński L, Szydło W, Rector B. 2013. The wheat curl mite *Aceria tosichella* (Acari: Eriophyoidea) is a complex of cryptic lineages with divergent host ranges: evidence from molecular and plant bioassay data. Biol J Linn Soc 109: 165-180.

[3] Skoracka A, Rector B, Kuczyński L, Szydło W, Hein G, French R. 2014. Global spread of wheat curl mite by its most polyphagous and pestiferous lineages. Ann Appl Biol. 165 (2): 222-235.

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The effect of herbal repellents in five rodent pest species

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The extensive damage in agricultural systems resulting in considerable harvest loss and damage to agricultural infrastructure through rodents leads to an enormous financial loss worldwide. Therefore, the request for antifeedants or repellents boosts in order to avoid rodent damage. The usually applied commercial chemical rodenticides, e.g. anticoagulants, might cause environmental knock-on problems. Plant compounds with a repellent odor could reduce environmental risk associated with the application of common rodenticides e.g. through minimizing unwanted effects on non-target species.

Our aim is to find plant secondary metabolites (PSM) or their derivatives that affect the feeding behavior of different rodent species by their repellent smell. We conducted laboratory feeding trials with common voles (*Microtus arvalis*), house mice (*Mus musculus*), Günther's voles (*Microtus guentheri*), Tristram's jird (*Meriones tristrami*) and pocket gophers (*Thomomys bottae*); all major vertebrate pest species in agriculture.

Our study demonstrates deterrent effects of some herbal substances/combinations causing reduced food intake in five rodent pest species. Interestingly, we found also substances with an attractive effect increasing food intake in some rodent species. However, there seems to be a different response to different PSM between the five rodent families. We conclude that the odor of PSMs as repellents might be helpful to minimize agricultural damage without negative impacts on the surrounding environment, while attractive substances could help to lure pest species away from their feed.

P NEPD 32

Chemotyping of the FHB pathogens in Lithuanian spring wheat grain

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Fusarium graminearum, main causal agent of *Fusarium* head blight (FHB) of wheat, was practically absent in Lithuanian spring crops until year 2012. Emergence of the new pathogen was accompanied by drastic increase in grain contamination with B trichothecene mycotoxins - nivalenol (NIV), deoxynivalenol (DON) and its acetylated derivatives 3-acetyl-DON (3ADON) and 15-acetyl-DON (15ADON). Usually, a particular *Fusarium* strain is able to produce only one type B trichothecene -NIV, 3ADON or 15ADON. The detection of gene for NIV, 3ADON or 15ADON production is used for detection of FHB pathogens in plant material and their chemotyping. The dominant chemotype is a subject of variation in different regions and chemotyping allows to track the changes in population of FHB pathogens. Also, since the disease is new to Lithuania, chemotyping could indicate the possible routes of its spread.

Objective was to quantify the *tri* genes in spring wheat samples from years 2013-2014 by quantitative real-time PCR (qRT-PCR) using specific primer sets for detection of *F. graminearum* and its NIV, 3ADON and 15ADON chemotypes.

The incidence of *F. graminearum* infection was higher in 2013 year grain samples. Both DON chemotypes were clearly dominant in grain on both 2013 and 2014. The 15ADON chemotype was detected at lower quantities than 3ADON. The NIV chemotype was detected in only few samples on both years.

Lower levels of qRT-PCR product could indicate lower aggressiveness of 15ADON strains in comparison to 3ADON strains. The 3ADON chemotype was somewhat more common than in other similar European studies.

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Local parameters driving anticoagulant rodenticide exposure in red foxes in Germany

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Anticoagulant rodenticides are regularly used to control rodent pests. The use of anticoagulant rodenticides can lead to exposure of non-target animals like small mammals that consume bait and mammalian and avian predators that prey on exposed rodents. Although there are several studies documenting secondary exposure to anticoagulant rodenticides in predators, there is only little systematic empirical knowledge about the influence of local factors and land use. The aim of our study was to determine local parameters that drive anticoagulant rodenticide exposure in red foxes (*Vulpes vulpes*) as a basis for the development of risk mitigation strategies.

Liver samples of red foxes were analyzed for residues of eight anticoagulant rodenticides using LC-MS/MS. Samples were provided by veterinary institutes and were originally sampled for rabies monitoring. Local parameters such as livestock density and the percentage of urban area were used to search for correlations to anticoagulant rodenticide exposure in liver samples of red foxes.

Residues of at least one rodenticide occurred in more than 50% of fox samples. Second generation anticoagulant rodenticides occurred more often and in higher concentrations than first generation anticoagulant rodenticides. We found good indicators for exposure to anticoagulant rodenticides in red foxes. Brodifacoum and bromadiolone occurrence was positively correlated to livestock density. Furthermore, the occurrence of brodifacoum and difenacoum in liver of red foxes was positively correlated to the percentage of urban area.

A high percentage of foxes carry residues of anticoagulant rodenticides. The relation to land use indicates that risk mitigation strategies for both farmland as well as urban areas are important when anticoagulant rodenticides are applied.

P NEPD 34

Solatenol™, the new tool to combat *Phakopsora pachyrhizi*

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Solatenol™ is the new Succinate DeHydrogenase Inhibitor (SDHI) fungicide Benzovindiflupyr, belonging to the chemical class of Benzonorbornenes. It derived from a focused research project and was optimized for outstanding soybean rust (*Phakopsora pachyrhizi* Syd.&P.Syd.) control. Due to the outstanding activity of Solatenol™ and beneficial behavior of the compound to protect important crops from this devastating disease, it took only 6 years until first registrations. In combination with azoxystrobin it was broadly introduced to one of the most important soybean markets, Brazil, in 2014 as Elatus™.

After application, the compound moves not only into the leaves but in addition builds a depot in the leaf wax layer, protecting the plant from *Phakopsora pachyrhizi* penetration into the leaf surface; Due to its intrinsically high activity the quantity moving into the leaf protects from mycelium growth even at very low rates. In detailed studies Solatenol™ showed a 5-fold increased activity against soybean rust compared to currently known best compounds of same mode of action.

Especially under severe soybean rust epidemics, Elatus™ has repeatedly shown outstanding activity, resulting in leaves remain green longer and allowing for best and longest soybean pod filling. This consistently results in higher and better quality yields.

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Occurrence of knot disease caused by *Pseudomonas savastanoi* pv. *savastanoi* on oleander in the eastern Mediterranean Region of Turkey

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Oleander (*Nerium oleander*) is a significant flowering outdoor ornamental plant grown in the Mediterranean Region in summer. Knot disease caused by *Pseudomonas savastanoi* pv. *savastanoi* is one of the most important bacterial diseases of oleander. The characteristic symptoms of the disease observe on oleander blossoms, trunks and branches. Initially, hyperplastic outgrowths become green resulted in grey color. Knots are mainly smooth, spongy, in time outgrowths lignify and harden. In this study, blossoms and branches of oleander with hyperplastic outgrowths were gathered from Adana, Hatay and Mersin in the eastern Mediterranean Region of Turkey. Fifty-three fluorescent, small, circular and smooth bacterial colonies were purified from the King's B medium. The identification of the strains were carried out using one-year-old oleander plants for pathogenicity, LOPAT and PsvF/PsvR, PsnF/PsnR and PsfF/PsfR primer pairs for molecular tests. After two months of inoculation, strains caused hyperplastic outgrowths on oleander branches typical to the first symptoms. All strains characterized in LOPAT Group 1b (----+). In PCR screening, all tested strains reacted with PsvF/PsvR primers and generated 388 bp bands identical to *Pseudomonas savastanoi* pv. *savastanoi*, but, do not react with PsnF/PsnR nor PsfF/PsfR primer pairs. According to classical and molecular tests, all strains were identified as *Pseudomonas savastanoi* pv. *savastanoi*. In this study, spreading of the oleander knot disease to several cities in the region was recorded. The studies on the emergence of the pathogen in other hosts, dispersal and the relationship of the strains are running.

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Novel management of non-native ambrosia beetles (Coleoptera: Curculionidae, Scolytinae) in North America

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Non-native ambrosia beetles (Coleoptera: Curculionidae, Scolytinae) are increasingly being recognized as challenging pests of ornamental, horticultural, and forestry products. Two species *Xylosandrus germanus*, and *Xylosandrus crassiusculus* are especially problematic in the United States. Adult females bore into the stems or trunks of trees and tunnels are then created within the sapwood and heartwood leading to a series of chambers for rearing the brood. Adult females leave their overwintering sites within trees in wooded areas from March to May depending on the latitude, and search for new hosts to colonize. Both *X. germanus* and *X. crassiusculus* have broad host ranges. Symptoms of ambrosia beetle attacks include sawdust "toothpicks" sticking out from the tunnel entrances. Identification of novel strategies to minimize losses to these pests is of critical interest to growers and producers.

Materials and methods: A series of laboratory and field studies were conducted to identify the factors in host tree selection by *X. germanus* and *X. crassiusculus*, and whether plant stressors affected the selection process of the insect. Laboratory studies compared antennal responses to volatile organic compounds, and compared results to the presence of compounds in trees. Field studies evaluated trapping techniques for monitoring insect emergence and if such could result in management strategies.

Results: Ethanol was identified as the compound eliciting the strongest response in EAG experiments. Volatile collections from living trees also identified ethanol as being emitted by trees subject to physiological stress. Such trees were preferentially attacked by *X. germanus* and *X. crassiusculus*.

Discussion: A variety of trapping methods successfully utilized ethanol release, and resulted in improved timing of pest management practices.

Conclusions: An attract and kill pest management strategy utilizing ethanol releasing baits, ethanol emitting bolts, or ethanol emitting artificially stressed trees could be implemented to eliminate the need for pesticide applications for these pests.

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Functional analysis of key genes in *Bactrocera dorsalis* (Hendel) wing development to achieve genetic control

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Oriental fruit fly (*Bactrocera dorsalis* (Hendel)), an important economic insect pest, threatens to a widely range of hosts including tropical and subtropical fruits and vegetables. It has been listed as one of the fifty-two key invasive alien species by Ministry of Agriculture in China and much attention has been paid to its control technology. Wing formation is conducive to the migration of agricultural pests and expansion of their survival scope, which highlights the spread risk of the oriental fruit fly. High reproduction and migration ability resulted in the limited control methods such as physical or chemical control. The study of oriental fruit fly wing development and differentiation can help us propose new ideas and methods to control this pest from the biological perspective. Previous research showed larval stage was the critical period of insect wing development, the wing in this stage called wing disc. Wing disc development depends on a variety of gene regulation. In the model insect *Drosophila melanogaster* wing disc, selector genes divide the wing disc into anterior/posterior (A/P) compartment and dorsal/ventral (D/V) compartment by giving cells different affinity. Cells in compartment boundary act as organizers through secreting signal molecules (organ morphogens) to promote cell survival and proliferation, control cell fate and differentiation. *Decapentaplegic* (*dpp*) and *apterous* (*ap*) have been known as two key transcription factors in *D. melanogaster* wing formation: *dpp* belongs to the organ morphogens, combined with other signaling pathways integrated regulation to ensure the correct cell morphology; *ap*, a selectors gene, decides the fate of the dorsal compartment cells. In this study, we analyzed expression features of the two genes from *B. dorsalis*, clarified their function in the wing development. From the transcriptome sequencing we got some gene segments, according to the known segments we used the rapid amplification of cDNA ends (RACE) to obtain genes' full-length. Gene interference technology (mainly RNA interference) was applied to cause gene silence by synthesizing double-stranded RNA (dsRNA) respectively, then feeding and injecting the oriental fruit fly. As a result, wing deficiency was observed in some individual adults after eclosion. In summary, *dpp* and *ap* act as the key genes in wing development, their normal expression play an important role in the formation of *B. dorsalis* wings.

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Pest interceptions in India on introduced cereals and millets germplasm

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Germplasm exchange for crop improvement programmes is essential though associated with the risk of introduction of exotic pests, their races or biotypes. Indian experience and the present study during 2010-13, proved that Quarantine examination using standard detection procedures is crucial for minimizing such risks. Crop germplasm (60133 samples of 450 consignments) including paddy-40907, wheat- 552, barley-10, maize-13197, sorghum-2644, pearl millet-1579 and small millets-1244 was received for quarantine processing at this station. The imported material included germplasm lines, elite breeding lines, wild accessions etc.

Seed samples were subjected to visual examination, standard blotter, washing, agar plate, growout tests etc. Several pathogens of quarantine importance, viz., *Alternaria padwickii*, *Drechslera oryzae*, *Tilletia barclayana* and *Aphelenchoides besseyi* on paddy; *Alternaria raphani* on wheat; *D. carbonum*, *D. maydis* and *Stenocarpella maydis* on maize; *Sporisorium cruentum* and *Colletotrichum graminicola* on sorghum, *A. solani* on sorghum and pearl millet; *D. nodulosa* on finger millet were intercepted (Table 1). In addition, *A. porri*, *Fusarium oxysporum*, *Rhizoctonia solani* and *R. bataticola* etc., were recorded on multiple hosts, including wild species. Literature search indicated that pearl millet and finger millet are the new host records for *A. porri* and finger millet for *F. oxysporum*. Most of the wild species turned out to be the new host records for *D. setariae* and *D. sorghicola* (Table 2).

The insect pest interceptions, viz., the lesser grain borer, *Rhyzopertha dominica* on paddy, sorghum and pearl millet; *Sitophilus granarius* on sorghum; rice weevil, *S. oryzae* on paddy; *Trogoderma granarium*, on maize; *Lasioderma serricorne* on maize and *Tribolium castaneum* on sorghum and maize were significant (Table 1). Infected samples were salvaged and healthy germplasm was released. These new host records, first time occurrence in a country, reveal the significance of quarantine as preventing mechanism to the entry of exotic pests and their races or biotypes.

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Table 1: Seed borne pest interceptions on cereals and millets germplasm during 2010-2013.

Table 1. Seed borne pest interceptions on cereals and millets germplasm during 2010-2013.

Pest interceptions	Samples infected	Exporting countries
Paddy		
<i>Alternaria padwickii</i>	15	China, Indonesia, Kenya
<i>Aphelenchoides besseyi</i>	3	Indonesia
<i>Drechslera oryzae</i>	3	Columbia
<i>Khuskia oryzae</i>	1	Indonesia
<i>Rhizoctonia solani</i>	1	Kenya
<i>Rhizopertha dominica</i>	3	Philippines
<i>Sitophilus oryzae</i>	5	Philippines, Vietnam
<i>Sitotroga cerealella</i>	42	Philippines, Vietnam
<i>Tillitia barclayana</i>	10	Belgium, China, Vietnam
Wheat		
<i>Alternaria raphani</i>	8	Australia, Hungary
Barley		
<i>Colletotrichum cereale</i>	1	USA
<i>Drechslera sativus</i>	1	USA
Maize		
<i>Drechslera maydis</i>	6	Kenya, Mexico, South Africa, USA, Vietnam
<i>Stenocarpella maydis</i>	2	USA
<i>Drechslera carbonum</i>	1	France
<i>Fusarium oxysporum</i>	6	USA
<i>Fusarium solani</i>	6	Argentina, USA
<i>Lasioderma serricorne</i>	3	Thailand
<i>Pestalotia macrotricha</i>	2	Mexico, USA
<i>Rhizoctonia bataticola</i>	1	Thailand
<i>Rhizoctonia solani</i>	46	France, Kenya, Mexico, South Africa, Thailand, USA
<i>Tribolium castaneum</i>	17	Kenya, Thailand
<i>Trogoderma granarium</i>	3	Kenya
Sorghum		
<i>Alternaria solani</i>	1	France
<i>Colletotrichum graminicola</i>	6	Mali, USA, Uganda, Zambia
<i>Corcyra cephalonica</i>	16	Zambia, Mali
<i>Cryptolestes pusillus</i>	1	Sudan
<i>Drechslera sorghicola</i>	7	Argentina, Bulgaria, Mali, Sudan, Tanzania, USA
<i>Rhizoctonia bataticola</i>	7	Argentina
<i>Rhizoctonia solani</i>	4	Bulgaria, France, Tanzania, USA
<i>Rhizopertha dominica</i>	8	Brazil, Mali
<i>Sitophilus granarius</i>	2	South Africa
<i>Sitotroga cerealella</i>	3	Brazil
<i>Sporisorium cruentum</i>	4	Ghana, Tanzania, Zambia
<i>Tribolium castaneum</i>	23	Zambia, Mali, Tanzania
<i>Tribolium confusum</i>	2	Zambia
Pearlmillet		
<i>Alternaria porri</i>	2	Uganda
<i>Alternaria solani</i>	2	Belgium
<i>Corcyra cephalonica</i>	7	Uganda
<i>Drechslera setariae</i>	35	Sudan, Uganda
<i>Fusarium oxysporum</i>	23	Niger
<i>Rhizoctonia solani</i>	4	Belgium, Ghana, Uganda
<i>Rhizopertha dominica</i>	11	Ghana
<i>Stegobium paniceum</i>	2	Ghana
Fingermillet		
<i>Alternaria porri</i>	1	Uganda
<i>Drechslera nodulosa</i>	5	Tanzania
<i>Fusarium oxysporum</i>	1	Uganda
<i>Rhizoctonia solani</i>	9	Uganda

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Table 2: Pathogen interceptions on wild species/other cultivated species

Table 2. Pathogen interceptions on wild species/other cultivated species

Pathogen interceptions	Wild species/other cultivated species	Exporting country
<i>Alternaria porri</i>	* <i>Eleusine Africana</i> ; * <i>Pennisetum procerum</i> ; * <i>P. ramosum</i>	[§] Uganda
<i>Drechslera nodulosa</i>	<i>Eleusine indica</i>	[§] Tanzania
<i>Drechslera setariae</i>	* <i>P. ciliare</i> ; * <i>P. meianum</i> ; * <i>P. polystachion</i> ; * <i>P. purpureum</i> ;	[§] Tanzania
<i>Drechslera sorghicola</i>	* <i>Sorghum cafforum</i> ; * <i>S. cernuum</i>	[§] Bulgaria
<i>Fusarium oxysporum</i>	* <i>E. africana</i>	Uganda
<i>Rhizoctonia solani</i>	* <i>S. cernuum</i> ; * <i>S. bentuorum</i> ; * <i>S. durra</i> ; <i>S. vulgare</i>	[§] Bulgaria
	* <i>P. purpureum</i> ; * <i>P. sphacelatum</i>	Tanzania

*New host record;

[§]New report of occurrence

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Molecular Characterization of *Pyrenophora tritici-repentis* Races in Syria Using AFLP Technique

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Tan spot, caused by *Pyrenophora tritici-repentis* - Ptr, is a common disease on wheat responsible for economic losses in some wheat growing areas worldwide. The study aimed to use AFLP technique determine variation between Syrian isolates. 29 Ptr were obtained from the durum wheat growing provinces in Syria (Aleppo, Homs, Hama, Hassakeh, Lattakia, Tartous, Idlib). There colony morphology on Potato Dextrose Agar were investigated. To identify the different races causing tan spot, AFLP templates were prepared by the digestion of Ptr DNA with *EcoRI* and *MseI* restriction. A total of 745 AFLP polymorphic bands were obtained using 3 primer combinations. The results showed that AFLP technique could determine the genetic variation in the Ptr population. This variation was low (9.87%) between sites within the same district, but has high (90.12%) within the same site. UPGMA cluster analysis jointly with PCoA analysis has helped to show the high variation within Ptr population as well as the possible similarity of some groups. Genetic similarity between some Ptr isolates was found between different geographical locations.

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Pests and pathogens observed on leaves of *Pyrus calleryana* urban trees in Warsaw

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Pyrus calleryana is one of several tree species recently planted along Warsaw streets due to its high vitality and tolerance to road-side harmful conditions. However, for 3-4 years there have been observed serious damages of leaves on numerous urban trees affecting their aesthetic value.

The aim of the study was to identify pests and/or pathogens responsible for the most serious leaf injuries and the main factors influencing pest and pathogen threats to urban trees.

The study was conducted in 2013 and 2014 on young *Pyrus calleryana* 'Chanticleer' trees planted in 2004-2005 along Warsaw streets in 4 locations (2 uptown, 2 downtown). 3-10 vigorous trees without any mechanical injuries were observed in each location. Tree leaves were examined four times a year during the growing season. Tree crowns were observed to assess the percentage of crown foliage injury. The presence of possible pest or pathogen hosts in the neighbourhood was analysed, as well as weather conditions and influence of road traffic measured by post-winter surface salinity of shoots.

The results showed that *Podosphaera leucotricha* and *Gymnosporangium sabiniae* were the pathogens which affected leaf vitality the most (30-100% injured leaves in a tree crown). *Podosphaera leucotricha* was present in the locations where old apple trees grew in the neighbourhood. Leaves of the trees affected by abiotic factors were injured the most severely. *Gymnosporangium sabiniae* was present in all the locations injuring up to 70% leaves in a tree crown. It should be explained by numerous *Juniperus sabiniae* plants in urban greenery. All the trees were infested by *Psylla piri* and *Panonuchus ulmi*, the highest degree of infestation (100% leaves in a crown) occurred in trees growing in extremely contaminated road-side locations. *Aphis pomi* and *Epitrimerus piri* occurred occasionally, as well as *Venturia pyrina*. *Mycosphaerella pyri* and *Eriophyes piri* were not noticed yet.

The biological threats to urban *Pyrus calleryana* trees include both pests and pathogens typical for *Pyrus* genus. The influence of abiotic stress factors, like post-winter shoot and bud salinity, increase the degree of infestation. The absence of potential source of infestation may eliminate the threat of certain pathogens or pests, as well as scattered locations of *Pyrus calleryana* plantations.

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Management, DNA Barcoding & Diversity of Three Date Palm Tree Insects: *Oryctes* spp., *Jebusaea hamerschmidtii*, and *Batrachedra amydraula* in UAE

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Insects cause major damage to crops and fruit trees worldwide. In the United Arab Emirates (UAE) the date palm tree is the most economically important tree because it is used for date production and as an ornamental tree. The longhorn stem borer (*Jebusaea hamerschmidtii*), the fruit stalk borer (*Oryctes* spp.), and the lesser date moth (*Batrachedra amydraula*) are three important insect pests causing damage to date palm trees in the UAE. The objectives of the study are: (1) studying population dynamics of the *B. amydraula* and *J. hamerschmidtii*, (2) exploring the potential biological control of *Oryctes* spp. by using entomopathogenic viruses, (3) DNA barcoding and studying genetic diversity of the populations of the three insect pests. Population dynamics of the two pests *J. hamerschmidtii*, and *B. amydraula* were studied by using light and pheromone traps, respectively, in Al-Ain, UAE. The first trap catch of *B. amydraula* adults occurred on 19 April and the insect population peaked on 26 April, 2014. The first trap catch of *J. hamerschmidtii* occurred on April 2014. The numbers increased over time and the population peak occurred in June. The impact of insect population dynamics data on integrated pest management is discussed. DNA was extracted from adult insects. PCR was carried out using universal barcoding primers (ITS1-2 and COX1). DNA similarity levels and their use in insect molecular identification and DNA barcoding are discussed. The study provides important and pioneer data, which can help in achieving a successful management of date palm insect pests.

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***Xylocopa pubescens* Spinola (Apoidea, Apidae), invasive species and potential pollinator of natural and cultivated plants in Algeria**

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Nearly 20,000 threatened plant species are still saved through the pollination action of bees. Indeed, many natural plant and crop flowers attract many groups of insects, including in particular the Apoidea, which are among the potential pollinators. *Xylocopa pubescens* Spinola, 1838 (Apoidea, Apidae), is one of the species of solitary bees most known for their pollination action. It is newly reported in Algeria. A study was carried out on the floral choice of this species in natural and cultivated environments region of Algiers. The results show that this species is oligolectic, it has a preference for some species of natural plants such as *Sinapis arvensis* and *Fumaria agraria* and the crop broadbean *Vicia faba* L. var. *major* (Fabaceae). As an invasive species, the bee *Xylocopa pubescens* was subjected to identification by the genitalia method. References: Solomon Raju and Purnachandra Rao, 2006 ; Leys *et al.*, 2002 ; Eardley, 1983 ; Lieftinck, 1956. Figures: *Xylocopa pubescens* female and male; Genitalia of *Xylocopa pubescens* male.

P NEPD 43

Diversity of Diatrypaceae species from grapevines and trees in the vicinity of vineyards in South Africa

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Introduction: Members of the family Diatrypaceae have for many years been considered to be saprophytic although a number are serious pathogens on economically important plants. On grapevines, *Eutypa lata* is the most important pathogen in the Diatrypaceae, causing Eutypa dieback. Recent studies on grapevines and surrounding trees in California and Australia have revealed an extensive diversity of Diatrypaceae spp., of which several have been found to be pathogenic on grapevines. These findings raised questions as to whether these newly discovered species are a threat to the sustainability of the grapevine industry and what the role of these species is in the development of Eutypa dieback. Little information is available regarding the diversity of Diatrypaceae species associated with declining South African vineyards.

Objectives: To identify diatrypaceous species occurring in diseased grapevines and tree hosts in the vicinity of vineyards in South Africa.

Materials and methods: Isolations were made from cankers, dying spurs, tree branches showing dieback and fruiting bodies. Fungal cultures resembling Diatrypaceae spp. were characterised based on their morphology and were compared in phylogenetic analyses, based on ITS and β -tubulin gene regions, to reference sequences.

Results: Several Diatrypaceae spp. were isolated. From grapevine, *Cryptovalsa ampelina*, *Eutypella citricola* and *E. lata* was the most abundant followed by *E. consobrina*, *Eutypa* sp., *Cryptovalsa rabenhorstii* and *Eutypella microtheca*. These species also occurred on various other trees. Further Diatrypaceae fungi isolated from trees (i.e. willow, pepper and black wood) are *Diatrypella vulgaris*, *Eutypella australiensis*, *Cryptosphaeria* sp. and *Eutypella* spp.

Conclusions: Several Diatrypaceae spp. may be involved in the development of Eutypa dieback in South Africa. Symptoms thought to be caused by *E. lata* may be caused by other Diatrypaceae spp. since at least two species occurred more abundantly than *E. lata*. The study revealed a high diversity in Diatrypaceae spp. and show that other trees act as reservoirs of inoculum to adjacent vineyards. Pathogenicity tests with these species will determine whether they are emerging as important grapevine pathogens in South Africa and what the implications are regarding efforts to manage Eutypa dieback.

P NEPD 44

Spread Prevention and Management of Cassava Pink Mealybug in the Greater Mekong Subregion

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Cassava is a major crop used for human consumption, animal feed and bio-energy production. In the Greater Mekong Subregion, about 3 million smallholder farmers derive their livelihoods from cassava production. In early 2008, incursion by *Phenacoccus manihoti* (Hemiptera: Pseudococcidae) or cassava pink mealybug, devastated extensive cassava areas in Thailand. Subsequently, it also invaded other neighbouring countries, namely Cambodia (2009), Indonesia (2010), Lao PDR (2011) and

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Vietnam (2012). To combat the invasive pest menace, Thailand introduced from Africa the parasitoid *Anagyrus lopezi* (Hymenoptera: Encyrtidae) for biocontrol of the mealybug. This, as well as releases of the local predatory lacewings, together with ecological pest management training efforts of field extension workers and farmers, have helped to effectively control this invasive pest problem in Thailand and elsewhere in the Asia region. FAO implemented a regional Technical Cooperation Project to support capacity building of Asian member countries to prevent spread and manage the cassava pink mealybug during the 2010-2013 period. This paper will outline project results and strategies employed for effective and sustainable spread prevention and management of the invasive cassava pink mealybug.

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Infestation of The Cassava mealybug, *Phenacoccus manihoti* (Matile-Ferrero (Hemiptera: Pseudococcidae), A Newly Invasive Pest in Indonesia

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Introduction: The cassava mealybug, *Phenacoccus manihoti* (Matile-Ferrero (Hemiptera: Pseudococcidae), was first detected in Bogor, Indonesia in 2010.

Objectives: Studies were conducted to determine perception of farmers, geographic distribution, extent of infestation, and potentials of cultural and biological control.

Materials and methods: Studies were consisted of farmer surveys and field observations.

Results: Nearly half the respondents mentioned that attacks by the cassava mealybug caused yield losses about 40-50%. The pest has spread throughout Java and Lampung. The infestation was worse in drier part of Indonesia. Heavy damage caused bunchy top, shortened nodes, and inhibition of plant growth. Field observations indicated that symptoms of bunchy top appeared as early as 8 weeks after planting (wap) and rose quickly started 16 wap, at the same time with the advent of the dry season (May-June). Level of infestation developed faster on variety Jimbul; at 18 wap all plants had bunchy tops. While on varieties Roti and Manggu, 100% infestation occurred at 30 and 36 wap, respectively. There was a correlation between early infestation with plant height and yield. Cassava plants infested during early stage were shorter and the yield lower, compared to those infested at further stages. Lower yields of variety Jimbul (0.94 kg/tree) than variety Manggu (3.16 kg/plant), was thought to be related to heavy infestation which occurred during early stage. The most abundant natural enemies in cassava fields infested by *P. manihoti* was the predatory lacewing *Plesiochrysa ramburi*. Population of *P. ramburi* usually increased at the end of dry season, when mealybug population had already reached its peak and cassava plants were severely damaged. Abundance of *P. ramburi* were higher on variety Jimbul coincided with higher mealybug infestation. Predator density on this plants reached 100 eggs, 80 larvae, and 70 pupae per plant at 24 wap. Parasitoid *Anagyrus lopezi* was introduced from Thailand in 2014 as complement to local natural enemies.

Conclusions: Efforts should be made to prevent further spreads of the cassava mealybug to other islands in Indonesia. Control strategies for the cassava mealybug include planting cassava at the onset of rainy season and use of predators and parasitoids.

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Use of root endophytic *Trichoderma* for *Psa-V* control in New Zealand kiwifruit

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The kiwifruit disease *Pseudomonas syringae* pv. *actinidiae* (*Psa-V*) was first identified in New Zealand in 2010 and currently affects over 85% of the country's kiwifruit orchard area. Our research focused on the use of *Trichoderma* isolates as a biological control option of *Psa-V*. These root endophytic fungi have the potential to colonise root surfaces and penetrate roots, and to limit the damaging effects of pathogens by releasing metabolic compounds, incl. antibiotics, inducing systemic resistance, and improving general plant health. Root colonisation with *Trichoderma* can also enhance plant growth, crop productivity, nutrient uptake and resistance to abiotic stresses.

To identify promising isolates for the control of *Psa-V*, root endophytic *Trichoderma* were isolated from healthy kiwifruit vines and other plant species in areas with high *Psa-V* incidence and assessed by inoculating young kiwifruit seedlings under environmentally controlled conditions with different isolates as mixtures or single isolates. 8 to 10 weeks after inoculation, seedlings were challenged with *Psa-V* by stab inoculation and disease progression and seedling survival were assessed over 4 weeks. To assess the effect of *Trichoderma* inoculation on roots of established plants with an existing microflora *Trichoderma* were re-isolated from the roots of orchard vines that had or had not received *Trichoderma*.

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Several promising mixtures and single isolates were identified as reducing PsA-V symptoms and increasing plant survivability and our results indicate that a single *Trichoderma* inoculation early during plant development has a significant effect on kiwifruit plant health and survival. Field trials in PsA-V infected kiwifruit orchards using container-grown kiwifruit also confirmed the efficacy of the best treatments alone and in combination with a plant elicitor and other microorganisms to reduce PsA-V symptoms on different kiwifruit cultivars. Re-isolating *Trichoderma* from orchard vines showed significantly higher numbers of *Trichoderma* in the roots of the treated compared to the untreated plants. The results suggest that inoculation of older orchard vines facilitates the establishment of a strong root endophytic *Trichoderma* community that may improve plant health and thus contribute to the control of PsA-V on kiwifruit.

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Whiteflies species (Hemiptera: Aleyrodidae) of Turkey

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Introduction: Whiteflies (Aleyrodidae) are small, usually inconspicuous, sap-sucking insects that contain many important agricultural pests. Sometimes they cause plant damages results from direct feeding, through contamination by excreted honeydew and secondary colonization by sooty mold, and by transmitting plant virus diseases. Faunistic studies on whiteflies in Turkey started with *Aleurolobus olivinus* in 1968 and have continued to present day. Although knowledge of the diversity of whiteflies and their distribution in Turkey is still incomplete, the current study represents important information about whiteflies fauna in Turkey.

Material and methods: A check list of whiteflies of Turkey was prepared base on the literature data and faunistic data from our own investigation. Browsing the literature of faunistic investigations of whiteflies in Turkey involved a period from 1968 to 2014.

Results and conclusion: In the reference, 15 species have been described. Thirty three different whitefly species were identified by our own faunistic studies and four species first recorded for Turkey in 2014, 7 of which are new for Turkey fauna and reported for the first time in this list. These are the following species: *Asterobemisia benata*, *Bemisia graminus*, *Bemisia tuberculata*, *Lipaleyrodes euphorbiae*, *Pealius misrae*, *Pealius rhododendri*, *Trialeurodes ricini*. The check list contains 40 whiteflies species from two subfamily (Aleyrodinae and Aleyrodicinae), categorized in 21 genera. We thought that continuing the faunistic survey in Turkey will be resulted to many other species and new country records.

Acknowledgements: We thank Dr. John Martin and Dr. R. Bink-Moenen for identification or confirmation of whitefly species found between 1987 and 2004.

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Peppermint leaf spot caused by *Alternaria alternata* in Iran

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Introduction: Peppermint (*Mentha piperita*), a herbaceous plant in the family *Lamiaceae*, is a medicinal herb widely grown in Iran. Symptoms of leaf spot were observed on more than 45% of 140 randomly examined peppermint samples during a survey in the fields of Kerman (southeast of Iran), in November 2012.

Objectives: This research was performed to study the etiology of peppermint leaf spot in Kerman.

Materials and methods: The leaves were washed with soap and running water, surface sterilized with 0.5% sodium hypochlorite for 1 min, rinsed with sterile distilled water, cultured onto PDA medium and incubated at 25°C for seven days. Pathogenicity of the isolates was confirmed by spraying a conidial suspension of 10⁴ conidia/ml onto the leaves of the three 2-months old plants for each of 5 selected isolates. The plants were incubated under greenhouse conditions for 10 days and the inoculated leaves were covered by plastic bags for 24 hours. Fungal DNA was extracted from seven day old mycelium using CTAB buffer. Universal fungal primers were used to amplify the internal transcribed spacer (ITS) region of the rRNA gene complex, incorporating ITS1, the 5.8S gene, and ITS2.

Results: Investigation of the infected leaves under stereo microscope revealed fungal sporulation on some of the spots. Dark olive colonies were observed in the plates which produced profuse golden brown, branched, and septate hyphae, and muriform conidia in long chains on straight and septate conidiophores. Based on the morphological characters, the fungus was identified as *Alternaria alternata*. Ten days after inoculation of plants, some spots similar to those observed in the fields were produced on 47.85% of the leaves, while the control plants showed no symptoms. An average of 40% of the leaf surface was covered by the spots and some of the leaves gradually fell off. *A. alternata* was re-isolated from the leaves of inoculated plants, thus fulfilling Koch's postulates. Amplification of the internal transcribed spacer (ITS) region of rDNA of a representative isolate using the universal primers ITS1F and ITS4 resulted in a 560 bp fragment which showed 99% similarity with *A. alternata* sequences and clustered with other *A. alternata* isolates in phylogenetic analysis.

Conclusion: This is the first study on the etiology of peppermint leaf spot which confirms the identification and placement of the Kerman isolate among other *A. alternata* isolates from Iran and the world.

P NEPD 50

Spread of *Dryocosmus kuriphilus* in Portugal, a new and very important Chestnut plague

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Dryocosmus kuriphilus is a species of gall wasp native from China and it is known in many other parts of the world, particularly the Northern Hemisphere, as an introduced and an invasive horticultural pest. It attacks many species of chestnut (genus *Castanea*), including most cultivated varieties. It is considered the world's worst pest of chestnuts. It causes disrupting of plant growth by inducing gall formation on new shoots and leaves and reducing fruit production drastically. In Europe, *D. kuryphilus* attacks *C. sativa* and the interactions with other factors such as drought, pests and fungal diseases, had contributed to a significant reduction of fruit production, up to 50-70%, giving high negative social and economical impacts.

D. kuriphilus could potentially spread throughout the range of *C. sativa* in Europe, but the areas currently considered at most risk for invasion by this pest species are northern Portugal, northern Spain and south-western France.

In 2006, the European Commission adopted provisional emergency measures to prevent the spread of the pest (Decision 2006/464/EC) but new countries started to be also affected like, Hungary, Switzerland, Croatia, Czech Republic, Spain, Germany, and finally in 2014 Portugal. The presence of this insect in Portugal was first reported in May 2014.

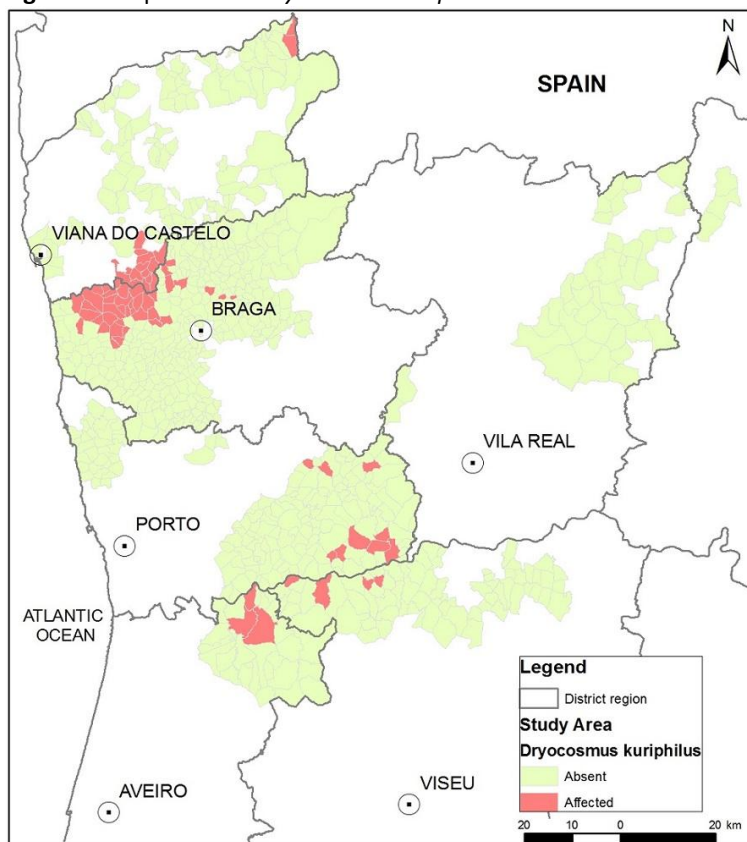
In June 2014 a task force involved governmental services, universities, local associations and farmers, was created in order to know the real situation the presence of this insect in Portugal. A lot of persons were involved, and between June to November 2014, a total of 749 parishes (small administrative district), with chestnut trees, were prospected. The observed area (approximately, 5583 km²) was distributed in 7 district regions (Fig. 1).

As result, were detected 71 parishes affected (425,8 km²), corresponding to 9% of the study area. Mostly of the *C. sativa* attacks were observed on Braga district (Fig. 1). This region, as well as, Viana do Castelo, Porto and Aveiro, have a low chestnut

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production. However, in the close districts (Viseu, Vila Real and Bragança) chestnut is a very important multifunctional tree. Also, the Portuguese specific chestnut stands, create difficulties to assessment and control the plague that could causes relevant economic losses in the future.

Figure 1: Propection of *Dryocosmus kuriphilus* in 2014.



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Current Status of Apple Scab (*Venturia inaequalis* (Cke.) Wint.) in India

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Apple Scab, caused by *Venturia inaequalis* (Cke.) Wint. (anamorph *Spilosea pomi* Fr.), is considered to be one of the most important fungal diseases of apple. In Bhatwari fruit belt, defoliation in apple trees started around mid August and continued for nearly 65 days. Maximum leaf fall (82%), however, was observed during the period October 15th - November 15th after which trees were completely defoliated. A single pre-leaf fall spray of 5 % urea was significantly decomposed the over wintered leaves, and were on par or superior in effectiveness as compared to the 100 % cow urine, antagonists and Carbendazim. Similarly, the application of urea at 3 and 5 percent was significantly proved better over others for reducing the pseudothecial formation (97.31, 78.72 %) and the discharge of ascospores (98.04, 95.66 %) from over wintered leaves. The ascospore emission period was 61-76 days and mean numbers of cumulative degree days for 50 and 85 per cent spore release from these observations were 456 and 960. On examination of the primary infection period of 17 years data from Bhatwari fruit belt, some differences were observed between our results and Mills table for ascospores infection. The observation revealed 2 day (light infection), 1 day (moderate infection) and 1 day (severe infection) delay in symptom expression under orchard conditions. The observation revealed 2 day (light infection), 1 day (moderate infection) and 1 day (severe infection) delay in symptom expression under orchard conditions. The PAD value was low during 1999 to 2001 (612 - 2192 ascospore/m²) and medium during 2002-2006 (4262 - 37848 ascospore/m²) due to the fact that the springs were early, dry and not more favorable for ascospores maturation. In 1996, 2008 and 2013, the PAD values were high because of the favourable weather conditions and increase of inoculum accumulation. The scabbed lesion and leaf litter density were approximately more than twice in Bhatwari fruit belt in 1996 2008 and 2013. PAD involves the elimination of unnecessary early-season sprays in orchards where the inoculum is below a specified level. PAD values were 50 times higher in the poorly managed orchards than in the integrated managed orchards. Warnings are issued mainly via a call in telephone, Agriculture Govt. department, and broadcasted through radio stations.

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Characterization of *Xanthomonas axonopodis* pv. *phaseoli* and *Xanthomonas fuscans* subsp. *fuscans* Isolated from Beans in Turkey

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The common blight caused by *Xanthomonas axonopodis* pv. *phaseoli* (Xap) and fuscous blight caused by *X. fuscans* subsp. *fuscans* (Xff) are devastating bacterial diseases of bean. In this study, Xap and Xff strains were isolated from the seeds of different bean cultivars, collected from 12 provinces in the Central Anatolia Region. All bacterial strains were identified by biochemical, physiological and molecular tests. Xap and Xff were determined on the bean seeds with the infection ratios of 11,11% and 1,51%, respectively. The mucoid appearance of Xap and Xff bacterial colonies was an indication of xanthan production, which is under the regulation of the diffusible factor DSF. Xff strains produced a brown pigment on tyrosine-containing medium, and these strains were highly aggressive on cv. Dermason. There were significant variations (p<0.05) with bean cultivars and locations. FAME profiles from whole cell fatty acids were identified in the MIS software package and the similarity indices ranged from 98.8 to 100%. PCR assays were performed using specific primer sets, Xf1 and Xf2, and X4c and X4e. A 450 bp amplification product was obtained only from all Xff isolates and the 730 bp product amplified by primers X4c and X4e were obtained only from Xap isolates. Using these primers were made Multiplex PCR and the primers succeeded to amplify a product of the expected size with Xap and Xff strains with exception two strains of Xap. Strain motility was tested in soft-agar (MOKA) assays. Specific primers were used to amplify of the five genes, chosen as markers of the flagellar cluster integrity. RAPD-PCR assays using 14 random primers from the primer kits (OP) were evaluated for their ability to differentiate 20 strains of Xanthomonads and the similarity more than 80% was obtained by suggesting a common origin. This study revealed that strains of Xap and Xff were genetically different and they grouped into six distinct genetic lineages. This result shows that Xap and Xff strains are genetically distinct and that strains of Xap were more heterogeneous than those of Xff. Understanding of the molecular mechanisms in pathogenicity will be essential for the development of new strategies for the control of the economically important diseases caused by Xap and Xff.

This study was supported by Selcuk University Coordinatorship of Scientific Research Projects

P NEPD 53

Study of the Spatiotemporal Evolution of grown-up individuals male and the females of the mediterranean fly of the fruit *Ceratitis Capitata* in an Orchard of citrus fruit in Mitidja, in Algeria

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Ceratitis capitata is a very wide-spread species and the harmfulness of which is one of the main obstacles to the production of healthy fruits and to their export. Our study is directed on the follow-up of the environmental actions on the spatiotemporal fluctuations in the populations of adults.

Our plot of land of study is an orchard of citrus fruit of rectangular shape of a surface of four hectare meadows; this orchard is surrounded with everything the highly-rated by dense one hedge of breezes wind, except highly-rated the North which remains exposed to the wind.

To understand the influence of the pressure of this hedge of breeze wind and the participation of the environment of the orchard on the dynamics of Medfly, we bounded the orchard studied in three levels of plantations in the shape of transects by installing it traps with pheromone on each one.

The obtained data are the results of the captures of the male grown-up individuals and the females of *Ceratitis capitata* obtained by the trapping with pheromone with periodical taking over all the period of sampling.

The superimposing of the curves of the spatiotemporal dynamics of the male adults with that of the females shows that fluctuations in the rates of capture between both sexes profile them follow relatively the same tendencies through time, with departures of flight coinciding in the same periods for every trap; nevertheless, peaks are more pronounced for males.

The evolution of the agro-ecosystems is strongly influenced by the environmental changes among which the impact of the bioaggressors and/or the anthropological activities including the diverse phytosanitary treatments, the fertilization and the other cultural practices. The extension of the contributions in knowledge on the lines of life story of the devastating of the cultures and echoed them are positive where denials of the various factors of their environment on their strategies of expansion and preservation undoubtedly turn out to be necessary for the improvement of the techniques of fight and the development of the alternative methods in the chemical fight.

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This present study is a contribution to the research and to the highlighting of the impact of certain ecological parameters on biological behavioral of the Mediterranean fly of fruits *Ceratitis capitata* (Diptera; Trypetidae), devastating one formidable which worries the producers and the exporters of fruits of a big economic importance, where from orange trees.

P NEPD 54

Management of blast, an emerging disease of pearl millet

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Introduction: Pearl millet blast, caused by *Pyricularia grisea* (Cooke) Sacc. (teleomorph - *Magnaporthe grisea* (Hebert) Barr.), has emerged as a serious disease of pearl millet hybrids in India in the past 5-6 years. Host plant resistance is the economical and viable disease management strategy to control pearl millet blast; however, resistance in the commercial hybrids being grown in India is not available as no efforts were made in the past to breed for blast resistance. Therefore, efforts are being made to identify resistance sources to different pathotypes of *M. grisea* as well as disease control through fungicides.

Objectives: The objective of this study was to identify resistance in the parental lines of pearl millet to diverse pathotypes of *M. grisea*, and fungicides effective against pearl millet blast.

Materials and methods: One hundred sixty two designated B-lines of pearl millet were screened for blast resistance against five pathotypes of *M. grisea*. Twelve-day old seedlings were inoculated with aqueous conidial suspension of *M. grisea* and blast severity was recorded after eight days of inoculation using a 1-9 rating scale. In another experiment, the efficacy of nine fungicides- Chlorothalonil, Tricyclazole, Hexaconazole, Kasugamycin, Benomyl, Carbendazim, Nativo (Tebuconazole 50% + Trifloxystrobin 25% WG), Tilt (Propiconazole 25% EC) and Ridomil was tested against pearl millet blast under field conditions.

Results: Of the 162 designated B-lines screened, eight (81 B, ICMB 88004, ICMB 92444, ICMB 02111, ICMB 06444, ICMB 07111, ICMB 09333, ICMB 09999) were found resistant (≤ 3 score) to all the five pathotypes tested. Three sprays of Nativo @ 0.4 g/l or Tilt @ 1 ml/l were found effective in controlling blast. Even two sprays of these fungicides were more effective than three sprays of other fungicides. Three sprays of Nativo @ 0.4 g/l also resulted in significantly higher fodder and grain yield of pearl millet line ICMB 95444.

Conclusion: Blast-resistant parental lines could be used to develop pearl millet hybrids resistant to this disease. The disease can also be effectively managed with the foliar sprays of Nativo or Tilt.

P NEPD 55

Identification and characterization of *Pseudomonas syringae* the causal agent of bacterial canker of sweet cherry (*Prunus avium*) in Algeria

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Bacterial canker is a damaging disease of sweet cherry tree (*Prunus avium* L.) in Algeria, destroying a thousands of sweet cherry trees every year. In our study over 50 *Pseudomonas* isolates were obtained from plant with bacterial canker symptoms collected during 2010 - 2012 from commercial orchards in Constantine and Khenchela. Based on the results of LOPAT tests twelve isolates were classified as *P. syringae*. Further phenotypic characterization using GATTa and L-Lactate tests showed that 7 of them belonged to *P. syringae* pv. *syringae* (Pss) and 5 to *P. syringae* pv. *morsprunorum* race 1 (Psm1). The results of PCR Melting Profile (PCR MP) technique used for genetic diversity showed that strains of *P. s.* pv. *morsprunorum* are highly homogeneous and similar to reference strain LMG2222 and Polish strains of Psm1. Also, the Algerian *P. s.* pv. *syringae* strains formed homogeneous group. However, they differ from reference strain LMG1247 and Polish Pss strains used for comparison.

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Invasive pathogens and pests in Germany - prevention and early detection strategies by the Plant Protection Service Bonn

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In Germany, protection against the introduction and distribution of non-native organisms which could damage plants and their ecosystems is regulated by the Plant Protection law and the Plant Inspection Ordinance. This poster gives you an overview of the prevention and early detection strategies undertaken by the Plant Protection Service in North Rhine-Westphalia (NRW) against invasive pathogens and pests. The Service is located in Bonn.

Recently, the non-native insect *Thrips palmi* was entrained to a local experimental station of the Chamber of NRW. Strategies of the Plant Protection Service to achieve the eradication are described.

2005 *Anoplophora glabripennis* was entrained to a small city near to Bonn. Strategies for eradication are illustrated.

Further examples for successful measures against invasive pathogens and pests in Germany are outlined.

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An International Plant Sentinel Network

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Introduction: The increase in the global trade and transport of plants and plant material increasingly creates new pathways for the entry of new plant pests and diseases; stressing the necessity of phytosanitary risk assessment (PRA). One problem lies in the fact that many damaging alien pests and diseases are considered as harmless in their native areas; thus are not identified by standard PRA practices.

Objectives: The International Plant Sentinel Network (IPSN) aims to use information derived from surveying plants living in collections in botanical gardens and arboreta outside of their native range ('sentinel plants'). Sentinels can provide valuable information on pests and diseases that can be used for early warning of newly emerging risks and inform the creation of control measures to manage introductions and/or outbreaks. It is developing a world-wide network of NPPOs, Botanical Gardens and Arboreta, and Research Institutes who will eventually survey, report and exchange information regarding newly arising pest risks.

Materials and methods: Botanical Gardens and Arboreta were approached using various methods; conferences, meetings, email, newsletters and through a newly developed website (www.plantsentinel.org). This work is ongoing, as the network is still recruiting, and will include the production of leaflets, translation of existing materials and expansion of the website.

Results: At present Botanical Gardens in 10 countries around the world are participating. In a first sampling period in late summer 2014, pest and pathogens were monitored in participating gardens. With an International Advisory Group newly established there are now IPSN participants in 6 continents.

Conclusion: The project has begun to establish a network, and initial surveys using the IPSN 'Plant Health Checker' (a form for recording change in sick trees) gained good levels of engagement from current participating members. Reactions of Botanical Gardens approached so far have been mostly very positive indicating general enthusiasm for the idea; potential participants have been easily convinced.

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Identification and epidemiology of *Pseudomonas syringae* on cherry and apricot trees

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This work was carried out to study the etiology and the epidemiology of Bacterial canker of sweet cherry (*Prunus avium* L.) and apricot (*Prunus armeniaca* L.) in Algeria. Samples of diseased plant material exhibiting bacterial canker symptoms (cankers and

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gummosis of branches , leaf spots ...) were collected from cherry and apricot trees grown in Constantine , Khenchela localities (Eastern Algeria) during 2008,2009 and 2010. A total of 42 , Gram negative, fluorescent, oxidative bacterial strains were isolated from margins of diseased and symptomless tissue. All investigated strains were levan and HR positive, and oxidase, pectinase and arginin dihydrolase negative (LOPAT+ - - +) . Based on positive pathogenicity tests on green immature sweet cherry fruitlets and differential GATTA tests, investigated strains were divided in two distinct groups: the first group consisted of strains with gelatin and aesculin positive, and tyrosinase and tartrate negative tests were classified as *Pseudomonas syringae* pv. *syringae*. The second group of strains with , gelatin and aesculin negative and tyrosinase and tartrate positive results were identified as *Pseudomonas syringae* pv. *morsprunorum*. Fatty Acid Methyl Esters analysis (FAME) confirmed the bacterial strains as *P. syringae* with similarity indices of 0.65 to 0.89. The results of the epidemiological study showed that the two pathogens of *Pseudomonas syringae* are present in cankers on shoots and branches as well as in diseased and symptomless leaves . Furthermore, bacterial epiphytic populations were maximal during cool and wet periods of the year and minimal during dry and hot periods.

P NEPD 59

Investigating the causes of strawberry decline disease which is an emerging threat to strawberry production in North America

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Strawberry [*Fragaria x ananassa*, Duch.] is an important berry crop in North America and Canada is a major supplier of strawberry transplants to US. Strawberry nurseries in eastern Canada supply bare-root transplants or plug plants to the commercial growers in Canada and US. However in 2012, growers in both countries noticed acute decline symptoms on strawberry plants in their fields such as uneven growth patterns, stunted foliage, reddening of older leaves, brittle roots, and small fruits. Most of the strawberry plants showing severe decline symptoms were found doubly infected with *strawberry mild yellow edge virus* (SMYEV) and *strawberry mottle virus* (SMoV). Both these viruses are vectored by aphids in a persistent or semi-persistent manner. Several strawberry plantings have already been discarded to prevent further spread and both strawberry fruit and nursery production sectors are at great risk to this disease. Recently, a new strawberry virus [strawberry polerovirus 1 (SPV1)] was also found associated with strawberry plants showing decline symptoms. A field survey of strawberry viruses indicated wide spread occurrence of this new virus on strawberry plants in Atlantic Canada, Quebec, and Ontario. In most cases, the new virus was detected in mixed infections with other two major strawberry viruses, SMYEV and SMoV. Mixed infections may be very common in nature as some strawberry samples showed mixed infections of six viruses. Analysis of soil samples from some of the infected fields in Nova Scotia also revealed presence of high numbers of root lesion and other plant-parasitic nematodes. A careful characterization and understanding of various components and their roles in acute decline disease, for instance interactions between strawberry viruses, host, aphid vectors, and other biotic and abiotic factors, would lead to development of disease management strategy to mitigate the economic losses to strawberry industry.

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Biological control of the allergen producer common ragweed (*Ambrosia artemisiifolia*)

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Common ragweed originated from North America, is an invasive weed spreading through Europe. This weed is mainly naturalized through contaminated sunflower- and bird seeds. This noxious weed establishes easily in disturbed areas such as roadsides. The pollen is highly allergenic causing the Austrian human health system an estimated 80 million €/year. Unfortunately, effective agents to control this weed are limited. Novel approaches for more environmental friendly and effective agents to combat this weed are demanded.

Application of plant-associated bacteria could be a successful strategy to win the battle against the invasive common ragweed. Because of their selectiveness of association with the host plant, such bacteria can be applied on agriculture land, without harming the crops as well as in areas where the use of synthetic herbicides are restricted. Another aspect of using natural occurring bacteria is the low risk of non-target effects which can be a great issue when introduction exotic biocontrol agents into a new environment. Taking previous research findings into account, this project will focus on the genera *Pseudomonas* as biocontrol agent.

In frame of this research project we are searching for deleterious rhizobacteria and endophytes of common ragweed. Therefore we collected ragweed from 3 different sampling sites in Austria (Burgenland/Lower Austria/Styria) and isolated around 1500

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bacteria. Bacterial isolates were taxonomic classified through 16S rRNA sequencing. Included in the screening for bioherbicidal effects are the production of hydrogen cyanide and antimetabolite toxins (Figure 1) like tabtoxin and coronatine.

After a rigorous screening 5 *Pseudomonas* isolates could be selected for in vitro testing. From these 5 isolates, 3 displayed a deleterious effect on ragweed (Table 1), which will be tested in the greenhouse. Furthermore, a suitable in vitro assay as well as an inoculation technique was established.

Up to this point, this leads us to the conclusion that plant-associated bacteria could be suitable for the application as bioherbicides. Especially, *Pseudomonas* isolates exhibited promising results.

Figure 1: Indicator test for antimetabolite producing *Pseudomonas*

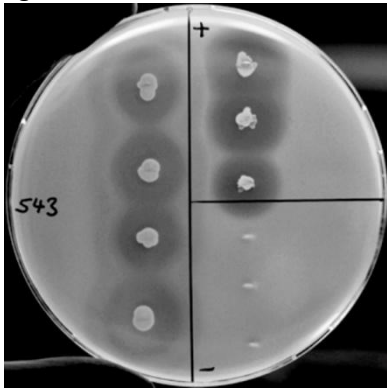
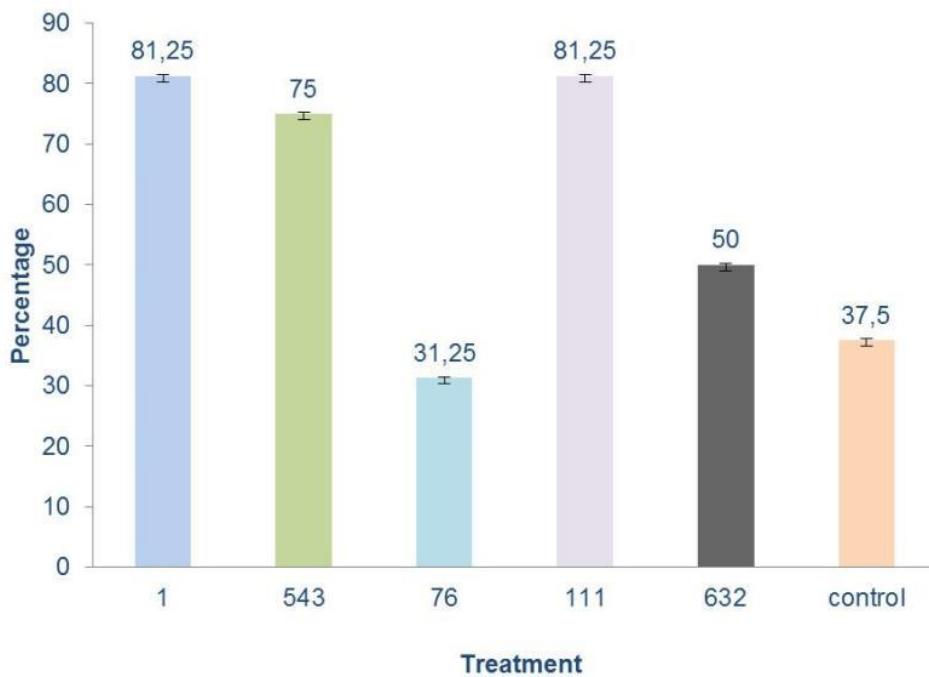


Table 1: Means of diseased plants 14 dpi



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Interaction between *Steinernema feltiae* and abamectin, azadirachtin and metaflumizone used for control of *Tuta absoluta*

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Introduction: *Tuta absoluta*, is a major pest of tomato plants. *Steinernema feltiae* may be useful as an alternate control agent for *T. absoluta*. The efficacy of entomopathogenic nematodes in controlling *T. absoluta* may be improved if they are combined with other control agents.

Objectives: The objectives of this study were to determine the interaction between abamectin, azadirachtin, metaflumizone and *S. feltiae* and determine feasibility of their use in integrated management of *T. absoluta*.

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Materials and methods: In this study one commercial formulation and a native isolate of *S. feltiae* were used. Effects of direct exposure of the chemical insecticides (at field rate) on survival of *S. feltiae* and infectivity to *Galleria mellonella* larvae were tested. Efficacy of these two *S. feltiae* isolates on 2nd instar larvae of *T. absoluta* was investigated. The interaction of the nematode stains and the insecticides in *T. absoluta* larvae was also assessed. In interaction tests, the nematodes were applied at LC₅₀ level, 0, 12, 24 and 36 h after larval treatment with LC₁₀ or LC₂₅ of the insecticides.

Results: The LC₅₀ values were 99.47 and 60.31 IJ/ ml for the native and commercial isolates of *S. feltiae*, respectively. Metaflumizone and azadirachtin did not reduce the survival of the nematodes to unacceptable level. However, since nematode survival was significantly reduced following exposure to abamectin, this insecticide was excluded from the infectivity test. On the other hand, azadirachtin significantly reduced nematode infectivity; but metaflumizone had the least adverse effect on the nematode infectivity.

Antagonism was observed when treatment with nematodes was done immediately after the insecticides application except for LC₁₀ of metaflumizone in both isolates. Also antagonism was observed when treatment with nematodes was done 12 h after abamectin and azadirachtin application. Additive effects were detected 12 h after metaflumizone treatment in both isolates. Combination of commercial isolate of *S. feltiae* 12 h after treatment with LC₁₀ of azadirachtin also caused additive effect. Additive effects were also detected in 24 and 36 h time intervals in the other treatments.

Conclusion: Based on the results obtained, *S. feltiae* can be a potential candidate for management of *T. absoluta*. The concomitant use of the insecticides tested and *S. feltiae* is not recommended for *T. absoluta* control, and an appropriate time interval must be allowed.

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Allelopathic effect of the invasive weed *Brassica nigra* (black mustard) on seed germination of agricultural crops

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The allelopathic potential of the invasive weed *Brassica nigra* (black mustard) was evaluated on seed germination of two common agricultural crops in Tunisia. As such, 100 g of finely powdered dry flowers, leaves, shoots, and roots were separately mixed with distilled water (1 L) and stirred for 24 h before filtration. Then, liquid tissue extracts were used for seed germination trials with *Triticum durum* and *Lactuca sativa*. For each tissue extract, five dilutions were prepared and 2 mL of each concentration were used to soak filter papers in Petri dishes. Then, 20 seeds of *L. sativa* and 10 seeds of *T. durum* were placed on water extract-soaked filter papers and incubated for 120 h at 25 °C in the dark. Distilled water was considered as control and all treatments were in triplicate. The phytotoxic effect of the four *B. nigra* parts on each plant species was estimated by determining the EC₅₀ of the root elongation inhibition (REI). The results revealed that *T. durum* was more sensitive to all *B. nigra* tissue extracts than *L. sativa* with flowers being the more toxic part (EC₅₀ = 0.4 and 9 g/L for *T. durum* and *L. sativa*, respectively). Root extracts were the less toxic for both plant species, mainly for *L. sativa* (EC₅₀ = 39 g/L). HPLC analysis of methanol extracts showed a possible relationship between seed germination and the allelochemicals identified in different *B. nigra* parts.

P NEPD 63

Occurrence of Pea Bacterial Blight caused by *Pseudomonas syringae* PV. *Pisi* in the Eastern Mediterranean Region of Turkey

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Pea bacterial blight caused by *Pseudomonas syringae* pv. *pisi* is one of the most significant disease of peas. The pathogen causes disease symptoms on pea shoots including leaflets, stipules, stem and young pods. Initially, the disease is characterized by water soaked lesions expanding to small, olive-green colored, irregular brown necrosis on leaflets and stems at the basal parts of the diseased plants. Under humid conditions, bacterial ooze can be seen coming out of the pods. In April of 2014, characteristic water soaked lesions becoming irregular brown spots and blight were observed on pea leaflets and pods (cv. Carina and cv. Jof) in the eastern Mediterranean Region of Turkey. Twenty-one gram-negative, grey to white, transparent, smooth bacterial strains were consistently isolated from diseased tissues onto King's B medium. The strains were identified by using pathogenicity tests on pea seedlings and lemon fruits, LOPAT, oxidative/fermentative reactions (Hugh-Leifson), utilization of homoserine and molecular identification using AN7F/AN7R primers. After ten days of incubation, leaflets inoculated with pea strains resulted in water soaked tissue, none symptoms were observed on lemon fruits. All strains classified as *Pseudomonas syringae* LOPAT Group 1a (+----), oxidative and used homoserine as carbohydrate source. In PCR tests, all strains generated 272 bp bands by

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using AN7F/AN7R primer pairs. According to pathogenicity, physiological, biochemical and molecular tests, all strains were identified as *Pseudomonas syringae* pv. *pisi*. In this study, the occurrence of pea bacterial blight were recorded in the eastern Mediterranean Region of Turkey. Studies on reaction of pea cultivars to disease is still undergoing.

P NEPD 64

Damages and psyllids populations dynamic of *Pseudophacopteron* spp. (Hemiptera: Phacopteronidae), pest of *Dacryodes edulis* (Burseraceae) in Yaounde-Cameroon

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Dacryodes edulis (Burseraceae) or safou is a fruit tree native to Africa, sometimes called African or bush pear or plum, Nsafu, bush butter tree, or butterfruit. This plant is gradually transferred in the agricultural landscape, where it is generally planted with cocoa and coffee trees. The oil of fruits of *D. edulis* is a rich source of amino acids and triglycerides. *D. edulis* has potential to improve nutrition, boost food security, and foster rural development and support sustainable landcare. This plant hosted various pests among which they existed, in Cameroon, a complex of four psyllids species: *Pseudophacopteron pusillum*, *P. serrifer*, *P. tamessei* and *P. eastopi*. These psyllids collected sap on *D. edulis* leaves, thus reducing its availability for the growth and the development of the plant. Additional, *P. tamessei* nymphs induced the formation of galls on leaves and, nymphs of *P. eastopi* produced large quantities of whitish flocculent waxy secretions recovering terminal buds of *D. edulis*. The factors regulating the population dynamics of these psyllids species remains unknown in Cameroon. From May 2010 to April 2011, the numerical variations of psyllids population showed three generations of *P. serrifer*, three of *P. tamessei* and five of *P. pusillum* and *P. eastopi*. The most important psyllids outbreaks were obtained in September, December, April and March for *P. tamessei*, *P. serrifer*, *P. pusillum* and *P. eastopi* respectively. From May 2011 to April 2012, we observed two generations of *P. serrifer*, three of *P. tamessei* and four of *P. pusillum* and *P. eastopi*. The most important psyllids outbreaks were obtained in September for *P. serrifer* and *P. tamessei*, in June and October for *P. pusillum* and in September for *P. eastopi*. Among the climatic factors studied, higher temperature induced a reduction of the number of psyllids counted. The number of buds and young leaves of *D. edulis* was positively correlated to the number of psyllids counted. These data of the population dynamic of psyllids pest of safou will be very important for an integrated pest management.

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A Tachinid Fly, the Potential Natural Enemy of the Brown Marmorated Stink Bug (*Halyomorpha halys*) in Korea

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The tachinid flies, *Pentatomophaga latifascia*, (Diptera: Tachinidae) were collected from the overwintering adults of the brown marmorated stink bugs (BMSB), *Halyomorpha halys* (Hemiptera: Pentatomidae), which has been introduced and became a destructive invasive pest in North America. In the original place of BMSB, we have recognized this tachinid parasitoid as the potential biological agent of BMSB. The morphological illustration and the parasitoid biology are presented.

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Optimizing the culture conditions for *Myrothecium roridum* Tode in relation to its virulence behavior against Bitter gourd

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The relationship between *Myrothecium roridum* physiology and virulence was investigated by conducting a series of interlined experiments on growth medium, temperature, pH and photoperiod whereas relation of culture age with virulence was measured by fungal development on young leaves of bitter gourd. Physiological response was measured on colony radial growth and sporodochia production. The optimum medium for mycelia growth and sporodochia production was PDA amended with pH 6.0 incubated at 25±2 °C with alternate light and dark photoperiod. While correlating mycelia growth and sporodochia production with virulence on bitter gourd young tender leaves it was highest in 5-6 day old culture. It was concluded that sporodochia production is more reliable tool for *in vitro* virulence evaluation of *M. roridum* against bitter gourd.

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Augmentative biological control of cassava mealybug, *Phenacoccus manihoti* Matile-Ferrero (Hemiptera: Pseudococcidae) in Thailand

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The cassava mealybug, *Phenacoccus manihoti* Matile-Ferrero (Hemiptera: Pseudococcidae) one of the most serious pests of cassava worldwide, has accidentally occurred for the first time in Thailand in 2008. More than 100,000 hectares of cassava plantations covering every part except Southern Thailand were reported its serious outbreak 2 years later which caused the catastrophic damage and worry to relating agro-industries. Hence, a biological control project was immediately initiated challenging the sustainable control of the pest. Later, approximately 3,300 hectares of cassava plantations infested by the mealybug were promoted for the use of natural enemies. With a total of 54.63 million native natural enemies in 3 highly potential species, namely, *Plesiochrysa ramburi* (Schneider) (Neuroptera: Chrysopidae), *Mallada basalis* (Walker) (Neuroptera: Chrysopidae) and *Allotropa suasaardi* Sarkar & Polaszek (Hymenoptera: Platygasteridae), were mass-produced. Dissemination and augmentative releases were attained. Subsequently, one hundred intensive training and technology transfer courses were provided for 1,758 cassava farmers' participation, to exhibit the application of the biological control agents. Additionally, four naturally occurring of the mealybug plots, eight acres each, were conducted to demonstrate the biological control applications, with paired treatments, release and unrelease (control) of the natural enemies. There was a significant lower population density of the mealybug in treated plots than those in untreated plots. Cost/benefit analysis of biological control was eventually completed, farmers' conventional practice gained maximum profit of 1,055 USD/hectare/year whereas the one obtained from release of natural enemy treatment did 1,430 USD/hectare/year with the annual difference of 375 USD/hectare. Final assessment of the project was accomplished both in the satisfaction, in terms of questionnaire, of the participating farmers and the control of the pest insects. Eighty three percentages of the participants adopted the biological approach rather than chemical control and mechanical control. Also after the training, they were interested in culturing the natural enemies for filed release. Satisfaction of the participants was moderate but it was significantly different ($P < 0.05$) between before and after the training.

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Vegetable insect pests of Ghana; New invasive insect species and implications for exports

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The current global trends of climate change and trade volume increment have led to the introduction of new insect pest species of both agricultural and health concerns in Ghana. Currently some of the major insect pests associated with vegetable production in Ghana are garden egg borer, *Leucinoides orbonalis* (Lepidoptera: Pyralidae), white flies, *Bemisia tabaci* (Homoptera: Aleyrodidae), Cotton aphid, *Aphis gossypii* (Homoptera: Aphididae), Cabbage moth, *Plutella xylostella* (Lepidoptera: Plutellidae), fruit flies, *Batrocera invadens* (Diptera: Tripitidae), *Thrips* (Thysanoptera: Thripidae).

Tomatoes, pepper, garden eggs and cabbage are some of the popular vegetables with serious insect pest concerns in Ghana. However, indications from preliminary studies indicate the possibility of new introductions into the ecological set-up of Ghana.

These newly observed insect pest species found on Ghanaian farms in 2014 are the melon thrips, *Thrips palmi* and the Eggfruit caterpillar, *Sceliodis cordalis* which is found mostly in Australia and New Zealand.

The complex nature of insect pest and its concomitant new introductions have given rise to serious safety concerns of Ghanaian vegetables, especially those for export.

The paper explains results of field studies from 2010 to 2014, examines the complex nature of insect pest fauna associated with crop production in Ghana, and draws implications for export of Ghanaian vegetables and fruits in future.

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Varietal differences in Fruit Flies responses of some fruits and vegetables in the forest ecological zone of Ghana

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Fruit and vegetable genetic resources are essential to ensure horticultural productivity, particularly in developing countries where numerous constraints affect production. Fruits and vegetables are among the high value crops that offer smallholders in developing countries opportunities to substantially boost income and alleviate poverty. However, their yields values in sub-Saharan Africa are low compared to worldwide figures. High insects' damage with respect to fruit flies amongst other insects are major causes of low yields.

Fruits and vegetable crop varieties for the lowland tropics should combine potential for high yields of good quality and nutritious fruit under multiple pests' resistance, particularly to fruit fly. Comparative field evaluation trials revealed differences in degree of fruit fly damage in some fruits and vegetables. Varietal differences were observed in terms of fruit fly damage hence both marketable and unmarketable yield. This study gives an insight into the degree of infestation and possible solutions to bring down the damages.

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Evaluating the Effects of Two Fungicides to manage Firstly Recorded *Alternaria radicina* on Date Palm Leaves in Wasit in the Middle of Iraq

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Three *Alternaria radicina* (Meier *et al.*) Neerg isolates (A1, A2, and A3) were isolated from date palm, *Phoenix dactylifera* L. leaves and identified as the causative pathogen of black spot. Two fungicides (Bavistin and Tachigazole) were used in vitro to measure their ability to control this pathogen. Study results revealed that both chemical fungicides expressed high level of inhibition in fungal radial growth especially at the concentration 100 ppm and the lowest growth was at the concentration 5 ppm among all three isolates in both two fungicides. The fungicide Bavistin revealed higher inhibition rate than Tachigazole with lowest LC50 (12.21) at the isolate A3. Tachigazole treatment showed high level of variation at all concentrations.

Figure 1

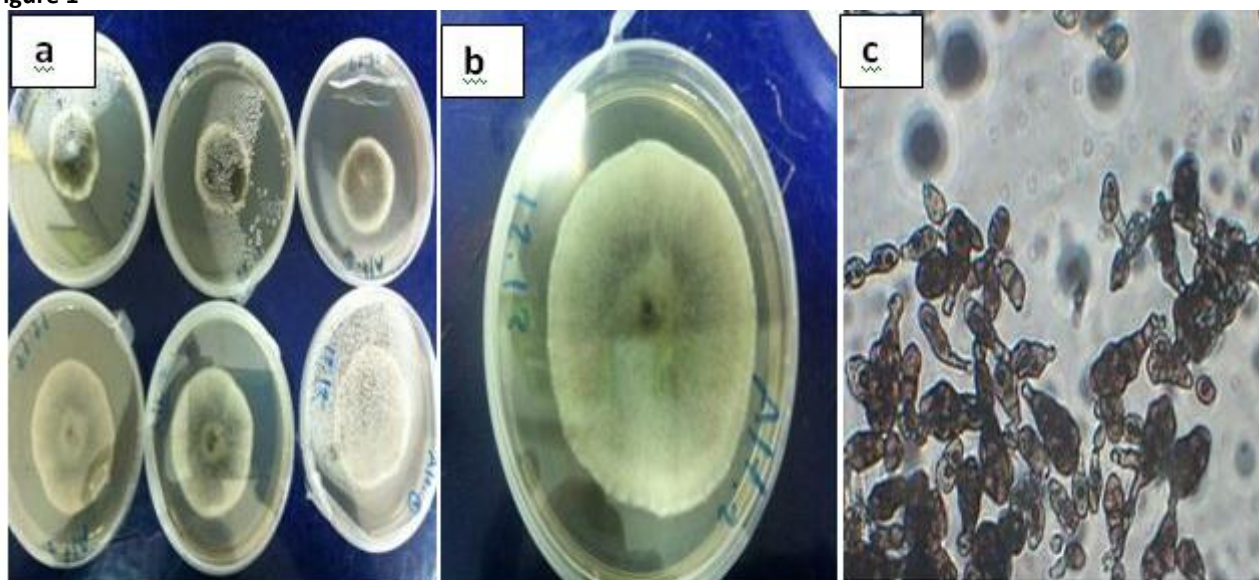


Image1: a) *A. radicina* (A1, A2, A3) single spore isolates b) *A. radicina* colony shape c) *A. radicina* spores under 40x microscope camera

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Figure 2

Table 1: Radiation growth of *Alternaria radicina* three isolates in 10 days time after treating them with the chemical pesticide Bavistin with five concentrations.

Date	% inhibition with Concentration ppm (A1)				
	5 ppm	25 ppm	50 ppm	75 ppm	100 ppm
2 days	15.6085 a	37.3016 a	37.3016 a	29.8942 a	100 b
4 days	40.6148 a	58.3084 b	61.9848 b	62.5408 b	90.224 c
6 days	40.3704 a	64.1481 b	62.6667 b	66.2222 b	90.4074 c
8 days	26.1425 a	63.8575 b	59.5954 b	65.265 b	89.8974 c
10 days	30 a	51.8519 b	59.2593 b	62.963 b	89.8148 c
(A2)					
2 days	25 a	38.0556 a	40.4167 a	38.1944 a	100 b
4 days	8.5101	41.5657	40.0758	47.2222	87.75
6 days	9.6342	33.9285	47.4825	49.2747	80.9464
8 days	25.1389	49.4775	56.8122	57.8571	83.2672
10 days	21.6021 a	45.5728 b	44.5306 b	57.8295 b	81.6753 c
(A3)					
2 days	17.2078 a	34.9928 a	21.9336 a	31.8543 a	100 b
4 days	26.7035 a	44.1909 a	38.8227 a	51.3227 a	94.0656 b
6 days	39.1532 a	54.8402 ab	53.2418 ab	62.5523 bc	80.6613 c
8 days	41.455 ab	62.5529 b	56.1905 ab	63.1614 b	83.2672 c
10 days	42.7821 a	57.2093 a	54.2636 a	62.7649 a	82.3652 b

Table 2: Radiation growth of *Alternaria radicina* three isolates in 10 days time after treating them with the chemical pesticide Tachigazole with five concentrations.

Date	% inhibition with Concentration ppm (A1)				
	5 ppm	25 ppm	50 ppm	75 ppm	100 ppm
2 days	15.6085 a	37.3016 a	37.3016 a	37.3016 a	100 b
4 days	40.6148 a	58.3084 b	61.9848 b	62.5408 b	87.626 c

6 days	40.3704 a	64.1481 b	62.6667 b	66.2222 b	81.8148 c
8 days	34.0741 a	61.1111 b	61.4815 b	64.8148 b	82.963 c
10 days	31.4815 a	60.3704 b	60.1852 b	64.0741 b	81.7407 c
(A2)					
2 days	35.7888 a	50.6238 ab	54.4372 abc	64.4269 bc	71.5037 c
4 days	38.4524 a	42.5053 a	52.8766 a	55.4644 a	80.1847 b
6 days	34.7277 a	39.6324 ab	44.9483 ab	54.872 b	76.2745 c
8 days	32.2222 a	39.6296 ab	47.037 bc	56.6667 c	77.4074 d
10 days	31.1111 a	37.7778 ab	47.037 bc	55.9259 c	76.5556 d
(A3)					
2 days	42.931 a	49.1879 ab	74.3817 bc	61.7017 c	97.656 d
4 days	40.1635 a	45.5111 a	67.8298 b	67.2783 b	77.5292 b
6 days	43.9499 a	41.1766 ab	58.1425 bc	71.7254 cd	77.0596 d
8 days	41.8519 a	41.8519 a	60 b	72.5926 c	78.5185 c
10 days	38.1481 a	40 a	58.7037 b	70.5556 c	77.963 c

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Winning the engame: Within-host interactions among three fungal parasites of *Brontispa longissima*

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The negative aspects of traditional pest control, which involves the use of harmful chemicals, have led to the scrutiny of alternative methods such as the utilization of non-hazardous biocontrol agents. *Brontispa longissima* (Gestro) is a noxious coconut pest, whose infestation has been responsible for the reduced fruit production and damage of more than three million coconut trees across the country. We evaluated the pathogenicity of entomopathogenic fungus (EPF) *Metarhizium brunneum* (Petch) (*M.b*) and two *Aspergillus* spp. of sections *Fumigati* (*Asp01*) and *Flavi* (*Asp02*) against *B. longissima* and determined the nature of interaction inside a single host. Single species inoculation was performed and dynamics of mixed infection was checked by formulating suspensions of the EPF with each of the *Aspergillus* spp. *M.b* and *Asp01* showed significant pathogenic effects toward *B. longissima*. However, the efficiency of *M.b* to infect larvae and adult *B. longissima* is altered in the presence of interacting fungal species, whereby *Asp01* acted as the EPF's antagonist while *Asp02*, though did not directly interact with *M.b*, out-competed the EPF in terms of proliferation.

The results gathered may pave the way towards exploitation of new and more efficient control strategy to specifically curb *B. longissima* infestation -the first study to report *Aspergillus* spp. infection on *B. longissima*

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First Report of *Neofusicoccum parvum* and *Diplodia* sp. Associated With Wood Canker and Dieback on Almond in Turkey

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During, a survey in 2014, plant specimens were collected from different almond trees from Adana and Hatay provinces showing defoliation, blighted and sunken necrotic bark lesions with gumming, which progressed into the trunk resulting in the death of large sections of almond. Transversal sections of branch and trunk from symptomatic trees revealed brown V-shaped and/or necrosis on xylem cankers of hard consistency. Transversally cut symptomatic branches were divided longitudinal into small pieces (5-8 mm) from the edge of necrotic and healthy tissue, then surface sterilized with 5% (v/v) sodium hypochlorite for 5 minutes. Later all pieces were rinsed three times with sterile distilled water and dried for 5. Pieces of symptomatic tissue (2 mm²) were plated onto potato dextrose agar amended with 0.01% tetracycline (PDA-tet) and incubated at 25°C for 5 days. All

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isolates were identified by morphological and molecular characteristics. Most isolates initially identified by morphological characteristics, such as growth pattern, speed of growth, and colony color, resembled those in the Botryosphaeriaceae (Slippers et al. 2006.) Sequences obtained from amplification of the internal transcribed spacer ITS1, 5.8S, and ITS2 region the β -tubulin gene were compared in a BLAST search in GenBank. Results identified isolates as *Neofusicoccum parvum* (identity of 100 % to KF778854-KF778860 and 99% to KF778949 for ITS and β -tubulin, respectively). The Sequences of *N. Parvum* were deposited in GenBank (KF494356, KF494357, KF494358, KF515953, KF515954, KF515955 for ITS Region and β -tubulin respectively). Colonies of *Neofusicoccum parvum* on PDA-tet is white, flattened with tufts of white mycelium, becoming plain bright greenish grey color after 2 weeks with the reverse side of the colonies greenish black. Conidia were ellipsoid with apex round and base flat, unicellular, old conidia becoming 1-2 septate hyaline, or light brown with middle cell darker than the terminal cells, and averaged 13,83 x 6 μ m (n=50). Colonies of *Diplodia* sp. on PDA-tet performed gray-brown with dense aerial mycelium producing brown cylindrical to ellipsoid conidia rounded at both ends and averaged 22,61 x 9,7 μ m (n=50). Black colored pycnidia were also observed on the cankered tissues. Young conidies are hyaline, old conidia becoming dark brown. Identity of the different taxa was confirmed by sequence analysis of the internal transcribed spacer (ITS1-5.8S-ITS2) region of the rDNA and part of the beta tubulin gene. Blast analysis of sequences indicated that 3 isolates were identified as *Neofusicoccum parvum* (GenBank: KF494356, KF494357, KF494358, KF515953, KF515954, KF515955).

Pathogenicity tests were conducted with all three isolates on detached shoots (15cm length) from healthy almond trees of the same cultivar/rootstock. One wound per shoot was made in the middle of the on one year-old stem cuttings using a 4-mm diameter cork borer and the wounded surfaces were inoculated with 4-mm diameter mycelial plugs of 10-day-old cultures of each isolate growing on PDA. Inoculated wounds were wrapped with parafilm. Control shoots were inoculate with sterile PDA plugs. Inoculated shoots were incubated at at 25 °C in moist chambers for 2 weeks. Lesions similar to those on the original infected shoots were observed on all inocuated shoots except the control treatments. Reisolation and symptom development on samples were examined for canker formation, the extend of vascular discoloration, and recovery of fungal isolates. *Neofusicoccum parvum* caused sunken necrosis both on the bark and xylem tissues longitudinally and widely. *Diplodia* sp. caused necrosis on the xylem tissue only. To our knowledge this is the first report of *Neofusicoccum parvum* and *Diplodia* sp. associated with almond dieback in Turkey.

References: (1) B. Slippers et al. Study. Mycol. 55:235, 2006.

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Estimation of Prevalence of Septoria Leaf Blotch Disease in The Eastern Meditteranean Region of Turkey

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In this study, 260 different wheat fields (8.270.000 m² area) were surveyed for the estimation of prevalence of septoria leaf blotch disease in the cities of Eastern Medditerranean Region. Out of 100 % of total wheat crop fields, 42.3% (5.478.000 m²) of wheat fields were infected with septoria leaf blotch disease. The mean value of septoria leaf blotch disease virulence was 11.62 %. Out of 257 diseased wheat crop samples, 74 isolates were obtained. Forty seven isolates from Adana, 3 isolates from Mersin, 3 isolates from Osmaniye, 14 isolates from Hatay and 7 isolates from Kahramanmaraş provinces were cultured on PDA-tet plates. The cause of disease agent was identified based on microscobic observation of morphological structure and molecular (PCR and sequences of ITS) analysis methods. Consequently, 15 isolates were sequenced for 5' and 3' ends of ITS and nblast of the ITS sequences has revealed that *Septoria tritici* was the agent of septoria leaf blotch disease. The highest disease prevalence (81.25%) was observed at Osmaniye, and then followed respectively Adana with 57.5 %, Mersin with 32.3 %, Hatay and Kahramanmaraş with 20.5 %. Based on septoria leaf blotch disease scoring scales, the highest disease prevalence (47.2 %) was found on scale 1, and then followed by 13.6 % in scale 2, 7.3 % in scale 3, 2.3 % in scale 4 and 1.25 % in scale 5.

References: (1) A.M. Prestes. Washington State University, Thesis (MSc. 53 (1974).

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Determination of Host Plants, Population Development and Infestation Ratio of Tomato Moth (*Tuta absoluta* Meyrick) (Lepidoptera: Gelechiidae) in Semi-arid Regions

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Tomato is the second most important vegetable crop next to potato in the world. In Turkey, Tomato Moth (*Tuta absoluta* Meyrick) (Lepidoptera: Gelechiidae) was first observed on tomato plants in 2010. This pest rapidly spread during the following years in all tomato growing areas and became an important pest of tomato plants. Host plants, population development and infestation ratio of Tomato Moth were identified by several researchers in Konya province which has a semi-arid climate. According to these studies, it was determined that the pests fed on potato and *Chenopodium album* L. beside tomato. The maximum adult numbers on pheromone traps in greenhouses and open-fields were found weekly as 640 and 455, respectively. On ferolite traps which are the combination of light and pheromone traps, in greenhouses and open-fields, maximum numbers of adults of Tomato Moth were recorded weekly as 1525 and 1000, respectively. The infestation ratios of the moth in greenhouses and open-fields were determined 80 and 37%, respectively. The pest rapidly spread and damages the tomato plants in semi-arid regions along with the Mediterranean climate regions.

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The Acar and Insect Fauna of Squash (*Cucurbita pepo* var. *pepo* L.) Area in Gulagac Town of Aksaray Province (Turkey)

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In Turkey, Cucurbitaceae species constitute 23% of the vegetable production. The squash, (*Cucurbita pepo* var. *pepo* L.), has considerable economic value and usually has been producing in the eastern part of Central Anatolia; Kayseri, Aksaray, Nevsehir provinces etc.

The present study was carried out in Gulagac Town of Aksaray where squash is nearly monoculture. In 2010 growing season, to determine the harmful and beneficial acar and insect fauna on the squash areas; surveys were started in June 13 and continued by six-eight day intervals for collecting; sweeping net and checking directly plant material were used as methods. As a result; 8 harmful insect species from 6 families of four order and 8 beneficial insect species from 5 families of 3 order were determined. From the pest species, *Empoasca decipiens* Paoli, *Tetranychus urticae* Koch., *Thrips tabaci* Lind., *Myzus (Nectarosiphon) persicae* Sulzer, *Aphis gossypii* Glover, *Aphis nasturtii* Kaltenbach and between the beneficial species *Coccinella septempunctata* L. *Adonia variegata* Goeze ve *Chrysoperla carnea* Step. were the common insect and acar species. *Empoasca decipiens* Paoli was the most important and dominant pest of squash in the area. Consequently It can be advised that the future work should be done on this species together vector aphids.

This study was summarized from MS thesis of Gulbeyaz Karakaya Keles

This study was supported by Selcuk University, Coordinatorship of Scientific Research Projects

P NEPD 76

The Cixiidae and Cicadellidae (Hemiptera) Species Harmful on Vegetables from Solanaceae in Konya Province' Turkey

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Due to the occurrence of stolbur diseases on Solanaceae plants in epidemic proportions every few years in Central Anatolia, this study was carried out in 2006-2007 to determine possible vectors of the diseases and like others from Hemiptera. In addition, the population development of dominant species on tomato, pepper, eggplant and potato plants from Solanaceae in Meram District which the vegetable production is highest in Konya Province of Turkey, were observed to collect some information useful for their especially early control. Collecting the samples was conducted in both years, but regular observations for population development were done only 2007. As a result; 1738 samples from Cicadellidae and 30 from Cixiidae were collected. While 15 species were determined from Cicadellidae, *Hyalestes obsoletus* Signored from Cixiidae was identified. According to the 2007 results, the most common species were *Zyginidia sohrab* Zachvatkin (53%), *Empoasca decipiens* Paoli (41%) and *Psammotettix striatus* (Linnaeus) (3%), respectively. These species were occurred on Solanaceae vegetable plants during June, July and August.

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Z. sohrab was peaked in mid-June. Whereas a drop population observed in July and August then it suddenly increased at the beginning of September. The most numerous insects was observed on Potato (50.6%), pepper (16.8%), eggplant (16.7%) and tomato (15.7%) from the tested Solanaceae plants, respectively. The high preference to the potatoes were probably due to its earliness and having soft and hairless leaves. In addition, different factors same as chemical contents of the plants can be mentioned. While, *Z. sohrab* was dominant species on other three plants, *E. decipiens* was on potato with the rate of 50.4% and then followed by *Z. sohrab* at level of 43.4%. *Solanum nigrum*, *Datura stramonium* and *Athropa belladonna* were determined as common weed species from Solanaceae in the district. *E. decipiens* (62%), *Hyalesthes obsoletus* (10%) and *Z. sohrab* (9.5%) were collected from them, too. *Chrysopa carnea* Sch (Chrysopidae:Neuroptera), *Coccinella septempunctata* (L.), *Coccinula quatuordecimpustulata* (L.), *Scymnus bivulnerus* Capra and *Adalia* sp. (Coccinellidae:Coleoptera) were dominant predators of leafhoppers. No parasitoid was found.

This study was summarized from MS thesis of Ertan Ahmed

This study was supported by Selcuk University, Coordinatorship of Scientific Research Projects

P NEPD 77

Biology of the black-headed caterpillar, *Opisina arenosella* Walker (Lepidoptera: Oecophoridae) the invasive species pest of coconut and its natural enemies in Thailand

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The black-headed caterpillar, *Opisina arenosella* Walker was native in India, Bangladesh and Sri-Lanka. The first recorded as invasive species pest of coconut was found in Prachuap Khiri Khan province, the southern part of Thailand in December 2008. Due to the devastating outbreak in 2010, gathering the information of this insect pest and natural enemies are essential. Accordingly the insects were reared on coconut leaves, to study their biology. Female adults laid yellowish creamy color eggs in mass. The newly hatched larva was orange-red and then turned to pale yellow, with dark brown head. Three brown lines ran along the lengthwise of the body. Thorax color was paler than that in head and legs. The oval shape pupae were dark brown. Head, antennae, wings and abdomen of adult were light gray. Female was larger than males. Average number of egg laid was 83.40+14.31 /female until death. Egg duration averaged 4.90+0.55 days. Larvae of *O. arenosella* had 10 to 13 instars with a larval period 57.67+9.25 days. The pupal period was 9.08+0.90 days. Longevity of male and female were 10.33+5.01 and 9.00+1.22 days, respectively. Mean duration life cycle was 80.45+10.46 days. Further study on alternative host plants of *O. arenosella*, disclosed a total of 11 species; 10 species of Palmae and one species of Musaceae. Additionally, five species of parasitoids and two species of insect predators were collected in the outbreak areas. Among of these natural enemies *Braconbebetor* Say (Hymenoptera: Braconidae) shown as a promising natural enemy. Its biology and comparative field study on the control tendency of *B. hebetor* as a biocontrol agent for *O. arenosella* was conducted in the lab at Natural Biological Control Research Center and in coconut plantations in the outbreak areas from January-July 2011. Results indicated that *B. hebetor* tends to be a potential parasitoid species in controlling *O. arenosella* in Thailand.

P NEPD 78

Epidemiology of carrot pathogen *Candidatus Liberibacter solanacerum* haplotype C in Finland

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Introduction: *Candidatus Liberibacter solanacerum* (CLso) has been detected in carrot psyllids (*Trioza apicalis*) and the psyllid-damaged carrots in Finland. Carrot psyllid was shown to transmit CLso bacterium to carrot in greenhouse, leading to psyllid feeding-associated leaf curling and CLso infection-associated leaf discolouration (Nissinen et al. 2014). Epidemiology of the CLso haplotype C occurring in the Nordic countries is still unknown. We studied the distribution of the bacterium in carrot fields and we are performing experiments on the effect of temperature on the multiplication of CLso. Carrot psyllid dispersal was also monitored during the psyllid flight peak in summer.

Objectives: The aims of this study were to assess the current distribution of CLso in Finland and to unravel the factors affecting the disease dispersal.

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Materials and methods: Plant sampling was performed in different locations in Finland, following the sampling scheme of the Finnish Food Safety Authority Evira. Carrot psyllid flight was monitored once a week during the growth season by yellow sticky traps placed in a grid at different distances from the carrot field edge. To study the effect of temperature to the colonization of carrots by CLso, carrot plants inoculated via carrot psyllid feeding are grown in greenhouse at three different temperature regimes. The distribution of the bacteria within the plants is tested at several time points. DNA was extracted from plant material by CTAB method and from insect material by DNeasy Blood and Tissue kit (Qiagen). The presence of CLso in the field samples of carrots and psyllids was tested by conventional PCR, and in the greenhouse experiments qPCR was used to determine the relative amounts of bacteria in the carrot and psyllid samples.

Results: CLso haplotype C was detected in carrots and carrot psyllids in several locations in the southern Finland, and the CLso occurrence in carrots correlated with the observed area of distribution of the psyllid vector. The results of this study will help to assess the risk of further spreading of this plant pathogen and to decide which measures need to be taken to restrict it from spreading.

Conclusions: CLso occurs in carrots in southern Finland in the areas where the vector *T. apicalis* is frequent, and thus special attention should be paid in the psyllid control.

P NEPD 80

Quantification and Feeding Performance of American bollworm, *Helicoverpa armigera* (Hub.) Against Different Cotton Cultivars

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Cotton, *Gossypium hirsutum* L., known as “white gold” is a vital backbone and cash crop of Pakistan. The average revenue of cotton in Pakistan is much lower than other cotton growing areas of the world. The low yield of cotton is attributed to many factors, but the most severe one is the intensity of insect pests attack. Besides sucking insect pests, American bollworm, *Helicoverpa armigera* (Hub.) cause considerable losses to this crop. The objectives of this study were to quantify the amount of plant tissue consumed and to check the feeding performance of this notorious pest. Three Bt (Bt CIM-599, Bt CIM-602 and CIM-616) and one non-Bt (CIM-554) cultivars were sown in field and tested. Three plants of each cotton cultivar were selected randomly and replicated thrice. American bollworms were reared in laboratory with standard rearing protocols. Larvae of same size and instar were used in this study. An upper and lower leaf of each plant was caged with cloth along with single larvae of *H. armigera*. The leaf areas were measured before and after feeding of larvae using CI-202 Portable Laser Area meter (CID Bio-Science, USA). The data were recorded after 48 and 72 hours to quantify the amount of leaves consumed. Non BT-CIM 554 was consumed the most. The BT cultivars were eaten less with BT-CIM 616 with least amount consumed. This study will be helpful in devising appropriate management techniques for the control of this notorious pest of cotton and will aid in recommending the suitable cotton cultivar for growers.

P NEPD 81

Occurrence of *Fusarium subglutinans* Causing Leaf Spot Disease on *Cymbidium* Orchids in Korea

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Introduction: During 2006-2010, some leaf spots were observed on *Cymbidium* species cultivated in greenhouses located in Taean, Gapyeong, and Gongju in Korea. Tiny yellow spots were initially detected and later turned dark brown to black with surrounding yellow halo on the upper side of the leaves. The lesions became larger and sunken with dark brown raised edges. In advanced stage, the centers of the leaf spots more than 50 mm in size fell out, leaving holes in the older lesions. The disease finally resulted in leaf deformation.

Objectives: The aim of this study was to identify the causal agent based on morphological characteristics, molecular analysis, and pathogenicity test.

Materials and methods: A total of five fungal isolates were obtained from different collections. Fungal structures were examined under light microscope. A portion of translation elongation factor 1-alpha (TEF1) gene was amplified with primers, EF1 and EF2. A neighbor-joining tree was generated based on the TEF1 sequences.

Results: Morphologically, conidiophores were unbranched or branched with bearing monophialides and polyphialides. Microconidia were aseptate, ellipsoid to allantoid, and 8-18 × 2.5-3 μm. Macroconidia were straight to slightly curved, 3-5-septate, and 32.5-75 × 2.5-4 μm. The morphological and cultural features of the causal fungus were consistent with the

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description of *Fusarium subglutinans*. A NCBI BLAST analysis showed high similarity (97-98%) to the sequences of *F. subglutinans* available in GenBank. In the phylogenetic tree, the present isolates were grouped into a clade consisting of *F. subglutinans* isolates. Accordingly, the molecular data verified the identity of the present isolates. Pathogenicity test carried out three times confirmed the Koch's postulate.

Conclusion: There have been no previous records of *Cymbidium* orchids associated with *F. subglutinans* in Korea, whereas the species has been known to occur on the plants in New Zealand and Japan. As the leaf spot disease may pose a serious threat to commercial *Cymbidium* growers, adequate disease control is required to reduce economic losses.

P NEPD 82

The Sprout/Seed-Potato Technology: Fourth Brazilian Quarantine Approval, for Canada Imported Sprouts Shipped for Seed-Potato Research-Risk Analysis Purposes

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Introduction: The Sprout/Seed-potato (*Solanum tuberosum*) technology (1), has been producing (mini)tuber/seed-potato stocks, just like tissue-culture "pre-basic" plantlets, similarly grown in isolation from soil, under protected/insect-proof greenhouses (2,3). After 3 successful years (2007-09) on Canada research-exports of sprout/seed-potato to Brazil (4), the sanitary agency of Brazilian Ministry of Agriculture (MAPA-DSV) has been undergoing mandatory sanitary risk analyses in order to commercial imports of sprouts/seed-potato. Therefore, a 4th import of Canada sprout/seed-potato was approved (permit # 314/08-07-2013), for sprouts originated from certified "ELITE": G-1 and G-2 tuber/seed-potato lots, i.e., the first 2 field generations, from Nuclear Stock or Pre-ELITE (5).

Methodology: On June 2014, in a non refrigerated box, 12 zipper clear "lunch" bags, 100 sprouts each (5 - 10 cm high) was FedEx shipped from Pommes de Terre Berube, Inc / Trois-Pistoles, Qc, Canada), to Importadora de Sementes Resultado Ltda, Santos, Sp (Brazil). Two out of the 12 bags contained G-1 and the other ten, G-2 certified sprout of potato cv Atlantic. Upon arrival (4-day transport), at Viracopos Airport, Campinas, SP; passing a regular inspections, the sprouts were taken to the accredited quarantine at APTA-IAC. About 50 days of growth in pots (0,5 kg substrate), all plants were inspected-tested by specialists on insects, bacteria, fungus, virus, and nematode. No quarantine nor regulated potato pathogens were detected (MAPA-IN 32/20-11-2012).

Results and discussion: Although unexpected loss of 30-35% of the sprouts in each bag, associated to near 2 months cold room storage, until import permit was issued, all 65-70% of the sprouts/bag (from G-1 and G-2) germinated similarly well: vigorous, healthy, with negative results to mandatory potato pathogen and pest analyzed; receiving the Quarantine Approval Certif. # 20533/2004 SVA/VCP. At 90-day growth, 2-3 tubers, sizing 2-6 cm were produced/sprout. From the three previous comparisons, no statistical differences are expected on tuber/seed-potato field production (1,4). These quarantine negative results for the 4th sprout/seed-potato evaluation from Canada, sustains similar risk analysis treatment as to conventional tuber/seed-potato. Advantages for import of sprouts as compared to tuber/seed-potato are: reduction on freight as well as on the risk of new and quarantine tuber-soil borne pathogens. Brazil is coming to pioneer a suitable plant protection technology on the export-import seed-potato marketing.

References:

(1)Souza-Dias et al., EAPR-2008. Brasov, Romenia, p. 184-187)

(2) http://www.unece.org/fileadmin/DAM/trade/agr/wgroups/ge_06/ncs_schemes/ncs_all_2004_03_31.pdf ;

(3)<http://www.potatoescanada.com/seed-potatoes.aspx>;

(4) Souza-Dias et al., 2010. Am. J. Pot Res (2010) 7:83-147);

(5) http://laws-lois.justice.gc.ca/eng/regulations/C.R.C.,_c._1400/page-17.html#h-38).

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Figure 1

At FEDex – Viracopos Airport, Campinas, SP Brazil, on the 22/07/2014 (5th day after arrival at the airport). Showing the Sanitary Inspection as report by local Ministry of Agriculture (MAPA) officer, addressed to IAC-Quarantine



Figure 2


 MINISTÉRIO DA AGRICULTURA, PECUÁRIA E ABASTECIMENTO
 SUPERINTENDÊNCIA FEDERAL DE AGRICULTURA EM SÃO PAULO
 SERVIÇO DE SANIDADE VEGETAL

LIBERAÇÃO DE QUARENTENA
148/2014

Interessado: Importadora de Sementes Resultado Ltda	CNPJ: 09.069.219/0002-64
Endereço: Rua Martin Afonso, 101 - 4andar- sala 45 - Santos/SP CEP 11010-000.	Telefone:
Mário Karl Imark	

Nos termos do artigo 12 do Regulamento de Defesa Sanitária Vegetal, do Decreto nº 24.114, de 12 de abril de 1934 e da Instrução Normativa nº 01 de 15 de dezembro de 1988 foi prescrita a quarentena do artigo regulamentado na Estação Quarentenária do Instituto Agronômico de Campinas. Durante a quarentena **não foram encontradas pragas quarentenárias**, conforme o laudo emitido. Desta forma, o artigo regulamentado abaixo discriminado está liberado para uso pelo interessado.

Nº de Processo: 21052.005628/2013-42	Nº de Autorização DSV: 314/2013 de 08/jul/2013
Prescrição de Quarentena/SVA: nº 20533/2014-SVA/VCP	Termo de Fiscalização/SVA: nº 20533/2014-SVA/VCP
Quarentena IAC nº 083/2014 ; (E-57.726 a E-57.727)	Início Qua: 23/jul/2014
Laudo de Quarentena IAC LN°148/2014	CDM: não
País de origem: Canadá	País de Procedência: Canadá

Artigo Regulamentado; referência/lote	Nº acessos	Quantidade
Broto Batata (<i>Solanum tuberosum</i>) Lote: Atlantic Elite G1 e G2	2 acessos	Importada: 5956 unidades (5,5kg)

Observações: Embarque único. Encerrar processo.

Campinas/SP, 03/out/2014.


 Rita Lourenço
 Fiscal Federal Agropecuário
 UTRA-Campinas/SFA-SP

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P NEPD 83

Assessment of damage, infestation level and susceptibility of Mango Cultivars to the Fruit Flies infestation in Shendi Area, Sudan

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Fruit flies are serious pests of Mango in Sudan; they cause substantial damage to Mango production, both qualitatively and quantitatively. This study is an effort to establish information about the pest and, to test the susceptibility of some Mango cultivars (Baladi, Kitchener, Shendi, Alphons, Abu Samaka and Bit abusamaka) to the pest infestation. A survey was carried out at Shendi area, River Nile State, during the period of May 2009 to July 2010, using a homemade trap resemble *lynfield* traps with attractants (Methyl eugenol, Trimedlure, cue lure and Nu lure) to identify the fruit flies species found in the area and to study the seasonal abundance of the pest species and to determine the infestation percentage of different Mango cultivars. Results indicated that the fruit fly *Bactrocera invadens* is present all year-round in the study area, with population peak July to September. The study showed that Guava (*Pseidium guajava*) represents the best alternative host for the pest. Abu samaka and Bit Abusamaka mango cultivars were the most susceptible, with 100% infestation, followed by Shendi 70 %, Kitchener 54%, Alphons and Baladi (30% ,28%, respectively) were the least susceptible. Also results revealed that females of both species *Ceratitis cosyra* and *Bactrocera invadens* out numbered males 4-to5 times.

P NEPD 84

***Phyllosticta* species from banana (*Musa sp.*) in Chongqing and Guizhou Provinces, China**

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Six *Phyllosticta* strains were isolated from diseased leaves of *Musa* species in Chongqing and Guizhou provinces, China. Morphological and molecular analysis of LSU and combined ITS, ACT, TEF-1, and GPDH gene sequences, identified these strains as *P. capitalensis* (3 strains), *P. musarum* (1 strain) and two isolates were distinct from known *Phyllosticta* species. These isolates are herein introduced as *Phyllosticta musaechinensis*.

Weakly pathogenic on leaves of *Musa* sp, slightly discoloring leaves yellow, with black, shiny conidiomata forming on healthy green, or yellowing parts of leaves. Sexual state: Unknown. Asexual state: Pycnidia 45-145 μm (93 μm) diam. subcuticular to erumpent, solitary or clustered in small groups, black, shiny, globose or subglobose, with a rounded ostiole at the center. Conidiogenous cells cylindrical or conical. Conidia 14-18 \times 8-12 μm (17 \times 10 μm), hyaline, aseptate, coarsely guttulate, ellipsoidal or clavate, thin- and smooth-walled, surrounded by a mucilaginous sheath 0.5-3.5 μm thick, apex tapering, straight to curved, appendage 4.0-18.5 μm (12 μm) long. Spermatial state: unknown.

Colonie on PDA bluish black to black, without aerial mycelium, irregular, raised to about 0.7 mm, reaching 14.2-12.5 mmdiam after 60 d at 28°C. Pycnidia solitary or aggregated in colony, black. Conidia 15.5-22.5 \times 8.5-13 μm (18 \times 11 μm), hyaline, aseptate, coarsely guttulate, ellipsoidal, clavate or irregular, thin- and smooth-walled, surrounded by a mucilaginous sheath or not, apex tapering, straight to curved 4-18 μm (11.6 μm). Spermata not formed.

P NEPD 85

***Metcalfa pruinosa* (Say, 1830) (Hemiptera: Flatidae), a new pest in kiwifruit orchards of Turkey**

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Citrus Flatid Planthopper, *Metcalfa pruinosa* (Say, 1830) (Hemiptera: Flatidae), an invasive pest, was found during the field surveys of kiwifruit orchards of Ordu Province (Turkey) between June and August 2014. Distribution of this polyphagous species, an important kiwifruit pest throughout the world, were given in kiwi production areas of Ordu. Results were discussed in terms of control methods against this pest.

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P NEPD 86

Occurrence of *Chilo partellus* in Turkey, a new invasive maize pest for Europe

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The spotted stemborer, *Chilo partellus* (Swinhoe, 1885) (Lepidoptera: Crambidae), an invasive pest of wild and cultivated grasses in Asia and Africa, was found during periodic surveys of maize fields in the East Mediterranean region of Turkey in September and October 2014. The pest was recorded in maize fields of three of four provinces surveyed (Adana, Hatay and Osmaniye; it was not detected in Icel province). The Mediterranean Corn Stalk Borer, *Sesamia nonagrioides* Lefebvre (Lepidoptera: Noctuidae), is the dominant maize pest in the East Mediterranean region of Turkey, followed by the European Corn Borer, *Ostrinia nubilalis* (Hübner) (Lepidoptera: Crambidae). The new invader comprised 4.89% of the total number of collected lepidopteran pests attacking maize stems and cobs in locations infested by *C. partellus*. No natural enemies of the new pest were recorded during our surveys. We discuss possible interactions among these three lepidopteran pests sharing the same habitat, prospects for control of *C. partellus* by the control methods currently used against *S. nonagrioides* and *O. nubilalis*, and also speculate on the path of invasion taken by *C. partellus* into Turkey.

P NEPD 87

Population dynamics and impact of Aeromycoflora on economic crops at selected locations of Rawalpindi, Pakistan

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Aeromycological study is important to comprehend the ecology, distribution and deposition of fungal propagules dispersed into the ambiance at significant distance from the sources. Most of the spores are killed during atmospheric dispersal as a consequence of exposure to atmospheric agents however, a number of spores remain viable, some of which may not be harmful while others can cause serious plants and human diseases. Monitoring of aeromycoflora is pivotal for better understanding of the epidemics of plant diseases. Occurrence and diversity of airborne fungal spores were monitored fortnightly, by employing two samplers viz. All Glass Impinger (AGI) and Gravimetric Plate Method (GPM) upon four media, exposed for 5-7 minutes, at plant (the height of the respective crop) and human height in the ambient air of three vegetable growing areas of Rawalpindi, Pakistan namely PMAS-AAUR, Sohan village and Adyalla for three years (2007-2010). A total of 20,409 fungal propagules belonging to fifteen genera viz. *Aspergillus*, *Alternaria*, *Cladosporium*, *Curvularia*, *Drechslera*, *Fusarium*, *Epicoccum*, *Helminthosporium*, *Rhizopus*, *Stemphylium*, *Mucor*, *Penicillium*, *Stachybotrys*, *Geotrichum* and *Trichoderma* were identified. *Aspergillus* being the most abundant with 16.73% relative contribution and *Fusarium* with 0.33% contribution in the total fungal spore count was established as the lowest in occurrence. Gravimetric plate method was outrival and statistically significant than AGI in capturing airborne fungal spores as regards selected area. Incidence of mean fungal spores was found significantly higher at plant height (4675) than the human height (2164). Maximum number of airborne fungal spores were trapped and isolated from Potato Dextrose Agar (PDA) medium. Out of 20,409 fungal propagules captured, 39 % were found in summer seasons and the minimum in winter season (11%). Significantly, ($P < 0.05$) the highest mean spore count was ascertained at Sohan followed by Adyalla and PMAS-AAUR. Out of 37 type isolates tested against eight hosts (Tomato, Okra, Cucumber, Squash, Spinach, Turnip, Radish, and Sugarbeet) only four isolates exhibited pathogenic behavior. *Alternaria solani* isolate AITS showed characteristic encircled leaf spots on tomato leaves. Mycelial growth rate of these pathogenic isolates at five temperature levels (15, 20, 25, 30 and 35°C) revealed that *Alternaria solani* exhibited maximum growth rate at 30°C whereas, *Cladosporium cucumerina*, *Cladosporium variable* and *Stemphylium lycopersici* exhibited maximum radial mycelial growth at 25°C. Among pathogenic fungal species early blight was most common disease of tomato in Rawalpindi. The severity of the early blight increased with the increase in the concentration of *Alternaria solani* propagules in the ambient air. Moreover, the increase in the severity of the *Alternaria* blight was always followed by the augmented *Alternaria* propagules in the atmosphere a few days in advance. The conidial liberation, dispersal in the air, transport and deposition to cause new infection on the host i.e. tomato; corresponds well with the disease progression curve.

P NEPD 88

Incidence and characterization of pathogens associated with loquat leaves

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Introduction: Loquat (*Eriobotrya japonica* Lindl.) is a perennial sub-tropical fruit tree belonging to 'Rosaceae' family. It is a perishable fruit with bright orange to yellow color and juicy fragrant taste and is an important evergreen fruit crop because of its ecological and economic attributes. The systematic study was designed which helped in documenting the prevailing causal agents responsible for foliar diseases that are prerequisite for the development of management strategies against disastrous pathogens of loquat.

Objectives:

- Survey to document the incidence of important foliar pathogens on loquat
- Morphological and molecular characterization of isolated pathogens

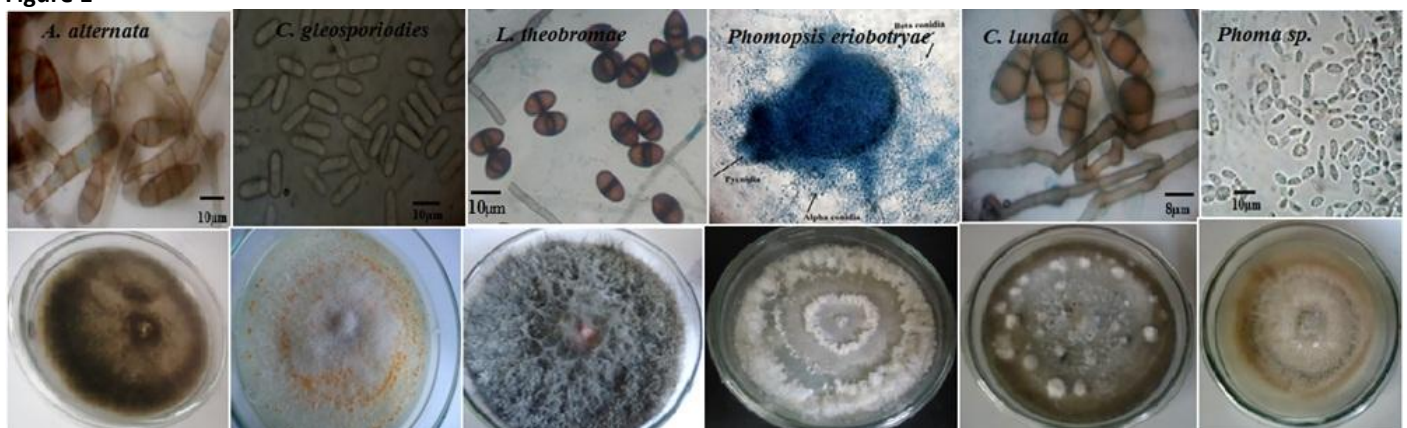
Materials and method: Survey of loquat orchard was conducted in Wah Gardens located at Wah Cantt (Punjab, Pakistan). Samples were collected on the basis of visual symptoms on the leaves and data on disease incidence was recorded. Small bits of surface sterilized tissues were placed on nutritional artificial medium and incubated for 7-8 days at 25±2°C. The pure cultures obtained were identified on the basis of their morphological and cultural characteristics by using fungal identification keys. The pathogenicity test was performed on healthy detached loquat leaves by using 10⁶ conidia/mL with 0.5% Tween20. Nucleic acid extraction was done by using phenol-chloroform-isoamyl alcohol method. The internal transcribed spacer (ITS) region of rDNA was amplified using the primers ITS1/ ITS4.

Results: Disease incidence in the surveyed orchard ranges from 88-100% and 6.3-11% on local and exotic varieties respectively. *Alternaria alternata* was the most commonly isolated pathogen from the infected leaves (51.85%) followed by *Colletotrichum gloeosporioides* (14.8%), *Lasiodiplodia theobromae* (12.96%), *Phomopsis eriobotryae* (9.3%), *Curvularia lunata* (7.4%) and *Phoma* sp. (3.7%) had the lowest percentage frequency of occurrence. In this work, we conducted pathogenicity assay by scoring symptoms and re-isolating fungi and 36 isolates tested were found pathogenic. No symptoms were recorded on control foliage. The identification of fungal species was confirmed by direct sequencing of the internal transcribed spacer ribosomal gene and relevant sequence information was submitted to GenBank.

Conclusion: Fungal leaf spot and blight was the major problem on loquat foliage. No bacterial pathogen was isolated.

Six fungal species belonging to six genera were found pathogenic. Among which; *Alternaria alternata*, *Colletotrichum gloeosporioides*, *Lasiodiplodia theobromae*, *Phomopsis eriobotryae*, *Curvularia lunata*, and *Phoma* sp. were confirmed based on their cultural, fruiting bodies, conidial morphology and molecular evidence (Fig.1).

Figure 1



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Effects of the Nematophagous Fungi *Arthrobotrys oligospora* Fresen on Nematodes infecting Lime Plants

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Nematophagous fungi are the fungi which attack nematodes, many of them are plant pathogens. These fungi worldwide in distribution and have been reported from many countries including Sudan. This study has been carried out to search for

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nematophagous fungi in Gezira soil and their capability to attack nematodes. Random samples collected from Gezira soil grown with banana and lemon crops were put on Corn Meal Agar media (CMA) for the growth of the fungi and nematodes. Using Digital Microscope many types of trapping nematodes had been seen, Such as those with adhesive nets, adhesive knob and presence of fungal spores inside the dead body of the nematode. The samples labeled, and kept in laboratory for further study in the glass house. The fungus has been identified as *Arthrobotrys oligospora*. The study investigated the capability of this fungus to attack and destroy nematodes. The nematophagous fungus *A. oligospora* was found to attack the nematode *Xiphinema sp.* It was also noticed that, the nematode had been captured by adhesive knobs and after that the nematode struggled until death. The fungus *A. oligospora* penetrates the nematode cuticle and consumed all the body content of the nematode. Also the same fungus had been seen capturing the nematode *Xiphinema sp.* by adhesive net and had been held at two points and sometimes at several points. Growing of lemon plants on soil artificially infested with *Xiphinema sp.* nematodes and treated with different concentrations of the fungus *A. oligospora* inocula (10^5 , 10^4 and 10^3) significantly increased the number of lemon plant leaves, stem length, root length and also increased the fresh and dry root weight compared to the same soil without fungal inoculation. So, Nematophagous fungi, if given more attention may be useful as biological control which can decrease cost of nematicides and conserve the ecosystem.

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Molecular genetic approaches for identification of *Cuscuta* species

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Cuscuta L. is cosmopolitan and taxonomically complex genus of the family *Cuscutaceae* Dumort., including 150-200 species in the world. All species of the genus belongs to quarantine plants for the Russian Federation.

The species of *Cuscuta* are distributed by fruits, seeds and stem fragments. Most of these species cannot be correctly defined without flowers, at the same time molecular genetic methods have not developed for the genus *Cuscuta*.

This work is devoted to investigation of trnL-trnF, non-coding region of the plastid genome, - for *C.campestris* Yunck., *C.monogyna* Vahl. and *C.approximata* Bab.

At first DNA was well extracted by method of Doyle & Doyle (1987) from stems and seeds of fresh samples (collected in 2014) and herbarium material (collected in the 1970s, 1980s and 2000s). All plant materials was previously identified by authour.

Then extracted DNA was used as template to amplify (by PCR) with primers B49317 and A50272 (Taberlet, 1991), after that PCR products were sequenced. The sequences of trnL-trnF region for three species of *Cuscuta* were compared to the NCBI database sequences for confirmation of species identification.

The sequences of 3 analyzed species and 10 species from NCBI database were investigated for detection of nucleotide substitutions and deletions. Sequence analysis was performed by the method of maximum likelihood with the construction of the dendrogram, the stability of its branches was determined by bootstrap analysis. In results the species were differentiated into 3 clusters, which corresponds to 3 subgeneric taxa (*Grammica* (Lour.) Engelm., *Monogyna* (Engelm.) Yunck. and *Cuscuta*) in the classical system of G. Engelmann (1859). The similar results were obtained in studies of other sites of chloroplast genome *Cuscuta* (Revill et al., 2005; McNeal et al., 2007; Stefanovic et al., 2007 et al.).

This data can be used to developing of molecular genetic methods to identify species of the genus *Cuscuta* and investigation their distribution and harmfulness in future.

P NEPD 91

Research Tuf gene of possibility applying for the diagnosis of phytoplasmas

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Phytoplasmas are pathogens of many cultures. They are ubiquitous, but some of them are quarantine objects. These phytopathogens are not cultivated on nutrient media, cause range of symptoms on host plants. Due to the low concentration of cells in plant tissues phytoplasmas accurate identification is possible only with the help of molecular methods. One of molecular diagnostic markers phytoplasmas is tuf gene. Phytopathological database "Q-bank" proposed method "nested"-PCR with a set of primers designed on the gene and subsequent sequencing. The aim of this study was to investigate the possibility of using the recommended primer systems for species identification of phytoplasmas. The objects of study were DNA samples *Candidatus* Phytoplasma following species found in Russia: *Ca. Ph. solani* Bois noir (Republic of Dagestan), *Ca. Ph. solani* Stolbur (Moscow), *Ca. Ph. pyri* (Stavropol region), *Ca. Ph. asteris* (Moscow region), *Ca. Ph. taraxanum* (Kaluga region), *Ca. Ph. rubi* (Moscow region). The reference sample of DNA was gold yellowing grapevine *Ca. Ph. vitis* Flavescence doree (France) - a quarantine facility

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for Russia. To perform multiplex PCR using two pairs of primers tuf340a,b / tuf890a,b,c and tuf400a,b,c,d,e / tuf835a,b,c. The size of the amplification product was 400 bp. Sequencing was performed with a pair of primers M13F (-20) / T7. Sequences were analyzed and compared with the data base NCBI. The results of the analysis tuf gene regions were informative and specific. Studies have shown that the proposed database «Q-bank» primer systems allow for species identification of phytoplasmas detected in the territory of Russia, as well as phytosanitary examination for the detection of quarantine species *Ca. Ph. vitis Flavescentiae*.

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Resistance of some eggplant genotypes to *Tuta absoluta* (Lepidoptera: Gelechiidae)

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Introduction: Tomato leafminer, *Tuta absoluta* Meyrick, has recently invaded some parts of Europe, Asia and North Africa. The feeding activity of this oligophagous insect can be extremely harmful to the crops of the family Solanaceae.

Objectives: Since the preliminary observations showed different levels of damage on different varieties of eggplant, *Solanum melongena* L., we examined some eggplant genotypes to evaluate their antixenosis and antibiosis resistance mechanisms to this pest.

Materials and methods: Fifteen genotypes of eggplant including Ghasri Dezphul, Paboland Yazd, Mahali Jahrom, Shend-Abad, Dastgerd Esfahan, Sarhun Bandar-Abass, Chahboland Neishabur, Ghalami Varamin, Black beauty, Blacky, Yalda, Lady, Linda, Lima and Kyme, were examined with free-choice and no-choice tests in laboratory and greenhouse.

Results: In free-choice test, the oviposition preference was significantly different on eggplant genotypes during all days of the study in both laboratory and greenhouse. In no-choice test, the eggplant genotypes showed significant differences in embryonic, larval and pupal development times, survival of eggs, larvae and pupae as well as survival rates of each larval stage; however pupal weight and sex ratio of progeny were not significantly affected by the eggplant genotypes (ANOVA, $p < 0.01$). Based on all examined characteristics, eggplant genotypes were clustered into three main groups: resistant (Kym), moderately resistant (Blackbeuty and blacky) and moderately susceptible (other genotypes).

Conclusion: The observed differences among the examined genotypes can be used in the Integrated Pest Management (IPM) of tomato leafminer in eggplant cropping systems.

P NEPD 93

Modelling invasive plant pests: why the abundance matters

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Introduction: Many modelling approaches have been proposed for invasive plant pests, however most of them consider state variables that are not directly linked to population abundance. This is a major hindrance in supporting quantitative pest risk analysis (PRA), since the abundance is important to foresee the outcome of the ecological interaction between a pest and its host plants.

Objective: A comprehensive modelling framework is proposed allowing the representation of the spatio-temporal dynamic of the invasive plant pest in terms of population abundance. This information can contribute to the quantitative assessment of the plant pests' potential spatial distribution, establishment and spread, their impact on crops and on the environment, and the comparative evaluation of risk reduction options.

Materials and methods: The framework relies on physiologically-based demographic models (PBDMs) which provide a mechanistic description of the weather driven biology of the species and of relevant interacting species in its food web. PBDMs are based on the estimation of rate functions describing the dependence of development, mortality, and fecundity responses on environmental variables. Bottom-up effects of plant growth and development and the top-down action of natural enemies can also be considered. Modules for continuous and stratified dispersal contribute to the representation of the pest spread. PBDMs are implemented in the context of a geographic information system providing a support for the representation of the suitable crops and the environment, as well as the pest's dynamics and its impact.

Results: PBDMs have been developed and successfully applied in several geographical areas and for many species. They have been used for PRA and as tools supporting decision making for IPM at tactical, strategic and policy levels. They have been

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recently used also by the EFSA Plant Health Panel to perform PRAs of species having impact on cultivated plants and the environment.

Conclusion: PBDMs are able to capture relevant ecosystem complexity using a modest number of measurable parameters. They provide an appropriate level of mechanistic synthesis producing detailed representation of the spatio-temporal pest population dynamics and interactions that underpin quantitative PRA and support decision making.

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Molecular characterization and mycotoxin profile of *Fusarium* species occurring on olive fruits in Apulia: an emerging issue

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Olive cultivation is one of the most important crop in Apulia, with 377,526 hectares cultivated and 10,102,300 quintals of olive production. In a survey aimed to evaluate the fungal colonization of olive fruits carried out in the whole Apulia Region, 5 fields for each of the 92 localities selected were investigated. From the survey, together with fungal strains belonging to *Botriosphaeria*, *Colletotrichum*, *Diplodia*, *Neofusicoccum* and *Penicillium* genera, several hundreds of strains belonging to *Fusarium* genus have been isolated mainly from olive fruits and, at a lesser extent, also from branches. The strains of *Fusarium* were identified at a morphologically level, resulting species able to produce a wide range of mycotoxins such as cyclohesadepsipeptides, moniliformin and trichothecenes. However, since each *Fusarium* species can have a specific mycotoxin profile, the toxicological risk related to their occurrence can be highly variable, according with the main species colonizing the olive fruits and must be accurately assessed. Confirmation of strain identification was carried out by using molecular approach. One-hundred and forty-eight representative strains were analyzed by sequencing a portion of calmodulin and β -tubulin genes, which have been proved to be effective for distinguishing species in *Fusarium*. Data have shown, for the first time, a wide genetic diversity within the population of *Fusarium* isolated from olives. In particular, *F. acuminatum*, *F. avenaceum*, *F. longipes*, *F. merismoides*, *F. oxysporum*, *F. proliferatum*, *F. solani*, and *F. torulosum* were identified. However, many strains could not be assigned to any species and therefore may represent new entities within the genus. The occurrence of some highly toxigenic *Fusarium* species suggests that a toxicological risk can occur in olive fruits highly contaminated by *Fusarium* and that such risk must be constantly monitored, also in order to evaluate possible influence of climatic changes on the *Fusarium* spread on this crop in Apulia.

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Screening of Biocontrol and Biodegrading potential of Oil Palm inhabiting Basidiomycete against *Ganoderma boninense*, and infected palm blocks

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Palm oil is a very important commodity which is hindered due to economic loss of oil palm (OP) caused by *Ganoderma* sp. The fungus degrades the lignin component of wood by deploying an arsenal of extracellular enzymes while leaving white cellulose exposed. Historically, the clean clearing methods including burning of oil palm biomass were opted to make way for replanting. In addition to reduced risks of potential pests and diseases the method pollutes the air and is costly. Consequently, the government of Malaysia had imposed a ban on open burning in under Environmental Quality Act (EQA) leading to the zero burning practice. Hence, in addition to abiding with the law, there is a need to treat and utilize these wastes in the most efficient and economical manner. The present study investigated the antagonistic activities of non-pathogenic basidiomycete fungi naturally occurring on oil palm trunks to assess their potential as biocontrol agents against *G. boninense* *in vitro*, further, their role as biodegrader(s) of infected oil palm blocks was also determined. Twenty-five fungal species belonging to the Basidiomycota were recovered using *Ganoderma*-Selective Medium (GSM) and were identified using ITS DNA sequencing. The three fungi showing significantly higher antagonistic activity against *G. boninense*, based on the percentage of inhibition of the radial growth measurements in dual culture were: *Pycnoporus sanguineus* (84%), *Trametes lactinea* (82%) and *Grammothele fuligo* (81%). In addition to these three fungi, *Lentinus tigrinus* and a *Rigidoporus* sp. were also able to successfully invade the sterilized oil palm blocks previously colonized by *G. boninense*. Colonization of the oil palm blocks by *G. fuligo*, *P. sanguineus*, *Rigidoporus* sp., *T. lactinea* and *L. tigrinus* resulted in mass losses of 32.50%, 30.78%, 27.64%, 25.20% and 19.33%, respectively at 120 days of biodegradation period. Most the biodegrading ability of these selected basidiomycetes owed to their ability to produce one or more lignocellulolytic enzymes namely laccase, Carboxymethyl cellulose (CMCase), amylase and xylanase.

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Further studies are required to determine their ability to degrade oil-palm trunks under natural conditions. These dual modes of action of the selected basidiomycetes will not only minimize the infection pressure caused by *G. boninense* but also, reduce the cost spend on managing oil palm generated debris in plantain.

P NEPD 97

A Survey of Wheat Rust Diseases in The GAP Region of Turkey

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Wheat (*Triticum* spp.), Due to having a large number of varieties that can be grown in almost any climate and soil conditions are grown in almost every part of the world. Wheat is the most needed in the world and in our country produced agricultural products. Therefore, it is exposed to many pests and diseases. This survey study was carried out to determine the prevalence and intensity of bread and durum wheat rust disease in all varieties grown total 19106 decar area during 2014 in the GAP region including Sanliurfa, Diyarbakir, Adiyaman, Mardin, Gaziantep and Kilis provinces. Sampling have been according to Bora and Karaca (1970) in a total of 70 fields and was controlled the fields every 10 km in the regions where the wheat areas are intense. As results of the survey; pathogen of Black Rust on wheat (*Puccinia graminis* f.sp *tritici*) was not detected. Frequency of Stripe rust (*Puccinia striiformis*) and Stem rust (*Puccinia triticina*) respectively % 12,86 and % 51,42 were found.

This study was supported by TAGEM (General Directorate of Agricultural Research And Policy) within the scope of National Project of Wheat Rust Diseases.

P NEPD 98

Challenges involve in eradications of Pest and Diseases in the Forestry Zones In Abuja ,Nigeria

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This paper look at the various challenges involve in the prevention and the eradication of various diseases in the different forestry zones in Abuja, Nigeria. The Findings shows that people cannot recognize the types of diseases that are affecting the different forestry trees within the nursery zones and in Plantation and this have being a serious challenges in solving the various problems and the type of best methodology to address the solution. Farm visit were made, questionnaires were administer and analyzed and pictures were taken to support the different findings from the field and different suggestions were made to proffer the solutions

Figure 2



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The effect of weather factors on the flight activity of the groundnut leaf miner (*Aproaerema* sp.), a new pest of groundnut in South Africa

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Groundnut leaf miner (GLM) has recently emerged as a major pest of groundnut (*Arachis hypogaea* L.) in Africa. The origin of this new pest is uncertain and there is also not much information on its ecology to facilitate the development of control strategies against it. The aim of the study was to monitor the flight activity of GLM in order to understand its dispersal and to predict its initial occurrence. The study was conducted at four localities including Vaalharts, Manguzi, Brits and Nelspruit from November 2010 to December 2012. Pheromone traps were used to monitor the moth's flight activity. Information collected included climatic data (rainfall, temperature and humidity) that were obtained from ARC weather stations placed at four planting sites. Though low in numbers, GLM moths were caught during winter at all locations other than Brits. At Nelspruit there was a significant negative association between temperature and GLM moth catches in pheromone traps, whereas at Vaalharts, there was a significant positive association between humidity and GLM moth catches. There was no significant correlation between any of the recorded environmental factors and GLM moth catches at Manguzi and Brits.

P NEPD 100

Potential biological control agents for the pea leafminer *Liriomyza huidobrensis*

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Liriomyza huidobrensis (Blanchard), the pea leafminer, is a highly polyphagous leaf miner capable of inflicting severe damage to crops. In Lebanon, farmers first noticed Leafminers' outbreak in Lebanon on Gerbera plants and a series of crops included leafy vegetables and many other greenhouse and field crops like cucumber and beans. Due to the wide range of insecticide resistance, the control of *L. huidobrensis* by chemicals remains a great challenge especially that it is difficult to implement biological control for this pest where it is not indigenous. In an attempt to find an environmentally safe control measure for the pea leafminer in Lebanon, the main objective of the present research aimed to assay *in vitro* two indigenous biological control agents for the control of the pea leafminer; the entomopathogenic nematode *Heterorhabditis indica* and entomopathogenic fungi *Beauveria bassiana*. As a first step in the evaluation of these species as biological control agents, assays consisted of placing Petri dishes containing sterilized soil and entomopathogenic nematode solution at 1000 IJs per mL in contact with *Liriomyza* pupae on one hand and pupae of *L. huidobrensis* in direct contact with *B. bassiana* at the rate of 5000, 500, 50 and 5 spores/pupa on the other hand. Results showed a mortality of 53±1.5% for the *Liriomyza* pupae following the application of entomopathogenic nematodes characterized by a red color and bioluminescence without any emergence of infective juvenile nematodes, one month following the infestation. Treatments with *B. bassiana* were able to kill 73-97% of the pupae and similarly treatments with *B. bassiana* and the surfactant Tween 80 were able to kill 73-93% of the pupae. Tween 80 was demonstrated to increase the rate sporulation in the first 7 days following the application of the spores of *B. bassiana*. According to the obtained results, *H. indica* and *B. bassiana* can be considered as good potential biological control agents for the pea leafminer and additional studies are required to design an effective biological control program against *L. huidobrensis* pupae in Lebanon.

P NEPD 101

Ecological study of *Tuta absoluta* (Lepidoptera, Gelechiidae) at Tolga, Biskra oasis, Algeria

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A survey on the *Tuta absoluta* (Lepidoptera, Gelechiidae), newly introduced in the oasis of Biskra, on the culture of the tomato under greenhouse to the level at Doucen, Biskra oasis, this last produces more than 45% of the national production in market culture.

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This survey is based on the installation of the traps TUTASON to sexual pheromone Pherodis to the level of 35 greenhouses installed according to the direction, North - South - Est - West.

The captured most elevated percentage of the male adults is recorded during the end of the month of March 2013, whereas the weakest percentage is recorded during the month of November

To shortcoming this survey, we mentioned the attacks and damages as well as the natural enemies found of manner accidental on culture of tomato during the period of sampling.

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Holistic view on Iranian populations of alfalfa weevil (*Hypera postica*)

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Introduction: The alfalfa weevil (*Hypera postica*) is a common Palearctic origin pest of alfalfa (*Medicago sativa*) that became an invasive species in many regions of the world (Summers 1998). Alfalfa weevil has widespread distribution in Iran and causes huge damages to alfalfa products annually. Local adaptation and variation between several Iranian populations are considerable.

Objectives: For getting a clue to inter-population differences of Iranian alfalfa weevil, we used several taxonomic markers (morphology, ecology & molecular) for Eastern and Western Iranian populations.

Materials and methods: A total of 200 specimens were collected from three localities (total 5 stations), including Karaj and Tuysarkan from Western and Jovein from Eastern of Iran in 2013-2014. 10 metric and 45 ratio variables measured on pronotum, elytra and rostrum. The shape of these anatomic parts had been analyzed by GM approach (outline method). Populations were reared in laboratory conditions for immature life stage analysis and CO1 barcode of these populations and two European populations were analyzed as well.

Results: The classical morphology failed to separate populations, but showed strong sexual size dimorphism (83%) based on discriminant analysis. Only the shape of pronotum showed strong population divergence. Immature life table analysis proved the separation between Eastern and Western populations based on the duration of the pupa and fourth instar larva and survival percentage of eggs ($P < 0.005$). High genetic variation has been observed in Iranian populations. According to molecular analysis, there was no distinct pattern of divergence among Iranian populations. In addition, high mtDNA differences (5-11%) were calculated between Iranian and European (Poland and Czech populations) strains.

Discussion: Our morphological analysis revealed SSD that advocated larger females than males. Increasing size in females is related to their ability to receive resources and produce more offspring (Liao et al. 2013) so larger female size is selected (fecundity selection). This introduced shape of pronotum as important taxonomic variable for study of *Hypera postica* populations. In spite of high genetic diversity, these populations cannot distinguish by understudies CO1 gens. The weak separation of Jovein population (Eastern) supported morphological and ecological research. The trade of alfalfa products between localities might have resulted in the admixture of populations in some parts and had prevented stronger pressures effect on evolutionary processes. Although the high calculated genetic distance between Iranian and European strains, more comparative study is needed for taxonomic decision.

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P NEPD 103

The brittle leaf disease of the date palm (*Phoenix dactylifera* L.): Study of Biochemical and Ultrastructural alterations in leaves

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Among the numerous diseases which affect date palm (*Phoenix dactylifera* L.), the brittle leaf disease (BLD) that appeared some years ago, is threatening palm groves in North Africa. The studies we achieved and whose results are presented here, aimed to know more about this affection which the causal agent is still unknown. The comparative analyses have unveiled the involvement of primary and secondary metabolism in the systemic response of the date palm to MFC. A strong membrane lipids peroxidation has been detected. It is followed by a high accumulation of oxylipin's precursors namely, linolenic acid and linoleic acid, and reduced levels of α -tocopherol. The effect of lipid peroxidation on the internal structure of chloroplasts and photosynthetic activity seems drastic. Indeed, several ultrastructural changes in chloroplasts of BLD-affected leaves were observed. The images show many changes in form, size and number of chloroplasts in mesophyll cells with a severely alteration

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of thylakoids. These changes are accompanied by significant reduction of chlorophyll contents and loss of photosynthetic capacity of chloroplasts. Further investigations on phenolic compounds using GC/EI-MS have also been fulfilled. An enhanced extracellular accumulation of *p*-hydroxybenzoic acid, *p*-hydroxycinnamic acid and *p*-hydroxybenzaldehyde, was highlighted in leaves presenting moderate symptoms. This accumulation was accompanied with a significant decrease in abundance of acetophenones, particularly 2'-Hydroxy-4',5'-dimethoxyacetophenone and acetosyringone. It was assumed that *p*-hydroxybenzoic acid and *p*-hydroxybenzaldehyde were formed as the major degradation products of *p*-coumaric acid. Further investigations and analyses have also shown that guaiacyl units have significantly decreased in supporting tissues. Withal, a progressive decrease of protein contents and peroxidase activity was registered. All these metabolic and ultrastructural changes affect the growth of the date palm and lead to the death of this plant after few years. Further investigations must be carried out in the aim to find the causal agent and to acquire better understanding concerning with date palm response to BLD.

P NEPD 105

Evaluation of different traps types for capture in field of *Xylotrechus arvicola* (Coleoptera: Cerambycidae), new pest in the vineyards (*Vitis vinifera*)

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Xylotrechus arvicola (Coleoptera: Cerambycidae) is a pest in vineyards (*Vitis vinifera*) in the main Spain wine-producing regions with Protected Denomination of Origin (PDO). The action of the larvae, associated to the spreading of wood fungi, cause a direct (by *X. arvicola*) and indirect damage (for fungal attack) especially in important varieties of *Vitis vinifera*. Females of *X. arvicola* lay the eggs, concentrated in cracks or under the rhytidome in the wood of vines. The location of the eggs enables the emerging larvae to get into the wood and make galleries inside the plant being then necessary to prune intensively or to pull up the bored plants.

The objective of the study was to evaluate different trapping types for capture of *Xylotrechus arvicola* adults in the field.

In 2013 trapping experiments were conducted in the wine-producing region of Spain with PDO Tierra de León in two varieties of *Vitis vinifera* (*Tempranillo* and *Prieto Picudo*), using three types of traps (interception, delta and screen with adhesive) in combination with one semiochemical attractive. The traps were checked every few days and the number of mean adults captured in the traps were compared using one-way ANOVA followed by Fisher's LSD *post-hoc* test (P<0.05).

Tempranillo variety had more *X. arvicola* adults captured per trap, significantly different from *Prieto Picudo* variety. The greatest days of captures were 2-July (55 adults in *Tempranillo* and 5 adults in *Prieto Picudo*) and 9-July (59 adults in *Tempranillo* and 7 adults in *Prieto Picudo*). Interception trap had higher total mean *X. arvicola* adults captured in both varieties, significantly different from Delta trap and Screen-Adhesive trap, and the greatest days of captures with this type of trap were 2-July (80 adults) and 9-July (95 adults). Interception trap had more *X. arvicola* adults captured in both varieties (28 in *Tempranillo* and 3 in *Prieto Picudo*), significantly different from Delta trap (2.55 in *Tempranillo* and 0.39 in *Prieto Picudo*) and Screen-Adhesive trap (0.17 in *Tempranillo* and 0 in *Prieto Picudo*).

Between varieties studied, *Tempranillo* is a variety more sensitive to be attacked by *X. arvicola*. The first days of July were the days which a higher number of *X. arvicola* adults were captured. Among traps studied, Interception trap is the best trap for capture *X. arvicola* adults in field.

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How seasonally shifted bud burst phenology can affect gypsy moth *Lymantria dispar* egg hatching and larval performance in simulated early/ late spring conditions? Experience with a practically tested local climate change scenarios

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Gypsy moth *Lymantria dispar* is serious worldwide known pest species. Taking in consideration high polyphagy of *L. dispar*, this species is considered as very dangerous for forestry, horticulture and city recreation green zones. Usually larvae of this pest species completely defoliates broadleaved trees and shrubs during intense outbreak periods. Excessive population growth of *L. dispar* negatively affects biodiversity. In Latvia, the first mass outbreak of this pest was recorded in 2008 in the city of Liepaja and second outbreak in 2011 near Engure's lake in nature reserve territory.

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Egg hatching success and mortality of *L. dispar* larvae are usually affected not only by natural enemies, but also by rapid weather change and seasonal transitions of phenological spring. It is hypothesised that sudden spring onsets leads to delay in bud burst process, leaving *L. dispar* early hatched larvae suffering from starvation. We presume that spring shifts in future during climate change can affect larval performance and therefore influence outbreak frequency of *L. dispar*.

Egg masses of two different *L. dispar* populations (near Liepaja - with growth phase, and Engure's lake - with decline phase; in Latvian territory) were selected for laboratory experiment with aim to carry out egg hatching success and larval performance in Latvia's possible future spring conditions. According to Uldis Bether's (U. Bethers 2012) climate change scenarios *L. dispar* eggs were reared in climate chambers with 3 different simulation settings - conditions of spring with noteworthy changes in far future (2071-2100) (day 12 °C - 16h cycle; night 9 °C - 8h cycle ,with weekly deviation of 5 °C), conditions of spring with noteworthy changes in near future (2021-2050) (day 9 °C - 14h cycle; night 6 °C - 10h cycle ,with weekly deviation of 5 °C) and spring conditions of nowadays (day 7 °C - 12h cycle; night 4 °C - 12h cycle ,with weekly deviation of 3 °C) as control. Each egg mass have been placed in separate Petri dish. Alongside with *L. dispar* eggs reared in 30 Petri dishes also water bowls with unblown branches of *Betula* sp. and *Quercus robur* were placed in climate chambers. All simulations were performed for 45 days in first experiment phase. In second experiment phase survived underfed *L. dispar* larvae were placed in 16 acrylic insectariums with differently bursted *Betula* sp. and *Quercus robur* branches. Larvae of *L. dispar* were weighted to estimate larval performance in comparison with bud burst phenology and source population status.

In result *L. dispar* larvae from Engure's lake region showed 30% lower growth rate compared to larvae from Liepaja. Also interesting connection was found between *L. dispar* egg hatching success and bud burst process.

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Determination of harmful important Mite (Acari) Species, their distribution and their control possibility on garlic growing areas in Kastamonu/Turkey

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Allium sativum L.(garlic) is the most common species of the *Allium* spp. And is produced at very high rate all over the world. The yield loss caused by pests is the most important problem in production of that crops. In the absence of control measures, yield loss would be around 35% on average. The yield loss sometimes depending on the pest species and population density can reach about 100%. Mites and nematodes are the most important pests of them. These pests that cause damage to *A. sativum* shows a wide range of taxonomic categories. The number of common pest mite and nematode species that cause damage to either *A. sativum* are over 20 species. In this study, detailed information on morphology, life cycle, management and symptoms of the economically most important harmful important mite (acari) and nematode species of garlic has been provided through careful survey of corresponding researches in Turkey and given informations about new practices and approaches on their controls. Keywords : garlic, pest, acari, control methods, Kastamonu, Turkey

P NEPD 109

First record and Identification of different *Eutypa* isolates from grapevine in Jordan using molecular analysis

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The genetic relationship among the *Eutypa* isolates tested was determined by the box PCR analysis with one oligonucleotide primer Box A1R. This primer and amplification conditions used in the study allowed generation of several weaker or stronger DNA bands varied from ca 600 to ca 800 bp in size. Each isolate was classified into Box type 1, 2, 3, 4 and 5. All *Eutypa* isolates tested in Box PCR assay produced 1-2 common bands. The entire Box-derived DNA fingerprint data generated with five *Eutypa* isolates were analysed using Jaccard's similarity coefficient and UPGMA-based dendrogram. These isolates could be clustered into two major clusters with sub-clusters within each. The results obtained showed that 30% of genetic relatedness among isolates no 1 and 4 and 35 % between isolates 2 and 3b was observed. However, a high degree of similarity (32%) was found among isolate no 1,4 and 2,3b, 3a. On the other hand, comparison of BOX PCR fingerprinting between isolates no 1,4 and 2,3 and 5 showed that these isolates generated two separate clusters with a low percentage of genotype similarity (40 % and 60 % respectively). From result section. *Eutypa dieback* is a serious disease of grapevine in Jordan which is caused by the fungal pathogen *Eutypa*. It is a worldwide threat to grape production. *E. maura* was reported for the first time in Jordan by morphological classification of ascospores obtained from remnants of pruned branches in grapevine fields (Almomany 2002). The number of ascospores in each ascus were uncountable under dissecting microscope. *Eutypa* enters the vine primarily through pruning wound and colonizes the plant vascular tissue, resulting in necrosis of the tissue in a wedge like shape,

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constriction of the vascular system and the reduction in flow of water and nutrients. The genetic relationship among the *Eutypa* isolates tested was determined by the box PCR analysis with one oligonucleotide primer Box A1R. This primer and amplification conditions used in the study allowed generation of several weaker or stronger DNA bands varied from ca 600 to ca 800 bp in size. Each isolate was classified into Box type 1, 2, 3, 4 and 5. All *Eutypa* isolates tested in Box PCR assay produced 1-2 common bands. The entire Box-derived DNA fingerprint data generated with five *Eutypa* isolates were analysed using Jaccard's similarity coefficient and UPGMA-based dendrogram. Amplification of 18S rDNA fragments prior to fungal community fingerprinting was done by using the primer pair NSO/EF3 in a PCR assay (AB Gene Amp PCR system). For the amplification step, the reaction mixture (25 l) consisted of ca 25 ng template DNA, 2.5µl Stoffel buffer, 0.2 mM dNTPs, 3.5 mM MgCl₂, 0.5 µl of 2 % DMSO, 0.2µM each primer and 0.2 U/µl Tag DNA polymerase. After 8 min of denaturation at 94 C°, 30 thermal cycles of 30 s at 94 C°, 45 s at 53 C° and 3 min at 72 C° were performed, followed by an extension step at 72 C° for 10 min. Electrophoresis was performed in TBE buffer at 100 Volt for 40-60 min with 1 Kb ladder to check the PCR product using 0.8% gel (Bio RAD gel system). DNA material was taken from samples extracted by FastDNA Spin kit method. The samples showed a clear band and free from any contaminants (Figure 7). The coming product by using Nested PCR for 18S r DNA was run again through 4 % NuSieve agarose gel for 3 hrs at 80 Volts with 100 bp ladder at both ends and 1 kb at the right side. 10 X reaction buffer was used (2µl) with Hinf 1 as an enzyme (1µl) and 10µl PCR product. The final volume was using Thermo mixer comfort for 2.5 hrs at 37 C with shaking at 350 rpm did 30 µl. Incubation. There were identical bands for samples No 1,2, 3a, 3b and different bands for sample No. 4. This confirmed that we have two different species among our isolates. Number 4 was identified a cording to ascospore formation as *Eutypa maura* while the other four isolates were *Eutypa lata*.

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