HIGHER EDUCATION
FOR SUSTAINABLE
Food Production

3rd Joint Meeting of Agriculture-oriented PhD Programs
UniCT, UniFG and UniUD
Giovinazzo, 11 - 15 OCTOBER 2021

Università di Catania

Università di Foggia

Università degli Studi di Udine

GIOVINAZZO

is a fortified village reflected in the Adriatic sea. The Arch of Trajan is the elegant welcome to the ancient center, where you can visit the "Palazzo del Marchese di Rende", the sixteenth-century "Palazzo Saraceno", seat of the Town Hall, the Church of Saint Domenico and the Church of the Holy Spirit with the "trullo" domes parade. The splendid "Fontana dei Tritoni" dominates Piazza Vittorio Emanuele II, the center of city life together with Piazza Costantinopoli. Going past the arches of via Cattedrale, you reach the Romanescue Cathedral of Santa Maria Assunta, which houses the Byzantine icon of the Madonna di Corsignano, the patron saint celebrated for the entire month of August. The promenade with shallow waters and low cliffs on the shore offers clean beaches and, at dusk, it lights up with bars and restaurants where you can taste the renowned cuisine based on fresh fish.
In the coastal Murgia, not far from the town, stands the dolmen of San Silvestro.

Higher education for sustainable food production : abstract of 3rd Joint Meeting of Agricolture-oriented PhD programs at Unict, Unifg and Uniud : Giovinazzo (BA), Italy, 11-15 October 2021

EDITORS AND ORGANIZERS

Coordinators of PhD courses

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Martina di Corcia
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Giuseppe Longo Minnolo
Marcello Franchini
Elisa Seffin

IMPRESSUM

Monday October 11

19:00 – 19:15 Welcome address

Giancarlo Colelli, coordinator PhD in Management of Innovation in the agricultural and food systems of the Mediterranean Region, UniFg

19:15 – 20:00 Opening lecture

Tuesday October 12

MORNING SESSION 1 (Chairpersons: Mahshad Maghoumi - Riccardo Braidotti)

09:00 – 09:40 Main lecture: Dai geni ai genomi, dalla struttura alla funzione. Una rincorsa fra scienza e tecnologia alla ricerca dell'identità dei viventi

Michele Morgante, Institute of Applied Genomics, Udine

09:40 - 10:05 Insurance effect on farmers' production and technical efficiency: the role of risk-aversion

Simone Russo, Unifg

10:05 - 10:30 Isolation and identification of microorganisms responsible of degradation in soil biodegradable mulching

Stefania Fontanazza, Unict

10:30 - 10:50

Hassan Fazayeli, Unifg

10:50 – 11:05 Chitosan nanoparticles doped with dsRNA as a tool for sustainable viticulture: preliminary results

Dora Scarpin, Uniud

11:05 - 11:30 Break

11:30 - 11:45 Efficient Extraction, Comparative Evaluation and Shelf Life Study Through Nano-encapsulation of Functional Compounds in Different Ginger Varieties

Muhammad Nouman Shaukat, Unict

11:45 - 12:05 Performance of cull cows fed diets supplemented with hempseed cake

Castro Ndong Ncogo Nchama, Uniud

12:05 - 12:20 Effect of growing factors on quality degradation kinetic and shelf-life of leafy salads

Aysha Saleem, Unifg

12:20 - 12:35 Organically grown multiline varieties of durum wheat for the development of a sustainable and functional pasta

Silvia Zingale, Unict

12:35 – 13:00 Impact of exogenous proteolytic treatments on meat tenderization

Martina di Corcia, Unifg

13:00 - 13:15 Study and selection of carpospheric microbial communities of citrus fruits in relation to biological management strategies for the use of microorganisms and biological products alternative to copper

Monia Federica Lombardo, Unict

13:15 - 13:30 Characterization of artisanal Sicilian Ricotta cheese varieties

Guido Mangione, Unict

13:30 – 15:00 Lunch Break

AFTERNOON ONLINE SESSION 1 (Chairpersons: Ilaria D'Isita – Mian Giovanni)

15:00 - 15:20 Anaerobic digestion for waste management and environmental impact control of marine fish farms with renewable energy production

Bartolome Owono Owono, Uniud

15:20 - 15:40 Red Mark Syndrome (RMS) in rainbow trout (*Oncorhynchus mykiss*): etiological investigations

Massimo Orioles, Uniud

15:40 - 16:05 Detection of Food Fraud in Commercial Fish products through DNA Barcoding

Ashraf Ali, Unifg

16:05 – 16:30 Break

16:30 – 16:45 Ochratoxin A and Aflatoxin B1 producing Aspergilli in pistachios from Bronte (Sicily). Detection and evaluation of ozone as an alternative control strategy

Wanissa Mellikeche, Unifg

16:45 – 17:00 *Toxoplasma* infection in goats in Pakistan: risk factors and public health significance

Muhammad Yaser Khan, Unifg

17:00 - 17:15 Insights on immune and inflammatory responses of rainbow trout (*Oncorhynchus mykiss*) affected by Lactococcosis or submitted to vaccination against *Lactococcus garvieae*

Sarker Mohammed Ibrahim Khalil, Uniud

17:15 - 17:35 Adding unprecedent economic and social values to the side - and by-products of Mediterranean fruit and vegetables by reshaping them in novel source of nutrients and tailored food products mediated by 3D printing technology

Mehmet Onur Oral, Unifg

General conclusions in plenary session.

Wednesday October 13

MORNING SESSION 2 (Chairpersons: Aysha Saleem, Muhammad Nouman Shaukat)

09:00 - 09:40 Main lecture: GIS modelling for precision and sustainable agriculture

Francesca Valenti, Department of Agriculture, Food and Environment, University of Catania.

09:40 - 09:55 On the relationship between durum wheat yields and weather events: reflections for the crop insurance systems

Marco Tappi, Unifg

09:55 - 10:20 Metabolomic and transcriptional profiling of oleuropein bioconversion into hydroxytyrosol during table olive fermentation by *Lactiplantibacillus plantarum* species

Amanda Vaccalluzzo, Unict

10:20 - 10:35 Screening of Different *Actinidia* Germplasm Resources in Relation to the Kiwifruit Vine Decline Syndrome

Mian Giovanni, Uniud

10:35 - 10:50 Environmental and economic advantages of Giant Reed harvesting in semi-arid climate conditions

Liviana Sciuto, Unict

10:50 - 11:05 Antioxidant and anti-inflammatory activity of the ketogenic diet and fat-soluble vitamins

Maria Ester La Torre, Unifg

11:05 – 11:30 Break

11:30 - 11:45 Nutritional value and functional properties of insect meal in fish feeds

Giulia Pascon, Uniud

11:45 - 12:00 *In vitro* inhibitory activity of JOHA HBS® polyphosphate used in processed cheese

Andressa Fusieger, Unict

12:00 - 12:20 Analysis of sequence variability and transcriptional profile of c*annabinoid synthase* genes with a focus on *cannabichromenic acid synthases* and their possible involvement in the chemical phenotype of *Cannabis sativa L.*

Flavia Fulvio, Unifg

12:20 - 12:40 Innovative *in vitro* rumen system for continuous measurements of methane and gas production

Matteo Braidot, Uniud

12:40 - 13:00 A look at the interaction among stress factors that may affect honeybee health

Elisa Seffin, Uniud

13:00 - 13:20 Determinants of precision agriculture technologies adoption in Italian wineries

Mubshair Naveed, Unifg

13:30 - 15:00 Lunch Break

AFTERNOON SESSION 2 (Chairpersons: Gaia Carminati - Milan Milenovic)

15:00 - 15:15 Actinomycetes come to rescue of viticulture sustainability

Marco Sandrini, Uniud

15:15 - 15:35 Biofortification of carrots using chelate and sulfate forms of iron and zinc

Camila Vanessa Buturi, Unict

15:35 - 15:50 Breeding for improving yield and grain quality of durum wheat in Southern Italy through the identification of ideal allelic combination of adaptation genes

Sanaz Afshari Behbahani Zadeh, Unifg

15:50 - 16:15 Characterization of new fungal diseases of Pistachio in Sicily and development of sustainable management strategies

Giorgio Gusella, Unict

General conclusions in plenary session

Thursday October 14

MORNING SESSION 3 (Chairpersons: Arianna Lodovici - Andressa Fusieger)

09:00 – 09:40 Main lecture: Management of Xylella fastidiosa in Apulia. Policies and instruments

Gianluca Nardone, Dipartimento agricoltura, sviluppo rurale ed ambientale, Regione Puglia.

09:40 – 10:00 Different approaches to identify genetic traits that control Flavescence dorée resistance phenotype in grapevine

Sofia Casarin, Uniud

10:00 – 10:15 Susceptibility of ancient and modern wheat varieties to the lesser grain borer, *Rhyzopertha dominica* (F.)

Ilaria D'Isita, Unifg

10:15 - 10:35 Evaluation of climate reanalysis as potential data source for estimating reference evapotranspiration at national scale

Giuseppe Longo-Minnolo*, Unict*

10:35 - 10:55 Sex pheromone components of the asparagus moth, *Parahypopta caestrum*: chemical analysis, electrophysiological study, and preliminary field tests

Onofrio Marco Pistillo, Unifg

10:55 - 11:10 One welfare experiences

Aloma Zoratti, Uniud

11:10 - 11:40 Break

11:40 - 12:05 Genome editing, a new biotechnological approach to produce lycopene-rich pigmented oranges

Fabrizio Salonia, Unict

12:05 - 12:25 "Bio-refinery" processes for the valorization of industrial waste from Tomato processing

Francesco Contillo, Unifg

12:25 - 12:50 Multilevel analyses of pest induced defenses in solanaceous plants

Simona Maria Tortorici, Unict

12:50 - 13:10 Grapevines subjected to different water regimes: a study of the physiological and molecular responses

Riccardo Braidotti, Uniud

13:10 – 13:30 Genomic selection for durum wheat improvement

Paolo Vitale, Unifg

AFTERNOON SESSION 3 (Chairpersons: Marco Tappi - Monia Federica Lombardo)

17:30 – 17:45Green solutions and innovative technologies for post-harvest management and safety of food products of the organic and zero-residue citrus production chain

Ermes Ivan Rovetto, Unict

17:45 – 18:00 Use of Meta-Analysis in Environmental Valuation

Laura Giuffrida, Unict

18:00 – 18:20 Exploring the molecular mechanisms underlying the pathogenesis of '*Candidatus* Phytoplasma solani'

Gaia Carminati, Uniud

18:20 – 18:40 Sweetpotato whitefly, *Bemisia tabaci* (Homoptera: Aleyrodidae), and its predator *Macrolophus pygmaeus* (Hemiptera: Miridae): what relationships and combined effects on the "plant system" they develop on?

Alessia Farina, Unict

18:40 – 19:00 Computer Vision System for non-destructive evaluation of quality traits in fresh and packaged fruits and vegetables

Michela Palumbo, *Unifg*

19:00 – 19:20 Ammonia and greenhouse gas concentrations for emission evaluation from an open-sided free-stall dairy barn in Mediterranean climate

Provvidenza Rita D'Urso, Unict

20:30 Social dinner

Friday October 15

MORNING SESSION 4 (Chairpersons: Mubshair Naveed – Dora Scarpin)

09:00 - 09:15 Post - harvest disease management of kiwifruit

Farwa Jabeen, Uniud

09:15 - 09:35 Human-carnivore conflict in Friuli Venezia Giulia: First data from north-eastern Italy

Marcello Franchini, Uniud

09:35 – 09:55 Exploring and predicting multitrophic interactions between host plants, whiteflies, parasitoids, and endosymbionts under changing climate

Milan Milenovic, Unict

09:55 - 10:15 Multi-mutualistic interactions for optimized management of the exotic fungus-farming beetle *Xylosandrus germanus*

Antonio Gugliuzzo, Unict

10:15 - 10:30 Multipurpose agricultural reuse of biomasses and their extracts from different sources

Emanuele La Bella, Unict

10:30 - 11:00 Break

11:00 - 11:15 Characterization of Lactic Acid Bacteria as Biological Control Agents for fresh-cut sector

Nicola de SImone, Unifg

11:15 - 11:40 Dietary tannins in extensive farming: how the effects on cow's milk and cheese change according to the season

Ruggero Menci, Unict

11:40 - 12:00 Physiological and molecular study of mechanisms involved in Iron and Nitrogen nutrition in crops

Arianna Lodovici, Uniud

12:00 - 12:20 Study on the effect of different storage temperatures on pomegranate husk scald

Mahshad Maghoumi, Unifg

12:40 - 13:00 Discussion

General conclusions in plenary session.

Session 1

Insurance effect on farmers' production and technical efficiency: the role of risk-aversion

Simone Russo – *simone.russo@unifg.it*

Antonio Lopolito

Agricultural productions have always had to cope with uncertainty. Farmers take their resource allocation decisions in a complex environment made of poorly controllable biological (diseases, insects, pests, weeds), environmental (i.e., weather, soil, and water conditions), and institutional (i.e., markets, legislation) factors. Additionally, economic and financial markets as well as the political and institutional environment can be sources of uncertainty. In the future, it is expected that the exposure to risk in agriculture is likely to increase due to upcoming challenges related to land degradation and climate change. Such a situation explains the wide array of farming practices and management approaches available to the farmers to manage their risks at the farm level. In this project, we specifically focus on crop insurance because of the increasing attention of policymakers especially in the European Union with the recent development of the Common Agricultural Policy (CAP). Crop insurance might cover all the different sources of uncertainty, supporting farmers in the process of adaptation to climate challenges. This project aims to investigate the insurance effect on production, technical efficiency, and input use of the Italian specialized quality grape growers. We apply a panel instrumental variable stochastic frontier approach to correct for the endogeneity due to insurance adoption. The results show that insurance has an enhancing effect on production and efficiency and reduces the use of intermediate inputs. This suggests that insurance may help to reduce the risk-averse farmers' sub-optimal input use due to the presence of uncertainty allowing them to behave as risk-neutral producers. It would lead farms to go in the direction of profit maximization in the production choices, also enhancing their specialization.

Isolation and identification of microorganisms responsible of degradation in soil biodegradable mulching

Stefania Fontanazza – *stefania.fontanazza@phd.unict.it*

Cristina Abbate

Biodegradable plastic (BPs) mulch films have become an important tool to reduce the plastic waste problem and pollution in agriculture, thanks to their beneficial effects, governed by many biotic and abiotic factors under field conditions.

An experimental field has been created in central Sicily for these specific studies in a Mediterranean apricot orchard, where are compared a conventional orchard management, the biodegradation of a commercial BP mulch with respect to a no-BP, a subterranean clover cover cropping with and without incorporating of *Trifolium subterranean* on the soil.

The aim of this research was isolation and identification of microorganisms responsible of degradation, with a combined approach, based on biodegradation and molecular tests.

We isolated and identified the mesophilic bacterium *Pseudomonas putida* from soil particles attached to the surface of BP mulching film, which was found to be a microorganism responsible of the biodegradation. Quantitative RT PCR analysis showed that *P. putida* was significantly higher in PB plots respect to other plot treatments.

These preliminary results are crucial because the isolation of plastic-degrading microorganisms could lead to their use for amendments to soil where plastics need to be degraded or to accelerate the degradation process, thus reducing the plastic disposal problem in agriculture.

Chitosan nanoparticles doped with dsRNA as a tool for sustainable viticulture: preliminary results

Dora Scarpin – *scarpin.dora@spes.uniud.it*

Enrico Braidot, Elisa Petrussa

Agriculture is recently undergoing a period of transition towards sustainability, with the aim of providing sufficient food for the growing population by reducing the environmental impact. In this context, nanotechnologies are arousing interest in research thanks to the versatility and peculiar properties of some nanomaterials, which appear promising to make some agronomic practices, such as nutrition and crop protection, more eco-sustainable. Chitosan (CH) is an interesting organic polymer to be used to obtain nanoparticles (NPs), thanks to its biocompatibility and to the possibility of sourcing it through the circular economy. CH is known for its ability to induce several biological responses in plants concerning their growth and their defense against diseases, and it shows also good performances as a shuttle for a variety of molecules. This opens the possibility both to profit from the CH carrier function and its protective action against external agents, and to obtain a synergistic effect between it and the transported molecule. In our case, the goal is the functionalization of CH-NPs with specific dsRNA sequences of grapevine pathogens to

exploit the RNA-interference (RNAi) mechanism, which has been suggested as an innovative strategy to limit pathogen infections.

Preliminary results will be here presented, concerning the development of a protocol for the synthesis of CH-NPs, their characterization and the first information regarding their interaction with dsRNA sequences. Two variants of NPs have been produced (from chitosan as it is, or treated with hydrogen peroxide), which were doped with dsRNA sequences of Esca disease pathogens. The difference in synthesis procedures determined opposite interactions with nucleotides, resulting in a lower dimensional size and greater retention of the doping agent by the NPs obtained with untreated chitosan.

Efficient Extraction, Comparative Evaluation and Shelf Life Study Through Nano-encapsulation of Functional Compounds in Different Ginger Varieties

Muhammad Nouman Shaukat - *nouman.shaukat@phd.unict.it*

Biagio Fallico, Rosa Palmeri

Functional foods have proved their worth due to their health promoting properties. Ginger (*Zingiber officinale*) is a rhizome which is largely consumed as a spice and also for medicinal purposes. Ginger contains two bioactive compounds named gingerol and shogaol. There are a lot of variables that affect the concentration, stability and availability of the functional ingredients such as varieties of the bio-material, cultivation and storage conditions and extraction methods. In the initial trails carried out in first year, different extraction techniques, using various extraction solvents, were utilized for the extraction of functional compounds from ginger. But, low extraction yield and no significant antimicrobial activity was observed which is much contrary to the literature reviewed. Optimization of varietal & cultivation approach, suitable storage conditions and efficient extraction method could be significantly overcome the qualitative & quantitative difference, storage losses and low extraction yield. Most of the functional food ingredients possess a lower shelf life and also prone to destabilization. Nanoencapsulation is an emerging field of nanotechnology which involves the enclosure of functional or active components within the encapsulation material. It increases stability, protects the aroma, improves shelf life and prevents evaporation of functional food ingredients. Thus, this PhD project will be fixated to encounter the above-mentioned variables which are adversely affecting the maximum availability of bioactive components from ginger and also to define the optimum conditions with maximal values of functional compounds. It will also involve nano-encapsulation technology for the advancement of shelf life and stability of efficiently extracted bioactive compounds.

Performance of cull cows fed diets supplemented with hempseed cake

Castro Ndong Ncogo Nchama – *ncogonchama.castrondong@spes.uniud.it*

Edi Piasentier

Cull cow (CC) fattening is infrequent because expensive, due to low daily weight gain (ADG) and feed efficiency. The effect of dietary hempseed addition on performance of CC was assessed on 18 Italian Simmental CC allocated into two groups, hay-based (HB, 10 animals) and corn silage-based (CS,8 animals) diet. CC within groups were randomly assigned to 2 dietary, isocaloric and isonitrogenous, treatments: soybean meal (SM) and hemp cake (HC, 5% DM basis), according to initial body weight 603±17.2 kg, and BCS 3.19±0.11points. The trial lasted 4 months after and adaptation period of 1 month. Data were analysed considering groups (HB, CS) and dietary treatments (SM, HC) as block and fixed factor, respectively. ADG was calculated every 3 weeks, carcass traits were recorded at slaughter, *longissimus thoracis* pHu was measured at 48h and meat quality parameters after 14d of ageing. Including HC had not influenced ADG (0,929 kg /d P>0.05) and dressing percentage (48.8%, P>0.05). Considering carcass conformation, 56% and 44% of the HC carcasses were classified as fair (O) and good (R), respectively, percentages similar to those observed for SM, 33% O and 66% (P>0.05). Considering carcass fatness 11% and 44% of the HC carcasses were classified as low (1), slight (2) and average (3), respectively, percentages similar to those observed for SM, 11% low, 33%, slight, and 56%, average (P>0.05). Almost all the samples had a normal pHu, within the range 5.40 to 5.59. HC had similar meat composition (e.g., EE 47.5±0.39 g/kg, P>0.05), WBSF (36.1±1.28 N) and cooking loss (26.9 ±0.87%) than SM. Including HC tended to increase L (33.4 vs.32.0, P=0.08) without affecting a*(16.6±0.40) and b*(16.7±0.28). HC can replace SM in the diet of CC without influencing *in vivo* performances, carcass traits and meat characteristics.

Effect of growing factors on quality degradation kinetic and shelf-life of leafy salads

Saleem Aysha - *aysha.saleem@unifg.it*

Leafy vegetables are not only a source of good nutrition but also provide us diversity of crops. During last few years baby leaf and cut lettuce are getting more attention of both (scientists and consumers) due to their convenience, attractive presentation (mixed varieties), bioactive compounds and freshness. Appearance is the primary criteria of horticultural products according to consumer perspective. Along with postharvest handling practices a wide range of pre-harvest factors e.g. genetic and environmental parameters are of

much importance while considering the shelf life of leafy salads. While the effect of agronomic practices on the quality at harvest is well known, but there is less information on the impact of growing conditions on the overall shelf-life of the product. The main objective of this study is to explore the effect of pre-harvest practices in order to have a better understanding of, effect of the modulation of main growing factors (water, light and fertilizer) on the quality degradation rate and postharvest shelf- life of leafy salads. A baby leaf (as lamb lettuce) and an adult leaf will (i.e. rocket) be choses as model for this study. A "vertical farming" prototype will be used to modulate the growing factors and for the definition of growth conditions of each species. During production cycle, quality parameters will be measured over time and up to the marketability limit. Moreover, hyperspectral images will be also acquired. Expected outputs will include degradation kinetic models and model for shelf-life prediction based on hyperspectral images. Expected positive outcomes of this project in the form of prediction model would be guide for growers consist of best pre-harvest practices for different baby leaf species in order to obtain more stable and marketable produce.

Organically grown multiline varieties of durum wheat for the development of a sustainable and functional pasta

Silvia Zingale – *silvia.zingale@phd.unict.it*

Paolo Guarnaccia, Alfio Spina

Durum wheat (*Triticum turgidum* subsp. *durum* (Desf.) Husnot) is the fourth most widespread cereal in the world (18 M ha area and 40.000 B t annual production) and plays a key role in human nutrition and global economy. Cereal breeding programs conducted to date addressed mainly higher yields and improved technological characteristics for industrial transformation, while neglected nutritional, functional, nutraceutical, digestibility, and potential allergenicity aspects. In this context, wheat landraces and old varieties could represent an optimal material to develop breeding programs aiming at food security and quality, since their genetic variability provides suitability to low input cropping systems and local environmental conditions and high healthy potential. Thus, this Ph.D. thesis research project is aimed at developing a sustainable and functional pasta involving the use of organically grown multiline varieties. In particular, the latter were originated within a participatory plant breeding project coordinated by the Universities of Catania, Bologna, and Firenze, which adopted Italian durum wheat landraces, previously studied for their nutraceutical traits, as starting materials. The above-mentioned genotypes were grown in the fields of three different Italian regions, namely Sicily, Apulia, and Tuscany, in 10 m^2 plots according to a randomized blocks experimental design with three replicates. The experimental bio-agronomic and merceological data of the studied varieties were measured according to official field samplings and

laboratory analyses and were subjected to one-way ANOVA and Tukey test. The results obtained highlighted an appreciable variability for the bio-agronomic and merceological parameters of the durum wheat genotypes under study. Further analyses on chemical-physical, technological, and functional quality traits of the whole-meal flours are still in progress.

Impact of exogenous proteolytic treatments on meat tenderization

Martina di Corcia – *martina.dicorcia@unifg.it*

Rosaria Marino

The meat industry has always focused its efforts on improving the palatability of beef with tenderness representing one of the most important traits. The improvement in tenderness occurs mainly during postmortem aging as a function of different factors that intervene in the disruption of the native structure of the muscle. However, traditional meat aging is long, expensive, and its effectiveness varies depending on the commercial cut and animals.

This research aimed to investigate the improvement of meat tenderness through exogenous proteolytic treatments as an alternative to the postmortem aging.

In the first phase the impact of exogenous enzymes and impregnation methods on meat tenderization was evaluated. Commercial papaya enzyme extract and ultrasound resulted the most effective treatments. Subsequently, on bovine S*emitendinosus* muscle, was evaluated the influence of the enzymatic and physical treatments in comparison with four different aging time. Organoleptic properties and proteomic profile using SDS PAGE and 2-dimensional electrophoresis (2DE) coupled with MS were evaluated on muscle

Experimental data indicate that exogenous proteolytic treatments, by weakening the structure of the meat, produced an improvement in the meat's functional properties. A significant increase in instrumental tenderness was observed in papain and ultrasound treated meats compared to the control, also confirmed by a high number of spots found in 2DE. Furthermore, the 2DE maps of the treated meat showed a greater number and intensity of troponin T spots and myosin fragments (fMHC).

Therefore, results demonstrate that the direct proteolytic action of papain together with the release of endogenous enzymes, caused by the sonic treatment, are able to improve the quality of meat.

Study and selection of carpospheric microbial communities of citrus fruits in relation to biological management strategies for the use of microorganisms and biological products alternative to copper

Monia Lombardo - *monia.lombardo@phd.unict.it*

Gabriella Cirvilleri

Plant microbiome is considered to play a beneficial role in protecting plant from potential pathogens, acquiring nutrients and improving growth and production, interacting closely with their host.

Citrus is a globally important perennial fruit crop and its production is faces many challenges, including plant defense. In fact, control of citrus plant pathogens still relies on the use of chemicals fungicides, including copper compounds. The use of copper is controversial and its application is limited in most European countries both in conventional and organic farming. Therefore, researches are needed to screen alternatives able to reduce or to phase out copper as a new sustainable tool for disease control. This represents a priority in Sicily, where organic farming is highly developed, representing the Italian region with the largest biological surfaces and where typical varieties of high quality, such as the pigmented oranges "Tarocco", are cultivated. Thus, the exploitation of natural occurring microbiome and the impact of different control practices on microbiome and beneficial microorganisms represents a starting point to face these challenges. In fact, citrus microbiome shows a promising potential for beneficial applications to combat citrus disease. Recently, there is a tremendous interest in exploring the structure and function of the citrus microbiome, also allowed thanks to new NGS sequencing analysis, but it still remains partially unexplored.

The aims of this study is to i) understand the composition and the role of carpospheric microbial communities of citrus fruits and how different control practices impact on native beneficial microorganisms; ii) to test and select potential biocontrol agents associated with citrus fruits to be used alone or in mixture as sustainable alternative to copper in order to minimize its use.

Characterization of artisanal Sicilian Ricotta cheese varieties

Guido Mangione – *guido.mangione@phd.unict.it*

Giuseppe Licitra

Sicilian dairy tradition, now thousands of years old, enjoys a wide range of products of renowned quality and excellence, strongly linked with the territories of production and among these stands out the artisanal Ricotta

cheese. For all cheesemakers, ricotta is an important source of income that provides immediate cash flow to the cheese factory.

The aim of this research is to provide a characterization of artisanal Sicilian Ricotta cheese varieties, to evaluate and extend shelf-life in order to export the product beyond the local market, to improve the knowledge, and inform consumers about the quality of these productions.

In the first part of the project a review on the Ricotta cheese production technologies, including differences between industrial and artisanal making reported in the literature, will be performed in order to highlight the main diversities between the two methodologies of production and also to distinguish the different ricotta cheese varieties produced. In the second part of the research, a characterization of the traditional Sicilian ricotta cheese varieties will be carried out, through the analysis of the products and on the different technologies of production. Each territory has its own traditions, so the variability of the product is significant, although using the same technological principles as the use of whey and heat, other ingredients can be integrated according to local customs and traditions (milk of enrichment, salt, acidifying agents). In the third part, will be developed an appropriate evaluation and extension protocols of the shelf-life, using new low-temperature technologies, allowing to formulate a product with longer shelf-life, well-preserved characteristics, and safe for the consumers.

The goal of the entire project is the enhancement of a unique product, strongly linked with the Sicilian tradition, promoting its production and marketing.

Ochratoxin A and Aflatoxin B1 producing Aspergilli in pistachios from Bronte (Sicily). Detection and evaluation of ozone as an alternative control strategy

Wanissa Mellikeche – *wanissa.mellikeche@unifg.it*

Giancarlo Colelli - Co-tutors: Anna Maria D'onghia, Alessandra Ricelli, Marilita Gallo.

Pistachios are mainly cultivated in Iran and Turkey and the USA. However, Italy produces an increasing amount of high-quality pistachios, mainly in Sicily in the Bronte area. The green pistachio of Bronte (Pistacchio Verde di Bronte) is officially registered as an Italian Protected Designation of Origin (PDO) and is a highly valued global trade commodity. Pistachio quality can be impaired in various ways, such as contamination by insects and/or fungi. In particular, post-harvest contamination by certain widespread fungi belonging to the genus *Aspergillus* can be a double-edged threat because these fungi can synthesize different toxins, e.g., ochratoxin A (OTA) and aflatoxin B1 (AB1), which are serious hazards to humans and animals.

This project aims to study such contaminations in several ways. The first consists of a survey of fungi present post-harvest in samples of pistachio nuts collected from Bronte. This will allow to determine the frequency of the presence of *Aspergillus* spp. and the relative abundance of some frequent mycotoxin-producing *Aspergillus* species such as *A. carbonarius* and *A. flavus*.

The second part aims to developing LAMP PCR primers which are specific to these species. Finally, this study relates to proposing an alternative means of controlling these contaminations. Thus, testing the effects of Ozone (O_3) on OTA- and AB1-producing species of *Aspergillus* isolated from pistachio kernels. In particular, fungal growth and conidia germinability, as well as the effects of O_3 treatment on OTA and AB1 production will all be evaluated. The overall aim of this project is to broaden understanding of Italian pistachios' contamination by *Aspergillus*-producing OTA and AB1, and to examine the possibility of using control agents such as O_3 as an alternative to pesticides and fungicides.

Anaerobic digestion for waste management and environmental impact control of marine fish farms with renewable energy production

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Francesco Da Borso

This study focuses on improving the sustainability of aquaculture applying the anaerobic digestion (AD) of effluents produced by intensive marine fish farming. The main goals of the experimental work were to evaluate the aquaculture effluents in relation to their methane potential and to identify the optimal operating parameters for anaerobic process implementation at a larger scale. Laboratory scale tests were performed in an automatic system (AMPTS) for the determination of the biochemical methane potential (BMP), adopting different ratios between inoculum and substrate (I /S) (from 50:1 to total absence of inoculum). The highest I/S ratio showed the highest BMP (564.2 NmL CH4·g-1 VS), while decreasing BMP values were obtained in relation to the lower amount of inoculum (319.4 and 127.7 NmL CH4·g-1 VS respectively for I/S = 30 and I/S = 0.3); in the absence of inoculum, the lowest BMP was recorded (62.2 NmL CH4·g-1 VS).The preliminary results obtained from the pilot scale test, not yet completed, with the bioreactor automatic system (BRS) for determining the conversion indices at two levels of salinity, showed that the effluent with the highest level of salinity (35 g·L-1) had the lowest performance, with an average yield equal to 172.4 NmL CH4·g-1 VS, compared to the brackish effluent (227.4 NmL CH4·g-1 VS and 235.0 NmL CH4·g-1 VS, with salinity between 10 and 13 g·L-1). By varying hydraulic retention times, organic loading rates and

number of weekly loads, it has been observed that in brackish conditions it seems possible to increase the organic load and decrease the hydraulic retention time without reducing anaerobic reactor performances.

Red Mark Syndrome (RMS) in rainbow trout (*Oncorhynchus mykiss*): etiological investigations

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Marco Galeotti

Red mark syndrome (RMS) affects farmed rainbow trout worldwide. Rickettsia-like organism (RLO), currently referred as Midichloria like organism (MLO), is supposedly involved in RMS.

RMS is generally associated with water temperatures below 16 C. However, given the long incubation phase and the temperature fluctuation on farms, the real effect of temperature on RMS transfer and symptom development remains elusive.

Studies of RMS so far mostly used diseased fish from farms, where the epidemiology and history of exposure is unknown.

This PhD project has been divided in three fundamental phases.

The first phase of this project consisted of a trial, which tested the effect of 3 temperatures on the transfer of MLO from RMS-affected fish to naïve SPF cohabitants at the DTU-AQUA facilities. This was completed in early 2020.

During the current second phase (2020-2021) of the project, samples for histology and electronic microscopy taken from experimental infection were analysed and classified using a recent histological grading system recently proposed by Prof. Galeotti and modified by Dr. Orioles, including regenerative phases of the disease. Preliminary results showed that RMS develops only in mild form and in fewer fish at 19°C and at 15.5°C the disease symptoms disappear more quickly compared to 12°C.

During 2021, our collaboration with Moredun institute of research (Scotland) made possible the drafting of the first review article on RMS disease. Furthermore, the cooperation between DI4A and human clinical pathology laboratory at the hospital of Udine resulted in the development and application of a sensitive droplet digital PCR for the detection of red mark syndrome infection.

During the current year, we started collecting epidemiological data from Italian farms through ad hoc questionnaire.

In the third phase of the project, we will further analyse all the data collected and aim to publish in peer review journals and international conferences.

Detection of Food Fraud in Commercial Fish products through DNA Barcoding

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Giovanni Normanno

Food fraud has existed for decades, and the fish industry is not an exception. Due to increased health benefits, awareness and wide acceptance in all religions and cultures demands for fish and fishery products are rapidly expanding in all continents. Species substitution, the use of a low-value fish in place of a high-value fish, is the biggest problem in international trade and the leading cause of commercial and sanitary fraud in the fisheries sector. Species identification is a key problem in fisheries research and control, as well as processed fish products in consumer protection. My study aims to evaluate the applicability of the mitochondrial genes, cytochrome b (*cyt* b), and cytochrome oxidase subunit I (COI) for the species identification of fish and processed fish products by techniques of "DNA barcoding". In the present study, universal primers for mitochondrial *cyt* b were used to discriminate fish species in raw and processed forms. The barcode primers were cross tested against 160 fish species and processed fish products. For this project, we collected 160 samples from different supermarkets and restaurants. DNA was isolated from each sample and amplified by PCR; the most intense amplified product was chosen for Sequencing. After obtaining the results with NCBI BLAST, species were identified and matched with the labeling of the products. Initial results have shown a minor level of mislabeling and we are analyzing the results of all samples. In the next phase Loop Mediated Isothermal Amplification (LAMP) will be developed for detection of Atlantic cod (*Gadus morhua*) and pollock (*Gadus chalcogrammus*) from processed fish products expecting that it will be more cost effective and less time consuming compared to other available methods. The proposed work will be very significant work in food control and the detection of food fraud in the fish and food industry.

Toxoplasma infection in goats in Pakistan: risk factors and public health significance

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Toxoplasma gondii is a parasitic protozoan infecting warm-blooded animals and humans. In small ruminants, this protozoan is a cause of fetal mortality; in humans, toxoplasmosis may pose a risk for immunocompromised individuals and during pregnancy leading to severe consequences, especially neonates. Infection occurs by ingestion of *i)* sporulated oocysts contaminating water or vegetables, *ii)* cysts in raw or undercooked meat, *iii)* tachyzoites in milk.

In Middle East countries, goats are the most common *livestock,* and consumption of unpasteurized goat milk is a traditional habit.

Due to the lack of epidemiological data in Pakistan, the present study aims to investigate the prevalence of *T. gondii in goat farms via* the evaluation of anti-*T. gondii* antibodies, the frequency of the parasite DNA in milk, and the risk factors influencing the infection.

One hundred and ten goat farms were selected in Layyah District (Punjab Province) and stratified according to size. Blood and milk from 12 goats per farm will be collected and processed for antibody detection (blood and milk) and molecular analysis (milk). Three farms with the highest *T.gondii* prevalence will be selected, and throughout the lactation period, milk collected from all animals will be molecularly analysed. A questionnaire will be administered to all owners to collect information on farm management. The detection of antibodies in blood and milk will be carried out using the ELISA test, and the results of the two biological samples will be compared. The parasite DNA in milk samples will be detected by qPCR (B1 gene), and the isolates genotyped.

Once completed, the study will provide original data on the circulation of *T. gondii* in goat farms and related risk factors for milk consumers.

Insights on immune and inflammatory responses of rainbow trout (*Oncorhynchus mykiss*) affected by Lactococcosis or submitted to vaccination against *Lactococcus garvieae*

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Marco Galeotti, Donatella Volpatti

Aquaculture is the fastest growing food-producing sector in the world. Among freshwater species farmed in Europe, rainbow trout (*O. mykiss*) is the most widely cultured and has a high economic value. However, the intensive farming conditions increase its susceptibility to different pathogens that lead to significant productive losses. In consideration of this, my PhD activity will be conducted in the framework of the EU PRIMA funded project SUPERTROUT (Improving SUstainability and PERformance of aquaculture farming system: breeding for resistance in rainbow TROUT), Ref: 2019-SECTION2-27. The mission of this project is to improve sustainability and profitability of small-scale farming systems facing the major challenge of infectious disease control in rainbow trout. A farmer centered approach involving multidisciplinary teams using genetics and immunology to breed for lactococcosis resistant animals and to cross different lines of broodstock, and developing a bio-safe vaccine based on recombinant proteins, will be adopted. Specifically, my study aims at improving the knowledge on the response of rainbow trout *vs Lactococcus garvieae*, which is one of the main

bacterial pathogens for this fish species. The main focus will be to evaluate the immune/inflammatory reactivity of the rainbow trout against viable *L. garvieae* throughout natural or experimental infections, as well as upon "in lab" or "in field" vaccination with *L. garvieae* antigens. The understanding of host-pathogen or host-vaccine interaction will be based on the analysis of different immunological parameters (humoral and cellular) and on the evaluation of immune gene expression. Data derived from lab experiments and field studies will be analyzed with a statistical package, presented in scientific conferences and published in reputed journals.

Adding unprecedent economic and social values to the side - and by-products of Mediterranean fruit and vegetables by reshaping them in novel source of nutrients and tailored food products mediated by 3D printing technology

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Carla Severinil

The first step of this doctoral research project is to characterize and optimize drying some waste or not edible parts of the Mediterranean fruits and vegetables without losing their nutritional value. The drying curve and isotherm have been mathematically modelled. The particle size of the dried powder will be optimized to create printable food formulas intended for innovative 3D printed food. After determining the nutritional value and appropriate conditions for the 3D printer nozzle, the food powder will be mixed with different gels and made suitable for printing with the 3D printer.

Artichokes and eggplants have been purchased locally, and the external leaves (for artichokes) or the skin (for eggplants) usually treated as food waste were reserved for the drying process. Klarstein-Arizona Jerky food dehydrator has been used for drying. At temperatures of 30°C, 40°C, 50°C. The samples were analyzed every 30 min for weight and moisture content. Water activity was measured by a water activity meter. The drying curve was created by plotting the moisture ratio (MR)instead of the moisture content to reduce the effect of the variance of moisture content for fresh food. The MR values as a function of time were modelled by using Page's model. The fitting was performed by using the packages of STATISTICA. Furthermore, the moisture content as a function of water activity was modelled by using the GAB equation.

As part of the doctoral project, the same study will be conducted on different fruits and vegetables. The optimal temperature and duration value will be calculated in nutritional content. In advanced stages, powders of such dried vegetables and fruits from waste ta different particle sizes and are mixed with a suitable gel. The appropriate parameters will be set for printing on a 3D food printer. 3D Printing will create

innovative food products nutritionally enriched by powders and personalized based on specific requirements of the consumer's group or their desires for sensory features.

Session 2

On the relationship between durum wheat yields and weather events: reflections for the crop insurance systems

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Rosaria Viscecchia - Co-tutor: Fabio Gaetano Santeramo

Agricultural production is closely related to the weather conditions and farmers need of implementing risk management tools to reduce the impacts on farming systems. Among several strategies available, crop insurance programs can play an important role, more specifically, weather index-based insurance may overcome problems associated with traditional insurances, however, they present the limit of basis risk: a farm may experience a significant yield loss, while the weather index does not trigger the payment, or a farm may obtain compensation without having any yield losses. Our case study is a part of a study on Italian territory that aims at deepening the knowledge on the linkages between crop yields and weather events that occur in susceptible phenological phases of crops. We focused on durum wheat crop in the Apulia region collecting 10-days frequency weather data (i.e., average minimum temperature, average maximum temperature, cumulative precipitation), and yearly yield data, from 2006 to 2019, for each province. According to the literature, the results show that maximum temperature and precipitation have a negative effect on durum wheat yields, while minimum temperature has a positive effect. However, only some phenological phases are influenced by weather variables (i.e., maximum temperature has a positive effect on yield during the germination stage), therefore, it emerges the need to further investigation, e.g., using more disaggregated data, to develop an effective risk management tool, also supporting the stakeholders and policymakers in forecasting, monitoring, and assessing climatic risks.

Metabolomic and transcriptional profiling of oleuropein bioconversion into hydroxytyrosol during table olive fermentation by *Lactiplantibacillus plantarum* species

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Cinzia Lucia Randazzo

The study aims to elucidate the mechanisms responsible for the bioconversion of oleuropein into low molecular weight phenolic compounds in two selected *Lactiplantibacillus plantarum* strains, under stress

brine conditions. For this purpose, an experimental strategy, combining high-resolution mass spectrometry, *in silico* functional analysis of GH1 candidates and gene expression study was adopted. Oleuropein hydrolysis products and underlying enzymatic steps were identified, as well as a novel putative *bgl* gene, responsible for the β-glucosidase activity, under low temperature conditions. According to biochemical analysis, both strains showed the ability to hydrolyse oleuropein and release hydroxytyrosol through the formation of an intermediate compound Hy-EDA. The strain C11C8 showed a more pronounced activity at 16 °C. At the genomic level, the presence of the β-glucosidase gene was detected by using the primer pairs proposed in this work (CS400_14770) and by primer pairs developed by Zago et al. (2013) (CS400_14765) and Spano et al. (2005) (CS400_15205). Both strains were able to transcribe the CS400_14770 gene under all tested conditions. In addition, the gene was expressed also with CS400_14765 gene, with the exception of strain C11C8 at 16 °C. The CS400_15205 gene was not transcribed, although it was detected genomically. In conclusion, strains C11C8 and F3.5 have shown different metabolite patterns at 16 °C and their genomes harbour different variants of the CS400_14770 gene. The difference in alleles of the gene locus responsible for differential β-glucosidase activity under low temperature conditions could have an important practical implication in brine fermentation of table olives and could guide future selection criteria for new oleuropein-free *L. plantarum* starter cultures.

Screening of Different *Actinidia* Germplasm Resources in Relation to the Kiwifruit Vine Decline Syndrome

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Guido Cipriani

Kiwifruit Vine Decline Syndrome (KVDS) is a sever disease, which causes dramatic yield losses and economic damage in *Actinidia deliciosa* and *A. chinensis* orchards. Currently, the aetiology is considers an interaction of both biotic and abiotic factors, primary soil-borne pathogens, and waterlogging conditions. Up to date, there is not any effective managing strategy. Nevertheless, the availability of a large *Actinidia* germplasm collection open up to the possibility of screening different species in order to find traits to contrast KVDS. In this context, we selected six accessions of *Actinidia* (*A. macrosperma 176* and *183*, *A. arguta cv. Miss Green*, *A. Polygama, cv. 'Bounty71'* and *A. deliciosa cv. Hayward* as control), planted in four KVDS inducing soils. Evaluation of the genotypes behaviour one year from planting was carried out on the root system, considering different agronomic parameters, along with the vegetative plant growth. Yet, a statistical method was developed to define *Actinidia* root system fitness. Furthermore, the capability of mineral up taking, and

protein content was evaluated in roots. The concentration of mineral elements was measured by using the Inductively Coupled Plasma-Atomic Emission Spectrometry, whilst the total protein content was measured through the Bradford assay. Indeed, discontinuous sodium dodecyl-sulfate polyacrylamide gel electrophoresis was performed in order to determine protein patterns. The results showed that significant differences exist among the genotypes, regarding both the radical systems parameters and vegetative growth. Moreover, there was a different content of mineral elements and proteins, without differences on protein's patterns. These first evidence demonstrated how some accessions were able to grow in KVDS inducing soils, showing a different behaviour for each analysis performed; in particular the best performing was A. macrosperma Only *A. polygama* was not able to grow, showing a progressive decline trend similar to *Actinidia deliciosa* cv. Hayward.

Environmental and economic advantages of Giant Reed harvesting in semi-arid climate conditions

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Giuseppe Cirelli, Feliciana Licciardello

The vegetation development in riverbeds creates obstructions to the regular water flow with flooding hydraulic risk increase. *Arundo Donax* L. or giant reed (GR) is one of the most successful invasive species of riparian ecosystem in Mediterranean areas. Due to its high biomass yield and adaption capacity to several conditions, GR is a very promising no-food crop to produce biogas by anaerobic digestion (AD). The research activity was carried out in the inner areas of Sicily (Calatino) in collaboration with SIMBIOSI Consortium. This company operates an AD plant fed with agricultural by-products from other close agro-industrial companies. The aim of the study was to estimate the surface covered by GR in the Calatino and evaluate its potential for biogas production, according to different harvest times. The integrated use of Remote Sensing techniques and of Geographic Information System (GIS) was essential to investigate the spatially distribution of GR. Since plant characteristics vary noticeably with their development stage affecting the Biochemical Methane Potential (BMP), it is important to estimate the BMP for each harvest times in order to evaluate how different cutting regimes can impact on the methane yield. The results showed that the surface covered by GR, computed in the GIS environment, was about 2 km^2. Moreover, the preliminary laboratory implemented tests demonstrated that the percentage of methane on the biogas product was on average 53%. The study could contribute to the development of a maintenance plan with the aim to reduce the risk of streams flooding in valley areas and at intersections with infrastructure works. The plan could be used by stakeholders to manage watercourses and to get economic benefits in inner areas.

Antioxidant and anti-inflammatory activity of the ketogenic diet and fat-soluble vitamins

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The aim of this research project is to evaluate the effects of ketogenic diet in which organic products are added.

The effects of ketogenic diet will be investigated, *in vivo,* by evaluating remodeling of body composition, improvement of blood indices, decreasing of ROS (Reactive Oxygen Species), markers of oxidative stress, and inflammatory species.

Free radicals, pro-inflammatory cytokines and oxidative stress play a pivotal role in many neurodegenerative disorders, due to this a diet that reduces these parameters may have a neuroprotective potential.

In the first phase of study we investigate, *in vitro,* the antioxidant and neuroprotective effects of a fat-soluble vitamins, Vitamin E, which is mainly present in foods consumed with a ketogenic diet by using microglia cell culture, BV2 cells. Cell viability after Vitamin E somministration was evaluated by MTT test, a colorimetric assay. We have tested different concentrations of Vitamin E (50uM, 100uM, 200uM, 400uM) founding that Vitamin E is not toxic for cells and the dose of vitamin E that induces an increase of cell viability is 100 uM. This dose was utilized to test the effects of Vitamin E on microglia polarization, after the stimulation with Liposaccharide (LPS) and we have observed that the addition of Vitamin E was able to polarize microglia toward the anti-inflammatory phenotype.

Moreover, we have performed cell migration assay to evaluate the ability of Vitamin E to influence microglia cell migration and we have also collected biological samples to investigate by Western blotting, qRT- PCR and ELISA how Vitamin E modulates the anti-inflammatory and antioxidant profile of BV2 cells.

Nutritional value and functional properties of insect meal in fish feeds

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Francesca Tulli

Aquaculture is expected to substantially support the protein supply for the human diet. Consequently, the industry and scientific community are asked to make great efforts to find eco-friendlier alternatives to fish-based ingredients to meet the expected increase in aquafeed production. Among the different innovative

and alternative ingredients, insects seem to be an ideal candidate. Indeed, their ability to renewal waste and their potential usage as feed in livestock and aquaculture production has attracted the attention of the European Council. The EC Regulation 893/2017 has defined seven breeding insect species that could be allowed in the fish diet. Insects have high protein content (up to 74.4% of dry matter) and a balanced amino acid profile that is similar to fish meal in the case of Dipterans. They are also rich in lipids (10–50% of dry matter), vitamins, and minerals such as potassium, calcium, iron, magnesium, and selenium.

The use of insect meal as a partial substitute for conventional protein sources has been investigated for several livestock and aquaculture species; promising results have been observed for fish growth performance, diet digestibility even if with different results. Insects are also reported to contain bioactive compounds that encourage their use as potential functional ingredients for animal feeding and food.

In this first year of research, the goal was the setup of the in vivo experimentation with rainbow trout juveniles. This experiment will investigate, especially, the functional properties of lauric acid and chitin (bioactive compounds that are naturally present in the insect meals) on the metabolism, gut morphology and physiology, and immunological traits of rainbow trout fed semi-purified diets under controlled experimental conditions.

In vitro inhibitory activity of JOHA HBS® polyphosphate used in processed cheese

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Polyphosphates are used as emulsifying agents in processed cheeses and their antimicrobial activity have been already suggested; however, for the *in vitro* studies a protocol standardization and validation are required. In this research we assessed different *in vitro* approaches to characterize the inhibitory activity of a commercial polyphosphate (JOHA® HBS) against bacteria strains. Selected strains (n=7) were streaked onto plates containing four different culture media (BHI, TSB, Nutrient, PCA) supplemented with agar (1.5%) + HBS (0.5, 1.0 and 2.0%). Then, selected strains (n=3) were transferred to the same culture media (no agar added) + HBS (0.5, 1.0 and 2.0%), incubated for 24h and drop-plated in PCA for enumeration. Streak assay showed greater inhibitory activity on Nutrient medium, three strains were inhibited by HBS at the lowest concentration (0.5%) and all strains were inhibited at 2.0%. Assay based on culture streaking (on agar) allowed a better inhibition when compared to culture enumeration (on broth). Finally, selected strains (n=21) were subjected to streak assay and through three diffusion protocols (spot-on-the-lawn, agar-spot, well-diffusion) using Nutrient medium + HBS (0.2 to 3.0%). Additionally, a quantitative protocol using Nutrient

agar + HBS was performed by culture drop-plated. Assays based on culture streaking and agar-spot were the most effective to demonstrate the inhibitory effect of HBS on 11/21 targets, at 0.4%, while the spot-on-the-lawn demonstrated the inhibitory effect exclusively for two strains, only at 3.0%; culture enumeration confirmed the inhibitory potential of HBS. We were able to demonstrate the inhibitory activity of HBS at low concentrations (0.2-0.4%) and the adequacy of using Nutrient medium associated to agar-based assays to confirm the potential of this polyphosphate on target bacteria of interest in the dairy industry.

Analysis of sequence variability and transcriptional profile of c*annabinoid synthase* genes with a focus on *cannabichromenic acid synthases* and their possible involvement in the chemical phenotype of *Cannabis sativa L.*

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Roberta Paris, Pasquale de Vita

Cannabis sativa has been long cultivated for its therapeutic properties due to the accumulation of phytocannabinoids in female inflorescences, and is now emerging for its promising use in numerous industries. Besides years of breeding, some hemp varieties consistently accumulate traces of residual tetrahydrocannabinolic acid (THCA) in inflorescences, close to the 0.20% dry weight limit set by E.U., despite the functional gene encoding for THCA synthase (*THCAS*) is lacking. Behind the presence of functional rather than non-functional *cannabinoid synthase* genes, resulting in the accumulation of THCA and/or cannabidiolic acid (CBDA), or in the prevalence of cannabigerolic acid (CBGA), there are some genes named *cannabichromenic acid synthase*, which role is controversial. In this work, the *cannabinoid synthases* have been isolated from a panel of thirteen Cannabis genotypes. Highly specific primer pairs were developed to allow an accurate distinction of different cannabinoid synthases genes at genomic level and the analysis of transcriptional profiles in hemp or marijuana. Based on results, hypotheses are given to explain the presence of low THCA levels in hemp varieties that do not carry any functional *THCAS* gene.

Furthermore, a genome wide identification of structural enzyme-coding genes for flavonoids, terpenes and cannabinoids synthesis and candidate regulatory genes is reported, providing valuable resources for further investigation of the *Cannabis* metabolic pathways.

A set of biochemical data concerning peculiar cannabinoids, terpenes and flavonoids for different Cannabis genotypes has been produced, in order to select the most interesting metabolites and to identify genotypes rich in specific compounds that will guide our future analyses aimed at characterizing the genes responsible for their synthesis.

Innovative *in vitro* rumen system for continuous measurements of methane and gas production

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Emissions in the atmosphere of gases that contribute to global warming are a serious problem to the planet's ecosystem. Public opinion has become aware of climate change problems and the EU has recently resolved to reduce 36% methane (CH_4) emission by 2030 compared to 2005 levels. Methane is one of the main greenhouse gas (GHG) and livestock production contributes approximately 10% of the total anthropogenic GHG production. Moreover, methane represents a loss of 2-12% of the gross energy intake. Methanogenesis is a physiological process to decrease hydrogen accumulation in the rumen and several strategies to mitigate methane production without depressing rumen fermentation have been studied. The present project has the aim to study rumen CH_4 yield through a new fully automated rumen *in vitro* equipment, which allows the simultaneous and continuous measurement of CH_4 and total gas production (MP and GP, respectively). In the first part of the work, the aim has been to compare the kinetics of MP emission to that of total GP using different feeds as substrates. The determination of the volatile fatty acid (VFA) profile in the fermentation fluid allowed to correlate the MP measured with that predicted by VFA concentrations. The second part of the work has studied the utilization of the *in vitro* system to evaluate the effect of different chemical substances suitable to reduce MP. Two additives having different mechanisms of action were studied: one is an electron sink (e.g. NO_3Na) able to accept H_2 from the environment, while the other (e.g. 2-nitroethanol) has a direct effect on methanogen bacteria and an inhibition effect on coenzyme M reductase used in the methane formation pattern from CO_2. The adoption of a continuous gas analyser permits to study the effects of additives during all fermentation processes and testing their efficacy at different time intervals.

A look at the interaction among stress factors that may affect honeybee health

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Honeybees (*Apis mellifera* L.) are important pollinators, contributing to plant biodiversity and agricultural production.

In the last decades, a worrying decline of bee colonies has been observed in many countries. The interaction among stress factors play a crucial role; in particular, nutrition can affect the capacity of bees to tolerate parasitic infections.

Pollen is the only source of proteins for bees and contains substances that positively affect bees' stress resistance. In previous experiments, we highlighted a beneficial effect of the pollen's polar fraction on bees infected by the Deformed Wing Virus (DWV). I therefore tested if, quercetin, one of the most common pollen's flavonoids, may account for that positive effect. Preliminary results suggest that quercetin is important but doesn't explain all the beneficial effects of pollen.

Moreover, pollen may also contain toxic compounds that must be detoxified and the honeybee's detoxification system (e.g., Cytochrome P450 monooxygenases) certainly plays an essential role. To investigate this aspect, honeybees were fed nicotine, pollen or both and treated or not with a common inhibitor of detoxification (i.e. piperonyl butoxide, a P450 inhibitor). The experiment was replicated early in the season when viral infection is limited or later when DWV is widespread to evaluate possible interactions with viral infection.

According to the preliminary results, the effect of nicotine is heavily affected by viral infection, instead, PBO shows a different trend according to the season.

Finally, to assess how other factors can shape the reaction to nutrition and infection, a multifactorial experiment was carried out in which bees were exposed to four different factors: the neonicotinoid sulfoxaflor, a low temperature and a parasitic infestation either in presence of pollen or not. The experiment suggested an interesting interaction between nutrition and toxic compounds that, again, could be mediated by the detoxification system.

Determinants of precision agriculture technologies adoption in Italian wineries

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Site-specific management (SSM) is the idea of doing the right thing, at the right place, at the right time. Precision farming provides a way to automate SSM using information technology, thereby making SSM practical in commercial agriculture. Precision agriculture includes all those agricultural production practices that use information technology (e.g., variable rate application (VRA), yield monitors, remote sensing). The global wine industry facing pressure from governments, different stakeholders, and the growing interest from consumers for green products and the higher commitment to export in countries with a strong attention

for "sustainable products" are among the "institutional drivers" to adopt sustainable agriculture practices. Therefore, the Italian wine sector also stepped up to the challenge and aiming to move towards adoption of sustainable agricultural practices to maximize potential market demand, sustainability objectives and mitigate climate change. At present little attention has been paid to the potential benefits deriving from the adoption of precision viticulture and its economic efficiency analysis in winery sector. Therefore, present research is aimed to highlight the factors, drivers, and challenges towards adoption of precision agriculture technologies in Italian wine sector.

Actinomycetes come to rescue of viticulture sustainability

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Walter Chitarra

A great number of recent researches have shown that Actinomycetes can be considered as promising beneficial bacteria improving the growth and the capacity of plants to face up both biotic and abiotic stresses. However, very few studies have been performed about the use of Actinomycetes in grapevine. Above this background, we decided to isolate bacteria from grapevine wood tissues characterizing a collection of 92 isolates, most of which belonging to the Actinobacteria phylum. Subsequently, they have been used in dual culture assay against some of the main grape pathogens such as B. cinerea and the etiological agents of esca syndrome. Additionally, regarding the isolates that previously showed a promising antagonistic activity against fungal pathogens, we have examined if the pathogen inhibition effects have been linked with the production of any volatile compounds (VOCs) through septate petri dish assays. On the basis of their pathogen's growth inhibition rate, 10 isolates were selected and used for subsequent in vivo test in order to asses their efficacy in controlling botrytis bunch rot of grape. We are also testing these bacteria on in vitro grapevine plants and on in vivo rooted cuttings. In the vitro assay we are evaluating their individual capacity to promote plant growth; with regard to the rooted cuttings experiment instead, we inoculated a microbial consortium testing the effect of these beneficial bacteria both alone and in combination with some mycorrhizae fungi. More studies are needed but preliminary results display an interesting attitude of these actinomycetes as promising tool to improve viticulture sustainability.

Biofortification of carrots using chelate and sulfate forms of iron and zinc

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Francesco Giuffrida, Rosario Mauro

Fresh consumed vegetables are healthy food products that can be nutritionally enhanced through agronomics strategies, such as mineral biofortification. Iron and zinc deficiency are the most common causes of micronutrient deficiency. Considering that carrot (*Daucus carota* L.) is one of the most consumed vegetables worldwide in this study we investigated the response of the early carrot crop to different forms of foliar applications of iron (Fe) and zinc (Zn). The aim of the research was to compare the effects of an inorganic salt ($FeSO_4$ and $ZnSO_4$) with a chelate of form of those elements (Fe-DTPA and Zn-EDTA). The Fe concentration in carrot showed an increase (+52%) in the treatment with $FeSO_4$ and a reduction (-35%) due to the treatment with $ZnSO_4$, no differences were found when Fe-DTPA was applied. With reference to the concentration of Zn, the application of Zn-EDTA resulted in an increase of 94%, compared to an increase of 57% obtained with applications of $ZnSO_4$. Furthermore, both antagonistic ($ZnSO_4$ on the Fe content) and synergistic ($FeSO_4$ on the Zn content) effects emerge in relation to the element and the chemical form considered. The application of Fe or Zn has in some cases resulted in an increase in the carrot fresh weight and size. In the case of dry matter, only the application of Zn-EDTA determined an increase compared to the other treatments. No significant differences were found with reference to color coordinates and carotenoid content. The results show that the studied applications of Fe or Zn are able to modify the mineral content of 'Dordogne' carrots, without compromising their main product and nutraceutical characteristics.

Breeding for improving yield and grain quality of durum wheat in Southern Italy through the identification of ideal allelic combination of adaptation genes

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Durum wheat is one of the most widely cultivated crops in the Mediterranean area, which is expected to be particularly vulnerable to climate changes. In face of this challenge, new varieties need to be developed to increase yield while maintaining quality and reducing agricultural inputs. Wheat phenology offers the opportunity to fight against drought by modifying crop developmental phases. The major genetic factors influencing such phenological characteristics are vernalization response genes (*Vrn*), controlling the requirement of a cold period to switch from the vegetative to reproductive phase, as well as photoperiod sensitivity genes (*Ppd*), determining plant response to day length. Phenology genes also regulate flowering time, plant height, tiller number, and spike characteristics. Due to the importance of these loci, the objective

of this research was to analyze the allelic variation at these loci in relation to plant phenology and architecture and determine which allelic combinations are most beneficial for plant adaptation to climate change. For this purpose, a large panel of 160 durum wheat genotypes, released for cultivation during the last two centuries in Italy, was genotyped. The analysis started from the allelic variation at the major *Vrn*, *Ppd* and *Rht* loci, using allele-specific molecular markers (i.e. *Vrn*-A1, *Vrn*-B1and *Vrn*-B3, *Ppd*-A1, *Ppd*-B1 and *Rht*-B1) loci and continued with the phenotyping of agronomically-important traits. The first results showed differences more qualitative than quantitative in genetic diversity among accessions investigated. The full characterization of the allelic variation will assist breeding programs to improve adaptation to local environments and to optimize wheat phenology and architecture, as well for climate change.

Characterization of new fungal diseases of Pistachio in Sicily and development of sustainable management strategies

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In Italy the pistachios production is condensed in the southern regions with Sicily considered as the first Italian producer. Aim of the project was to identify and characterize fungal isolates associated with canker, fruit and foliar diseases of pistachio. Isolations from cankered tissues and fruit spots samples consistently yielded fungal species in the *Botryosphaeriaceae* family. At the same time, presence of trees showing characteristic septoria-like leaf spot were investigated. Identification of all collected isolates was conducted using morphological and molecular analyses. Morphological characterization was based on colony morphology, growth on different artificial media, conidia measurements and on the effects of temperatures on mycelial growth. DNA data derived from sequencing the ITS, *tef1*-α and *tub2* gene regions were analyzed via phylogenetic analyses (Maximum Parsimony and Maximum Likelihood). Regarding cankered diseases, results of the analyses confirmed the presence of *Botryosphaeria dothidea*, *Neofusicoccum hellenicum* and *Neofusicoccum mediterraneum*. Regarding foliar disease, results showed that our isolates clustered with *Septoria pistaciarum.* Pathogenicity tests were conducted for all the recovered fungal species under laboratory and field conditions using the mycelial plug and spore suspension techniques. The inoculation experiments revealed that all the fungal isolates resulted pathogens to pistachio. Among the *Botryosphaeriaceae,* species identified in this study *N. hellenicum* (occasionally detected) and *N. mediterraneum* were the most aggressive based on lesion length on shoots and fruits. *N. mediterraneum* was the most widespread among the orchards while *B. dothidea* can be considered a minor pathogen

involved in this complex disease of pistachio. Moreover, to our knowledge, this is the first report of *N. hellenicum* in Italy. Regarding Septoria leaf spot, this study represents the first update on *S. pistaciarum* in Italy since its first identification in 1934.

Session 3

Different approaches to identify genetic traits that control Flavescence dorée resistance phenotype in grapevine

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Flavescence dorée (FD) is one of the most destructive grapevine yellows (GY) diseases, and a quarantine pest in the European Community. It is caused by phytoplasmas, which are transmitted in vineyard by the leafhopper *Scaphoideus titanus*. Grapevine varieties completely resistant to FD have not been uncovered yet, however inter and intraspecific differences in susceptibility have already been observed. The different behavior among the grapevine varieties suggests the presence of genetic traits in grapevine germplasm related to high or low susceptibility to FD. The aim of this work is to find out these genetic traits. The first approach consists in the identification of Quantitative Trait Loci (QTLs) by genotyping and phenotyping of a segregant population generated by crossing between Tocai friulano and Chardonnay, two varieties with very different susceptibility. 184 individuals were genotyped by Genotyping by Sequencing (GBS) and the Single Nucleotide Polymorphisms (SNPs) obtained were used to create a linkage map. At the same time, F1 individuals were planted in two experimental vineyards and are undergoing to phenotyping. The SNPs highlighted by GBS were also associated with genes differentially expressed between Chardonnay and T. friulano during FD infection to check if a SNP may be responsible for the different phenotype. Another approach to uncover the genetic differences concerns three Chardonnay clones that showed different susceptibility to FD. From one side the clones are being sequenced by accurate whole genome techniques (PacBio), from the other side the transcriptomic profiles of two of them are studied by RNAseq performed in the early stage of FD infection. The RNAseq data first analysis showed a different kind of response against the pathogen between two clones.

Susceptibility of ancient and modern wheat varieties to the lesser grain borer, *Rhyzopertha dominica* (F.)

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The increasing demand for healthy cereal-derived products and organic food is giving to ancient cereal varieties a new chance of survival in marginal agricultural areas and is providing a way to safeguard these precious genetic resources. The Ph.D. programme mainly aims to define the susceptibility of ancient and modern wheat varieties originating from Southern Italy towards primary stored-cereal pests in order to identify less-susceptible or resistant genotypes useful in breeding programs. Rhyzopertha dominica (F.) (Coleoptera, Bostrichidae) is one of the major primary pests of stored cereals worldwide. The susceptibility of seven wheat varieties, including 3 ancient genotypes (Saragolla Antica, Dauno III, Senatore Cappelli), 3 modern durum wheat varieties (Ofanto, Svevo, Faridur) and one bread wheat variety (susceptible control) to R. dominica was assessed during the first year of Ph.D. activity. For each genotype, kernel (60 g) samples (n = 5) were placed in cylindrical polypropylene containers (Ø 9.8 x 10.8 cm) and infested with 12 twoweek-old unsexed adults. Containers were closed by screw cap with a central hole (1 cm) screened by a metallic net (mesh 0.5 mm) to allow air exchange and maintained in the dark at 28 ± 2°C and 60 ± 5% R.H. After 15 days, insects were removed and the number of dead specimens recorded.

Emergence of F1 progeny was monitored every 2 - 5 days. For all varieties, a low adult mortality was recorded after 15 days exposure. The lowest progeny production was obtained from the Dauno III variety (274.6 ± 39.8) and it was significantly lower than that observed for the Faridur variety (539.6 ± 52.8) ($P < 0.05$, Tukey test). The average development period (D) of R. dominica in kernels of Senatore Cappelli (61.04 ± 0.09) was significantly higher than in those of the remaining varieties. According to the susceptibility index (S.I. = 100 * lnF1/D) (Dobie, 1974), the different

wheat varieties were classified as "susceptible" (S.I. from 8 to 10).

Evaluation of climate reanalysis as potential data source for estimating reference evapotranspiration at national scale

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Simona Consoli, Daniela Vanella, Juan Miguel Ramírez-Cuesta

Accurate estimates of reference evapotranspiration (ET_0) are essential for an optimal irrigation management. ET_0 is traditionally computed using the FAO-56 Penman-Monteith equation through in situ agro-meteorological observations. However, this information is not evenly spatially distributed and often presents

substantial time gaps. In this context, climate reanalysis offers consistent time-series of multiple meteorological parameters, around all the globe.

The aim of this study was to assess the reliability of ERA5-Land reanalysis to predict the main agro-meteorological variables (solar radiation S_r, air temperature T_{air}, relative humidity RH, wind speed u_{10}) and estimate the ET_0 at national scale. All parameters were compared at daily and seasonal scales with in situ agro-meteorological observations from 63 automatic weather stations over several irrigation districts in 6 different Italian regions, during the reference period 2008-2020.

The results showed a good agreement between observed and reanalysis T_{air} and S_r, with R^2 and RMSE values ranging from 0.93 to 0.96 - 1.31 to 2.60 °C, and from 0.80 to 0.88 – 31.96 to 44.98 $W \cdot m^{-2}$, respectively. On the other hand, moderate overestimation and underestimation of reanalysis RH and u_{10}, were detected, respectively.

Globally, a good performance of ERA5-Land reanalysis was observed in estimating ET_0, with a slight underestimation ranging from 2 to 14% and with R^2 and RMSE values ranging from 0.81 to 0.94 and from 0.57 to 0.88 $mm \cdot d^{-1}$, respectively. Moreover, a seasonal pattern was observed, being ET_0 estimates more accurate in spring/summer than in autumn/winter.

These results confirm the potential of ERA5-Land reanalysis as alternative data source to estimate ET_0, overcoming the unavailability of observed agro-meteorological data.

Sex pheromone components of the asparagus moth, *Parahypopta caestrum*: chemical analysis, electrophysiological study and preliminary field tests

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Parahypopta caestrum (Hübner) (Lepidoptera, Cossidae) is the most serious pest of *Asparagus* spp. in the Mediterranean basin. To contribute to the knowledge on the sex pheromone of this pest further chemical, electrophysiological and field tests were carried out during the second year of the Ph.D. programme. In the previous year, gaschromatography coupled with mass spectrometry and electroanntennography (GC-MS-EAD) analyses have revealed the presence of four EAG-active peaks in the gland extracts of calling virgin females whose mass spectra matched those of three monounsatured C_{14} aliphatic acetate and one monounsatured C_{14} aliphatic alcohol, respectively. In the second year, isomerism and double bond position in the structure of these EAG-active compounds were fully elucidated by comparing their GC retention times and MS fragmentation patterns with those of authentic standards. Double-bond position and configuration

of sex pheromone candidates were also confirmed by comparative EAG experiments with series of monounsaturated C_{14} aliphatic acetates and alcohols. To define the relative ratio of different sex pheromone components in female glands, quantitative analyses of different gland extracts were performed. The sensitivity of male antennae to each sex pheromone component was characterized by calculating the corresponding EAG dose-response curves. In preliminary field trapping trials, individual compounds were not attractive. Some binary and even more ternary mixtures of acetates were attractive to *P. caestrum* males. The addition of the monounsaturated C_{14} alcohol to the attractive blends did not improve male trap catches. Next year activities will focus on the optimization of pheromone lure (blend, dosage, dispenser) and traps to be used for monitoring and mass trapping applications and on the identification of asparagus volatile compounds possibly involved in insect host-plant interactions.

One welfare experiences

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Edi Piasentier

The One Welfare concept recognises the interrelation between animal welfare, human well-being and the environment. For animals, health and welfare are not separable and, as highlighted by the One Health concept, health means the same for both humans and animals. Two One Welfare experiences, referred to both a long and a short supply chain ones, will be studied by the PhD activity.

The transport of domestic animals through and within European countries is a very widespread and numerically consistent practice that highly concerns European citizens. Long journeys (EC Reg. n. 1/2005) have more harmful effects on animal welfare than short journeys. Horses can be transported for a maximum of 24 hours, after that they must stop in official resting places, Control Posts (CP) and Collection Centers. The aim of this study is to verify the influence of the transport conditions (size of the consignment, environmental temperature) and animal category (breed, size, age, sex) on horses' well-being during the period spent inside a CP. The measures and their degree of expression will be collected following the indications of AWIN welfare assessment protocol for horses.

The consumer citizen, through food choices can support agri-food production processes, inspired by One Welfare and One Health, when they are reliable and traceable. For short supply chains, blockchain technology can be a great opportunity to give value to their traced products. Indeed, this technology consists of a shared and immutable ledger for recording transactions, keeping track of assets and consolidating a relationship of

trust. During the PhD, a feasibility study will be carried out on the blockchain system applied to the regional short supply chain of antibiotic free beef "Filiera CarnePRI".

Genome editing, a new biotechnological approach to produce lycopene-rich pigmented oranges

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Fruits and vegetables rich in anthocyanins and lycopene are highly appreciated by consumers because of their healthy properties. Among citrus, there are no high evidence on the accumulation of both pigments in the same fruit. Breeding programs have been addressed to that scope, even though they need a large use of resources and time. Cisgenesis and genome editing represent the most advanced genetic technologies that overcome traditional breeding limits, particularly challenging for woody plants. Two conditions are requested to use those: the knowledge of the gene controlling the trait of interest, the ability to regenerate transformed plants.

A preliminary study was focused on the evaluation of quantitative expression of genes involved in the lycopene metabolism occurring in pink-fleshed citrus varieties. Phenotypic and expression data showed that the high content of lycopene is correlated with a low expression of β-LCY2 gene.

Therefore, we have used a CRISPR/Cas9 approach to enrich in lycopene 'Doppio sanguigno' (DS) pigmented orange, deactivating the β-LCY2. Carrizo citrange (CC) was used as model species in transformation experiments. Genome editing vector was realized using the Golden Braid technology; it includes two sgRNAs for β-LCY2, the cassettes for *Cas9* and *nptII* genes. After *Agrobacterium*-mediated transformation, 149 CC resistant shoots and 66 DS are *Cas9*-and-*nptII* PCR positive; CC have been self-rooted, DS were mini-grafted onto Carrizo seedlings. Moreover 5 CC and 4 DS plantlets showed a large deletion between the sgRNAs, all the others are presumably mutated in one or both sgRNAs. Illumina sequencing will elucidate which kind of mutation has occurred on each plant.

This study represents the first attempt to use CRISPR/Cas9 genome editing approach to improve qualitative traits in citrus varieties.

"Bio-refinery" processes for the valorization of industrial waste from Tomato processing

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Mariangela Caroprese - Co-tutor: Matteo Francavilla

Climate change and growing populations are a threat to sustainable development around the world. The EU is adopting a series of strategies to improve global sustainability, including incentivizing bio-refinery in the agro-food sector. Biorefineries, designed to minimize waste and transform every fraction of raw materials into chemicals with high added value with a low environmental impact, will lead to the achievement of the objectives described in the European Green Deal. With these premises, this study focused on the valorization of tomato pomace (TP) from the industrial processing of tomatoes through an integrated biorefinery process to improve economic and environmental sustainability. The investigated biorefinery model was centred on a *" one-pot "* process for the simultaneous extraction of lipids (lycopene, FAMEs, phenolics) and pectin from TP, through a biphasic system (BPS). The BPS was made of two immiscible green and safe solvents (ethyl acetate and water). The remaining solid by-product (SR) was further valorized by microwave-assisted extraction (MAE) of cutin. Different temperatures (25, 40, and 60 °C) and time ranges (1, 5, and 10 minutes) were tested for the one-pot extraction process. It generated a three phasic system composed of a Red Phase (RP, ethyl acetate), a White Phase (WP, hydrogel in water), and a Solid Residue (SR, pellet), corresponding to 7.5% dw, 17.8% dw, and 74% dw of TP respectively. The extraction (MAE) of cutin from SR was optimized by varying temperature (from 100 °C to 160 °C) and time (from 10 to 30 minutes). The highest yield of cutin (20% dw of TP) was found operating MAE with an alkaline solution (0.5M NaOH) at 120 °C for 30 minutes. In conclusion, the proposed biorefinery process seems to demonstrate the effectiveness of green sequential extractions of high-value compounds from tomato pomace, converting this by-product/waste into a multi-products source.

Multilevel analyses of pest induced defenses in solanaceous plants

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Plants are able to respond to abiotic and biotic stresses, including insect herbivores, with complex defense responses. Defense mechanisms in cultivated plants represent an important tool for their integrated pest control packages. In particular, plant volatiles (VOCs) and induced volatiles (HIPVs) can play a key role in indirect plant defense, involving multitrophic levels. VOCs and HIPVs are important to allow plant-plant and plant-natural enemy communications. In this context, I conducted olfactory bioassays, I identified VOCs and HIPVs, and I evaluated the gene expression of tomato and aubergine plants primed by *Tuta absoluta* (Lepidoptera: Gelechiidae) larvae. In the tests, for tomato var 'Delizia', var 'Cikito', var 'Marinda', var

'Optima', var 'Rovente', var 'San Marzano nano'' and var 'Tyty' were used and for aubergine var. 'Black beauty'. Adults of *T. absoluta* and *Encarsia formosa* (Hymenoptera: Aphelinidae) were used in olfactory bioassays to understand how the key tomato pest and a model parasitoid are attracted to VOCs and HIPVs emitted by primed plants. The evaluation of the gene expression of three genes were done to highlight defense plant pathways, i.e., *jasmonic acid*, *salicylic acid* and *B-phellandrene*. The results showed that in the identification of volatiles there was a significant increasing of production of primed volatiles. This explains the pest and parasitoid olfactory responses that varied among primed and non-primed tomato and aubergine varieties. The results of the gene expression analyses provided an important evidence of defense induction in the plant. These results are useful for understanding plant defense mechanisms and, above all, can provide volatile compounds involved in the defense of plants that could be used in integrated pest control, for example through attractive dispensers for natural enemies, and in multitrophic relationships.

Grapevines subjected to different water regimes: a study of the physiological and molecular responses

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Paolo Sivilotti

It has been ascertained that future global warming scenarios will increase the drought risk in most cultivated regions in the next several decades. Drought can seriously affect crop yield and quality, and, in exceptional cases, crop survival. Where applicable, irrigation practices are largely adopted to compensate seasonal water deficit, even though such practices may also generate negative environmental aftermaths. In this context, agricultural research must deal with this emerging problem by either optimizing water usage or creating more tolerant/resistant breeds. To achieve these objectives a deeper understanding on the responses and on the mechanisms implemented by plants under stress conditions is needed. Grapevine (*Vitis vinifera* L.) has demonstrated a high phenotypic plasticity and is considered as a model perennial plant in many studies in plant science.

In the present work we aimed to: 1) better understand the physiological mechanisms related to the plant responses under water shortage; 2) study the molecular mechanisms that drive these responses. In the experimental farm of the University of Udine, we carried out water stress trials on different grapevine cultivars subjected to different conditions. During all the experiments, plants water status and gas exchanges were monitored, and leaf were sampled. Grapevine was demonstrated to adopt physiological responses able to avoid excessive plant dehydration levels. In particular, grapevine adaptations are closely related to a

different carbon allocation. In fact, stressed vines tend to preserve reserves accumulation in perennial organs, sacrificing mainly the shoot growth. Moreover, on two varieties that showed different behavior under stress, a multiple hormonal profile on leaf samples was performed by HPLC-MS/MS. The results of this study are still under processing, but a preliminary analysis is encouraging. The final step of this research will be the identification of target genes involved in the plant drought resilience.

Genomic selection for durum wheat improvement

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Durum wheat (*T. turgidum* ssp. *durum* Desf.) is one of the most important staple crops in the Mediterranean, with areas of cultivation spread worldwide, often in drought-prone areas. Genomic selection is an attractive breeding method used to improve complex quantitative inheritance traits. In this study, prediction accuracy for grain yield (GY) and related trait such as heading date (HD), plant height (PH) and thousand kernel weight (TKW) was investigated in a panel of 250 durum wheat varieties using several GS models, then the most fitting model was chosen to select the best parents to cross. The durum wheat panel was phenotyped for the trait of interest in two consecutive growing seasons at the CREA-CI, Foggia, under two different field conditions: “Not stressed” (with nitrogen and water supply), and “Stressed” (without any supply). The entire panel was genotyped using the “Axiom TaBW420” SNP array. Best linear unbiased estimates (BLUEs) between year and a filtered SNP matrix were used to verify prediction accuracy using several models such as RR-BLUP, BayesA, BayesB, BayesC, and Bayes LASSO. Traits including GY, HD and TKW showed an high-moderate accuracies for each model up to ~0.7. Otherwise, low accuracy was detected for the trait PH using both ridge regression based and Bayesian models (<0.4). Prediction accuracies resulted similar among different models for each trait in both treatments. All models showed higher accuracies for the treatment “not stressed” than “stressed” for each trait. In this work, the accuracy of five models was estimated to select the best fitting model that will be used to predict the best crosses for grain yield and related traits in low input and standard field conditions.

Green solutions and innovative technologies for post-harvest management and safety of food products of the organic and zero-residue citrus production chain

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Quality standard, health of the consumers and a long shelf-life are fundamental aspects affecting the competitiveness of citrus fruits produced by Mediterranean countries on both domestic and international markets. Rots caused by fungi are one of the main causes of post-harvest losses (average of 30%) of citrus fruits and may consistently reduce their shelf life. Diseases by these pathogens occur before or during harvesting and appear after an incubation period and often they are visible only during storage, transport and marketing. Some post-harvest fungal pathogens produce toxins that can also be found in juices and are, therefore, a concern for human health. Prevention of post-harvest rots of citrus fruits is usually carried out with synthetic fungicides; but their use has often determined the selection of resistant pathogenic strains that reduce the effectiveness of some active ingredients. However, increasingly restrictive laws and regulations have reduced or prohibited the use of pesticides and promoted eco-friendly post-harvest fruit treatments, based both on the use of environmentally friendly substances and bio-products obtained from natural microbiota associated with citrus fruit, aimed at extending fruit shelf-life.

The project aims at detecting i. the non-toxic green substances, such as GRAS, biostimulants, natural substances and bioproducts to the manage citrus postharvest rots; ii. develop new sensitive, specific, cost-effective and easy to handle diagnostic kits based on RPA to detect quarantine pathogens infecting citrus fruits; and finally iii. develop practical and sensitive analytical methods to detect mycotoxins in citrus fresh fruit peel and juice as parts of an integrated and sustainable post-farming management strategy of organic citrus fruits.

Use of Meta-Analysis in Environmental Valuation

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Maria De Salvo, Giovanni Signorello

Over the past 50 years, environmental economists have put a great deal of effort into the conceptualization and valuation of nonmarket benefits supplied to the society by environmental goods and natural capital. Numerous case studies and applications have been produced worldwide. This huge empirical literature, which is now easily searchable on line or still partially collected in specific and accessible databases, facilitates the use of the Meta-Analysis (MA) techniques, either for taking stock of values and their moderators, or for benefit transfer purposes (Johnston et al., 2015; Bergstrom and Taylor, 2006). In this presentation, first I shortly review the main features of MA, and how MA has been applied so far in environmental valuation

area. Then, I provide a practical example to illustrate how existing MAs can be used for assessing and scaling up nonmarket benefits. Finally, I point out which new MAs of environmental valuation studies I am planning to carry on, and which econometric issues of the meta statistical regression functions I would like to explore for taking into account the panel structure of data and the spatial correlation among the observations.

Exploring the molecular mechanisms underlying the pathogenesis of '*Candidatus* Phytoplasma solani'

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Phytoplasmas are wall-less, phloem-limited prokaryotes belonging to the class Mollicutes. '*Candidatus* Phytoplasma (*Ca*. P.) solani' (16SrXII-A) is associated with bois noir (BN) of grapevine and stolbur disease of herbaceous plants and is primarily transmitted by the planthopper *Hyalesthes obsoletus* Signoret. The aim of our study was to investigate the interactions established in tomato (*Solanum lycopersicum* L. cv. Micro-Tom) by several strains of '*Ca*. P. solani'.

Strains of '*Ca*. P. solani' were acquired by individuals of *H. obsoletus* captured on bindweed and stinging nettle in vineyards of northeastern Italy with high incidence of BN and forced to feed on tomato plants. The infection was then maintained through grafting. All the strains were characterized on the basis of symptom development, ultrastructural modification of the sieve elements, molecular typing based on *tuf*, *secY, vmp1* and *stamp* genes as long with genome sequencing by MinION (Oxford Nanopore Technologies, ONT) and Illumina followed by genome structural and sequence analysis.

Molecular typing allowed us to distinguish our strains in three main clusters: one including strains of tuf-a genotype (transmitted by insects from stinging nettle) and two including strains of tuf-b genotype (from bindweed). Genome analysis on assembled ONT sequences highlighted molecular and structural differences in the genome of our strains, confirming the three clusters. The quite different symptoms as well as the sieve-element cytological modifications induced on tomato by the strains of the two *tuf* genotypes suggested a higher virulence of tuf-a strains.

Our results propose that '*Ca*. P. solani' species holds unexplored genomic variability associated with different virulence and symptoms in tomato. Ongoing studies are genome hybrid assembling and *in planta* experiments for evaluating differences in pattern of colonization and phytoplasma gene expression.

Sweetpotato whitefly, *Bemisia tabaci* (Homoptera: Aleyrodidae), and its predator *Macrolophus pygmaeus* (Hemiptera: Miridae): what relationships and combined effects on the "plant system" they develop on?

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Carmelo Rapisarda

Sap-sucking insects are important herbivorous, building up a rich group of pests of crops. In Mediterranean environments, the whitefly *Bemisia tabaci* (Gennadius) is one of the key pests in greenhouses and, among its natural enemies, the predatory bug *Macrolophus pygmeus* (Rambur) is a main candidate. Anyway, while being an active predator, this bug often shows also a phytophagous habit. In the present study, the impact of *B. tabaci* and *M. pygmeus* on morphology and physiology of two species of solanaceous plants, namely *Solanum lycopersicum* L. (tomato) and *S. melongena* L. (eggplant), was analyzed under laboratory conditions. The investigations were carried out in two separate experiments, by completing one whitefly generation in the first and after nymphs emergence of *M. pygmaeus* in the second. In both experiments, data on the main agronomic parameters of plants, processed through analysis of variance (ANOVA) and Fisher's LSD test, show significant difference at $P<0.01$ and $P<0.05$ levels. In the first experiment, significant differences were observed in height, dry weight of aerial part, leaf area and indirect chlorophyll content, with a reduction percentage in infested plants of -61%, -32%, -61% and -63%, respectively. Significant differences were observed also in the second experiment, in which reduction percentages of plant height, roots dry weight and SPAD in infested plants were -11%, -32% and -11%, respectively. For all biological stages of *B. tabaci* the analysis indicates no significant differences in the insect preference between leaf positions. In view to reduce the use of pesticides and promoting biological control, the results of this work can improve our capability to forecast plant growth and development, managing potential inoculative release of the predator according to pest infestation.

Computer Vision System for non-destructive evaluation of quality traits in fresh and packaged fruits and vegetables

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In green vegetables, leaf chlorophyll content is a key indicator of the physiological status of plants and the retention of green colour during postharvest, strictly related to the chlorophyll loss, is the most common index used to evaluate the external quality and freshness of this kind of products. Among the most developed non-destructive instruments widely used for chlorophyll content measurement, Computer Vision System (CVS) provides a good solution for its low cost, rapid and consistent inspection. It has been widely demonstrated that CVS can assess the quality level of fresh and fresh-cut commodities through the prediction of chemical parameters related to the quality. Nevertheless, there are very few works about its application on packed products.

During my second PhD year, five experiments were carried out on rocket leaves to measure destructive quality parameters along the cold storage (colour, chlorophyll and ammonium content, electrolyte leakage) and, at the same time, to acquire images of the same packed and unpacked samples by CVS. The aim of this research activity was to: a) identify a marker parameter (conventionally assessed by destructive methods) able to assess the shelf-life loss of rocket leaves, and b) develop and validate a CVS that can measure the shelf-life of the product by the prediction of the marker parameter, even through the package. Main results showed that the CVS is a valid non-destructive technology for the chlorophyll content prediction of rocket leaves, recording a performance loss of only about 2% due to the presence of packaging (accuracy of 84% on packed product instead of 86% on unpacked one).

Finally, four trials were conducted on four fresh-cut apple varieties and Barattiere melon to predict their quality through the packaging material by the use of colour information measured non-destructively by CVS. The results are still being evaluated.

Ammonia and greenhouse gas concentrations for emission evaluation from an open-sided free-stall dairy barn in Mediterranean climate

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Ammonia (NH_3) and greenhouse gas (GHG) emissions from the dairy sector are responsible of harmful impacts on the environment. In order to improve the design and the choice of mitigation techniques, the first step is the accurate evaluation of emissions. Starting from the values of gas concentrations, this study aims at: (i) analysing the influence factors on gas concentrations and emissions; (ii) comparing different measurement strategies; (iii) estimating emissions through a systematic approach. Gas concentrations of NH_3, carbon dioxide (CO_2) and methane (CH_4) as well as climatic parameters and cow's behavioral activity

were monitored in an open-sided free-stall dairy barn located in Sicily (Italy) during different experimental periods. NH_3 and GHG emissions were estimated through the application of the CO_2 mass balance method. Data acquired were organised in different datasets and statistical analyses were applied.

The results showed that both daily gas concentrations and emissions changed in time and space. In detail, the horizontal and vertical variability of gases was not homogeneous in the barn. Besides the spatial distribution of sampling locations, the number of measurement repetitions increases data reliability. Among the main influencing factors, microclimatic parameters (i.e., air flow velocity, temperature, relative humidity), cow behaviour (i.e., activity, lying) and barn management (i.e., cooling system, milking frequency, cleaning of the barn) statistically affected ($P<0.05$) the gas concentrations in the barn environment. Although these parameters statistically influenced gas emissions, the daily trend of gas emissions was different than that of gas concentrations due to the variability of the ratio between the concentrations of pollutants (i.e., NH_3, and CH_4) and the tracer gas (i.e., CO_2).

Session 4

Post - harvest disease management of kiwifruit

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Skin-pitting is a post-harvest disease of kiwifruit caused by Cadophora luteo-olivacea, which has been recently reported from different Italian packing houses. Symptoms of skin-pitting, characterized by depressed and elliptical areas associated with brown-violet skin and brown pulp, appear on fruits after about 3 months of cold storage. Skin-pitting causes great economic losses for different stakeholders; moreover, its control through the application of chemical pesticides is not advisable since these products are harmful to both the environment and humans. Thus, the use of biocontrol agents (BCAs) against post-harvest diseases together with other alternative strategies, is considered to be potentially sustainable, cost-effective and efficient. Indeed, the first objective of this work aims to evaluate potential biological control agents (BCAs) for their efficacy against skin-pitting in Kiwifruit. A total of 8 BCAs were selected according to the literature and tested in-vitro for their antagonistic activity against C-luteo-olivacea. Three of them seemed to have a stronger activity and will be used for in-vivo assays in the near future. The second aim is to improve the knowledge regarding the epidemiological characteristics of skin-pitting and comprises the molecular characterization of C. luteo-olivacea isolates recovered from symptomatic kiwifruit during a survey (2021) in FVG region (Italy). PCR amplified internal transcribed spacer (ITS) regions were analyzed by RFLP analyses and fungal identities were confirmed with previously characterized strains. β-tubulin (BT) gene will also be used in further molecular analyses. In addition, a reproducible protocol for estimating the amount of C. luteo-olivacea on fresh kiwifruit surfaces, was set-up in order to understand if there is a correlation between the quantity of its propagules and symptom development. This correlation would allow to plan different strategies for the storage and commercialization of the fruits.

Human-carnivore conflict in Friuli Venezia Giulia: First data from north-eastern Italy

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Human-carnivore conflict is one of the key issues which have been leading to the decline of several carnivore species across the globe and to the abandonment of traditional livestock practices in those areas in which conflicts are more intense. In Friuli Venezia Giulia (FVG), the two main carnivores that come into conflict with livestock activities are the brown bear (*Ursus arctos*) and the grey wolf (*Canis lupus*). Both species were almost extirpated within the area. However, their recent return has led to an increasing number of attacks on livestock. Using predation data collected in FVG, the main purpose of the present work was to determine the main factors which may increase the likelihood of attacks towards domestic species. Our results showed that bears were involved in a higher number of attacks (n = 74, 64.35%) compared to wolves (n = 41, 35.65%). Bears were also responsible for killing a higher number of individuals (n = 189, 57.62%) than wolves (n = 139, 42.38%). Nevertheless, if we compare the ratio between number of attacks and number of killed individuals per species (bear = 0.39, wolf = 0.29) the difference resulted as non-significant. Sheep were the most predated species (n = 267, 81.40%), followed by goats (n = 50, 15.24%) and cattle (n = 11, 3.36%). We did not observe a significant reduction in terms of number of killed individuals between farms which used mitigation measures (n = 42, 36.84%) and those which did not (n = 72, 63.16%). Based on the results presented the synergistic collaboration between local authorities and research institutions assumes remarkable importance to find the most adequate solutions aimed at improving coexistence in the long-term.

Exploring and predicting multitrophic interactions between host plants, whiteflies, parasitoids, and endosymbionts under changing climate

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The impact of elevated CO_2 levels, temperature and humidity on multitrophic interactions between plant, sap-sucking insects, their antagonists, and symbionts has not been studied in detail so far. The whitefly *Bemisia tabaci* is a worldwide important sap-sucking pest on a wide range of host plants, including tomato, to which it causes direct and indirect damage by feeding and vectoring viruses. Biological control using parasitoids, is a standard practice in Europe. In addition, bacterial endosymbionts of *B. tabac*i play an important role in the interaction between plant, whitefly, parasitoids, and the environment. Currently, studies investigating the endosymbiont role in whitefly biology under future climate scenarios do not exist. We investigate the effects of physical consistent elevated CO_2 levels, relative humidity and temperatures derived from multi model ensembles of regional climate projections on multi-trophic interactions, including effects on the tomato plant, *B. tabaci* pest, its natural enemy, and endosymbionts. We peer into the

underlying mechanisms using RNA sequencing and identify differentially expressed genes in both tomato and whitefly to gain a better understanding of how the plant-whitefly system responds to climate change, parasitoid presence, and endosymbiont composition. Further, we combine information on metabolic changes with life parameters of plant, whitefly, and parasitoids to get a comprehensive picture of multitrophic interactions under changing climate. The outcome of this study is a prediction of climate change effects on this important pest. A detailed molecular understanding of plant's and insect's biochemical responses to whitefly colonization under current and future climate, will help create novel whitefly control methods and refine climate change adaptation strategies.

Multi-mutualistic interactions for optimized management of the exotic fungus-farming beetle *Xylosandrus germanus*

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Among invasive wood-boring beetles, polyphagous *Xylosandrus* (Coleoptera: Curculionidae: Scolytinae) species are now common in several European regions. Generally, different microorganisms can be found in association with ambrosia beetles, including mutualists, commensals and antagonists. However, their different relationship with the beetle behavior has not been fully investigated. Ethanol is used by these beetles as one of the main host finding cue, likely because the growth of co-evolved *Ambrosiella* mutualists benefit by an ethanol enriched substrate. Other semiochemicals produced and/or elicited by beetle associated microorganisms could also play a role in host choice and fungal recognition by the beetles. In this context, I assessed under laboratory conditions the role of ecologically relevant semiochemicals, i.e., the volatile organic compounds (VOCs) produced by *Xylosandrus germanus* (Blandford) associated microorganisms and affecting the beetle behavior. First, laboratory beetle colonies were established, and beetle associated microorganisms isolated. Then, different fungal species were identified and characterized. Multiple-choice and no-choice experiments were carried out for evaluating the beetle response to different microbial sources. VOCs belonging from the different beetle associated microorganisms were collected at different timing by means of polydimethylsiloxane (PDMS) sorbents, before storage at -20 °C for upcoming analysis of related semiochemical profiles. Several fungal species were isolated from dispersing *X. germanus* females, including the beetle mutualist *Ambrosiella grosmanniae*. In the behavioral bioassays, *X. germanus* exhibited a positive response to volatiles of *A. grosmanniae* but not for some of the other beetle associated fungi. Dispersing adults also exhibited attraction to volatiles of branch sections previously infested by another beetle female. Overall, these results suggest that VOCs emitted by *A. grosmanniae* represent short-range olfactory cues involved in the host selection of dispersing *X. germanus* females.

Multipurpose agricultural reuse of biomasses and their extracts from different sources

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The pollution of wastewaters with organic and inorganic compounds, such as nitrates, phosphates, heavy metals, etc. is one of the most critical and common environmental problems. The excessive presence of

pollutants causes ecosystem problems, subsequent eutrophication of waterbodies and alteration of water system health.Wastewater, for its composition, can provide the essential nutrients for microalgae growth, and some microalgal species are able to efficiently remove the pollutants, in variable percentages, from different origin wastewater. The adoption of microalgae-based treatment of wastewater represents a good alternative to conventional purification methods. The aim of the research project is the development of a new sustainable approach in the management of urban wastewater through "phycoremediation", and the study of microalgae-based products that could be applied for agricultural purposes.

Samples of urban wastewater from an already active wetland were collected, and the isolation and cultivation of autochthonous microalgae species at laboratory scale were performed. The morphological and molecular identification of each species is currently ongoing. The remediation performances of isolated species were evaluated in a pilot laboratory-scale open purification system and compared to the action of well-known microalgae species as Chlorella vulgaris and Scenedesmus quadricauda. To test multipurpose agricultural applications of microalgae biomasses, for an eco-sustainable chemical-free agronomy, a preliminary test with a Chlorella vulgaris extract as biostimulant was performed on lettuce seedlings. The results showed that the C. vulgaris extract positively influenced the growth of lettuce seedlings, by increasing the fresh and dry weights, chlorophylls, carotenoids, protein content, and ashes at shoot level. At the root level, the extract increased dry matter, proteins, and ash content. Furthermore, both primary and secondary metabolisms at shoot level, in particular nitrogen metabolism, were positively influenced.

Characterization of Lactic Acid Bacteria as Biological Control Agents for fresh-cut sector

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Undesired microflora negatively impacts the fresh-cut sector leading to a reduction of safety, shelf-life and marketability. For this reason, synthetic protectants are often used to reduce microbial contamination of these products. However, the increasing consumers' concern about the toxicity of residues is leading to a general reduction of the use of chemicals in the sector. In this context, the use of Biological Control Agents (BCAs) based on antagonistic microorganisms has assumed international relevance as a promising eco-friendly alternative to chemical interventions. Lactic Acid Bacteria (LAB) are food-associated bacteria with the strain-specific ability to control harmful microorganisms and decay agents but also to improve shelf-life, quality, and safety of fruits and vegetables. In addition, they are often used in the production of functional foods, such as probiotic and biofortified products. In this work, five LAB strains isolated from the surface of

wild fruits and identified as *Lactiplantibacillus plantarum* (formerly *Lactobacillus plantarum*) by 16 S rRNA gene sequencing were characterized for their antimicrobial activity against a panel of 6 fungal strains responsible for fruit decay and 3 food-borne human pathogenic bacteria. The ability to survive in an alginate-coating and counteract the decay development on the surface of artificially contaminated table grapes cv. Italia was evaluated during the shelf-life. The application of the selected strains in *ready-to-eat* fruits and vegetables will also be discussed.

Dietary tannins in extensive farming: how the effects on cow's milk and cheese change according to the season

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Extensive ruminant farming depends on the seasonal availability of pasture for grazing. The strategies adopted by farmers may not have the same effect in different seasons, because of the differences in the basal diet. Therefore, this thesis aimed to assess the effect of dietary tannins on cow's milk and cheese quality in two different seasons in extensive farming. A first *in vitro* experiment showed that tannin extracts had different effects on rumen fermentation and biohydrogenation according to the basal diet (i.e., herbage *vs* hay). In a subsequent *in vivo* experiment, 14 dairy cows were divided into two groups: a control group and a group (TAN) supplemented with 150 g/head/d of tannin extract. Throughout the feeding trial (23 d), the individual milk was sampled and analyzed for proximate composition, urea, color, cheesemaking aptitude, antioxidant capacity, and fatty acid (FA) profile. Also, the cheeses produced with individual milk at the end of the trial were analyzed for proximate composition, proteolysis, color, rheology, FA profile, and aromatic compounds. The experiment was performed twice: in spring, with pasture availability, and in summer, with cows grazing on dry stubble. In spring, tannins had negligible effect on milk and cheese quality. Conversely, in summer, TAN milk had 10% lower urea and slightly higher antioxidant capacity, compared with control. Dietary tannins reduced branched-chain FA, *trans*10-18:1/*trans*11-18:1, and *cis*9*trans*11-18:2/*cis*9*cis*12-18:2 in milk. Also, TAN cheese had lower *trans*10-18:1 concentration and *n*-6/*n*-3 polyunsaturated FA, compared with control. The effect of tannins on rumen metabolism seems enhanced in seasons in which pasture is not available. This could have practical implications for a more conscious use of tannin sources such as tannin-rich extracts, forages, and agro-industrial co-products.

Physiological and molecular study of mechanisms involved in Iron and Nitrogen nutrition in crops

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Plants require at least 17 mineral elements, divided in macro- and micro-nutrients, for their nutrition. Mineral nutrition has an essential role in crop production due to their crucial roles in numerous aspects of plant metabolism, growth and development.

Among nutritional stresses, nitrogen (N) and iron (Fe) deficiencies are widespread limiting factors for plant growth in agricultural fields since they are involved in innumerable physiological processes in living organisms and their poor bioavailability in soils frequently exerts a strong constraint on plant growth and yield.

My project focuses on N acquisition in crops, characterizing plant response when different N sources, such as nitrate, ammonium, urea, and biostimulants are available alone or in combination in the root external media. Furthermore, it focuses on how different N forms and the presence or absence of biostimulants affect Fe acquisition when Fe-sufficient and Fe-deficient condition subsist.

Plants response was studied at both, physiological and molecular level. Ionomic profiles were analyzed and showed different elemental content patterns between treatments.

Metabolomic analyses were carried out and revealed different modulations of plant metabolism among treatments. These analyses were supported by morphometric measures and expression analyses of genes involved in N and Fe metabolism.

The obtained results and the ongoing analyses will help to define the interactions between the plant nutritional pathways of these two nutrients that could give clues on how to optimize the use efficiency of N and Fe sources in crops.

Study on the effect of different storage temperatures on pomegranate husk scald

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Pomegranates (*Punica granatum* L.) have high nutritional value and health benefits and can be cold - stored for several months. However, skin superficial browning that is called husk scald (HS) in particular is a physiological disorder limiting pomegranate marketability during long-term storage. The aim of this study was to analyze the relationship between the incidence of HS and wight loss (WL), respiration rate (RR), total

phenolic content (TPC), antioxidant activity (AA), anthocyanins (AT) electrolyte leakage, (EL) and malondialdehyde (MD), as well as color values (CIEL*a*b*), ΔE and browning index (BI) of pomegranate skin during long-term storage. A total 324 fruits were stored at 3.5, 7 and 11 °C and 95% RH. Evaluations were performed on individual fruits at harvest and at monthly intervals for a period of up to 4 months plus 2 days of ambient temperature after each sampling time. The results showed that HS incidence is strongly related to the storage temperature, especially when shelf life was prolonged for more than 3 months. In addition to ΔE and BI, HS, WL, RR, EL and MD were significantly higher at 11 °C, whereas TPC, AA and AT were higher at 3.5, 7 °C storage after 16 weeks. Findings suggest that higher storage temperature as an abiotic stress more likely triggers a cascading oxidative change which weakens the antioxidant defense system and consequently interrupts cell integrity along with membrane injury. Eventually, cell disruption manifests itself as wounding, which results in the enzymatic browning known as HS.

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