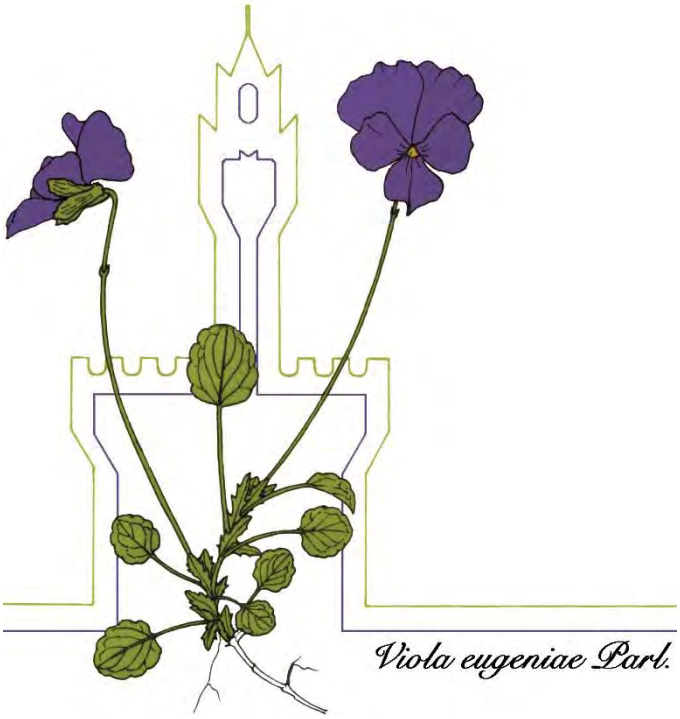
116° Congresso della Società Botanica Italiana

VII INTERNATIONAL PLANT SCIENCE CONFERENCE (IPSC)

ONLINE, 8 - 10 SEPTEMBER 2021



ABSTRACTS

KEYNOTE LECTURES, COMMUNICATIONS, VIDEO ABSTRACTS

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VII INTERNATIONAL PLANT SCIENCE CONFERENCE (IPSC)

Online, 8 - 10 September 2021

Programme

Wednesday 8 September 2021

BRIDGING SESSION BETWEEN THE ITALIAN BOTANICAL SOCIETY AND MEDPALYNOS
on platform Clickmeeting
(Chairpersons: A. Chiarucci and A.M. Mercuri)

9:00-9:20 Opening of the 116th SBI Congress - announcement of the MedPalynoS closing ceremony

9:20-9:50 **Scott Mensing**, Edward Schoolman, Adam Csank, et al., University of Nevada, Reno, USA (30 min)
"The challenge of combining historical archives with paleoenvironmental data to create robust explanations of environmental transformation through time"

9:50-10:00 Questions

The 116° Congress of the Italian Botanical Society continues on platform Webex

SYMPOSIUM

"ENVIRONMENTAL AND PRODUCTIVE CHALLENGES IN PLANT CELL BIOLOGY AND BIOTECHNOLOGY"
(Chairpersons: L. Navazio and M.S. Lenucci)

Key words plant cell biology and molecular biology, plant biotechnology, plant growth and differentiation, plant-environment interactions, productive challenges

10:00-10:30
- **Salma Balazadeh**, Leiden University, The Netherlands; Max Planck Institute of Molecular Plant Physiology, Potsdam, Germany. (25 + 5 min)
"Mechanisms of thermomemory in plants"

10:30-13:06 **Communications**

- **Erika Bellini**, Luca Paoli, Stefania Bottega et al. (10 + 2 min)
"New insight on thiol-peptides: are they involved in cadmium extracellular secretion?"
- **Ignacio Ezquer**, Maurizio Di Marzo, Vívian Ebeling Viana et al. (10 + 2 min)
"New insights into the role of cell wall modifications induced by α-XYLOSIDASE1: The impact in seed and fruit size in *Arabidopsis thaliana*"
- **Lavinia Mareri**, Claudia Faleri, Luigi Parrotta et al. (10 + 2 min)
"Insights into the mechanisms of priming and thermotolerance in tobacco pollen"
- **Antonella Muto**, Emanuela Talarico, Ernesto Picardi et al. (10 + 2 min)
"Genome-wide analysis of the H3K27me3 epigenome and transcriptome in the ovule developmental stages during pollination of *Ginkgo biloba* L."
- **Lorenzo Ferroni**, Andrea Colpo, Alessandra Molinari et al. (10 + 2 min)
"Quantitative assessment of the thylakoid membrane appression in the chloroplast of *Selaginella martensii* Spring (Lycopodiophyta)"

Coffee Break

- **Federica Della Rovere**, Camilla Betti, Laura Fattorini et al. (10 + 2 min)
"Roles of Brassinosteroids on *Arabidopsis thaliana* rooting in the presence of Cadmium"
- **Enrico Cortese**, Alessio G. Settimi, Mattia Franceschi et al. (10 + 2 min)
"Effects of plasma-activated water on signalling pathways underlying plant self-defence responses"

- **Daniela Zuzolo**, Carmine Guarino, Alessia Postiglione et al. (10 + 2 min)
  "Overcome the limits of multi-contaminated industrial soils bioremediation: insights from a multi-disciplinary study"
- **Mattia Terzaghi**, Angela Cicatelli, Francesco Guarino et al. (10 + 2 min)
  "Soil amendment with compost and biochar in a 50-year old olive grove affects soil enzymatic activity, rhizosphere microbiome and fine root production"
- Ilaria D'Ippolito, Alba Mininni, **Adriano Sofo** et al. (10 + 2 min)
  "Alteration of the anatomical and morphological structure of kiwifruit roots subjected to soil anoxic conditions"
- **Andrea Crosino**, Ivan Sciascia, Mara Novero et al. (10 + 2 min)
  "Digital image analysis to quantify arbuscular mycorrhizal root colonization"
- **Veronica De Micco**, Antonello Bonfante, Giovanna Battipaglia et al. (10 + 2 min)
  "Multiscale and multitemporal approach to evaluate resource use efficiency in vineyard towards sustainable cultivation of Greco grapevine: the GREASE Project"
- **Cristina Votta**, Valentina Fiorilli, Jian You Wang et al. (10 + 2 min)
  "The effect of zaxinone, a natural metabolite derived from carotenoids, on tomato plants"

SYMPOSIUM

"MEDICINAL PLANT SECONDARY METABOLISM: FROM PLANT-ENVIRONMENT INTERACTIONS TO HEALTH PROPERTIES"

(Chairpersons: L. Menghini and F. Conforti)

Key words medicinal plants, bioactive principles, secondary metabolites, plants and environment, plants and health

15:00-15:30 • **Milen I. Georgiev**, Bulgarian Academy of Sciences, Bulgaria (25 + 5 min)
"Metabolomics: Just Another OMICS or an Ideal Platform for Accelerated Lead Finding?"

15:30-16:42 **Communications**

- **Ilaria Chiocchio**, Cinzia Sanna, Elisabetta Grillini et al. (10 + 2 min)
  "Seasonal variation in the metabolome of *Arbutus unedo* L. leaves collected in different areas of Sardinia (Italy)"
- **Giuliana Donadio**, Valentina Parisi, Valentia Santori et al. (10 + 2 min)
  "Antimicrobial activity of extracts, fractions and compounds from leaves of *Psiadia punctulata* (DC.) Vatke growing in Saudi Arabia"
- **Valeria Iobbi**, Roméo Arago Dougué Kentsop, Giuliana Donadio (10 + 2 min)
  "Abietane diterpenoids from the hairy roots of *Salvia corrugata* Vahl."
- **Mariangela Marrelli**, Massimo Tacchini, Maria Rosaria Perri et al. (10 + 2 min)
  "*Lavandula* spp. from the Pollino Massif: anti-arthritic potential of essential oils and bioactive components"
- **Andrea Mastinu**, Giulia Abate, Leilei Zhang et al. (10 + 2 min)
  "Phytochemical analysis and anti-inflammatory activity of different ethanolic phyto-extracts of *Artemisia annua* L."
- **Eleonora Spinozzi**, Filippo Maggi, Riccardo Petrelli et al. (10 + 2 min)
  "A focus on jambù: from the Brazilian folk medicine to the development of novel botanical insecticides"

SYMPOSIUM

"BEYOND DISTRIBUTION DATA OF ALIEN SPECIES: WHAT'S NEXT?"

(Chairperson: G. Brundu)

Key words species distribution models, plant invasions, prevention, management, risk analysis

17:00-17:30 • **Joana R. Vicente**, A.S. Vaz, J. Honrado, Universidade do Porto, Portugal (20 + 10 min)
"Species distribution models in invasion Ecology: challenges and applications"

17:30-18:42 **Communications**

- **Leopoldo de Simone**, Emanuele Fanfarillo, Tiberio Fiaschi et al. (10 + 2 min)
  "Patterns of alien species in a riparian ecosystem of southern Tuscany: is there any news?"
- Francesco Maria Raimondo, Enrico Bajona, **Gianniantonio Domina** et al. (10 + 2 min)
  "Xenophytes and anthropogenic vegetation in Sicily: the case of *Opuntia stricta* (Cactaceae) in the protected area of Capo Rama (Terrasini, Palermo)"
- **Flavio Marzialetti** Ludovico Frate, Walter De Simone et al. (10 + 2 min)
  "UAV-based mapping of *Acacia saligna* invasions in the Mediterranean coastal dune"
- **Francesco Boscutti**, Elisa Pellegrini, Massimo Buccheri et al. (10 + 2 min)
  "From local to landscape disturbances: new perspectives in alien plant invasions across multiple spatial scales"
- **Lorenzo Lazzaro,** Michele Giunti, Giulio Ferretti et al. (10 + 2 min)
  "Management of *Carpobrotus* spp. within the Life project LETSGO GIGLIO on Giglio Island (Tuscan Archipelago): control actions and monitoring of flora and vegetation"
- **Luigi Cao Pinna,** Marco Malavasi, Alicia T. R. Acosta et al. (10 + 2 min)
  "Plant invasion in Mediterranean Europe, current hotspots and future scenarios"

Thursday 9 September 2021

SYMPOSIUM

"IMPORTANCE OF FIELDWORK AND PLANT COLLECTING FOR FLORISTIC AND SYSTEMATIC RESEARCH"

(Chairperson: G. Domina)

Key words Biodiversity, taxonomy, primary data, herbaria

9:00-10:45 **Communications**

- **Juri Nascimbene**, Renato Benesperi, Elisabetta Bianchi et al. (9 + 1,5 min)
  "Don't stop exploring and collecting! Field work and herbaria still play a fundamental role in the analysis of lichen diversity: some case-studies from Italy"
- **Giulia Albani Rocchetti**, Thomas Abeli (9 + 1,5 min)
  "Rediscoveries and systematic research bring back 17 European endemics from extinction"
- **Lorenzo Peruzzi**, David Dolci, Brunello Pierini et al. (9 + 1,5 min)
  "Climate change and elevational shifts in plant distribution. A case study from the vascular flora of Tuscany across the last century"
- **Gianmarco Tavilla**, Antonia Cristaudo, Gianpietro Giusso del Galdo et al. (9 + 1,5 min)
  "*Anthemis messanensis* (Asteraceae) a neglected endemic species: new data on distribution, ecology, and conservation status"
- **Paola De Giorgi**, Antonio Giacò, Giovanni Astuti et al. (9 + 1,5 min)
  "An integrated taxonomy approach points towards a single-species hypothesis for *Santolina* (Asteraceae) in Corsica and Sardinia"
- **Zohreh Hosseini**, Flavia Bartoli, Emanuela Cicinelli et al. (9 + 1,5 min)
  "Checklist of the archaeological site of Pasargadae, World Heritage Site in Iran"
- **Giulio Barone**, Stephen Mifsud, Gianniantonio Domina (9 + 1,5 min)
  "Plant conservation through use: field surveys on four case studies in Sicily and Malta"
- **Jacopo Franzoni**, Giovanni Astuti, Angelino Carta et al. (9 + 1,5 min)
  "An integrative approach shows independent local genotype and phenotype variation in *Dianthus virgineus* (Caryophyllaceae)"
- **Anahi Elena Ada Bucchini**, Lisa Brancaleoni, Donata Ricci et al. (9 + 1,5 min)
  "The third phase of the project to enhance the Botanical Garden of the University of Urbino: the recovery of the *Herbarium Athenaei Urbinatis*"

- **Emilio Di Gristina**, Alfredo Carratello, Giuseppe Certa et al. (9 + 1,5 min)
  "Recovery of historical duplicates of the *Herbarium Siculum* in Palermo"

SYMPOSIUM
"BIODIVERSITY AND CONSERVATION OF BRYOPHYTES"
(Chairperson: M. Puglisi)

Key words checklist, diversity, ecology, red list, threatened species

11:00-11:30 • **Marko S. Sabovljević**, Aneta D. Sabovljević, University of Belgrade, Serbia (25 + 5 min)
"Introducing bryophytes to conservation physiology and vice versa"

11:30-12:00 • **Rosalina Gabriel**, Cecília Sérgio, Manuela Sim-Sim, University of the Azores, Portugal (25 + 5 min)
"The importance of different habitats for bryophytes: common threats to species living on the Azores Islands (Macaronesia)"

12:00-12:36 **Communications**

- **Silvia Poponessi**, Michele Aleffi (10 + 2 min)
  "New Checklist of the Bryophytes of Italy twelve years apart from the first"
- Marta Puglisi, Francine Valérie Navillod, **Luca Miserere** et al. (10 + 2 min)
  "Rare bryophytes of the Alps: monitoring and analysis of the current situation with a focus on the Aosta Valley"
- **Antonio De Agostini**, Annalena Cogoni, Pierluigi Cortis et al. (10 + 2 min)
  "Strategies of heavy metal tolerance in metallicolous and non-metallicolous populations of mosses: the role of photoprotective mechanisms"

SYMPOSIUM
"A JOURNEY AMONG AQUATIC PHOTOTROPHS: FROM BIODIVERSITY TO APPLICATIONS"
(Chairperson: R. Pistocchi)

Key words floristic studies, biodiversity, algae and environment, biotechnology and application

14:00-14:30 • **Javier Cremades Ugarte**, University of A Coruña, Spain (25 + 5 min)
"Marine agronomy as a pillar of the aquaculture with an ecosystem approach in southern Europe"

14:30-15:42 **Communications**

- **Mara Simonazzi**, Laura Pezzolesi, Franca Guerrini et al. (10 + 2 min)
  "Insight into the use of chlorinated oxidants for the removal of cyanobacterial toxins produced by *Microcystis aeruginosa*: good practice for drinking water plants"
- **Simona Armeli Minicante**, Nathan Beda, James T. Melton et al. (10 + 2 min)
  "Progress on compiling a DNA barcode inventory of the genus *Ulva* Linnaeus in the Mediterranean Sea"
- **Anna Maria Mannino** (10 + 2 min)
  "Bioactive compounds from brown algae inhabiting the north-western Mediterranean Sea"
- **Leone Ermes Romano**, Giovanna Aronne (10 + 2 min)
  "The key role of Duckweed (Lemnaceae) in human space flight"
- **Michele Maglie**, María José Ibáñez González, Elvira Navarro López et al. (10 + 2 min)
  "Waste-to-value approach: the production of lipids from the microalga *Tisochrysis lutea* using a reject brine"
- **Giulia Santunione**, Alberto Muscio, Elisabetta Sgarbi (10 + 2 min)
  "Biodeterioration of surfaces: the development of an experimental protocol involving *Nostoc commune* as pioneer organism"

SYMPOSIUM

"RELEVANT HABITATS NEGLECTED BY THE DIRECTIVE 92/43 EEC: MAY VEGETATION SCIENCE CONTRIBUTE TO THEIR CONSERVATION?"

(Chairperson: G. Giusso Del Galdo)

Key words conservation, Red List of ecosystems, vegetation mapping, vegetation classification, geobotany

16:00-16:30 • **Borja Jiménez-Alfaro**, Universidad de Oviedo, Spain (25 + 5 min)
"Habitat typologies for the conservation of high-mountain Mediterranean vegetation"

16:30-17:30 **Communications**

- **Michele Dalle Fratte**, Stefano Armiraglio, Marco Caccianiga et al. (10 + 2 min)
"Spatial distribution and conservation status of species-rich Nardus grasslands in Lombardy region (Northern Italy)"
- **Simona Casavecchia**, Giampiero Ciaschetti, Daniela Gigante et al. (10 + 2 min)
"Neglected habitats in central Apennines"
- Giovanni Spampinato, **Valeria Tomaselli**, Luigi Forte et al. (10 + 2 min)
"Relevant but neglected habitats by the Directive 92/43 EEC in southern Italy"
- **Riccardo Guarino**, Giuseppe Bazan, Alessandro Crisafulli et al. (10 + 2 min)
"Relevant habitats neglected by the Directive 92/43 EEC: the contribution of Vegetation Science for their reappraisal and protection in Sicily"
- **Mauro Fois**, Gianluigi Bacchetta, Maria Carmela Caria et al. (10 + 2 min)
"Discovering neglected vegetation in Sardinia: from knowledge to conservation"

18:00-19:00 General assembly of the Members of the Italian Botanical Society

Friday 10 September 2021

SYMPOSIUM

"ECOLOGY OF DISTURBANCE EVENTS"

(Chairpersons: C. Wellstein and S. Chelli)

Key words extreme weather events, fire, flooding, large-scale human disturbance, succession

9:00-10:36 **Communications**

- **Giulia Capotorti**, Laura Zavattero, Riccardo Copiz et al. (10 + 2 min)'
"'Ecosystems under fire': assessment of anthropogenic fire threat for compiling the Red List of Ecosystems in Italy"
- **Elisa Carrari**, Patrizio Biagini, Federico Selvi et al. (10 + 2 min)
"Post-fire management and forest type affect species diversity, composition and resprouting during early recovery stages in a Mediterranean vegetation area"
- **Andrea Coppi,** Lorenzo Lazzaro, Federico Selvi et al (10 + 2 min)
"Plant mortality on ultramafic soils after an intense heat and drought event"
- **Michele Di Musciano**, Walter De Simone, Valter Di Cecco et al. (10 + 2 min)
"Monitoring vegetation dynamics after wildfire events in Mediterranean mountain using satellite data"
- **Giovanni Iacopetti**, Filippo Bussotti, Elisa Carrari et al. (10 + 2 min)
"Understorey changes after an extreme drought event are modulated by overstorey tree species mixtures in thermophilous deciduous forests"
- **Davide Mosanghini**, Giuseppe Oriolo, Francesco Boscutti (10 + 2 min)
"Different ways to success: plant community trajectories along time and a soil moisture gradient in restored wetlands"

- **Ludovica Oddi,** Edoardo Cremonese, Mirco Migliavacca et al. (10 + 2 min)
  "European larch growth and CO2 flux responses to heat and drought"

- Marco Vuerich, Paolo Cingano, **Francesco Boscutti** et al. (10 + 2 min)
  "Effects of flooding stress on the saltmarsh halophyte *Salicornia fruticosa* (L.) L.: upscaling perspectives"

SYMPOSIUM

"FUNGI AS A TOOL TO FACE THE GREAT SOCIETAL CHALLENGES IN A CHANGING WORLD"

(Chairpersons: S. Tosi and S. Di Piazza)

Key words biodiversity, ecosystem services, bioremediation, circular economy, next generation EU

11:00-12:50 **Communications**

- **Ilaria Filippi**, Federica Spina, Viktoria Ilieva et al. (8 + 2 min)
  "Identification of bioplastic degrading fungi"

- **Andrea Zanellati**, Federica Spina, Luca Rollé et al. (8 + 2 min)
  "The potential of fungi to enhance the yields and sustainability of anaerobic digestion"

- **Grazia Cecchi**, Simone Di Piazza, Laura Cutroneo et al. (8 + 2 min)
  "Myco-booms application in Port waters for bioremediation of heavy metals: the GEREMIA and QUALI-PORTI projects experience"

- **Andrea Ceci**, Flavia Pinzari, Anna Maria Persiani (8 + 2 min)
  "Metabolic responses of soil fungi to the presence of hexachlorocyclohexane isomers"

- **Marta Elisabetta Eleonora Temporiti**, Lidia Nicola, Chiara Daccò et al. (8 + 2 min)
  "Screening methods to detect potential bioremediation fungi"

- **Simone Di Piazza**, Michela Mazzoccoli, Grazia Cecchi et al. (8 + 2 min)
  "Fungal mining of precious elements from urban mine: the MYRAEE project experience"

- **Francesco Dovana**, Gabriel Moreno, Roberto Para et al. (8 + 2 min)
  "The genus *Laccaria*: is it possible to get the correct identification of the different specimens? *Laccaria macrocystidiata*, a case study"

- **Carolina Elena Girometta**, Rebecca Michela Baiguera, Francesco Bracco et al. (8 + 2 min)
  "*Fomitiporia punctata* missing: ITS-LSU analysis in Lombardia detects the polyphagous pathogen *F. mediterranea* only"

- **Paola Angelini**, Roberto Maria Pellegrino, Giancarlo Angeles Flores et al. (8 + 2 min)
  "Metabolomic profiling and biological activities of *Pleurotus columbinus* Quél. cultivated on different agri-food by-products"

- **Elisa Moscato**, Federica Spina, Iolanda Perugini et al. (8 + 2 min)
  "Mycoprotein: a way for a more sustainable food system"

- **Matteo Florio Furno**, D. Ferrero, A. Poli et al. (8 + 2 min)
  "The marine mycobiota as a possible bioresources: the fungal biodiversity associated with microplastics"

SYMPOSIUM

"FROM INTRODUCTION TO INVASION: AN ARCHAEOBOTANICAL AND PALYNOLOGICAL STORYTELLING"

(Chairpersons: L. Sadori and A. Travaglini)

Key words archaeophytes, neophytes, pollen, seeds/fruits, public health

14:00-14:30 • **Jordina Belmonte**, University of Barcelona, Spain (20 + 10 min)
"Aerobiology as an alert and control tool in front of invasive plants"

14:30-15:00 • **Jaromír Beneš**, University of South Bohemia, Czechia (20 + 10 min)
"How archaeobotany contributes to understanding the Holocene plant movement in the Old World"

15:00-15:48 **Communications**

- **Claudia Moricca**, Francesca Alhaique, Lia Barelli et al. (10 + 2 min)
  "New species from the New World: early archaeobotanical and archaeozoological evidence from the Santi Quattro Coronati complex in Rome (Italy)"
- **Alessia Masi**, Fanny Gaveriaux, Lucrezia Masci et al. (10 + 2 min)
  "Beech and stone Pine, the Italian landscape modelled by valuable ritual trees"
- **Jordan Palli**, Assunta Florenzano, Alessia Masi et al. (10 + 2 min)
  "*Cannabis* pollen records in Italy: when the *taxon* actually entered the Mediterranean?"
- **Alessia D'Agostino**, Gabriele Di Marco, Mauro Rubini et al. (10 + 2 min)
  "Environmental implications and evidence of natural products from dental calculi of a Neolithic–Chalcolithic community (central Italy)"

Post-congress

(Chairperson: S. Loppi)

16:00 **EVENTO "COMUNICARE LA BOTANICA"**

Dopo la recente rivoluzione digitale della comunicazione abbiamo bisogno di cambiare anche il nostro modo di comunicare la botanica: l'evento è dedicato a chi ha interesse a condividere idee ed esperienze per trovare proposte per migliorare la capacità di disseminare i temi della botanica al grande pubblico mantenendo il rigore scientifico, ma allo stesso tempo trovando soluzioni per incuriosire i non-specialisti. Brevi interventi precederanno le discussioni che, auspichiamo, porteranno a proposte e azioni concrete da adottare da parte della SBI per promuovere la diffusione della cultura delle scienze botaniche e delle loro applicazioni.

- **Silvana Munzi**, Università di Lisbona
  "Licheni e internet al tempo della pandemia"
- **Gabriele Gheza**, Università di Bologna
  "Superare le difficoltà nel comunicare habitat aridi e organismi negletti: il caso Life Drylands"
- **Fabio Attorre**, Università La Sapienza Roma
  "L'importanza degli orti botanici tra comunicazione, educazione ambientale e ricerca"
- **Mario De Tullio**, Università di Bari
  "The Sound of Science - Suoni per la didattica delle scienze e la comunicazione scientifica"
- **Stefano Martellos**, Università di Trieste
  "Comunicazione e Citizen Science: arruolamento e fidelizzazione dei volontari"
- **Giovanni Rivieccio**, Università di Sassari
  "Manifestazioni scientifiche e social media: un'occasione da non perdere?"
- **Marco Galaverni**, WWF Italia
  "Tra correttezza scientifica e coinvolgimento: nuove sfide della comunicazione scientifica"

Modera le discussioni

- **Marco Gisotti**, Agenzia di studi e comunicazione ambientale Green Factor; Consulente per la Comunicazione del MITE; Docente di Teorie e Linguaggi della Comunicazione Scientifica presso l'Università di Roma Tor Vergata

Conclusioni

- **Gianni Bedini**, Università di Pisa
- **Mario De Tullio**, Università di Bari
- **Michela Marignani**, Università di Cagliari

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Keynote Lectures

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FREE TOPIC

KEYNOTE LECTURES

The challenge of combining historical archives with paleoenvironmental data to create robust explanations of environmental transformation through time

Scott Mensing[1], Edward Schoolman[2], Adam Csank[1], Gianluca Piovesan[3]
[1]Dept. of Geography, University of Nevada, Reno, USA; [2]Dept. of History, University of Nevada, Reno, USA; [3]Department of Science, Agriculture and Forestry (DAFNE), Tuscia University, Viterbo, Italy

It is widely acknowledged that people have had a major impact on changing the environment through time, and yet demonstrating human influence as a primary causal factor in landscape transformation, as opposed to climate, is challenging. Among environmental scientists, climate is often given primacy in explanations of both landscape change, and societal collapse. One reason for this is the general absence of interdisciplinary teams of social scientists and historians working with environmental scientists to examine more holistic and nuanced explanations of environmental change. Another reason is that combining social science and historical data with paleoclimate and paleoecologic data is not trivial, and designing studies from the outset with both data sets in mind is still fairly uncommon. However, modern forest patterns and structure are a legacy of both human land use practices and climatic change, and if we are to plan for the future, we would be advised to have a clear understanding of how both societal and climatic change interacted in the past to create our existing landscapes. These landscapes have been shaped over periods of decades to centuries, so our examination must take into account change at multiple temporal and geographic scales.
This plenary presentation seeks to present a model for interdisciplinary research that addresses the interaction between society and climate in causing environmental change, at three different scales (local regional and hemispheric or global), that we think are most appropriate for answering specific types of questions. Our goal is to clarify the strengths and weaknesses of differing types of datasets for answering questions of causality in landscape change. For example, physical scientists now have access to Europe-wide datasets of tree rings, pollen, charcoal and other paleoecologic proxies. These analyses identify broad regional patterns of environmental change through time. However, once local data are amalgamated into hemispheric datasets, they become essentially aspatial, and the broad physical patterns seen in the data can no longer be used to explain landscape change at any local site. Thus, using such analyses to explain change in any given place is specious. The opposite problem exists with very local data. When linked with local social dynamics, they may provide clear causal relationships between climate, human activity and land use change; however, the explanation may not be appropriately scaled up to a regional level. Our challenge is in defining the strengths and limitations of these different scales of analysis, both geographically and temporally, so that interdisciplinary teams can design their data collection and analysis appropriately for the scale of analysis they are using, and not extend their interpretations beyond what their data are suitable for. A further challenge is how to nest these different types of studies so that we can eventually scale up local studies to make more robust regional and hemispheric interpretations.

Mechanisms of thermomemory in plants

Salma Balazadeh[1,2]
[1]Leiden University, The Netherlands; [2]Max Planck Institute of Molecular Plant Physiology, Potsdam, Germany

Global warming due to climate change adversely affects crop yield, jeopardizing food supply for a growing world population. Breeding stress-resilient cultivars is, therefore, an urgent need. An exciting, but poorly understood phenomenon is ´thermomemory´ whereby plants ´remember´ a high temperature from the past to robustly withstand a later – and even more extreme – heatwave. During the memory period, several but not all molecular and biochemical heat stress (HS)-induced changes are maintained which prepares, or 'primes', the plant to respond more effectively to future HS events. However, the molecular machinery that underlies the memory and forgetfulness of stress in plants is so far largely unknown. During my talk I will summarize current understanding of the mechanisms underlying thermomemory. Furthermore, I will highlight our recent findings on the importance of protein stability control for the regulation of thermomemory.

Metabolomics: Just Another OMICS or an Ideal Platform for Accelerated Lead Finding?

Milen I. Georgiev
[1]Laboratory of Metabolomics, Institute of Microbiology, Bulgarian Academy of Sciences, Plovdiv, Bulgaria; [2]Center of Plant Systems Biology and Biotechnology, Plovdiv, Bulgaria; e-mail: milengeorgiev@gbg.bg

Since time immemorial plants have long had a central role in the treatment and management of a wide variety of disorders, hence continuously supporting the health of humans. Nowadays, in excess of quarter of modern medicines are derived, either directly or indirectly, from plants. Artemisinin (antimalarial), paclitaxel (antineoplastic), codeine and morphine (analgesic), and galanthamine (reversible cholinesterase inhibitor) are remarkable examples in this direction and amongst the best-selling drugs worldwide. At the same time the development of new drugs is rather costly, laborious and time-consuming, hence platforms for accelerated lead finding/drug discovery, quality control assessment and mode of action of healing herbs, and their sustainable production are continuously sought [1-3, and the literature cited therein]. Metabolomics represents a comprehensive holistic approach, comprising of systematic identification and quantification of all metabolites in an organism, at given conditions. Since the rise of the OMICS age several platforms for high throughput analyses of targeted metabolites have been developed accordingly. Nuclear magnetic resonance (NMR) appears very suitable and adequate platform to carry out metabolomics analyses, as it allows simultaneous detection of diverse range of abundant (primary and secondary) metabolites, which opens novel avenues to fully explore the total biochemical machinery of plants. A great advantage of NMR-spectrometry over the other analytical platforms is the possibility for quantification and hence the direct comparison of concentrations of moelcules, present in the sample [4].
Here an overview of the metabolomics and pharmacological (in vitro and in vivo studies) aspects of research on selected medicinal plants towards accelerated lead finding will be given and discussed [4-8].

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Species distribution models in invasion Ecology: challenges and applications

J Vicente, A S Vaz, J Honrado

Models have been used in invasion ecology for a variety of purposes, ranging from fundamental research to the management of established populations. Examples include the anticipation of new invaders, identification of priority areas for surveillance, control of ongoing invasions, the identification of key drivers of invasion success, or the prioritization of invasive species based on their potential impacts on biodiversity and ecosystem services.
The successful development and application of species distribution models in invasion ecology and management requires taking a sequence of decisions regarding the input data, statistical procedures and methods, the reporting of uncertainties, and the ommunication of model outputs. In this talk we will address such decisions and their challenges, with an emphasis on the importance of data, both in terms of species occurrence (from traditional field work to citizen science and social media information) and of the socio-environmental drivers of invasions (traditional geographic or statistical data vs new opportunities provided by remote sensing Earth observations). We will also discuss the application of socioeconomic and environmental change scenarios to anticipate future invasions and their impacts.

Introducing bryophytes to conservation physiology and vice versa

Marko S. Sabovljević[1], Aneta D. Sabovljević[1]
[1]Institute of Botany and Botanical Garden, Faculty of Biology, University of Belgrade, Takovska 43, 11000 Belgrade, Serbia

Conservation physiology is a novel branch of conservation science, introducing functional traits of threatened entities with aim to enable self-sustainability of taxa highly endangered of extinction.
Belgrade Bryophyte Biology Group (BBGB) is dealing with various aspects of bryophyte biology and among other also introduced conservation physiological studies for the purpose of bryophyte species preservation. The living collection of axenically cultured bryophytes *in vitro* includes over 260 species from all over the world. Nearly 60% are globally or regionally threaten, and of some conservation interest.
Many problem in axenical culture establishment of rare and threaten taxa will be discussed and solution during ex situ conservation will be presented. The achievements with some of those species will be showed and case studies on European species of high conservationist interest will be communicated. The short review of the best achievements in active conservation of bryophytes will be discussed with positive and negative experiences, problem faced and solution found.
The bryophyte conservation science includes many aspects and receive rather low public interests, thus the networking in saving bryophyte diversity will be highlighted.

The importance of different habitats for bryophytes: common threats to species living on the Azores Islands (Macaronesia)

Rosalina Gabriel[1,2], Cecília Sérgio[3], Manuela Sim-Sim[3]
[1] cE3c/ABG - Centre for Ecology, Evolution and Environmental Changes/Azorean Biodiversity Group; [2]FCAA/DCEA - University of the Azores, Rua Capitão João d'Ávila, sn. 9700-042 Angra do Heroísmo, Açores, Portugal, [3]cE3c/ABG - Centre for Ecology, Evolution and Environmental Changes/Azorean Biodiversity Group

The recent classification of European bryophytes with IUCN criteria shows that mosses, liverworts and hornworts are (at least) as much endangered as other biological groups. In order to ensure the sustainability of life on Earth, different areas and habitats need to be assessed and preserved. Islands, are an important part of the conservation effort. In spite of their small size, islands have often served as refugia for many distinct species and may include well preserved habitats. The Azores archipelago, set in the northern part of the Macaronesia biogeographical region, is a case in point. The nine islands harbour more than 480 bryophyte species, about a quarter of all the bryophytes recorded in Europe, in a small fraction of the continental area (0,02%). Moreover, bryophytes in the Azores represent an important biomass in different ecosystems, and include a number of restricted endemic species. The aim of this talk is to explore how different ecosystems (Coastal habitats, Mesic areas, Cave entrances, Native forests, Aquatic habitats, Peat bogs, Semi-natural grasslands, Intensive pastures, Mountainous areas, Urban habitats, Parks and Gardens and Exotic plantation forests), compare in terms of total richness of species, richness of conservation concern' species, and threats affecting each habitat. Bibliographic surveys, Herbarium material and field studies were used to assess species presence and distribution on the Azores. Native forests were the richest ecosystems sampled (Fig. 1), however each habitat adds something and all need to be secured in order to guarantee the conservation of the species, particularly those which are have stringiest requirements, such as Conservation Concern' species and Endemic species (Fig. 2, 3). Out of the 25 Macaronesian endemic bryophytes present in the Azores, 19 are threatened (critically endangered, endangered or vulnerable), although all of them exist in at least one protected area. Nevertheless, with global changes, such as climate change or substitution and fragmentation of land use, invasive species, additional measures should be taken in order to guarantee the survival of the rich communities still found in the Azorean Islands.

Fig. 1.
Azorean natural forest, Terceira Island (RG-Azores BryoLab).

Fig. 2.
Bazzania azorica, Terceira Island (RG-Azores BryoLab).

Fig. 3.
Isothecium prolixum, Pico Island (RG-Azores BryoLab).

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Marine agronomy as a pillar of the aquaculture with an ecosystem approach in southern Europe

Javier Cremades Ugarte
Coastal biology research group (BioCost). Advanced Scientific Research Centre (CICA). University of A Coruña. 15071 A Coruña, Galicia, Spain

Almost half of the world's aquaculture production is dedicated to seaweed farming used mainly to obtain various phycocolloids or for human consumption. However, this production is concentrated in Southeast Asia in countries such as China, Indonesia, Korea, the Philippines, Japan, and Malaysia, listed by order of magnitude of figures of production. Today the world production of cultivated seaweed is 34,679,134 Tonnes (wet weight), Asia being responsible for the production of 99.1% of them (FAO). The global seaweed production has been growing almost 10% per year over the past decade, however, at first glance, it looks like the European seaweed industry has not been invited to the party, since it only contributes 0.8% to seaweed production of the world. And yet, it is still believed there is a massive opportunity for the European seaweed industry to thrive in the next decade.

The renewed interest in the culture of seaweeds in the western world is due to: i) their possible cultivation in Integrated Multi-Trophic Aquaculture systems (IMTA), in which they are a key component to recover dissolved inorganic nutrients from other aquaculture practices, ii) the emerging understanding of the ecosystem services they provide, and iii) the development of novel uses and new applications for seaweed.

Western world groups working on IMTA have spent two decades developing small-scale / pre-commercial IMTA operations by modifying fish sites to co-cultivate invertebrates and seaweeds (the FIS approach). The modifying site approach of IMTA systems in Asian countries has been diametrically opposed: seaweed sites have seen the development of smaller invertebrates, and later fish infrastructures (the SIF approach).

Until now, seaweeds (and other extractive species) have been cultivated mainly for their biomass and food trading values. However, to calculate IMTA's full value, they also need to be seen for the ecosystem services they provide in a circular economy approach. Seaweeds are excellent nutrient scrubbers, in IMTA systems they can be cultivated without fertilizers, do not need to be irrigated nor arable soil. Moreover, seaweeds as an aquaculture component providing O_2, while the animal and microbial components consume it, and sequestering CO_2 and slowing down global warming and could also reduce coastal acidification. Finally, the cultures of seaweeds can be used for habitat restoration and for increasing biodiversity. All this makes marine agronomy a key component in the development in Europe of aquaculture practices with an ecosystem approach throughout the implementation of IMTA multi-crop systems that hence may also help increasing the resilience of the aquaculture sector.

Seaweed cultivation (and IMTA systems) could be associated also with wind farms, in integrated food and renewable energy parks (IFREP), for a reduced cumulative footprint by combining the two activities. To increase the benefit of seaweed cultivation on European coasts developing a system of nutrient trading credits is necessary, since there is more money to be made with nutrient trading credits (NTC) than with carbon trading credits (CTC). The recognition and implementation of NTC would give a fair price to seaweed and extractive aquaculture.

There are four main reasons to believe in the European potential for seaweed farming: i) ideal growing conditions, ii) burgeoning innovation community, iii) existing and fast-growing demand for seaweed-based products and ingredients, and iv) strong alignment with European Green Deal.

This communication reviews the main initiatives and its bottlenecks that emerged in the last years in southern Europe for the development of IMTA systems that include primary producers (seaweeds & halophytes) in the different scenarios in which this activity can be carried out: offshore installations, open inland systems, IMTA-RAS (water recirculating) systems, and aquaponics.

Habitat typologies for the conservation of high-mountain Mediterranean vegetation

Borja Jiménez-Alfaro
Universidad de Oviedo, Spain

The conservation of natural and semi-natural ecosystems strongly depends on classifications to develop legal instruments and regional management actions. In Europe, phytosociological concepts and EUNIS habitats are generally linked with the habitat types of the Habitats Directive to provide tools for identification and mapping. However, the accuracy of these tools is not homogeneous across habitats and regions, with a general bias towards Central and Western Europe. One example of habitat typologies needed of further refinement refers to high-mountain ecosystems of southern Europe. These ecosystems are unique refugia for plant lineages and vegetation types adapted to cold and dry conditions, supporting micro-hotspots of biodiversity in the Mediterranean global hotspot. Using vegetation data at the European scale, I illustrate current gaps in the classification of alpine and oromediterranean vegetation, suggesting further directions for improving their understanding. I also provide an example of refinement of vegetation and habitat types for fine-scale mapping at the regional scale, with the ultimate aim of generating a multi-scale approach for monitoring the conservation status of these ecosystems.

Aerobiology as an alert and control tool in front of invasive plants

Jordina Belmonte
Departament Biologia Animal, Biologia Vegetal i Ecologia, Universitat Autònoma of Barcelona

Aerobiology is a rather recent discipline derived, in its origins, from the application of palynology to the knowledge of the pollen grains and fungal spores present in the air (aeropalynology) and enlarged to the study of any living organism and biological particle airborne (bacteria, viruses, microscopic algae, micro-arthropoda, seeds, butterflies, birds).

The first aerobiological studies on pollen aimed at the establishment of the airborne pollen spectra for the studied site and how the different pollen types registered varied their concentrations along the year, from one year to another and between localities. These studies showed clearly that the airborne pollen spectra reflect the plant species growing in a region, mainly those pollinating through the air, and that species with other pollination strategies may appear but in very much lower quantities.

Since its beginnings, aerobiology has also been focused on the study of the relationship of pollen in the air and meteorological parameters, showing the effects of precipitation, temperature, radiation, on pollen concentrations. And, with the time passing and the experience growing, aerobiologists showed that sometimes pollen grains travel long distances and are registered in remote places from the source vegetation. The name for this is pollen transport.

Aerobiology is then a useful tool for the study of the vegetation and if the flora of a locality counts with allochthone invasive species their pollen may appear in the airborne pollen spectra. Of course, this is easier in the case of plants using the air as pollen transport media.

The prolonged study in time of the airborne pollen in a locality can show the progression (and the regression!) of an invasion. There are numerous studies dealing with the presence of the bioinvader *Ambrosia artemisiifolia* L. in Europe and most of them are based on pollen analysis of air samples. This plant is a serious problem in the cultivated fields, causing important economic losses, but its pollen is a very aggressive respiratory allergen, adding important health problems to the population of the invaded localities.

Ailanthus altissima (Mill.) Swingle is another example of invasive plant which airborne pollen has recently been demonstrated to cause allergy problems. Other recent examples of invasive plants that contribute to the airborne pollen spectra and can aggravate the allergenic panorama moreover of the environmental problem they produce are *Humulus japonicus* Siebold & Zucc. (in France), *Amaranthus palmieri* S. Watson (in Spain).

The studies on atmospheric pollen transport have revealed that the pollen itself acts as a sporadic invader. And the two main effects of these pollen invasions remain not sufficiently studied and open the door to future research: the risk of suffering from allergies in unexpected periods of the year and the benefit of gene flow between populations.

How archaeobotany contributes to understanding the Holocene plant movement in the Old World

Jaromír Beneš[1]
[1]Laboratory of Archaeobotany and Palaeoecology, Faculty of Science, University of South Bohemia, České Budějovice, Czechia

The lecture will be divided into two parts. The first part deals with the difference between growing wild grasses and legumes on the one hand and fruit trees on the other. Since the end of the last Ice Age stadial, human's influence on nature has intensified. This is observable not only from the growing number of evidence of plant use but above all from the current knowledge that even Paleolithic humans needed a larger share of plant food for his life than previously thought. In several parts of the Old World, pre-domestication and domestication hot-spots were created (Levant - hulled wild species of the genus *Triticum, Hordeum, Pisum, Lens*; Inner Mongolia - species of the genus *Setaria*). In other areas, long-term use of specific plants led to human societies based on the combined use of hunting and wild plant procurement and later the early cultivation practices (West Africa - *Penisetum glaucum, Oryza glaberrima*; central China, *Oryza rufipogon / sativa*). These groups of plants have a short life cycle. The domestication syndrome could have developed rapidly here, especially in a specific environment (Cyprus). The second group of plants was just as important, but so far it has not received so much attention. They are woody plants whose domestication is little known. Their domestication requires a stable human society in pre-state and early state formations. Undoubtedly, the most important areas of the Old World in this respect are central-eastern China (*Armeniaca vulgaris, Amygdalus persica*; and western Asia – *Ficus carica, Vitis vinifera*). The example of common fig is focused on the issue of domestication of trees. The prehistory of the *Ficus carica* domestication in the Levant is shown, special attention is paid to the spread of figs in Europe on the basis of a comparison of large archaeobotanical databases. The issue of the spread of cultural species in Europe is concluded by the topic of synanthropisation of entire biomes during Holocene.

COMMUNICATIONS

New insight on thiol-peptides: are they involved in cadmium extracellular secretion?

Erika Bellini[1], Luca Paoli[1], Stefania Bottega[1], Carmelina Spanò[1], Daniela Valensin[2], Carlo Sorce[1], Monica Ruffini Castiglione[1], Andrea Andreucci[1], Claudio Varotto[3], Luigi Sanità di Toppi[1]
[1]Department of Biology, University of Pisa, Via Luca Ghini 13, 56126 Pisa, Italy; [2]Department of Biotechnology Chemistry and Pharmacy, University of Siena, Via Aldo Moro 2, 53100 Siena, Italy; [3]Department of Biodiversity and Molecular Ecology, Fondazione Edmund Mach, Research and Innovation Centre, Via Mach 1, 38100, San Michele all'Adige (TN), Italy

Plants have evolved a set of mechanisms that control and respond to the uptake and accumulation of both essential and non-essential metals, including chelation and sequestration of these trace elements by thiol ligands, such as glutathione (GSH) and phytochelatins (PCn). Indeed, both GSH and PCn can chelate some metals, quickly form thiol-metal complexes, and compartmentalize them in the vacuolar environment. Reasonably, similar mechanisms can be assumed to be responsible for the transport of thiol-metal complexes - in particular with Cd - through the plasma membrane, with consequent release of the toxic metal in the extracellular environment. We verified such hypothesis in axenically-grown gametophytes of the liverwort *Marchantia polymorpha*, exposed to three different Cd concentrations (10, 20 and 36 µM) under five exposure-times. In such system, the presence of thiol-peptides in the extracellular environment was assayed. The HPLC-ESI-MS-MS revealed the presence of extracellular GSH and PCn, the latter up to PC_4. Interestingly, the GSH and PCn concentrations trend in the culture medium followed the intracellular ones. No Cd-induced damage of membranes in the gametophyte cells was revealed, thus ruling out that the extracellular presence of thiol peptides was attributable to plasma membrane leakage caused by Cd-treatment. Furthermore, the pH-dependent ability of GSH to form complexes with Cd ions was demonstrated by NMR analysis. The overall results suggested that GSH-Cd complexes were formed intracellularly, and then secreted into the extracellular environment, where the pH would cause the complexes cleaving and releasing free Cd ions out of the cell. If these data were further confirmed, even with the identification of possible plasma membrane thiol-metal transporter(s), a new mechanism for extracellular detoxification of Cd could be certainly postulated.

https://drive.google.com/file/d/16nmVfB-6TtNE-aS0o92XohFq313kfX09/view?usp=sharing

New insights into the role of Cell wall modifications induced by α-XYLOSIDASE1: The impact in seed and fruit size in *Arabidopsis thaliana*

Ignacio Ezquer[1], Maurizio Di Marzo[1], Vívian Ebeling Viana[1,2], Camilla Banfi[1], Valeria Cassina[3], Roberta Corti[3,4], Humberto Herrera-Ubaldo[5], Andrea Guazzotti[1], Edward Kiegle[1], Veronica Gregis[1], Francesco Mantegazza[3], Javier Sampedro[6], Stefan de Folter[5], Lucia Colombo[1]
[1]Dipartimento di BioScienze, Università degli Studi di Milano, Via Giovanni Celoria 26, 20133 Milan, Italy; [2]Plant Genomics and Breeding Center, Federal University of Pelotas, Capão do Leão - RS, Brazil; [3]School of Medicine and Surgery, Nanomedicine Center NANOMIB, University of Milan-Bicocca, Monza, Italy; [4]Department of Materials Science, University of Milan-Bicocca, Milan, Italy; [5]Unidad de Genómica Avanzada (UGA-LANGEBIO), Centro de Investigación y de Estudios Avanzados del Instituto Politécnico Nacional (CINVESTAV-IPN), Km. 9.6 Libramiento Norte, Carretera Irapuato-León, CP 36824 Irapuato, Guanajuato, Mexico; [6]Universidad de Santiago de Compostela, Departamento de Fisiología Vegetal, Facultad de Biología, Rúa Lope Gómez de Marzoa, s/n. Campus sur, 15782 Santiago de Compostela, A Coruña Spain

Cell wall modifications are of pivotal importance during plant development. Among cell wall components, xyloglucans are the most abundant hemicellulose in primary cell walls, and can connect the cellulose microfibril surface to affect cell wall mechanical properties. Changes in xyloglucan structure are known as the major factor regulating cell growth. Therefore, the degradation of xyloglucan is an important modification that alters the cell wall. The α-XYLOSIDASE1 (*XYL1*) gene encodes the only α-xylosidase acting on xyloglucans in *Arabidopsis thaliana*. Here, we show that mutation of *α-xyl1* strongly influences seed size, seed germination, and fruit elongation. We found that the expression of *XYL1* is directly regulated in developing seeds and fruit by the MADS-box transcription factor SEEDSTICK (STK). We demonstrate that *XYL1* complements the *stk* smaller seed phenotype. Furthermore, using Atomic Force Microscopy (AFM) combined with cell wall composition analysis, we discuss the role of XYL1 activity and xyloglucan deposition in controlling tissue stiffness and growth.
Overall, in this work we provide new insights into the current models for plant organ growth concerning the role of cell wall modulation in shaping organs.

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Insights into the mechanisms of priming and thermotolerance in tobacco pollen

Lavinia Mareri[1], Claudia Faleri[1], Luigi Parrotta[2], Iris Aloisi[2], Stefano Del Duca[2], Giampiero Cai[1]
[1]Department of Life Sciences, University of Siena, Via P.A. Mattioli 4, 53100 Siena, Italy; [2]Department of Biological, Geological and Environmental Sciences, University of Bologna, Via Irnerio 42, 40126 Bologna, Italy

Global warming poses major challenges for plant survival and for agricultural productivity. Plants, as sessile organisms, are particularly threatened by temperature changes and they have developed a remarkable number of strategies to cope with heat stress (HS). Among such mechanisms there is the acquired thermotolerance (ATT) that is the acquisition of thermotolerance after a pre-induction event, also known as heat priming. This latter consists in the pre-exposure of plants to a sub-optimal temperature that stimulates a more active response when the organism will be subjected to a next stress exposure. Up to now many studies focus their attention on heat priming on plants but as far as we know, there is a lack of information about heat priming in pollen, the male gametophyte shown to be more sensitive to high temperatures than the female counterpart.
The present research aims to investigate the effects of heat priming (and the consequent acquisition of thermotolerance) on the functioning of tobacco pollen grains. In addition to evaluate how a heat pre-exposure influences pollen viability, germination and pollen tube length and growth, a series of analyses were performed to understand the possible mechanisms underlying heat priming. First, we analysed the accumulation of stress-related proteins such as HSP70, osmotin and dehydrins. Then, we focused on Ca^{2+} distribution because this ion is fundamental to initiate and promote the polarization of pollen and pollen tube and we also evaluated ROS level and distribution. Indeed Ca^{2+} in combination with ROS modifies the dynamics of the cytoskeleton thus affecting intracellular transport. To verify the cytoskeleton status and organization we performed microscopical analysis and a series of blotting for actin, tubulin, tyrosinated tubulin and acetylated tubulin. In addition to this, we also investigated pollen sugar metabolism. This latter is of great importance for a correct cell wall deposition by providing the activated substrates for polysaccharide elongation (both pectin and cellulose). Among the many sugar-metabolizing enzymes, sucrose synthase was considered because it provides UDP-glucose, an energetic reservoir as well as the substrate for cellulose and callose synthesis.
Results herein obtained confirmed that a pre-exposure to moderate stress (priming) can positively affect pollen overall performance by modifying its metabolism. Indeed, heat pre-exposure causes a series of metabolic adjustments that are able to partially restore the physiological level of all the analysed molecules leading to a normal pollen tube growth when pollen is subjected to acute heat stress. On the contrary, pollen subjected to a drastic temperature stress is characterized by a deep alteration of the main metabolic processes that result in an abnormal pollen tube growth. Our data represent an important step toward the understanding of the mechanisms at the basis of heat priming and of the acquisition of pollen thermotolerance giving a wide overview of the principal processes that regulate pollen tube length.

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Genome-wide analysis of the H3K27me3 epigenome and transcriptome in the ovule developmental stages during pollination of *Ginkgo biloba* L.

Antonella Muto[1], Emanuela Talarico[1], Ernesto Picardi[2], Adriana Ada Ceverista Chiappetta[1], Maria Beatrice Bitonti[1], Fabrizio Araniti[3], Barbara Baldan[4,5], Lucia Colombo[6], Leonardo Bruno[1]
[1]Department of Biology, Ecology and Earth Sciences, University of Calabria, Via Ponte P. Bucci 6b, 87036 Arcavacata di Rende Cosenza, Italy; [2]Institute of Biomembranes, Bioenergetics and Molecular Biotechnologies, Consiglio Nazionale delle Ricerche, Bari, Italy; Department of Biosciences, Biotechnology and Biopharmaceutics, University of Bari A. Moro, Bari, Italy; [3]Dipartimento di Scienze Agrarie e Ambientali -Produzione, Territorio, Agroenergia, Università degli Studi di Milano, Via Celoria 2, Milano, 20133, Italy; [4]Botanical Garden of Padova, University of Padova, Padova, Italy; [5]Department of Biology, University of Padova, Padova, Italy; [6]Dipartimento di BioScienze, Università degli Studi di Milano, Via Celoria 26, Milano, 20133, Italy

Molecular network controlling ovule development and its transformation into seed, extensively investigated in *Arabidopsis thaliana*, is still largely unexplored in non-model plants and mainly in Gymnosperms, This prompted us to extend such studies to *Ginkgo biloba* L., whose ovule at morpho- histological level has been precisely described exhibiting several unique and primitive characteristics. In particular, we focused our attention on pollination event which in *G. biloba*, as in most Gymnosperms, is separated by fertilization through a very long interval, during which ovule growth proceeds and gamethophyte differentiation occurs, suggesting that the pollen arrival could trigger the signal for the transformation of the ovule into seed.
In this respect, it is important to consider that Polycomb repressing complex 2 (PRC2) proteins are rated among the master regulators in several aspects of plant development. They are responsible for the tri-methylation of lysine 27 of Histone 3 (H3K27me3), causing the transcriptional repression of target genes. For example, in *Arabidopsis thaliana*, the differentiation of the ovule integument into seed coat which is dependent on the fertilization of the central cell is repressed by PRC2 whose subunits belong to the VERNALIZATION (VRN)/EMBRYONIC LOWER (EMF)PRC2 complex.
In this context, the focus of the present work was to identify the target genes involved *G. biloba* ovule development with respect to the pollination stage, mediated by H3K27me3 epigenetic mechanisms.
Pool of pollinated ovules was collected from ten female plants at the Botanical Garden of the University of Calabria (Cosenza, Italy) in the pollination period (from March to April 2020). Namely, four stages were considered: the pre-pollination stage (PRE_DROP), the pollination drop stage (DROP), and two post-pollination drop stages, respectively 6 and 8 days after the emission of the pollination drop (POST_DROP_1, POST_DROP_2). Moreover, to evaluate the effect of pollination signal on the ovule growth, no pollinated ovules were also collected from female and isolated plants, located in the Coretto Garden (CS) Calabria. First, to precisely determine the location of H3K27me3- marked regions across the genome, we performed a ChIP-seq analysis on the pollinated ovules. The obtained results were correlated with the transcriptomic data, performed in the same stage of ovule development. 4307 Differential Methylated Genes (DMGs) and 4838 Differentially Expressed Genes (DEGs) were identified among the steps. These results evidence that H3K27me3 is present in a large number of genomic locations across the *G. biloba* genome. Moreover, along ovule development–H3K27me3 correlates with low transcript levels especially after pollination event, indicating that H3K27me3 plays an important role in the regulation of gene expression. Weighted gene co-expression network analysis (WGCNA) was then applied to construct molecular networks involved in this event. DMGs were then compared with RSM to discern those modules that may be biologically regulated as a group by trimethylation of histone H3lysine27. KEGG enrichment analysis of the significant RNA-Seq Modules (RSM) by WGCNA revealed that “Plant hormone signal transduction” and “Flavonoids biosynthesis” were the most representative pathways. Interestingly, most of the Flavonoids and Auxin DEGs were also target of PRC2. So far, the expression levels of Flavonoids and Auxin metabolism related genes on both pollinated and not pollinated ovules were evaluated by RT-QPCR. Taken together, a model can be drawn with PRC2 complex playing pinpointing roles for the determination of flavonoid and auxin accumulation profiles at defined developmental phases.

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Quantitative assessment of the thylakoid membrane appression in the chloroplast of *Selaginella martensii* Spring (Lycopodiophyta)

Lorenzo Ferroni[1], Andrea Colpo[1], Alessandra Molinari[2], Simonetta Pancaldi[1]
[1]Department of Life Sciences and Biotechnology, University of Ferrara, Corso Ercole I d'Este 32, 44121 Ferrara, Italy; [2]Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, Luigi Borsari 46, 44121 Ferrara, Italy

The chloroplast organization in lycophytes belonging to the genus *Selaginella* exhibits unique variations as compared to the lenticular morphology typical of the great majority of land plants. The presence of a gigantic chloroplast in the upper epidermal cells of the microphyll is considered a special adaptation to the understory environment, where the available light has a low intensity and is enriched with far red components of the solar spectrum. Inside the organelle, the adaptation to shade can also result in a zonation of the thylakoid system – granal vs. lamellar. In *Selaginella martensii*, a shade-adapted species of American rainforests, transmission electron micrographs revealed a gigantic cup-shaped chloroplast filled with a very abundant thylakoid system. The degree of thylakoid appression is very high and gives rise to large grana, which, rather than appearing as individual stacks connected by stromal lamellae, are often in continuity with each other. The grana size is also very variable within the same organelle. Such a complex membrane organization does not allow accurate morphometric analyses, to define in particular the ratio between appressed and non-appressed thylakoid domains.
The aim of this study is to ground a basis for the quantitative assessment of the thylakoid membrane appression in the chloroplast of *S. martensii*. The plants used for experiments were cultivated in the warm humid greenhouse of the Botanical Garden of Ferrara under the natural shade of above-growing plants. Thylakoid membranes were isolated from dark-acclimated plants and used for analyses. The thylakoid appression degree was evaluated by solubilising the thylakoids with mild detergents. Digitonin in bis-tris-HCl (BTH) buffer allowed the selective solubilisation of non-appressed membranes. The results were compared with two conditions potentially allowing an almost complete solubilisation: β-dodecylmaltoside in BTH buffer or digitonin in aminocaproic acid buffer. Because Photosystem I (PSI) is confined in the stroma-exposed regions, while Photosystem II (PSII) is enriched in the appressed membranes together with most of the chlorophyll *b*-containing light-harvesting complexes II (LHCII), other thylakoid samples are currently being used for the electron paramagnetic resonance (EPR) measurements. The EPR detection of the $Y_D^{\cdot}$ and $P700^+$ radicals, which are generated stoichiometrically in PSII and PSI, one spin per reaction centre respectively, can lead to the precise determination of the PSII/PSI ratio.
The results obtained so far show that in *S. martensii* the stroma-exposed membranes account for ca. 25% of the thylakoid system, and their low chlorophyll *a*/*b* ratio indicates that they must be unusually rich in LHCII. This inference is consistent with previous evidence about the extensive association of LHCII with PSI in *S. martensii* even when dark acclimated. Because the thylakoid architecture is intimately connected with the lateral heterogeneity in photosystem distribution, the thylakoid solubilisation results will be checked for consistency with the quantification of PSII and PSI concentrations, as obtained by ongoing EPR measurements.

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Roles of Brassinosteroids on *Arabidopsis thaliana* rooting in the presence of Cadmium

Federica Della Rovere[1], Camilla Betti[2], Laura Fattorini[1], Nicoletta Girardi[1], Diego Piacentini[1], Giuseppina Falasca[1], Maria Maddalena Altamura[1]
[1]Department of Environmental Sciences, "Sapienza" University of Rome, Piazzale Aldo Moro 5, 00185 Roma, Italy; [2]Department of Medicine, University of Perugia, Piazzale Menghini 8/9, 06132 Perugia, Italy

Brassinosteroids (BRs) are plant hormones that belong to the class of polyhydroxy steroids. BRs can regulate multiple physiological functions, such as embryogenesis and seed germination, cell division and elongation, microspore germination and growth of pollen tubes, differentiation of tracheary elements and polarization of cellular membranes. However, BRs not only regulate different physiological and morphogenetic responses in plants, but also act in various biotic and abiotic stresses through a modulation of both synthesis and signalling. The role of BRs in stress management, including heavy metal stress, has been explored in many species. In particular, BRs treatments can reduce cadmium (Cd) accumulation and toxicity in *Brassica juncea*, which belongs to the same family of the model plant, *Arabidopsis thaliana*. The global aim of this research is to demonstrate whether BR hormones, still little known for their effect on rhizogenesis, have a positive role on root development and if this also occurs in the presence of a heavy metal pollutant, such as Cadmium (Cd). To this aim, Arabidopsis wild type seedlings were grown *in vitro* in the presence of different concentrations (1nM to 1µM) of the most active BR, epi-brassinolide (eBL) and in the presence of absence of 60 µM $CdSO_4$, known to cause damage to the Arabidopsis root system. To favor AR formation, the plants were grown vertically for 9 days in continuous darkness and then exposed to 16 h light/8 h dark photoperiod for 7 further days. At the end of the cultural period every component of the root system (primary root, PR; lateral roots, LRs; and adventitious roots, ARs), were morphologically and anatomically examined. In particular, PR and hypocotyl length, as well as LR and AR densities, were measured and an anatomical analysis of LR and AR apices have been conducted.
The results show that BL plays an important role in the development of the root system in Arabidopsis by stimulating the formation of LRs and ARs, thus helping to increase the root system extension in the substrate thereby increasing the plant functional well-being. In particular, the lowest BL concentrations used (i.e. 1nM and 10 nM) promoted root induction both by the PR and the hypocotyl, responsible for the formation of LRs and ARs respectively. However, anatomical analyses showed that the optimal BR concentration for rooting induction (10 nM) negatively interferes with the regular construction of the stem cell niche and its quiescent center (QC) in the AR, differently from what is observed in the LR where a regular organization of the niche is observed. The 10 nM eBL concentration perfectly counterbalances the inhibitory effect that $CdSO_4$ has on root production, restoring the self-promoting ability of both ARs and LRs production. However, this mitigating effect of BL hormone is not observed in the organization of the stem cell niche. Therefore, further analyses will be carried out using QC fluorescent markers. In addition, to precisely dissect BR effect on rhizogenesis, the research will also focus on the possible link between BRs and nitric oxide (NO), other important mediator molecule of plant development and response to abiotic/biotic stresses. Experiments in absence and presence of $CdSO_4$ are being carried out also using the NO-donor compound sodium nitroprusside (SNP), followed by NO epifluorescence tissue localization using the NO probe, DAF-FM DA, and NO quantification.

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Effects of plasma-activated water on signalling pathways underlying plant self-defence responses

Enrico Cortese[1], Alessio G. Settimi[2], Mattia Franceschi[2], Luca Cappellin[3], Alessandro Galenda[4], Alessia Famengo[4], Manuele Dabalà[2], Vanni Antoni[5], Lorella Navazio[1]
[1]Department of Biology, University of Padova, Via U. Bassi 58/B, 35121 Padova, Italy; [2]Department of Industrial Engineering, University of Padova, Via F. Marzolo 9, 35131 Padova, Italy; [3]Department of Chemical Sciences, University of Padova, Via F. Marzolo 1, 35131 Padova, Italy; [4]CNR ICMATE, Corso Stati Uniti 4, 35127 Padova, Italy; [5]Consorzio RFX, Corso Stati Uniti 4, 35127 Padova, Italy

Water activated by plasma discharge, termed as plasma activated water (PAW), has been attracting a great deal of interest in the last few years. The interaction between low-temperature plasma and water creates a unique environment that results in changes of the redox potential, conductivity and promotes the formation of several reactive chemical species – such as reactive oxygen species (ROS) and reactive nitrogen species (RNS) – that may render PAW suitable for a large variety of potential applications. Promising reports suggest the potential application of PAW as anti-cancer, anti-metastatic, regenerative medicine for blood coagulation and dental treatment agent. Cold plasma and PAW also represent alternative methods for microbial disinfection that may be optimally applied in the food sector and agriculture. In plant biology, PAW has been demonstrated to increase the seed germination rate, even under osmotic and saline stresses, as well as to promote plant growth. Moreover, PAW irrigation of tomato plants has been reported to induce defence gene expression and accumulation of the defence hormones salicylic acid and jasmonic acid. These studies suggest that PAW can play beneficial roles in agriculture by promoting plant growth and pre-alerting plant defence prior to a potential subsequent attack by pathogens, a phenomenon defined as “priming”. Nevertheless, studies addressing the signalling pathways activated in plants in response to PAW are lacking so far, and the possibility that PAW may induce a wider spectrum of plant defence responses, *e.g.* an increased resistance to abiotic stresses, is still largely unexplored.
In this work PAW was generated by two plasma torches, operating in a range of power 700-1100 W and with pressure from 1 to 3 bar. Different distances (from 8 to 1 cm) of the nozzle from the water surface were used, taking the resulting pH as a reference. PAW was immediately transferred to the biological set up or cryogenically frozen and then transferred. PAW chemical composition was analysed by UV/Vis absorption spectroscopy and ion chromatography. The pH of PAW, flash-frozen in liquid nitrogen upon generation and stored at -80°C for different time intervals was found to be stable for over two months. The total energy transferred to the water has been estimated to vary from 1.8 kJ to 9 kJ according to the duration. This was used as a proxy for the duration and intensity of the water treatment to compare PAWs generated in different ways.
As experimental system for PAW treatments, we used entire seedlings and cell suspension cultures of the model plant *Arabidopsis thaliana*. Viability assays on plant cell suspension cultures demonstrated the lack of cytotoxic effects of PAW. To investigate the signalling mechanisms underpinning the beneficial effects played by PAW on plants, and in particular to analyse the potential involvement of calcium as ubiquitous and versatile intracellular transducer, we used Arabidopsis lines stably expressing the Ca^{2+}-sensitive photoprotein aequorin. Ca^{2+} measurement assays demonstrated the Ca^{2+}-mediated perception of molecules contained in the PAW by Arabidopsis seedlings. Monitoring intracellular Ca^{2+} dynamics provided evidence for the dependence of the recorded Ca^{2+} signals upon different parameters, such as the type of PAW source, the operational conditions of the torches (energy, etc.), temperature, duration of PAW storage, and dilution. Interestingly, the single administration of nitrates, nitrites and H_2O_2 at the same doses as those measured in the PAW was not found to trigger any detectable Ca^{2+} responses in Arabidopsis. These data suggest that the unique mixture of different ROS and RNS contained in the PAW is responsible for the peculiar Ca^{2+} signatures that may lead to the downstream plant responses. Unveiling the signalling mechanisms underpinning plant perception of PAW may allow to finely tune its generation for application in agriculture, with potential advantages in the perspective of a more sustainable agriculture.

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Overcome the limits of multi-contaminated industrial soils bioremediation: insights from a multi-disciplinary study

Daniela Zuzolo[1], Carmine Guarino[1], Alessia Postiglione[1], Maria Tartaglia[1], Pierpaolo Scarano[1], Antonello Prigioniero[1], Maria Maisto[1], Roberto Terzano[2], Carlo Porfido[2], Rosaria Sciarrillo[1]
[1]Department of Science and Technology, University of Sannio, via de Sanctis snc, 82100 Benevento, Italy; [2]Department of Soil, Plant and Food Sciences, University of Bari "Aldo Moro", via Amendola 165/A, 70126 Bari, Italy

Soil contamination is an extensive problem worldwide and the number of contaminated sites is expected to hugely rise. Hence, finding environmental sustainable solution for soil remediation is a current issue and a future global challenge. In this context, phytoremediation (a nature based solution strategy) of polluted soils is receiving renewed and significant attention as a non-invasive and environmentally safe approach. However, at field level, this tool can be limited by many issues. Thus, achieving an exclusively biological approach to remediate contaminated soils is not an easy task but is fundamental for increasing environmental sustainability. Herein, we combined native plant species under different cover type (mono and polycultures) with a microbial consortium (composed of fungi and bacteria) in an in-field mesocosm experiment for the remediation of Potentially Toxic Elements (PTEs) multi-contaminated industrial soils. Five perennial herbaceous species and an arboreal specie naturally occurring on the site have been chosen to implement our contaminated mesocosms (treatment) and uncontaminated ones (control): (i – Poaceae) *Festuca arundinacea* Scherb. (Fes), *Piptatherum miliaceum* (L.) Coss. (Pip), *Dactylis glomerata* L. (Dac) (ii – Fabaceae), *Lotus corniculatus* L. (Lot), *Medicago lupulina* L. (Med), (iii – Salicaceae) *Salix purpurea* L. (Sal). Our experimental design included a preliminary assessment of the physical, chemical and biological features of the soils and subsequently the design of a suitable rhizosphere network for phytoremediation (considering plant functional traits). Then we assessed plants performance for PTEs (As, Cd, Hg, Pb, V and Zn) removal over time (240 days) and simultaneously evaluated the plants redox biology. A final follow-up of the most suitable species was performed by SEM and TEM microscopy and transcriptome analysis of the rhizosphere to assess the plant traits and gene expression induced by tolerance to toxic trace elements in soil living environment.
The physical-chemical profile of the analyzed soil showed that these soils held few nutrients, and were likely to be subject to nutrients leaching. Therefore, fertilization interventions have been carried out to ensure a good supply of macronutrients for plants and edaphic micro-flora. The PTEs accumulation rates and patterns highlighted that the selected plants are perfect candidates for phytostabilization. The statistical analyses revealed that changing the cover type significantly affected the PTEs removal from soil, founding that polycultures had better responded in terms phytostabilization efficiency with a multi-PTEs reduction in soil of 50% on average. It was observed that plant assemblages better adapted also from a stress tolerance perspective, maybe due to facilitative interactions between species. Among plant species, *F. arundinacea* Scherb. resulted in the best multi-element remediation ability (both in mono and polycultures) due to the overexpression of metal transporters able in both PTEs influx and sequestration from the cytoplasm. TEM analyses confirmed the presence of metals in the root of *F. arundinacea*, as particles and intracellular aggregates (Fig. 1). Moreover, SEM images depicted the spatial organization of the rhizoplane suggesting that rhizoplane adhesion was as a key feature for microbial proliferation. With this study, we provided a comprehensive view of a complex phenomenon that is, in our opinion, the right path to make effective and truly sustainable the nature based solutions for soil remediation. The knowledge of the biotechnological potential of nature-based systems are still to be explored but are of fundamental importance for the sustainable and fast recovery of large areas.

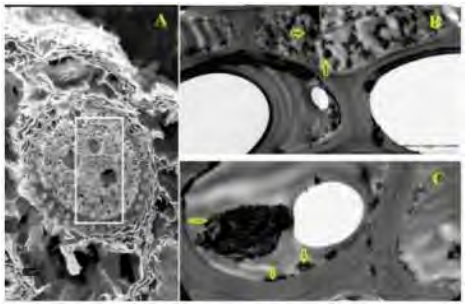

Fig. 1.
Festuca arundinacea roots observed at SEM (A) and TEM (B, C).

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Soil amendment with compost and biochar in a 50-year old olive grove affects soil enzymatic activity, rhizosphere microbiome and fine root production

Mattia Terzaghi[1], Angela Cicatelli[1], Francesco Guarino[1], Carmen Tammone[1], Paola Iovieno[2], Stefano Castiglione[1]
[1]Department of Chemistry and Biology "A. Zambelli", University of Salerno, Fisciano (SA), Italy; [2]Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria (CREA), Pontecagnano (SA), Italy

In the last decades the need to find a new agricultural *paradigma* that meets sustainability and the concept of circular economy grew exponentially. In this context, agriculture has to switch from being a system that overexploits resources and decreases environmental health to a system that takes care of the environment and protects it. Biochar and compost, used as amendment in agricultural soils, are promising candidates to contribute to this change. Biochar, a product of pyrolysis of biomass in partial or complete absence of oxygen, has been studied for its potential of enhancing soil carbon sequestration, improving soil quality, and increasing crop productivity. Compost, obtained by recycling agri-food related wastes, showed a promising fertilization effect enhancing soil chemical, physical and biological properties. The present study, funded by the National Operational Programme on Research and Innovation, aims to investigate the effects of the use of biochar and/or compost in an olive grove. The experimental site is located in the municipality of Fisciano, in South of Italy, (Lat. 40°46'35.15''N; Long 14°47'29.30''E; elevation 269 m.a.s.l.). Olive trees are spaced with plant-row spacing of 4.0 m and 4.5 m, respectively, rows orientation is North-South. A preliminary genetic characterization of the grove was carried out: leaves from each tree (25) were collected, DNA extracted and 6 nuclear and 4 chloroplast microsatellites were investigated. Results showed that the study site is a monovarietal grove (no genetic differences was detected). Therefore, in February 2020, the olive trees were selected keeping a minimum spacing between each other of 6 m. A randomized plot experiment, with five treatments and five replicates was setup. The treated area around each tree was delimited by two boundaries 0.5 and 1.5 m away from the tree stump, corresponding to an area of 9.16 ±0.15 m^2. Treatments (April 2020) were as follows: biochar (B) at a rate of 20 Mg ha^{-1} (Figure 1), compost (Co) at a rate of 38 Mg ha^{-1}, biochar plus compost (BCo) at a rate of 20 and 38 Mg ha^{-1} respectively, commercial mineral fertilizers [NPK(SO3) / 15-5-6(26)] (M) at a recommended rate of 3.3 Mg ha^{-1}, and control (C, no treatments). Seven months after application of treatments, (November 2020) shallow soil (15 cm depth) was sampled for enzymatic activity analyses. FDA hydrolysis, N-acetyl-β-D-glucosaminidase, arylsulphatase, β-glucosidase, alkaline and acid phosphatase and dehydrogenase activities were measured. Results showed a general increase of all enzymatic activities for B treatment, especially the FDA hydrolysis and the β-glucosidase. Ten months after amendments, (February 2021) fine roots from the first 15 cm of soil were collected from each tree and a metagenomic analysis of rhizosphere bacterial community was performed by 16S rDNA PCR and NGS (Next-Generation Sequencing) analysis. Preliminary results showed that the macro-groups were not affect by the different treatments. However, some differences were observed in the relative frequencies of the less representative bacterial genera. Moreover, some significant differences in β-diversity were found. For the quantification of fine roots, on May 2021 two 15-cm-soil cores were sampled for each plant (10 cores each treatment). Fine roots were estimated in terms of biomass and morphological traits. Preliminary results show an increment in the fine root web (total biomass and length) when biochar and/or compost were used. Being an ongoing project, these first results will be enriched in the near future by new analyses: the late-spring enzymatic activities, time scale microbiome characterization, oil quality in terms of saponifiable and unsaponifiable fractions and soil analysis.

Fig. 1.
Olive tree with soil amended with biochar

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Alteration of the anatomical and morphological structure of kiwifruit roots subjected to soil anoxic conditions

Ilaria D'Ippolito[1], Alba Mininni[1], Bartolomeo Dichio[1], Giovanni Scillitani[2], Maria Mastrodonato[2], Evangelos Xylogiannis[3], Adriano Sofo[1]
[1]Department of European and Mediterranean Cultures: Architecture, Environment, Cultural Heritage, University of Basilicata, Via Lanera 20, 75100 Matera, Italy; [2]Department of Biology, University of Bari, Via Orabona 4, 70126 Bari, Italy; [3]Zespri Fresh Produce Italy Srl. Via delle Margherite 121, 04011 Aprilia (LT), Italy

Italy, the third largest producers of kiwifruit producing in the world, has lost 10% of its production in recent years because of the spread of the Kiwifruit Vine Decline Syndrome (KVDS / moria), which leads to a gradual deterioration of the plant that starts from the roots. KVDS symptoms have been observed in different areas and have often been associated with water stagnation and anoxic soil conditions, resulting in root rot. Kiwifruit is a species adapted to hot-humid environments and requires large amounts of water. At the same time, it is also extremely sensitive to waterlogging, whose deleterious effects can arise if a rapid drainage of excess water does not occur in the soil, with the consequent establishment of anoxic conditions in the soil. Kiwifruit roots, and in particular meristems, have a high oxygen consumption and are therefore more sensitive to anoxia. Therefore, at low oxygen concentrations, they are the first tissues to be damaged. Early symptoms associated with KVDS are root browning, disappearance of absorbent roots, morphological and anatomical alterations, and tissue rupture and decomposition, resulting in blockage of xylem vessels, followed by late symptoms, such as leaf necrosis, shoot wilting and, in its acute phase, plant death. Unfortunately, the causes of KVDS are still unknown. On this basis, an experimentation was started in a kiwifruit orchard (*Actinidia chinensis* (A. Chev.) CF Liang & AR Ferguson, 1984, *var. deliciosa*) affected by KVDS and located in Latina (Lazio Region, Italy), in order to investigate the potential causes and suggest solutions to counter this physiopathy. Root samples from healthy plants were collected and compared with samples from plants with KVDS. Macroscopically, the roots affected by KVDS were rotting and with reddish sections, showing a loss of rhizodermis and cortical parenchyma (Fig. 1). For microscopic analysis, the roots were fixed in 10% formalin, dehydrated in alcohol, cleared and finally embedded in paraffin. Each individual sample was sectioned into 5 µm thick sections and stained with Safranin-Fast Green (Fig. 2) and PAS (Fig. 3) for light microscopy, or prepared for fluorescence microscopy (Fig. 4). Damage to the root system was observed, with tissue breakdown and decomposition, rhizodermis flaking, cortical area with loss of cell turgor, initial decay of the stele, and clear detachment of the cortex from the central conducting tissues. In the control samples, the roots exhibited a 13 µm thick rhizodermis and a mean parenchymatous cell size of 44.5 µm, unlike the KVDS sample, where the rhizodermis was 8.3 µm thick and the average cell size 34.7 µm. Waterlogging and the resulting decrease in oxygen around the roots had a negative, rapid and significant effect on the physiological state and on kiwifruit growth. To ensure optimal growth of kiwifruit plants, innovative soil management will be applied to reduce compaction and increase aeration.

With the support of the Zespri Innovation project "Water and soil management of G3 in Italy" Gi21020.

Fig.1. (a) Control and (b) KVDS roots.

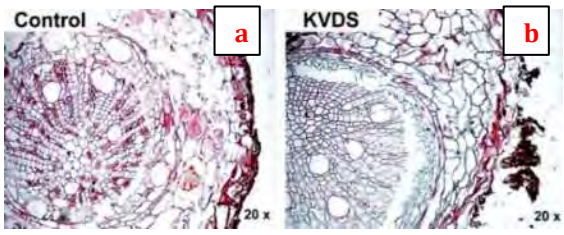

Fig. 2. Cross section of (a) control and (b) KVDS roots (Safranin-Fast green stain)

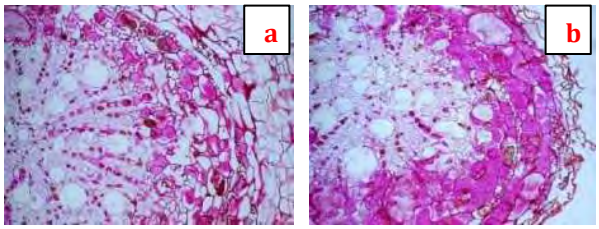

Fig. 3. Cross section of (a) control and KVDS roots (PAS stain).

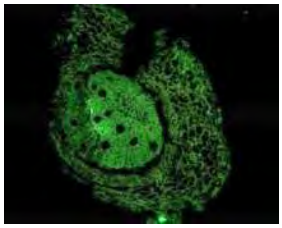
Fig. 4. Root affected by KVDS in fluorescence.

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Digital image analysis to quantify arbuscular mycorrhizal root colonization

Andrea Crosino[1]*, Ivan Sciascia[1]*, Mara Novero[1], Mara Politi[1], Andrea Genre[1]
[1]Department of Life Sciences and Systems Biology, University of Turin. Viale P.A. Mattioli 25, 10125 Turin, Italy
* These authors equally contributed to the work.

Arbuscular mycorrhizas are the most widespread plant symbiosis and involve the majority of crop plants. This interaction between plant roots and Glomeromycetes grants the green host a preferential access to soil mineral nutrients and water, supporting plant health, biomass production and resistance to both abiotic and biotic stresses. The nutritional exchanges at the core of this symbiosis take place inside the living cortical root cells, which are diffusely colonized by specialized fungal structures called arbuscules. For this reason, the vast majority of studies investigating arbuscular mycorrhizas and their applications in agriculture require a precise quantification of root colonization intensity. To this aim, several manual methods have been used for decades to estimate the extension of intraradical fungal structures, mostly based on optical microscopy observations and individual assessment of fungal abundance in the root tissues. Such methods are extremely time consuming, based on the ability of trained operators and subject to errors.
We propose a novel semi-automated approach to quantify AM colonization based on digital image analysis, comparing two methods based on image thresholding and machine learning to manual classification. Our results indicate in machine learning a very promising tool for accelerating, simplifying and standardizing this critical type of analysis, with a direct potential interest for applicative and basic research.

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Multiscale and multitemporal approach to evaluate resource use efficiency in vineyard towards sustainable cultivation of Greco grapevine: the GREASE Project

Veronica De Micco[1], Antonello Bonfante[2], Giovanna Battipaglia[3], Angelita Gambuti[1], Carmen Arena[4], Sheridan Lois Woo[5], Simona Castaldi[3], Arturo Erbaggio[6], Pierpaolo Sirch[7], Luigi Pagano[7], Chiara Cirillo[1]
[1]Dipartimento di Agraria, Università degli Studi di Napoli Federico II, Via Università 100-80055, Portici (Napoli); [2]Dipartimento di Scienze Biologiche ed Agroalimentari DiSBA, Istituto per i sistemi agricoli e forestali del mediterraneo, CNR, Piazzale Enrico Fermi 1 - Loc, Porto del Granatello, 80055, Portici (Napoli); [3]Dipartimento di Scienze e Tecnologie Ambientali, Biologiche e Farmaceutiche, Università della Campania Luigi Vanvitelli Via Vivaldi, 43- 81100, Caserta; [4]Dipartimento di Biologia, Università degli Studi di Napoli Federico II, Via Cinthia 21, 80126, Napoli; [5] Dipartimento di Farmacia, Università degli Studi di Napoli Federico II, Via D. Montesano 49, 80131, Napoli; [6]Libero professionista; [7]Feudi di San Gregorio Società Agricola S.p.A., Contrada Cerza Grossa, 83050, Sorbo Serpico (Avellino)

Climate change is one of the main challenges for future viticulture in the Mediterranean region where climatic models forecast a significant increase in temperature with more frequent and severe drought events. Such environmental changes are expected to severely affect plant growth and development, ultimately aggravating some critical issues in the production of the autochthonous Greco grapevine. This grape is widely cultivated in the Campania Region (southern Italy) and used alone or blended in many quality wine labels. Farmers and stakeholders denounce the high economic risk for the sustainability of Greco cultivation due to reduced vine productivity and low selling price of grapes, that leads to the abandonment of small/medium-sized farm production, with consequent fragmentation of the territory. It is likely that Greco cultivation will continue to be performed as a rainfed production, due to specifications and physical constraints of the territory; therefore, there is a need to introduce innovative and integrated management of cultivation techniques to improve the resource use efficiency, especially water, in vineyards.
The GREASE project, funded by Campania Region within the Rural Development Programme 2014-2020, falls within the framework of sustainable management of vineyards in the light of increasing drought. The general objective of the project is to realize a specific model of cultivation including vine pruning and soil management to improve the potential of Greco grapevine, that will achieve a good vegetative and reproductive balance, improve farm profitability, grape and wine quality, and finally environmental sustainability.
This project is conducted in a vineyard (Feudi di San Gregorio) of *Vitis vinifera* L. subsp. *vinifera* 'Greco' located in southern Italy. The project is based on three main inter-disciplinary activities: A1) to determine the effect of diverse vine pruning systems on plant resource use, through the reconstruction of vine eco-physiological history (dendro-anatomical and -isotopic analyses); A2-A3) to analyse the effect of soil management (SM) and of vine training systems (VTS) on the continuum soil-plant-atmosphere system. Specific activities include the comparison of vine traits in the six combinations of SM and VTS, and more specifically: pedoclimatic, vegetative and reproductive, physiological and hydraulic characterization; microvinification and characterization of grapes and wine; evaluation of resources use efficiency, diseases and pests, footprint family markers; model development. The applied analyses range from the microscopic scale (to unravel vine acclimation strategies to changing environmental/cultivation factors) to the territorial scale up to the application of UAV techniques. The experimental trials will be monitored for three years.
The integration of overall results is proving that the applied multiscale and multitemporal approach allows gaining a deep understanding of vine functioning in the different conditions, useful to design simple modifications to the presently used agronomical practices, to achieve production and economic gains without long-term structural investments.

Fig. 1. Logo of the PSR-GREASE Project and view of the experimental Greco vineyard at Feudi di San Gregorio farm.

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The effect of zaxinone, a natural metabolite derived from carotenoids, on tomato plants

Cristina Votta[1], Valentina Fiorilli[1], Jian You Wang[2], Silvia De Rose[1], Alessandro Silvestri[1], Teresa Mazzarella[1], Aparna Balakrishna[2], Muhammad Jamil[2], Salim Al-Babili[2], Luisa Lanfranco[1]
[1] Department of Life Sciences and Systems Biology, University of Torino, Viale Mattioli 25, 10125 Torino, Italy; [2] King Abdullah University of Science and Technology, Division of Biological and Environmental Science and Engineering, the BioActives Lab, Thuwal, 23955-6900, Saudi Arabia

Carotenoids are precursors of several plant hormones and signaling molecules, which are involved in plant growth and development and contribute to abiotic stress response and communication with surrounding organisms, including arbuscular mycorrhizal (AM) fungi (1, 2).
Zaxinone, a product of the rice carotenoid cleavage dioxygenase (*OsZAS*), was recently described as a novel natural metabolite that is required for rice growth (3). The exogenous application of zaxinone increased rice growth and biomass, while the corresponding mutant (*oszas*) showed growth retardation. Moreover, the hormone quantification analysis revealed that *oszas* roots and root exudates have a lower zaxinone content and an enhanced strigolactones (SLs) content compared to wild type.
This novel apocarotenoid is present in other plant species and the discovery of putative *Oszas* homologous genes in the majority of plants suggests that the biosynthetic pathway is conserved (1, 3). In this context, we have considered another important crop, tomato (*Solanum lycopersicum*) to analyze the effect of exogenous zaxinone on plant development, mycorrhization and responses to water stress.
The biometric characterization of plants showed that zaxinone has an impact on growth, not only on the root apparatus, but also on the shoot, with an increased length and biomass. Moreover, we carried out a transcriptomic analysis (RNAseq) in roots and shoots after 6 hours exposure to zaxinone: a few genes, including nitrate and phosphate transporters, turned out to be differentially expressed in roots, highlighting the rapid activation of plant responses.
To evaluate the impact of zaxinone on SLs we performed the *Striga hermonthica* seed germination assay and analysed the expression of SLs biosynthetic genes. The results indicate that in tomato exogenously supplied-zaxinone acts as a negative regulator of SLs production, possibly acting through the down-regulation of biosynthetic genes, in analogy to what has been described in rice (2). Furthermore, we investigated the role of zaxinone during the AM symbiosis. Morphological and molecular analyses showed that zaxinone application reduces AM colonization, confirming the results obtained in rice. We also studied the zaxinone effect on water stressed plants; a decrease of leaf transpiration rate after spray treatment with zaxinone was observed, suggesting that this apocarotenoid could be involved in stomata regulation and in drought stress acclimatization.
In their whole, these data represent a first contribution to understand the biological effect of zaxinone on tomato plants and open perspectives for future applications in agriculture.

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Seasonal variation in the metabolome of *Arbutus unedo* L. leaves collected in different areas of Sardinia (Italy)

Ilaria Chiocchio[1], Cinzia Sanna[2], Elisabetta Grillini[1], Valentina Maria Daniele[1], Manuela Mandrone[1], Ferruccio Poli[1]
[1]Department of Pharmacy and Biotechnology, University of Bologna, Via Irnerio 42, 40129 Bologna, Italy; [2]Department of Life and Environmental Sciences, University of Cagliari, Via Sant'Ignazio da Laconi 13, 09123 Cagliari, Italy

Arbutus unedo L. is an evergreen shrub or small tree native to the Mediterranean regions and Western Europe; strongly versatile, dry-adapted, low temperatures and fire resistant, it grows on a wide variety of soil types. *A. unedo* and its metabolites proved endowed with numerous biological activities and the use of different organs to treat a large number of illness has been reported in traditional medicine of Iberian Peninsula and Sardinia (Italy). This work was aimed at studying the metabolomic profile of *A. unedo* leaves across different seasons. A further investigation was dedicated to establish whether metabolomic differences could result in a significant variation of samples *in vitro* antioxidant activity. Considering that in addition to seasonal variations, other environmental factors might strongly affect plant metabolome, samples were harvested in different locations. This study was focused on Sardinia, which is a hotspot for biodiversity being characterized by a wide range of habitats and high degree of endemism. *A. unedo* leaves were collected in ten different locations of Sardinia during the four seasons and underwent ^{1}H NMR-based metabolomic analysis. In the winter season, leaves from flowering and fruiting branches were kept separate.
Thirty mg of dried leaves were extracted with 1 mL of mixture (1:1) of phosphate buffer (90 mM; pH 6.0) in D_2O (containing 0.01% TMSP) and CD_3OD by ultrasonication followed by centrifugation, then 700 µL of supernatant were transferred into NMR tubes and analyzed. The ^{1}H NMR-based metabolomic profiles of samples were measured and treated through multivariate data analysis performing both unsupervised (PCA, Fig. 1) and supervised (PLS-DA) analysis. Total phenolic and flavonoid content and *in vitro* antioxidant activity of the samples were also evaluated and compared through statistical analysis.
The multivariate analysis showed that seasonal variation has an impact on *A. unedo* leaves metabolomic profile and antioxidant activity. Moreover, slight differences between leaves harvested on flowering branches and fruiting branches (during winter) were also highlighted by PCA. Leaves in spring were characterized by higher content of sucrose while in summer resulted more enriched in quinic acid and glucose. Interestingly arbutin, the most known active principle of *A. unedo*, increased in autumn and an overall increasing of amino and organic acids, in particular malic acid, was registered in winter, together with an increment of unknown aromatic compounds, most likely conjugated with rhamnose. Focusing on the winter season, it emerged that leaves from fruiting branches have higher content of arbutin compared to the one collected on flowering branches, but lower levels of the other metabolites probably because more resources are invested in fruit formation. Phenolic content was higher in spring than other seasons and winter leaves from fruiting branches showed the lowest phenolic content. Regarding flavonoids, they were found particularly abundant in winter leaves collected on flowering branches, while the lowest content was found again in samples harvested on fruiting branches and this pattern was reflected also in samples antioxidant activity. Our results show that also the collection site has influence on plant's phytochemical features and biological properties. This was evident for samples total phenolic content and antioxidant activity which both vary significantly across different locations. Interestingly the sample exerting the lowest antioxidant activity was collected at the highest altitude (957 m a.s.l.) providing further insight into the influence of this environmental parameter on plants metabolome.

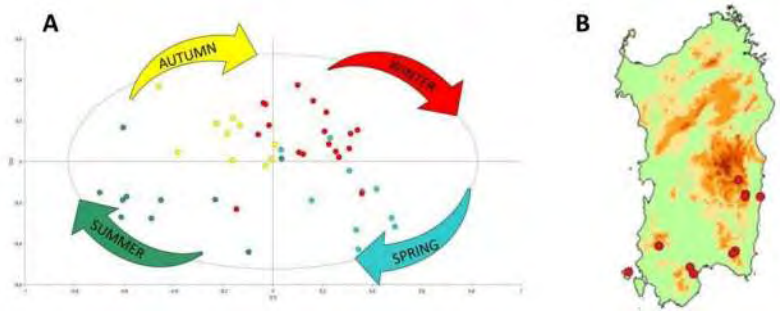

Fig. 1. ^{1}H NMR-based PCA of *A. unedo* leaves samples harvested in different seasons (A) and harvesting locations (B).

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Antimicrobial activity of extracts, fractions and compounds from leaves of *Psiadia punctulata* (DC.) Vatke growing in Saudi Arabia

Giuliana Donadio[1], Valentina Parisi[1], Valentina Santoro[1], Angela Bisio[2], Ammar Bader[3], Fabrizio Dal Piaz[4] and Nunziatina De Tommasi[1]
[1]Department of Pharmacy, University of Salerno, 84084 Fisciano, Italy; [2]Department of Pharmacy, University of Genova, 16148 Genova, Italy;[3]Faculty of Pharmacy, Al-Zaytoonah Private University of Jordan, P.O. Box 130, 11733 Amman, Jordan; [4]Department of Medicine and Surgery, University of Salerno, 84082 Baronissi, Italy

The analysis of the dichloromethane surface extract of a specimen of *Psiadia punctulata* (DC.) Vatke (Asteraceae) collected in Saudi Arabia revealed the presence of polymethoxylated flavonoids that exhibit antimicrobial activity against selected bacteria and fungal (*S. aureus, C. albicans*).
Based on this previous paper *P. punctulata* can be considered a source of bioactive compounds. In this work the leaves of *P. punctulata* were ground to homogenous powder for solvent extraction.
The extracts were then tested on bacterial strains: *Staphylococcus aureus, Escherichia coli*, *Streptococcus mutans* and *Treponema denticola,* by broth dilution methods to determine their minimal inhibitory concentration (MIC). *Streptococcus mutans* is considered the most relevant bacteria in the transition of non-pathogenic commensal oral microbiota which contribute to the dental caries process, and *Treponema denticola* is a spirochete bacterium associated with progression of periodontal diseases, infectious diseases that destroy attachment of teeth. Chloroform extracts of *P. punctulata* showed antimicrobial activity (MIC values ranging from 500 to > 1500µg/ml) (Table 1. A) against *Staphylococcus aureus, Streptococcus mutans* and *Treponema denticola*. No activity was detected against *E. Coli.*
The extract was subsequently separated by column chromatography on silica gel, yielding nine fractions.The fractions were evaluated for their antimicrobial activity at doses ranging from 20 to 100 µg/ml on all previous bacteria except *E. Coli. F*ractions,G and I were active against all the species tested, while H fractions were active only against *Treponema denticola* (Table 1.B*)* . Fractions x were then separated by RP-HPLC to yield thirteen compounds (1–13) along with known diterpenes and flavonoid; structures were elucidated by 1D-and 2D-NMR Spectroscopy (^{1}H, ^{13}C, DQF-COSY,HSQC, HMBC, ROESY) and confirmed by mass spectrometry. The phytochemical screening revealed that compound **4** (Psiadin), **5** (Propsiadin) and **9** (Calcycoptein) showed antimicrobial activity, other compounds were not active.

A

Extracts	Parameters	*S.Aureus*	*S.Mutans*	*Treponema*
Methanol	MIC	-	-	-
Chloroform	MIC	1500µg/ml	500µg/ml	-
Chloroform Methanol	MIC	1500µg/ml	500µg/ml	1000µg/ml

B

Fractions	Parameters	*S.Aureus*	*S.Mutans*	*Treponema*
D	MIC	>100µg/mL	>100µg/mL	-
F	MIC	100µg/mL	100µg/mL	-
G	MIC	>100µg/mL	75µg/mL	100µg/mL
H	MIC	-	-	>100µg/mL
I	MIC	>100µg/mL	100µg/mL	100µg/mL

C

Molecole	Parameters	*S.Aureus*	*S.Mutans*	*Treponema*
Psiadin	MIC	125 µg/mL	140µg/mL	100µg/m
Propsiadin	MIC	-	250µg/mL	125µg/mL
Calcicoptein	MIC		130µg/m	200 µg/m

Tab. 1.
Antimicrobial activity conducted with *Staphylococcus aureus,Pseudomonas aeruginosa*. *Streptococcus mutans* and *Treponema denticola*. (A) MIC assay of Extract from *Psiadia*; (B) MIC assay of selected fractions from *Psiadia*; (C) MIC assay of isolated molecules.

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Abietane diterpenoids from the hairy roots of *Salvia corrugata* Vahl.

Valeria Iobbi[1*], Roméo Arago Dougué Kentsop[1,2*], Giuliana Donadio[3], Barbara Ruffoni[2], Nunziatina De Tommasi[3], Angela Bisio[1]
[1]Dipartimento di Farmacia, Università di Genova, Viale Cembrano 4, 16148 Genova, Italy; [2]Consiglio per la Ricerca e la Sperimentazione in Agricoltura – CREA Centro di ricerca Orticoltura e Flo-rovivaismo, San Remo (IM), Italy; [3]Dipartimento di Farmacia, Università di Salerno, Via Giovanni Paolo II 132, 84084 Salerno, Italy

Common metabolites of the roots of *Salvia* species are diterpenoids with abietane skeleton that have role in plant chemical defence and display a wide spectrum of biological properties including antimicrobial, antifungal, anti-leishmanial, antiplasmodial, antiviral, antioxidant, antitumour, cytotoxic and anti-inflammatory activity. In previous work we isolated abietane compounds from the aerial parts of *Salvia corrugata* Vahl., along with icetexanes. *In vitro* regenerated shoots and micropropagated plants produced the same specialized metabolites. The aim of the study was to obtain transformed roots of *S. corrugata* and to evaluate the production of diterpenoids in comparison with untransformed root. Hairy roots initiated from leaf explants by infection with ATCC 15834 *Agrobacterium rhizogenes* onto hormone-free Murashige and Skoog basal solid medium. Transformation was confirmed by polymerase chain reaction analysis of rolC and virC1 genes. The biomass production was obtained in hormone-free liquid medium using Temporary Immersion System bioreactor (RITA).
The chromatographic separation of the methanolic extract of the untransformed roots afforded horminone, ferruginol, 7-O-acetylhorminone and 7-O-methylhorminone. From the hairy roots ferruginol and agastol were isolated. Ferruginol shows important bioactivities such as antimicrobial, cardioactive, antioxidative, antileishmanial and nematocidal, and antiulcer properties, antimalarial and against prostate cancer. The antiviral activity of this compound has been underlined, and interestingly against coronaviruses *i.e.* SARS-CoV $3CL^{pro}$. Owing to the increasing interest of natural compounds active against coronaviruses, specifically of terpenoids, *in vitro* culture technologies can represent efficient systems of production of secondary plant antiviral metabolites. A HPLC-DAD determination was then performed, and showed that the relative amounts of ferruginol and agastol (% w/w) in the extract were 31.2±3.3% and 33.6±2.8%, respectively, corresponding to theoretical extractive yields of 10.8% and 11.6% from the hairy root biomass, respectively, thus indicating that hairy roots of *S. corrugata* are a good source of these compounds.

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Lavandula spp. from the Pollino Massif: anti-arthritic potential of essential oils and bioactive components

Mariangela Marrelli[1], Massimo Tacchini[2], Maria Rosaria Perri[1], Valentina Amodeo[1], Alessandra Guerrini[2], Filomena Conforti[1], Gianni Sacchetti[2], Giancarlo Statti[1]
[1] Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, Via Pietro Bucci, 87036 Rende (CS), Italy; [2] Department of Life Sciences and Biotechnology, Pharmaceutical biology Lab., University of Ferrara, P.le L. Chiappini 3, 44123 Ferrara, Italy

Lavandula species, mainly *L. angustifolia* Mill., are aromatic and medicinal plants with a great economic value, due to their use in perfumery, cosmetics, and aromatherapy. Lavender populations from the Pollino National Park (the locally so-called "loricanda" lavender), were recently analyzed by a morphological and phytochemical point of view by Passalacqua and coworkers, who proposed to classify them as a new species, *Lavandula austroapennina* N.G. Passal., Tundis & Upson. *L. angustifolia* essential oil has been traditionally utilized for the treatment of painful conditions and its anti-inflammatory potential has been demonstrated both *in vitro* and *in vivo*.
The aim of this study was to evaluate the potential anti-arthritic activity of essential oils (EOs) from both *L. austroappennina* and true lavender *L. angustifolia* from the Pollino Massif together with their main metabolites. To this goal, the *in vitro* inhibitory activity on protein denaturation was assessed using bovine serum albumin (BSA) as a protein model. Tissue protein denaturation is a major cause of arthritic diseases, and chemical compounds able to prevent protein denaturation could be useful for the development of new drugs. Furthermore, the *in vitro* inhibitory effect on the production of nitric oxide (NO), a pro-inflammatory mediator also involved in the progression of osteoarthritis, was verified in LPS-stimulated RAW 264.7 macrophages.
Lavender EOs were characterized using GC-MS. Linalool was the most abundant compound, with *L. austroapennina* showing a higher content compared to *L. angustifolia*. Both essential oils induced BSA anti-denaturation effects, showing a concentration-dependent inhibitory activity (Fig. 1). The essential oil obtained from *L. austroapennina* showed better activity compared to *L. angustifolia*, with IC_{50} values equal to 260.4 ± 4.2 and 480.0 ± 2.6 µg/mL, respectively. In order to relate the observed results to the most interesting identified phytochemicals, some major components of the EOs were also tested, linalool and terpinen-4-ol being effective in protecting bovine serum albumin from denaturation.

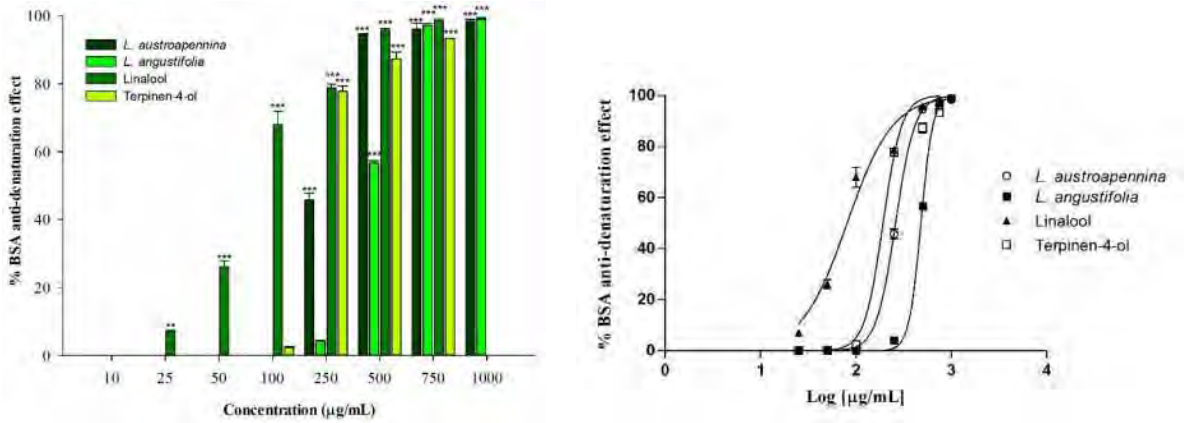

Fig. 1.
Inhibition of BSA denaturation induced by lavender EOs and their main bioactive components.

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Phytochemical analysis and anti-inflammatory activity of different ethanolic phyto-extracts of *Artemisia annua* L.

Andrea Mastinu[1], Giulia Abate[1], Leilei Zhang[2], Mariachiara Pucci[1], Giuseppina Maccarinelli[1], Giovanni Ribaudo[1], Alessandra Gianoncelli[1], Daniela Uberti[1], Luigi Lucini[2], Maurizio Memo[1].
[1] Department of Molecular and Translational Medicine, Division of Pharmacology, University of Brescia, 25123 Brescia, Italy; [2]Department for Sustainable Food Process, Università Cattolica del Sacro Cuore, 29122 Piacenza, Italy

Artemisia annua L. (AA) has shown for many centuries important therapeutic virtues associated with the presence of artemisinin (ART). The aim of this study was to identify and quantify ART and other secondary metabolites in ethanolic extracts of AA and evaluate the biological action in the presence of an inflammatory stimulus. In this work, after the extraction of the aerial parts of AA with different concentrations of ethanol, ART was quantified by HPLC and HPLC-MS. In addition, anthocyanins, flavonols, flavanones, flavonols, lignans, low molecular weight phenolics, phenolic acids, stilbenes and terpenes were identified and quantified by UHPLC-QTOF-MS. Finally, the viability of human neuroblastoma cells (SH-SY5Y) was evaluated in the presence of the different ethanolic extracts and in the presence of lipopolysaccharide (LPS). The results showed that ART is more concentrated in AA samples extracted with 90% ethanol. Regarding the other metabolites, only the anthocyanins are more concentrated in the samples extracted with 90% ethanol. Finally, ART and all AA samples showed a protective action towards the pro-inflammatory stimulus of LPS. In particular, the anti-inflammatory effect of the leaf extract of AA with 90% ethanol has been confirmed also at molecular level since a reduction in TNF-α mRNA gene expression was observed in SH-SY5Y treated with LPS.

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A focus on jambù: from the Brazilian folk medicine to the development of novel botanical insecticides

Eleonora Spinozzi[1], Filippo Maggi[1], Riccardo Petrelli[1], Loredana Cappellacci[1], Giovanni Benelli[2], Angelo Canale[3], Roman Pavela[3]
[1]Dipartimento di Scienze Chimiche, University of Camerino, Via Sant' Agostino 1, 62032, Camerino, Italy; [2]Department of Agriculture, Food and Environment, University of Pisa, Via del Borghetto 80, 56124, Pisa, Italy; [3]Crop Research Institute, Drnovska 507, 16106, Prague, Czech Republic

Even if the research on innovative safe and sustainable insecticides is continuously growing, the management of mosquito vectors still represents a great challenge. Nowadays, an increasing interest is focusing on novel botanical pesticides, due to their good bioavailability, sustainability and eco-friendly characteristics. In this scenario, *Acmella oleracea* (L.) R.K. Jansen, also known as jambù, represents a novel candidate for the development of new insecticides. Native from Brazil, but cultivated worldwide, *A. oleracea* (Fig. 1) is an edible and medicinal plant, presenting several applications in food, cosmetics, pharmaceutics, and pest management science. Its biological properties are mainly linked to the presence of *N*-alkylamides, among which spilanthol is the most representative one. The plant biomass deriving from the large-scale cultivation is also used to yield an essential oil (EO), which is mainly characterized by mono- and sesquiterpenes, besides the presence of spilanthol in minor amount. To maximize the content of spilanthol in the *A. oleracea* EO, the aerial parts obtained from a cultivation in central Italy were subjected to microwave-assisted extraction (MAE). This procedure led to the achievement of a higher yield and spilanthol content when compared with traditional hydrodistillation (0.47 v 0.22%, and 13.31 vs 2.24%, respectively). To improve its stability, which is one of the major limits of the employment of these natural products at an industrial level, *A. oleracea* EO (6%) was encapsulated into a nanoemulsion (NE), through a high-energy method. The formulation physical stability was assessed by optical microscope and DLS analyses at different timepoints showing stability up to one year of storage. The *A. oleracea* EO, its NE, and the isolated spilanthol were evaluated for acute toxicity against the 3rd instar larvae of *Culex quinquefasciatus*, a filariasis vector of public health importance, showing LC_{50} values of 16.1, 407.5, and 3.1 µL/L, respectively. The interaction with the cholinergic system was also evaluated for the explanation of the larvicidal activity, with both *A. oleracea* EO and spilanthol exhibiting high IC_{50} values in the anti-acetylcholinesterase (AChE) assay. In addition, *A. oleracea*-borne products showed a significant impact on larval development, fecundity, fertility and potential natality of *C. quinquefasciatus* testing LC_{30} values. At these values, *A. oleracea* EO-NE displayed higher efficacy than spilanthol, reducing the adult fertility, in terms of egg hatchability (%) and the overall abundance of F_1 larvae. These results can be considered as promising in terms of new mosquito larvicide development. The safety use of *A. oleracea* EO, NE and spilanthol was also demonstrated in assays on mammalian fibroblasts and microglia cells and low level of cytotoxicity coupled with protective effects against inflammation were detected. All the data obtained represent a starting point for the development of safe, effective, and eco-friendly botanical products to be used against several insect pests and vectors.

Fig. 1.
Acmella oleracea flower.

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Patterns of alien species in a riparian ecosystem of southern Tuscany: is there any news?

Leopoldo de Simone, Emanuele Fanfarillo, Tiberio Fiaschi, Simona Maccherini, Claudia Angiolini
Department of Life Sciences, University of Siena, Via PA Mattioli 4, 53100 Siena, Italy

Riparian areas host among the most fragile, threatened, and at the same time biologically valuable ecosystems in the world. Alien plant invasions have considerable impacts on riparian vegetation, which is often under severe human pressure. The artificialization of river courses due to human-induced transformations of the surrounding landscape led to increasing management requirements for riparian plant communities. Increasing disturbances such as cutting, clearing, and ground movement paved the way to major invasions of alien plant species, often totally changing the original features of riverscapes.
The Arbia river stretches for about 65 km across a North-South direction in the province of Siena, in southern Tuscany, at elevations decreasing from about 550 m a.s.l. to about 150 m a.s.l. The water course is mainly inserted into a human-haltered riverscape, especially in the middle and lower parts. In the context of a study aiming at finding sustainable management strategies for riparian vegetation, we began a vegetation survey along the entire length of the river. The river was initially divided into sectors of 500 m-length and 80 m-width, transversally centered on the river channel (40 m per side). The sampling was then stratified according to the bank (left/right) and into an inner and outer section of 20 m per bank, to broadly represent two strips with an increasing distance from the riverbed. This sampling design resulted in 4 sets for each sector (two outer and two inner sets for each river bank). Thirty-two sectors were selected along the entire river course, with an equal density. One plot was then randomly selected per each set (4 plots per sector). A total of 128 rectangular plots were thus randomly placed along the river. In each plot, we recorded all the species of vascular plants and their percentage cover, plus a set of structural and environmental variables. We investigated both the floristic assemblage and the rates of occurrence of alien species in each plot in relation to plot position along the water course, distance from the water channel and slope. An analysis of about a half of the plots revealed that 22 species out of about 300 are alien, among which *Artemisia verlotiorum* and *Robinia pseudoacacia* are the most frequent ones. The rates of occurrence of alien plant species gradually increase moving from the river head to the lower part. On the contrary, no significant differences in the rates of invasion were detected between the inner and outer plots (Fig. 1), indicating that neither inner or outer sections act as a buffer zone for alien species. The PCA biplot graph in Fig.1 shows the distribution of 25 species with a fit higher than 0.2. This analysis confirmed that, along the river, *A. verlotiorum* shows an increasing cover along a gradient of increasing distance from the source, according to its ecological requirements, whereas *R. pseudoacacia* is able to outcompete native woody riparian vegetation all along the river, showing an unexpected distribution pattern being not linked to the longitudinal gradient. Our results suggest that in the Arbia river, in contrast to previous findings in other Tuscan riparian ecosystems, the spread of *R pseudoacacia* reached the headwater area, probably due to the lack of two constraints usually containing its invasion: elevations over 900 m a.s.l. and surrounding natural areas covered by native trees and mostly situated in mountain hillsides. Our vegetation survey highlighted that the remnant well-preserved patches of riparian ecosystems need measures of protection considering their fragmented nature. This evidence provides the basis for the definition of guidelines for a sustainable management of riparian vegetation, given the lack of natural limits to the invasion of alien species.

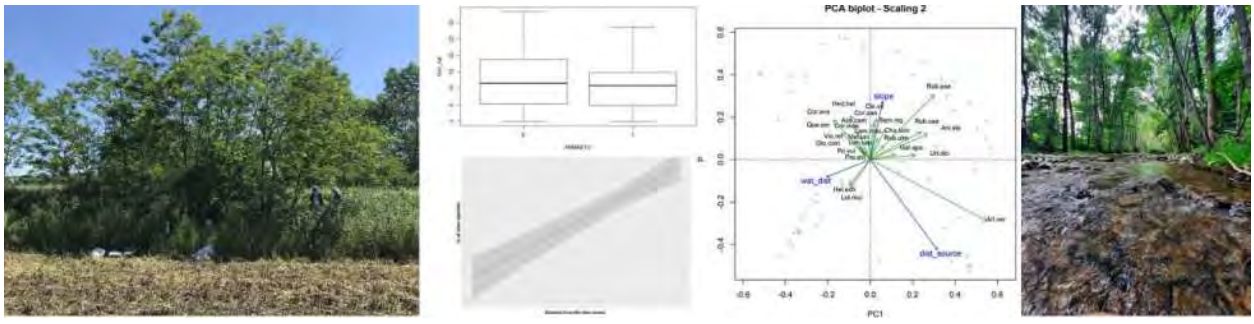

Fig. 1. clockwise: vegetation invaded by *Robinia pseudoacacia*; boxplots for the percentage occurrence of alien species in plots located in the inner and outer sectors (t test, p = 0,3); relationship between the percentage occurrence of alien species in the plots and the distance from the river source ($p<0.001$; $R^2=0.47$); ordination (PCA) of the plots; riparian vegetation with *Alnus glutinosa*.

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Xenophytes and anthropogenic vegetation in Sicily: the case of *Opuntia stricta* (Cactaceae) in the protected area of Capo Rama (Terrasini, Palermo)

Francesco M. Raimondo[1], Enrico Bajona[1], Emilio Di Gristina[2], Gianniantonio Domina[2]
[1]*PLANTA*/Center for Research, Documentation and Training, Via Serraglio Vecchio n. 28, I- 90123, Palermo, Italy; [2]Department of Agricultural, Food and Forest Sciences, University of Palermo, Viale delle Scienze, bldg. 4, I-90128 – Palermo, Italy

Since the last postwar period to today, the Sicilian territory has witnessed helplessly the invasion of some exotic plants adventitious or introduced to be cultivated and then spontaneously grown. Mainly along the coast, the abandonment of some lands that are climatically suitable for hosting neotropical species has favored the settlement and subsequent spread of some of them that have become integrated into the indigenous communities, ending - in some cases - by physiognomizing them. Thanks to their biological characteristics and suitable reproductive and dispersive strategies, such as hardiness and reproductive biology, as well as the undisputed competitive power, in many cases these taxa have ended up supplanting native species. Extensive and worrying cases are those offered by perennial herbaceous elements such as *Pennisetum setaceum* (Forssk.) Chiov. and *Boerhavia repens* subsp. *viscosa* (Choisy) Maire. Among the woody elements, apart from the chronic and widespread cases of *Ailanthus altissima* (Mill.) Swingle and *Robinia pseudoacacia* L., cause great concern *Acacia saligna* (Labill.) H. L. Wendl. and *Vachellia karroo* (Hayne) Banfi & Galasso. Apart from *Carpobrotus edulis* (L.) L. Bolus and some exotics historicized in the Sicilian cultural landscape, such as *Agave americana* L. and *Opuntia ficus-indica* L., among the arborescent succulents in recent decades, several other species of *Opuntia* have started to spread, including *O. amyclaea* Ten., *O. dejecta* Salm-Dyck. *O. dillenii* (Ker-Gawl.) Haw., *O. engelmannii* Salm.-Dyck (*s. l.*), and above all *O. stricta* (Haw.) Haw. The latter species is more widespread than the others and in some places it assumes an invasive character, as in the protected coastal area of Capo Rama (Terrasini, Palermo). In this context it constitutes a serious threat to native plant associations in which, in addition to the most common elements of the Mediterranean Maquis, infrequent species on the island find their habitat. Among these there are *Asparagus horridus* L. (=*A. stipularis* Forssk.), *Ephedra distachya* L. and *Quercus calliprinos* Webb. They differentiate special surviving communities in the few stretches of coast spared from urbanization, thanks to the imposition of environmental constraints such as the creation of a nature reserve (R.N.O. Capo Rama). As happened for other invasive xenophytes, the undisturbed settlement of these species in the spaces occupied by the autochthonous phytocoenosis, in many cases determines their integration with the other species of natural phytocoenosis, in other cases, however, ends with their total replacement. With the advantage of the single invasive species that will almost completely occupy the colonizable space. In both cases, they diversify the pre-existing indigenous phytocoenosis or create new, clearly anthropogenic ones.
The case of Capo Rama, with dominant *Opuntia stricta*, is one of the latter. Common and partly characteristic species of this new association dominated by *O. stricta* are: *Artemisia arborescens* (Vaill.) L., *Asparagus acutifolius* L., *A. horridus* L., *A. pastorianus* Webb & Berth., *Asphodelus ramosus* L. subsp. *ramosus, Beta vulgaris* subsp. *maritima* (L.) Arcang., *Brachypodium retusum* (Pers.) P. Beauv., *Cachrys pungens* Jan ex Guss., *Calendula arvensis* subsp. *bicolor* (Raf.) Nyman, *C. suffruticosa* subsp., *fulgida* (Raf.) Ohle, *Chamaerops humilis* L., *Clematis cirrhosa* L., *Clinopodium canescens* (J. Presl) Melnikov, *Cynara cardunculus* L. subsp. *cardunculus*, *Cytisus infestus* (C. Presl) Guss. subsp. *infestus, Dactylis glomerata* subsp. *hispanica* (Roth) Nyman, *Echium arenarium* Guss. ,*Euphorbia ceratocarpa* Ten., *Ferula communis* L. subsp. *communis*, *Galactites elegans* (All.) Soldano, *Hyparrhenia hirta* (L.) Stapf subsp. *hirta, Malva sylvestris* subsp. *ambigua* (Guss.) Tell., *Mandragora autumnalis*Bertol., *Medicago marina* L., *Micromeria nervosa* (Desf.) Benth., *Olea europaea* subsp. *sylvestris, Onopordon illyricum* L. subsp. *illyricum, Phillyrea latifolia* L., *Pistacia lentiscus* L., *Plantago crassifolia* Forssk., *Prasium majus* L., *Ranunculus bullatus* L., *Rubia peregrina* L. subsp. *peregrina, Rubus ulmifolius* Schott, *Salvia verbenaca* L., *Scolymus hispanicus* L., *Silene colorata* Poir., *Smilax aspera* L., *Stipellula capensis* (Thunb.) Röser & H.R. Hamasha, *Tapsia garganica* L. subsp. *garganica*, *Teucrium fruticans* L., *Thymelaea hirsuta* (L.) Endl., *Thymbra capitata* (L.) Cav., *Trifolium campestre* Schreb., *T. nigrescens* Viv., *T. stellatum* L., and some aliens as *Pennisetum setaceum* and *Opuntia engelmannii*, plus *Solanum linneanum* Hepper & P. –M.L. Jaeger. The syntaxonomic framework of the new association - in some ways problematic - will follow the same criterion used for the steppe grasslands with *Pennisetum setaceum* at Monte Pellegrino in Palermo, starting from the assumption of a floristic and quantitative-qualitative evolution suffered by the pre-existing community.

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UAV-based mapping of *Acacia saligna* invasions in the Mediterranean coastal dune

Flavio Marzialetti[1], Ludovico Frate[1], Walter De Simone[2], Anna Rita Frattaroli[2], Alicia Teresa Rosario Acosta[3], Maria Laura Carranza[1]

[1]Envix-Lab, Departement of Biosciences and Territory, Molise University, Contrada Fonte Lappone, 86090 Pesche (Is.), Italy; [2]Department of Life, Health and Environmental Sciences, University of L'Aquila, Piazzale Salvatore Tommasi, 67100 L'Aquila, Italy; [3]Department of Sciences, Roma Tre University, Viale G. Marconi 446, 00146 Rome, Italy

Invasive Alien Species (IAS) are one of the major drivers of global change and biodiversity loss. Remote Sensing (RS) data offers considerable support for alien plants detection and mapping and could play a key role in managing and preventing IAS. Using satellite or airborne data with very high spatial resolution, is effective for mapping woody species with a distinct spatial pattern with respect to the surrounding landscape and native vegetation. On the other hand, adopting an Unmanned Aerial Vehicle (UAV) with ultra-high spatial resolution data (< 5 cm per pixel) could represent a good alternative for IAS early detection and mapping invasions on highly heterogeneous and dynamic mosaics.

In this work, we explored the potential of RGB and multispectral data acquired by UAV for detecting and mapping the invasive plant *Acacia saligna* on coastal dunes dynamic landscapes. *A. saligna* is an evergreen tree native to Western Australia introduced into Europe during the last half century and has become one of the most aggressive IAS in the Mediterranean basin (included in the EU Regulation 1143/2014). In particular, we analyzed the potential of using UAV RGB data and the combination of RGB and multispectral data collected during pre-flowering and flowering phenological stages for mapping *A. saligna*.

We acquired the UAV images using DJI Phantom 4 Pro equipped with RGB Camera (CMOS 1'' sensor, 20 Mpx) and multispectral camera (Parrot Sequoia composed by bands Green, Red, Red Edge, Near-Infrared, 1.2 Mpx) on two dates (pre-flowering period – 17/02/2021, flowering – 26/04/2021) along an invaded coastal dunes of 11 ha inside the SAC Foce Trigno – Marina di Petacciato - IT7228221 (Molise region). We derived the Digital Surface Model (DSM) and the Hue-Intensity-Saturation values (HIS) from RGB images, and we calculated the Normalized Difference Vegetation Index (NDVI) from Red and Near-Infrared bands (multispectral data). All variables (DSM, RGB, HIS, NDVI) were rescaled to a common range of values (0-255).

For *A. saligna* mapping, we used an automated Object-Based Image Analysis (OBIA) as follows: a) image segmentation by the Large Scale Mean Shift algorithm (parameters settings: 5 of Spatial Radius, 5 of Range Radius and 100 of Minimum Segment Size), b) photo interpretation of the 2% of the segments in six classes (*A. saligna*, Woody vegetation, Herbaceous vegetation, Sand, Shadow, Deadwood), the 70% of these segments was successively used as a training set for classification and the 30% for accuracy analysis, c) image classification using two algorithms (Random Forest: RF and Support Vector Machine: SVM) and four different combinations of variables (DSM + RGB, DSM + HIS, DSM + RGB+ NDVI, DSM + HIS + NDVI), d) accuracy assessment (overall accuracy, kappa statistic, and producer's and user's accuracies of *A. saligna*) using confusion matrices for the two classification algorithms in pre-flowering and flowering periods and in all the combinations of variables.

RF comprehensively performs better than SVM; the classification based on UAV images collected during the flowering period showed higher overall accuracy and kappa statistic concerning those produced using pre-flowering RS data. *A. saligna* is better detected using the combination of variables DSM+HIS+NDVI and showed a higher producer's (RF: 78.79%, SVM: 69.70%) and user's (RF: 89.66%, SVM: 87.34%) accuracies using RS data collected during the flowering period. However, the combination of variables derived only by RGB images (DSM+HIS) showed similar accuracy values, producer's accuracy with 77.78% in RF and 70.71% in SVM and user's accuracy with 92.77 in RF and 87.50 in SVM. Our results gave evidence about the effectiveness of UAV data for mapping *A. saligna* using both RGB data and a combination of RGB and multispectral data and identified the flowering period as optimal to detect this IAS.

Using UAV with ultra-high spatial resolution data, *A. saligna* invasion can be detected and mapped at a very fine scale over management-relevant extents. The proposed allows frequent surveys, making it particularly suitable for early detection, mapping and monitoring of IAS on highly heterogeneous landscapes as coastal dunes.

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From local to landscape disturbances: new perspectives in alien plant invasions across multiple spatial scales

Francesco Boscutti[1], Elisa Pellegrini[1, 2], Massimo Buccheri[3], Fabrizio Martini[4], Lorenzo Marini[5]
[1]Department of Agricultural, Food, Environmental and Animal Sciences, University of Udine, via delle Scienze 91, 33100 Udine, Italy; [2]Department of Biology, University of Copenhagen, Universitetsparken 4, 3rd floor, 2100 København Ø, Denmark; [3]Museo Friulano di Storia Naturale di Udine, via Cecilia Gradenigo Sabbadini, 22-32, 33100 Udine, Italy; [4]via Fortunio 10, I-34141 Trieste, Italy; [5]DAFNAE, University of Padova, Viale dell'Università 16, 35020 Legnaro, Padova, Italy

Exotic plant invasions are considered one of the major threats to biodiversity causing important impacts at the population, community, and ecosystem level. Unveiling the mechanisms driving exotic plant invasion represent a central issue to contain the loss of biodiversity and related ecosystem services. Understanding the drivers of plant invasions and underlying processes require a multiple spatial and temporal scales approach. Generally, at a broad spatial scale, climate, soil and habitat types are expected to be more influential in determining species distribution while at finer scale, disturbances and biotic interactions should become more important. We here report a synthesis of the findings from two studies conducted from local to regional scale relying on a large floristic survey undertaken in North - Eastern Italy with over 300.000 floristics records. We considered the effect of landscape composition and configuration, focusing on the two most impacting anthropogenic land uses, namely agricultural and urban areas.
At regional scale (Figure 1A), we found that both extent of urban and agricultural land uses greatly affected the spread of alien plant invasion, but the occurrence of unimodal patterns showed that small percentage of extensive agricultural land may concurrently curb the richness of exotic plant while sustaining native plant diversity. The results suggest that a shift of intensive to extensive agricultural landscapes, by implementing green infrastructures, could be a win-win solution favouring native species while controlling plant invasion.
At landscape scale (Figure 1B), we found that increasing urbanization coupled with high shape complexity of urban elements were major drivers of exotic plant invasions across heterogeneous productive landscapes. In particular, shape complexity seemed to be a key driver of plant invasions at large spatial scale whereas the type of recipient habitat and urban cover determined the exotic success at the patch level. High shape complexity of urban areas is expected to increase the exchange surface that exotic plants use to spread their propagule across the landscape mosaics. These findings suggest that urban planning aimed at curbing urban fragmentation by both reducing shape complexity and urban sprawl might greatly improve the resistance of landscapes to biological invasions.

(A) (B)

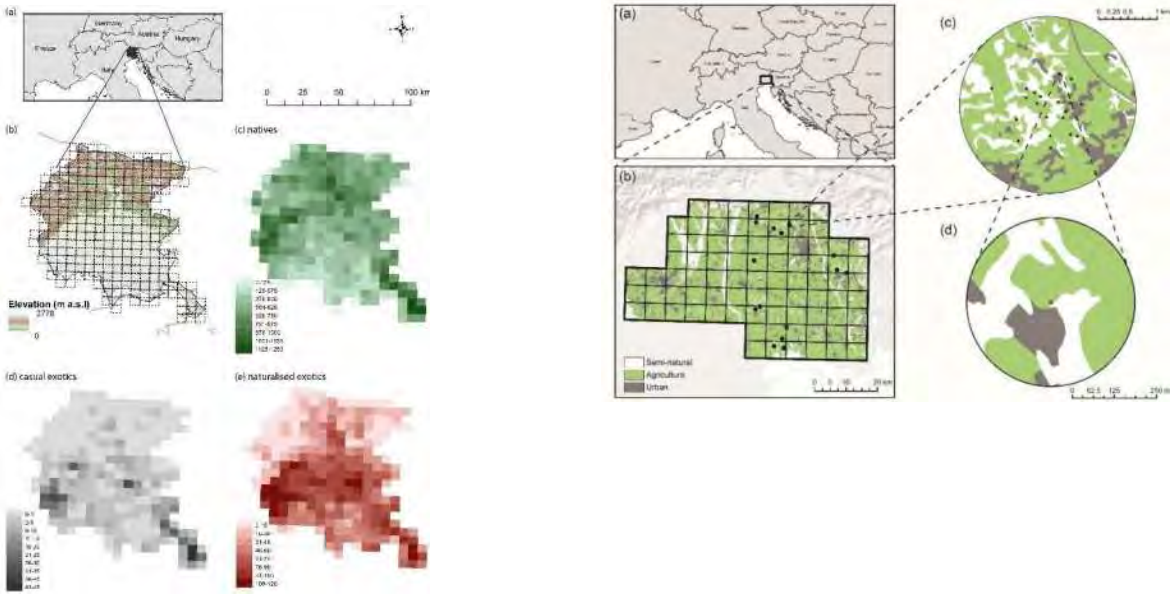

Fig. 1. Sampling design of the studies conducted at regional (A) and landscape (B) scales.

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Management of *Carpobrotus* spp. within the Life project LETSGO GIGLIO on Giglio Island (Tuscan Archipelago): control actions and monitoring of flora and vegetation

Lorenzo Lazzaro[1], Michele Giunti[2], Giulio Ferretti[1], Michele Mugnai[1], Francesca Giannini[3], Renato Benesperi[1]
[1]Department of Biology, University of Florence, 50121 Firenze, Italy; [2] Nature and Environment Management Operators s.r.l., 50136 Firenze, Italy; [3]Parco Nazionale Arcipelago Toscano, 57037 Portoferraio, Italy

The project LETSGO GIGLIO "Less alien species in the Tuscan Archipelago: new actions to protect Giglio island habitats" is a Life Natura project co-funded by the European Commission (www.lifegogiglio.eu). The project sees the collaboration of the Tuscan Archipelago National Park, the Department of Biology of the University of Florence, and the Company NEMO srl, for different actions on Giglio Island (Tuscan Archipelago) towards the conservation of habitats protected under Dir. 92/43/EEC, thanks to the reduction of important threat factors, mainly represented by invasive alien species. Indeed, one of the project actions is focused on the control of *Carpobrotus* spp. invading the habitats 1240: Vegetated sea cliffs of the Mediterranean coasts with endemic *Limonium* spp., 1430: Halo-nitrophilous scrubs (*Pegano-Salsoletea*) (note that 1430 is represented at Giglio Island by the alliance *Artemision arborescentis*) and 5320: Low formations of *Euphorbia* close to cliffs. Within this contribution, we present the final executive project for the control actions and the first results from the monitoring of flora and vegetation.
According to detailed mapping of the distribution of *Carpobrotus* spp. conducted during the spring/summer of 2020, this species is currently invading about 50,000 m^2 at Giglio Island. About 30,000 m^2, corresponding to the main extent in natural contexts, will be the object of the control activities within the project. The mapping was aimed also at individuating the constraints limiting the application of the different control methods planned: manual removal and use of mulching sheet. In fact, the choice of the method is strongly linked to several factors to be taken into account simultaneously: slope and terrain roughness; accessibility by means; stand extension; compactness and thickness of mats and presence of non-target species (especially if of conservation interest). According to these factors, we individuated the specific interventions for the control actions, which will involve covering with mulching sheets for the main extent and manual removal in case of excessive slope and presence of native species.
Together with the phase of mapping of *Carpobrotus* spp., planning and development of the executive project for the control actions in May 2020 and 2021 we conducted before-intervention monitoring of the habitats invaded by *Carpobrotus* spp. This monitoring represents the first set of surveys that will be repeated throughout the project. To assess the impacts of *Carpobrotus* spp. we surveyed the presence and abundance of native species on 44 permanent square plots sized 2 meters, in invaded and uninvaded areas, in each of the 3 habitats mentioned. The preliminary results clearly show, as easily predictable, a decrease in plant species richness in the invaded areas. The difference between control and invaded areas is particularly high for the habitats 5320 and 1430, while it is less pronounced as to habitat 1240. The first two are habitats that usually host a greater species richness, so here the effect of the disappearance of species caused by *Carpobrotus* spp. is particularly evident. Habitat 1240 naturally has few species at Giglio Island, due to the characteristics of the granitic substrate, so the effect appeared less marked. These preliminary results allowed us to outline the actual impact of *Carpobrotus* spp. on the plant communities of Giglio Island. These impacts are confirmed not only on sand dune and sea cliffs habitats (mentioned most frequently in the literature), but also on more structured habitats such as 5320 and 1430.


Fig. 1. *Carpobrotus* spp. at SW slopes of Giglio Island

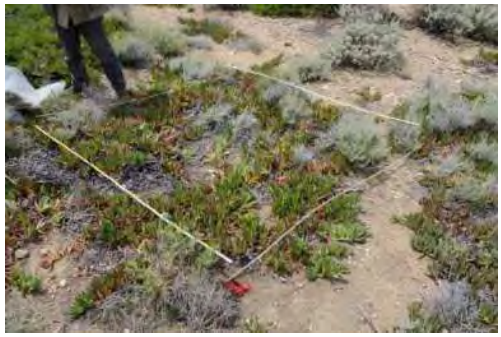
Fig. 2. Monitoring plot on invaded hab. 5320

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Plant invasion in Mediterranean Europe, current hotspots and future scenarios

Luigi Cao Pinna[1], Marco Malavasi[2], Alicia T. R. Acosta[1], Marta Carboni[1]
[1]Department of science, Roma Tre University, Viale Marconi 446, 00146 Rome, Italy; [2]Department of Applied Geoinformatics and Spatial Planning, Czech University of Life Sciences, Kamýcká 129, 165 21 Prague 6, Czech Republic

Alien species and climate change are among the major causes of ecosystem alterations and biodiversity loss. This is particularly true in Mediterranean Europe, a biodiversity hotspot in terms of species, endemisms and ecological functions, which has been historically subject to biological invasions and is expected to be particularly threatened by climate change. After the introduction, alien species can locally adapt to the new environmental conditions, even though few of them may expand and fill their potential niche. However, to date, we have a limited understanding of the fate of alien species under climate change and we still do not know whether alien species will be able to spread, filling their potential niche, or are expected to be hampered by climate change. Therefore, understanding alien species spread in Mediterranean Europe under climate change is a pressing conservation goal, also questioning basic assumptions about the fate of native species.
To address this goal, we analyzed 325'000 vegetation plots from the European Vegetation Archive (EVA), and identified 93 naturalized alien plants in Mediterranean Europe. Since alien species may not be at ecological equilibrium in the invaded range, we also estimated their potential niche. To this scope, we collected 3.5 million presences worldwide from GBIF. To capture potential scenarios of invasion, we then fitted species distribution models and projected them in the current and future environmental conditions (2050). Finally, we evaluated if changes in the invaded area are predicted by niche unfilling or exposure to climate change.
Results demonstrated that for 42% of species the potential and realized niches were more different than expected by chance, due to unfilling in the realized niche. Overall, we found that for the analyzed alien species, the invasion will decrease in 2050 even though few aliens are predicted to greatly enlarge their range. For example, Poaceae will spread more than other families according to the potential niche model. Invasion hotspots maps showed a similar pattern for both potential and realized niche scenarios, also predicting that the highly invaded areas will remain stable. Finally, we found out that species with higher niche unfilling will expand more and that exposure to climate change is a significant driver of range loss. In a nutshell, most of the analyzed alien species are going to reduce their range affected by the severity of climate change, also defining an unfavorable scenario for native species.

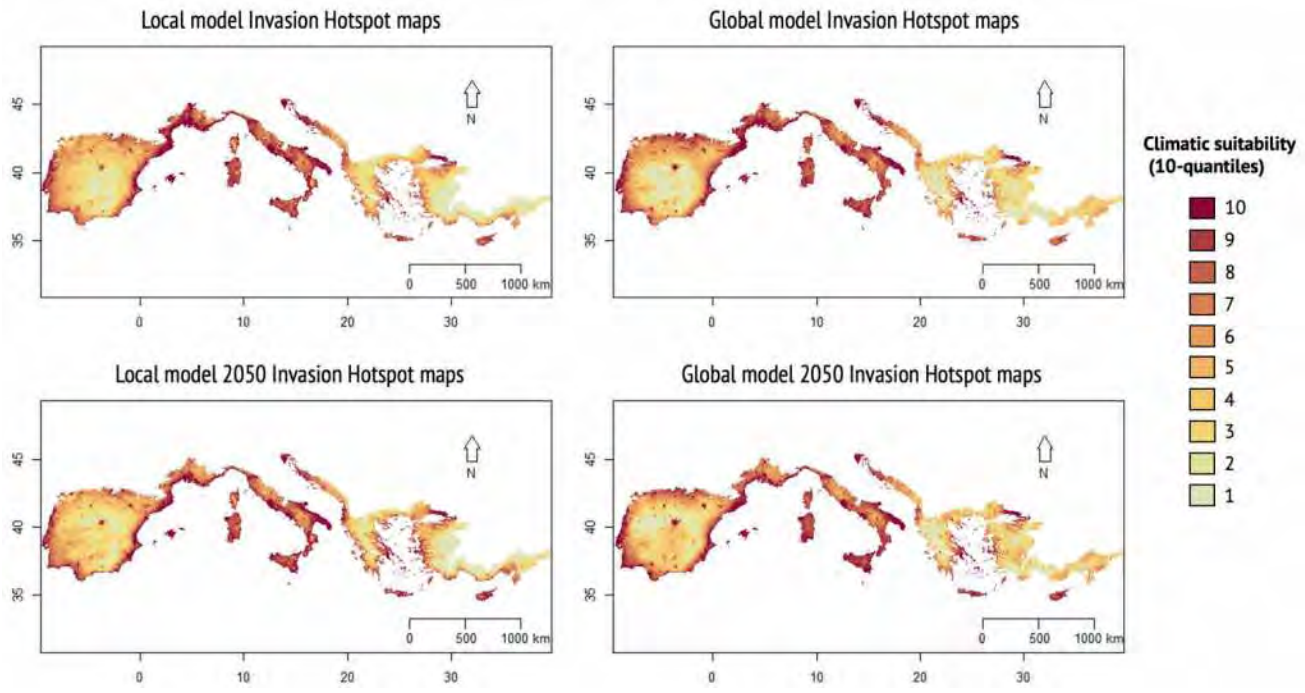

Fig. 1. Invasion's hotspot map for current and future scenarios.

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Don't stop exploring and collecting! Field work and herbaria still play a fundamental role in the analysis of lichen diversity: some case-studies from Italy

Juri Nascimbene[1], Renato Benesperi[2], Elisabetta Bianchi[2], Luca Di Nuzzo[2], Gabriele Gheza[1], Paolo Giordani[3], Lucia Muggia[4], Chiara Vallese[1], Pier Luigi Nimis[4]
[1]BIOME Lab, Department of Biological, Geological and Environmental Sciences, Alma Mater Studiorum University of Bologna, via Irnerio 42, 40126 Bologna, Italy; [2]Università di Firenze, Dipartimento di Biologia, Via la Pira 4, 50121 Firenze, Italia; [3]Università di Genova, Dipartimento di Farmacia, viale Cembrano, 4, 16148, Genova, Italia; [4]Department of Life Sciences, University of Trieste, 34121 Trieste, Italy

The lichen biota of Italy is among the best known worldwide, thanks to a long tradition of lichenological studies that has experienced a strong boost after the publication of the first modern checklists and of the first computer-aided keys. However, new records are constantly being published every year, which indicates that the exploration of the lichen biota of Italy is far from being complete. Additional field work, coupled with the analysis of collections (including historical herbaria), is of paramount importance to obtain a clearer picture of lichen diversity within the country. Field work (even when focused on biomonitoring or ecological issues), and specimen collections are interconnected, given the impossibility of recognizing most of the species in the field. The increase in the number of herbarium specimens, provides material that can be re-evaluated in the light of phylogenetic–taxonomical advances and contributes to a better knowledge of biogeographic patterns. This situation is exemplified by case-studies focused on the lichen biota of Italy, e.g. the study of cryptic taxa in freshwater Verrucariaceae or in some epiphytic Parmeliaceae, the hundreds of recently discovered new records by coupling field work and data mining in herbaria across Europe, and the study of type material in historical herbaria.

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Rediscoveries and systematic research bring back 17 European endemics from extinction

Giulia Albani Rocchetti[1], Thomas Abeli[1]
[1]Department of Science, University of Roma Tre, Largo S. Leonardo Murialdo, 1, 00146 Roma, Italy

According to the IUCN Categories and Criteria "a taxon is Extinct when there is no reasonable doubt that the last individual has died". To know whether a taxon is extinct is of key importance in conservation biology because an extinct species is automatically removed from conservation frameworks; de-extinction, the new frontier of conservation aimed at resurrecting or creating proxies of extinct species, is currently (and likely for long) only theoretical.
Inspired by the impressive global analysis of modern extinction in plants (Humphreys et al. 2019, https://doi.org/10.1038/s41559-019-0906-2) and its subsequent update (Knapp et al. 2020 https://doi.org/10.1038/s41477-021-00878-1), together with an international team of experts we reviewed the status of seed plant species endemic to Europe (including the Canary Islands, Azores and Madeira Archipelago) listed as extinct (EX) from several authoritative sources including scientific publications, red lists and floras and found that 17 out of 36 species (*i.e*. 47%) should be delisted. We did not consider the subspecies level.
The classical reason for changing the status of an extinct species is its rediscovery due to ad hoc exploration campaigns or fortunate encounters. This applies to three plant species endemic to Europe (*Astragalus nitidiflorus* Jiménez Mun. & Pau, *Ligusticum albanicum* Jávorska and *Ornithogalum visianicum* Tomm. ex Vis.), which adds on to previous well-known cases of species rediscoveries. It is noteworthy that plant rediscovery has happened in an area of the world well known and widely explored by thousands of botanists and citizen scientists.
An important contribution to delisting extinct taxa derives from changes in taxonomic status. Seven extinct species are now considered synonyms of as many extant taxa. Finally, three plant species have been erroneously identified in the past and should be ascribed to different taxa, endemic to Europe. Two species have been subject to ex situ conservation activities before their extinction in the wild and are now cultivated in botanic gardens, *i.e*. *Hieracium hethlandie* (F.Hanb.) Pugsley recently and possibly *Armeria arcuata* Welw. ex Boiss. & Reut, as ex situ material is preserved at the Utrecht University Botanic Gardens, but its taxonomy is doubtful. Morphological and genetical comparison with the type specimens of *A. caespitosa* (Ortega) Boiss. is still ongoing. These species have been therefore erroneously declared extinct when they should have been considered "extinct in the wild" (EW). The importance of fieldwork and plant collecting goes beyond their main aims, and these cases are a prime example. In fact, these activities could also strongly affect taxa and groups of taxa conservation, and therefore their conservation decisions and frameworks, as the changing status has important implications for the conservation policies and targets of the hosting countries (*e.g.* species that were rediscovered or reassigned to the EW category should urgently be included in fast-track conservation frameworks to avoid re-extinction). We demonstrated that rediscoveries and knowledge improvements from fieldwork and plant collecting activities have a key role in plant conservation and may produce a turnover in lists of extinct species, with implications for conservation policy and the development of a conservation framework for the delisted species. Periodic reviews of the status of extinct species, fieldwork and plant collecting are therefore required.

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Climate change and elevational shifts in plant distribution. A case study from the vascular flora of Tuscany across the last century

Lorenzo Peruzzi[1,2], David Dolci[1], Brunello Pierini[3], Giulio Ferretti[4], Francesco Roma-Marzio[5], Giovanni Gestri[6], Gianmaria Bonari[7], Lorenzo Pinzani[1], Marco D'Antraccoli[5], Giuliano Pacifico[8], Jacopo Franzoni[1], Daniela Ciccarelli[1,2], Angelino Carta[1,2], Gianni Bedini[1,2]

[1]Department of Biology, University of Pisa, Via Derna 1, 56127 Pisa, Italy; [2]CIRSEC, Centre for Climatic Change Impact, University of Pisa, via del Borghetto 80, 56124 Pisa, Italy; [3]Via Zamenhof 2, 56126 Pisa, Italy; [4]Department of Biology, University of Firenze, Via Giorgio La Pira 4, 50121 Firenze, Italy; [5]Botanic Garden and Museum, University of Pisa, Via Luca Ghini 13, 56126 Pisa, Italy; [6]Via Bartolomeo Bonfiglioli 30, 59100 Prato, Italy; [7]Faculty of Science and Technology, Free University of Bozen-Bolzano, Piazza Università 5, 39100, Bolzano, Italy; [8]Via Ponte del Vescovo 32/d, 54100 Massa-Carrara, Italy

Climate change can drive different changes in vascular plants, including shifts in phenology and distribution, either in latitude or elevation. According to historical and current climatic data, the climate of Tuscany (Central Italy) became slightly warmer and drier during the last 100 years (1860–1908 vs. 1970–2021 periods). Thus, we could hypothesize that the vascular flora experienced elevation shifts towards higher elevations. To check for these potential shifts, we used the data stored in the free online database Wikiplantbase #Toscana (http://bot.biologia.unipi.it/wpb/toscana/index.html). We extracted all georeferenced floristic records of the periods 1860–1908 ('past' hereafter) and 1970–2021 ('present' hereafter) and retained only those species shared by the two datasets. Species with less than 5 floristic records for each period were discarded. The resulting joined dataset comprised 2,018 shared species (about 49% of the total vascular flora of Tuscany) and 182,633 records, of which 49,480 referred to past and 133,153 to present. Based on these records, mean elevations in the two periods and putative elevation shift (difference between past and present mean elevations) were calculated for each species.

There is an overall significant difference between past and present by comparing the 2,018 mean elevations (ANOVA, $df = 1$, $F = 9.508$, $p < 0.01$), with a mean elevation shift of +39 m. The variation of elevation shifts is normally distributed (Shapiro-Wilk test $W = 0.9633$, $p < 0.01$), with a median centered on slightly positive values (+37.5 m). Most of the species (25^{th} percentile–75^{th} percentile) experienced a shift ranging from -43 m to +118 m, while 502 species showed a present mean elevation lower ($\leq$ -43 m, up to -700 m in *Rumex arifolius* All.) than that referring to the past, and 512 species showed a higher present mean elevational range ($\geq$ +118 m, up to +829 in *Anchusella cretica* (Mill.) Bigazzi, E.Nardi & Selvi).

Elevation shifts are slightly negatively correlated with past mean elevations ($r = -0.09$, $p = < 0.01$), while they are positively correlated with present mean elevations ($r = 0.27$, $p < 0.01$). Interestingly, the past mean elevations are not significantly different between the group of species showing an elevation shift below -43 m (lower quartile) and that showing an elevation shift above +118 m (upper quartile), with mean elevations 561 and 512 m a.s.l., respectively. On the contrary, the interquartile central group of species (-43 m < shift < +118 m) shows a significantly lower past mean elevation, namely 393 m a.s.l. (Mann-Whitney pairwise, $p < 0.01$). This implies that species occurring at lower elevations in the past possibly experienced minor elevation shifts with respect to those occurring at higher elevations, while the latter undergone the largest positive or negative elevation shifts. By analysing each single species, we found that not all of them undergone statistically significant elevation shifts. However, many species undergone a statistically significant positive elevation shift, in agreement with the general trend shown above and with our initial expectations. However, unexpectedly, we found that a relevant number of species also undergone statistically significant negative shifts.

With this study, we provide the first evidence of plant elevation changes for the Tuscan flora as a whole during the last century. Further research is needed to assess the relevance of climate change to elevation shifts versus other factors as, e.g., land use or plant-pollinator interactions, known to affect plant distribution. Indeed, by further explicitly considering current and past climate variables, as well as by evaluating potential shifts among functional groups, we will disentangle general from specific patterns in distribution changes, providing support in the management and conservation of the Tuscan flora.

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Anthemis messanensis (Asteraceae) a neglected endemic species: new data on distribution, ecology, and conservation status

Gianmarco Tavilla, Antonia Cristaudo, Gianpietro Giusso del Galdo, Veronica Ranno, Pietro Minissale, Saverio Sciandrello
Department of Biological, Geological and Environmental Sciences, University of Catania, via A. Longo 19, I-95125 Catania, Italy

Plant conservation strategies are crucial for preserving the extraordinary diversity of Mediterranean flora, especially in the precence of severe and long-lasting human pressures which often led to the loss of some endemic species.
Nowadays, several plant species need targeted protection actions due to their poor conservation status from both natural and artificial causes. In particular, *Anthemis messanensis* Brullo (Fig.1), a narrow endemic species of Peloritani Mountains (NE Sicily), requires attention. This chamaephyte grows on metamorphic rocks and is known only in the isolated peak of Dinnammare (about 1.000 m a.s.l.). Considering its extremely restricted distribution range and low number of mature individuals, *A. messanenis* was recently classified as Critically Endangered.
During recent field investigations carried out in the Peloritani area, a new interesting sub-population of *A. messanensis* was found close to Mt. Poverello (Fiumedinisi), about 12 Km away from its *locus classicus* (Dinnammare). This important finding highlights two aspects related to the field researches: the first concerns improved knowledge of the ecological requirements of the species, which is particularly useful and relevant for any potential actions aimed at reinforcing the natural population; the second regards the distribution and consequent updating of the conservation status of the species. Regarding its phytosociological role, *A. messanensis* was considered a characteristic species of the shrubby vegetation of the *Calicotomo infestae-Adenocarpetum commutati* Bartolo, Brullo & Pulvirenti 1994 (*Cytisetea scopario-striati* Rivas-Martìnez 1975).
Our results highlight the rupicolous character of the species that shares the rocky habitat (Fig.2) with other casmo-mesophilous species, such as *Silene sicula* Ucria, *Hypochaeris laevigata* (L.) Ces., Pass. & Gibelli, *Micromeria graeca* subsp. *consentina* (Ten.) Guinea., *Hypocheris cretensis* (L.) Bory & Chaub., *Galium lucidum* var. *peloritanum* Nicotra, *Centaurea gussonei* Raimondo & Spadaro, as well as some mosses. Based on the new data, chiefly regarding the vegetation structure and floristic composition, we refine the ecological and syntaxonomic framework of the plant communities featuring by *A. messanensis*. Finally, thanks to the new distribution data and population consistency, we reassess its conservation status.

Fig. 1. *Anthemis messanensis* Brullo (Dinnammare).

Fig. 2. Rocky cliff with *A. messanensis.*

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An integrated taxonomy approach points towards a single-species hypothesis for *Santolina* (Asteraceae) in Corsica and Sardinia

Paola De Giorgi[1], Antonio Giacò[1], Giovanni Astuti[2], Luigi Minuto[3], Lucia Varaldo[3], Daniele De Luca[4], Alessandro De Rosa[4], Gianluigi Bacchetta[5], Marco Sarigu[5], Lorenzo Peruzzi[1]
[1]Department of Biology, University of Pisa, Via Derna 1, 56126 Pisa, Italy; [2]Botanic Garden and Museum, University of Pisa, Via Luca Ghini 13, 56126 Pisa, Italy; [3]Department for the Earth, Environment and Life Sciences (DISTAV), University of Genoa, Corso Europa 26, 16132 Genoa, Italy; [4]Department of Biology, University of Naples Federico II, Via Cinthia, 26, 80100 Naples, Italy; [5]Centre for Conservation of Biodiversity (CCB), Department of Life and Environmental Sciences, University of Cagliari, V.le S. Ignazio da Laconi 13, 09123 Cagliari

Santolina corsica Jord. & Fourr., *S. insularis* (Gennari ex Fiori) Arrigoni, and *S. chamaecyparissus* L., three species belonging to the *S. chamaecyparissus* complex, show high morphological and kariological affinity. The first two species are endemic to the Corsican-Sardinian insular system. In particular, *S. insularis* is reported as a narrow endemic hexaploid species endemic to Sardinia, and *S. corsica* as a tetraploid species endemic to Corsica and Sardinia. *S. chamaecyparissus*, instead, is a cultivated pentaploid species of unknown origin. The objective of this study is to test the taxonomic distinctiveness of these three putative species through an integrated approach including morphometric, seed morpho-colorimetric, karyological, molecular, and niche similarity analyses. While the latter analysis was restricted to the two native species *S. corsica* and *S. insularis*, by obtaining distribution data from literature, data for other analyses were obtained by sampling flowering individuals and seeds from 8 populations. For morphometric analyses, we measured 45 features and analysed them through PCoA and univariate analyses in 20 individuals for each population (9 in *S. chamaecyparissus*). Random Forest was used as tool to quantify the discriminant power of morphometric features with respect to groups of populations. Concerning seed morpho-colorimetry, we analysed 100 seeds per accession using 124 morpho-colorimetric variables, then subjected to Principal Component (PCA) and Linear Discriminant Analysis (LDA). For karyological analyses, chromosome number (2*n*), total haploid length (THL), and karyotype asymmetry indices (CV_{CL} and M_{CA}) were calculated. Concerning molecular systematics, we sequenced ITS region and six cpDNA markers (*trn*H-*psb*A, *psb*M-*trn*D, *trn*Q-*rps*16, *trn*F-*trn*L, *trn*S-*trn*G, and *rps*15-*ycf*1) from three individuals for each population, in order to build a Bayesian phylogram. Finally, niches were quantified with 19 bioclimatic variables downloaded from the WorldClim dataset website, and niche overlap was estimated with Schoener's D index and PCA.
Four overlapping groups can be observed in the PCoA scatter plot: a first group is represented by *S. chamaecyparissus*, a second group by *S. corsica* from its *locus classicus* (Mont Pigno near Bastia, Corsica), a third group by *S. insularis* from Buggerru (south-western Sardinia), and a fourth group by all the remaining populations of *S. insularis* and *S. corsica* from Sardinia. Random Forest did not correctly classify *S. corsica*, due to possible confusion with *S. insularis*. A slightly better classification is obtained if *S. insularis* from Buggerru is considered as a distinct group. Morpho-colorimetric analysis shows that seeds of *S. corsica* are misidentified with *S. insularis* in 46% of cases. Within *S. insularis*, central-eastern Sardinian populations are very similar, and slightly distinct from those from south-western Sardinia. Tetraploid *S. corsica* from *locus classicus* is more similar to this latter group than to the putative *S. corsica* from central-eastern Sardinia (also tetraploid). We confirm the chromosome numbers already reported in literature for *S. chamaecyparissus*, *S. corsica*, and *S. insularis*, and the latter two species also share a strikingly similar karyotype structure. ITS sequences are identical in all the investigated populations. The phylogram of cpDNA markers shows instead three clades: a first one is represented by *S. chamaecyparissus*, a second one by *S. insularis* from south-western Sardinia, and a third one by all remaining populations of *S. corsica* and *S. insularis*. The results of Niche similarity test show slight overlap among the two species, and in particular *S. insularis* prefers environmental conditions of *S. corsica* ($D = 0,09$; $p < 0,05$), but not vice versa.
Overall, *S. chamaecyparissus* is a very similar, but distinct species, while a two-species hypothesis for *Santolina* (Asteraceae) in Corsica and Sardinia is not supported by our data. Accordingly, we deem more opportune to recognise a single variable species endemic to Corsica and Sardinia, namely *S. corsica*.

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Checklist of the archaeological site of Pasargadae, World Heritage Site in Iran

Zohreh Hosseini[1], Flavia Bartoli[1], Emanuela Cicinelli[1], Ferdinando Lucchese[1]
[1]Department of Science, University of Roma Tre, Viale G. Marconi 446, 00146 Roma, Italy
Pasargadae WHS Research Center - Madar Soleyman City, Fars, Iran"

Archaeological sites present a clear interaction of the natural environment and human activity and are considered as one of the habitats for plant communities due to the natural dynamic of vegetation. These areas create a hot spot for plant biodiversity since archaeological sites have reduced external disturbance and the management practices of the site are often compatible with plants; while the surrounding areas provide a combined landscape of agricultural, rural, or semi-natural areas. Comprehensive floristic surveys are of great value for both biodiversity management, exploring general patterns in ecological preferences, evaluating biodeterioration aspects, and valorization of archaeological sites. In Iran, despite the significance of plant colonization in archaeological areas, floristic assessment in these areas is neglected in the research studies. Due to the lack of information on plant communities of archaeological sites of Iran, we selected Pasargadae World Heritage Site (6th century BC) due to its location in Zagros geobotanical zone of Iran and a vast area (160 hectare) with high biodiversity of Irano-Turanian and endemic species. The bioclimate of the Pasargadae region belongs to the Mediterranean Xeric Continental climate that is characterized by hot, dry summers and relatively cold winters.
In this context, the main aim of this research is to describe the floristic composition of vascular flora in Pasargadae WHS, to proceed with its floristic analysis, and to address an update to the information achieved from the previous references. The plant species were collected in multiple field surveys from 2019 to 2020, during Spring, Summer, and Autumn, to cover the flora growing in the archaeological area. The field survey covered a total of 76 transepts (200 to 500 m^2) considering different habitats (plain, riverbed, hill). The collected species have been analyzed at the biology department of Tehran University and at the Herbarium of Roma Tre University, where their taxonomical identification has been carried out following the *Flora Iranica by Rechinger 1966* and through the comparison with the interactive herbarium samples available on GBIF. We referred to Plantlist.com for nomenclatural updates. The natural and conservation value of the species was evaluated using the red data book of Iran, scientific literature and International Directives CITES (Convention on International Trade in Endangered Species of Wild Fauna and Flora). At last, we created a database that includes the referred species information and identification code of Roma Tre Herbarium, where the samples are stored.
Our preliminary results indicate the high variety and richness of flora in Pasargadae. We identified multiple species in the site with an endemic distribution and, some of them were previously described by Rechinger (1966) for the site and the region. Based on results, the 250 species recognized in the site belong to 39 families and 146 genera. The richest families are Asteraceae (66 species), Poaceae (32), and Fabaceae (19). More than 50% of species are Therophyte and Hemicryptophyte, indeed we identified only a few Phanerophyte species: *Ficus johannis* Boiss., *Daphne mucronata* Royle and *Amygdalus erioclada* Bornm (Fig. 2). The last one was previously recognized by Rechinger (1966) in Pasargadae with Endemic distribution and, it was described by Bornm in *rupestribus* of Persepolis.
Our work is not yet complete, but with our preliminary data, we can conclude that the wild flora of the site is well conserved. The current presence of species previously described more than 50 years earlier denotes good conservation of the context despite the changes undergone in the time. The dominance of therophytes is not a risk for the ruins, so with a good management and conservation plan it will be possible to protect and enhance the endemic flora, adding natural value to the cultural one of the site.

Fig 1. the wide area of the site.

Fig. 2. *Amygdalus erioclada* Bornm.

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Plant conservation through use: field surveys on four case studies from Sicily and Malta

Giulio Barone[1], Stephen Mifsud[2], Gianniantonio Domina[1]
[1]Department of Agricultural, Food and Forest Sciences, University of Palermo, Viale delle Scienze, bldg. 4, I-90128 – Palermo, Italy; [2]EcoGozo Regional Development Directorate, St. Francis Square, VCT 1335 – Victoria, Gozo, Malta

One of the most important factors affecting plant conservation is overharvesting from wild populations. This is recognised as one of the drivers of the mass extinction which is occurring at present. Conservationists have currently developed several strategies to mitigate the pressure on wild populations *in situ*, while preserving a backup of this diversity in Botanical Gardens as living collections, seed banks, herbaria, or other *ex situ* structures.
Nevertheless, harvesting plant species from the wild is supported by international conventions such as the Convention on Biological Diversity when it is practised by local communities in a sustainable way. This is justified because there is an increasing acknowledgement by policy makers and other conservationists, for the importance to engage and involve local communities living in close contact with wildlife as key partners in the development of conservation strategies. Also, CITES (the Convention on International Trade in Endangered Species of Wild Fauna and Flora), supports a sustainable harvest and the artificial propagation of endangered species in order to regulate the trade. Farming over harvested species in structures *ex situ* is a recognised approach that can alleviate the pressure *in situ*, especially by answering the market demand and, thus, reducing the necessity to harvest them from their natural habitats. This is particularly true for medicinal and ornamental plants which are among the groups most affected. However, there is an ongoing debate on whether cultivation can really release pressure on wild populations. The commercialisation of cultivated plants could stimulate the search for rarer forms of the individuals in the wild. Moreover, from an ecological point of view, the possibility of using cultivated populations to perform conservation translocations presents several constraints that need to be considered. For example, a decline in the genetic variation of *ex situ* populations due to a small starting sample or inbreeding, or the effect of cultivation which is known to influence the evolution of *ex situ* plant populations (especially on short-lived ones). Taking all these aspects in consideration, the present work is aimed to reflect on what is happening in our territory which could bring a different operational hypothesis for the conservation of these populations by balancing advantages and disadvantages. Could ornamental collections not managed by scientific authorities but entrusted to private hobbyists and horticulturists function as a boost of gene flow and diversity on populations strongly affected by anthropic pressures?
We analysed some cases of native plants cultivated for ornamental purposes: *Cytisus aeolicus* Guss. (Fabaceae) and *Matthiola incana* (L.) W.T.Aiton subsp. *incana* (Brassicaceae) in Sicily, *Cheirolophus crassifolius* (Bertol.) Susanna (Asteraceae) in Malta, and *Iris sicula* Tod. in Sicily and Malta. For each of these taxa we have reasoned if cultivation for ornamental purposes in the indigenous areas has favoured their conservation.
Cytisus aeolicus is endemic to the Aeolian islands. In Alicudi it is rare and localised on inaccessible cliffs; in Stromboli, on the Eastern slope, it is in expansion; in Vulcano it is rare on cliffs but a rich population, of cultivated origin, on the edge of arable land and in the gardens of the Piano and Cardo territories, exists. It exhibits a low intrapopulation variability and hence there is no difficulty regarding the origin of reproductive material so that any attempts to reinforce the population can be applied.
Matthiola incana subsp. *incana* is a steno-Mediterranean taxon occurring on cliffs. It is commonly cultivated in small villages of Sicily, and it is characterised by a low genetic variability, possibly due to the reintroduction in nature of germplasm that has undergone a bottleneck following cultivation.
Cheirolophus crassifolius is rather rare, endemic to the Maltese archipelago, and assigned as the National plant of Malta. It is a strictly chasmophytic and rupestral species, distributed on vertical coastal cliffs or secondary escarpments close to the coast up to 250 m above sea level, yet it can grow when cultivated inland in fertile non-clayey soil. It is widely cultivated in Malta in parks, central strips, roundabouts, side of streets, public gardens, etc.; it is propagated exclusively by cuttings. The wild population do not form many viable seeds and their germination rate proved to be not successful *ex situ*.
Iris sicula is a species whose taxonomy and chorology are still controversial. In Sicily and Malta, it occurs, scattered, in the inland on rocky banks. In the provinces of Agrigento and Caltanissetta it is commonly grown in gardens and in pots. The genetic variability of *Iris sicula* needs further investigation, but it is known that spontaneous populations and cultivated individuals sporadically produce viable seeds; it is propagated vegetatively using rhizomes.

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An integrative approach shows independent local genotype and phenotype variation in *Dianthus virgineus* (Caryophyllaceae)

Jacopo Franzoni[1], Giovanni Astuti[2], Angelino Carta[1], Simone Fior[3], Lorenzo Peruzzi[1]
[1]Department of Biology, University of Pisa, Via Derna 1, 56127 Pisa, Italy; [2]Botanic Garden and Museum, University of Pisa, Via Luca Ghini 13, 56126 Pisa, Italy; [3]Institute of Integrative Biology, ETH Zürich, Universitätstrasse 2, 8092 Zürich Switzerland

Integrating independent datasets to analyse affinities and diversity in vascular plants is crucial to assess the basis of the variation in plant lineages undergoing rapid evolutionary radiations. In the Eurasian genus *Dianthus* L. (Caryophyllaceae), the southern-central European complex of *D. virgineus* L. is one of the most variable groups, characterized by extensive morphological diversity and a complex taxonomy. In Tuscany (Central Italy), three putative species have been traditionally recorded, mostly on a geographical-elevational basis, supported by qualitative morphological variation. In order to quantitatively survey the degree of phenotypic and genotypic variation across this geographical region, we sampled 12 Tuscan populations, spanning from the coasts of the Tuscan Archipelago (40 m a.s.l.) up to the Apennine mountains (1875 m a.s.l.). Genetic, karyological, morphometric, and functional (seed germination) analyses were performed to detect patterns of differentiation among populations.
Genotyping of 654 single nucleotide polymorphisms (SNPs) and chromosome counts reveal the presence of a single diploid ($2n = 2x = 30$) group, genetically structured along a latitudinal gradient. Multivariate analysis of 25 morphological variables shows that this group is differentiated in two main phenotypes: one including Apennine populations marked by a smaller plant size, thinner leaves, few-flowered inflorescences, and 2–6 epicalyx scales, and the other including Mediterranean populations marked by larger plant size, broader leaves, many-flowered inflorescences, and 4–8 epicalyx scales. Whilst seeds of all populations are not dormant and germinate promptly at most temperatures tested, they prefer cool temperatures (< 20 °C) reflecting a typical Mediterranean seed germination syndrome. However, lower temperatures (< 10 °C) negatively affect the germination of seeds from montane populations (> 600 m a.s.l.), likely to avoid frost damage during the regeneration stage.
Mantel test shows that morphometric variation is not correlated with the genetic fixation (pairwise F_{ST}) among populations, but several morphological variables are negatively correlated with elevation according to Spearman correlation tests. On the contrary, genetic fixation is not correlated with elevation, but is positively correlated with geographic distances among populations. As the seed germination variation among population do not follow the genetic distances, but rather shows an elevation pattern, this variation seemingly highlights phenotypic plasticity (modulation) in seed germination responses.
We conclude that elevation is a plausible driver of the phenotypic divergence observed among populations, while genetic relationships follow an isolation-by-distance model. This highlights a major role played by environmental conditions, more than genetic isolation, in shaping phenotypic variation at local scale in *Dianthus virgineus*.

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The third phase of the project to enhance the Botanical Garden of the University of Urbino: the recovery of the *Herbarium Athenaei Urbinatis*

Anahi Elena Ada Bucchini[1*], Lisa Brancaleoni[2], Donata Ricci[1], Laura Giamperi[1*]
[1]Botanical Garden, University of Urbino, Department of Biomolecular Science, University of Urbino, Via Bramante 28, 61029 Urbino, Italy; [2]Department of Life Sciences and Biotechnology, University of Ferrara, Corso Ercole I d'Este 32, 44121 Ferrara, Italy. *Equal contributors

Herbarium Athenaei Urbinatis (Fig. 1) is the name given to the historical herbarium located at the Botanical Garden of the University of Urbino. Until now, it has never been subject of maintenance or specific research studies. Since the 16th century, the herbaria have been considered irreplaceable tools for the study, consultation and dissemination of botanical knowledge. Today, the scientific community also recognises their great value in the study of biodiversity changes over time and as an irreplaceable cultural asset.
The recovery of the exsiccata represents the third step of the project, started in 2009, to highlight the Botanical Garden of Urbino.
A first analysis of exsiccata, thanks to the advice of Dr. Lisa Brancaleoni and Prof. Renato Gerdol (Botanical Garden of Ferrara), showed a rather marked heterogeneity. On the basis of historical references we have assumed that the herbarium dates back from the mid-to-late 19th to the beginning of the 20th century. The recovery project, based on scientific and methodological research carried out by the technical staff, has just begun and it has been structured so as to include several phases of work.
The initial phase of biodeterioration control will be followed by the cleaning and recovery phase, carried out together with the collection of photographic data and the cataloguing of the exsiccata.
The *Herbarium Athenaei Urbinatis* represents a precious historical, cultural and scientific documentation and could play an essential role in:
- botanical research, in particular in the systematics, phytogeography, ecology and genetics fields;
- evaluations of the environmental quality of the specific geographical areas, both current and past;
- research aimed at studying changes in biodiversity resulting directly or indirectly from human activities;
- education, particularly for the younger generations, on the importance of the plants in today's world, on the variety of the plant world and on the knowledge of useful plants.

Fig. 1. *Herbarium Athenaei Urbinatis* specimens

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Recovery of historical duplicates of the *Herbarium Siculum* in Palermo

Emilio Di Gristina[1], Alfredo Carratello[2], Giuseppe Certa[2], Natale Surano[2], Giulio Barone[1], Gianniantonio Domina[1], Rosario Schicchi[1, 2]
[1]Department of Agricultural, Food and Forest Sciences, University of Palermo, Viale delle Scienze, bldg. 4, I-90128 – Palermo, Italy; [2]SIMUA Sistema Museale di Ateneo, Via Lincoln, 2 (Orto Botanico), I-90133 Palermo, Italy

The herbarium of the Botanical Garden of Palermo was set up with the foundation of the Botanical Garden in 1790. Its early collections including specimens by Giuseppe Tineo, Bernardino da Ucria, Constantine S. Rafinesque, Antonino Bivona Bernardi were destroyed during the Sicilian uprising of 1820-1821. Since 1821 Vincenzo Tineo re-established the herbarium with a series of capillary collections throughout Sicily and by asking its correspondents to send specimens to Palermo. The same did Agostino Todaro, his successor. Todaro deposited his and his collaborators' collections and improved exchanges by preparing centuries of Sicilian plants for the main European herbaria. In the second half of the 19th century, further remarkable collections by A. Borzì, G. Gibelli, C.C. Lacaita, G. Nicotra, H. Ross, S. Sommier, G. Strobl, N. Terracciano, etc. contributed to the enrichment of the herbarium.
Each of these collections was kept separated inside the *Gymnasium* building. Since 1939 the Sicilian herbaria plus the Sicilian specimens extracted from the General Herbarium were assembled into a coherent regional collection named the "*Herbarium Siculum*". This herbarium has been increased with new collections since the second World War to the present day. Currently, it consists of 51,131 specimens coming from all over Sicily by about 80 different collectors.
The relocation of the *Herbarium Siculum* from the Gymnasium in via Lincoln n. 2 to the Department of Botany in Via Archirafi n. 38 was completed in 1954. In 1995 the whole collections were moved to the newly acquired buildings of via Lincoln n. 4, on the occasion of a restoration of the Gymnasium. The packages considered duplicates, never included in the *Herbarium Siculum* because damaged, were placed in a warehouse in via Lincoln in large cardboard boxes. The re-evaluation of these exsiccata began in 2010 when 850 of them were extrapolated, restored, and included in the *Herbarium Siculum*.
This year an organic work has been completed for the recovery of all the exsiccata of this collection still in good condition which is actually classified as “historical duplicates”.
On the whole, 101 folders on 150 were examined. The dried plants were unmounted inside herbarium sheets. Only some of them had handwritten labels and were followed by numerous unlabelled duplicates. In many cases these packages were damaged by humidity and insects. The restoration began with the sterilization in freezer and the separation of the exsiccata too deteriorated to be housed in an herbarium. The sheets were numbered by pencil and a first databasing of their metadata was done.
Altogether 1053 specimens and their 3,693 duplicates were recovered this year. The labels, when present, are quite heterogeneous, some report the scientific name only, others include the locality, date, and collector. All the main families of the Sicilian flora are included. The surroundings of Palermo are the most represented localities but also specimens from the Madonie, the Etna Mt., the Nebrodi Mts, Ispica, Porto Empledocle, the Aeolian islands, Pantelleria, etc. occur. The specimens date back to the last years of the 19th and the very first ones of the 20th century. There are collections by A. Todaro, A. Borzì, H. Ross, G. Gussone, F. Patti Chachon (Duke of Sorrentino), F. Minà Palumbo, M. Lojacono-Pojero, A. Porcari, N. Citarda, A. Riccobono, G. Reina, G. Bonafede, etc. Probably many specimens were new Todaro's centuries in preparation that remained unfinished. Just under half of the collections are already present in the *Herbarium Siculum*. The other half concerns taxa already documented but from other localities. The exsiccata are now being restored by fixing them on cardboards and placing them in new unprinted newsprint. We will proceed with the scanning, complete databasing and their inclusion into the *Herbarium Siculum*. Good quality duplicates with photocopied labels will be exchanged with other institutions.

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New Checklist of the Bryophytes of Italy twelve years apart from the first

Silvia Poponessi[1], Michele Aleffi[2]
[1]Department of Department of Environmental and Life Science, University of Cagliari, Via S. Ignazio da Laconi 13, 09123 Cagliari, Italy; [2]School of Biosciences and Veterinary Medicine, Plant Diversity & Ecosystems Management Unit, Bryology Laboratory & Herbarium**,** University of Camerino, Via Pontoni 5, 62032 Camerino, Italy

In the last years, bryological knowledge increased both at national and global scale thanks to the many taxonomic studies that updated current nomenclature and changed the set-up of many species, genera and even of entire families, in particular in pleurocarpous mosses and liverworts. As a consequence, it became necessary to update the catalogue of Italian bryophytes accordingly to new 2020 data, since the most updated version of the catalogue of Italian bryophytes dates back to 2008. In order to evaluate any increase in the number of *taxa* in the country it is necessary to analyse ecological and chorological aspects of new records, since it is impossible to make a simple comparison between the number of species in past and present checklists, precisely because of the many taxonomic changes occurred. In addition, 1968 and not 1950 (as in the old version) was chosen as temporal threshold in the analysis of the different reports. As a result, bryophytes taxa so far recorded in Italy comprises 1191 species, 18 subspecies and 35 varieties. Among them, the hornworts and liverworts list 297 species, 5 subspecies, and 4 varieties included in 99 genera and 54 families. Mosses include 894 species, 13 subspecies, and 31 varieties, grouped into 253 genera and 77 families. The present checklist also provides critical annotations on some important misidentifications, nomenclatural and taxonomical problems, as well as those cases of species for which only one report is known. Critical review of the old version of the checklist resulted in 37 species of liverworts and 13 species of mosses excluded from the Italian flora thanks to the revision of herbarium specimens, due the impossibility of tracing and checking their herbarium specimens, or due their doubtful ecology and chorology.

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Rare bryophytes of the Alps: monitoring and analysis of the current situation with a focus on the Aosta Valley

Marta Puglisi[1], Francine Valérie Navillod[3], Santa Tutino[3], Luca Miserere[2]
[1]Department of Biological, Geological and Environmental Sciences, University of Catania, Via A. Longo 19, 5125 Catania, Italy; [2]Via Melchiorre Voli 14 – 10135 Torino, Italy; [3]Aosta Valley Autonomous Region, biodiversity, sustainability and protected natural areas Office, Efisio Noussan Regional Natural History Museum, Le Grand Chemin 46, 11020 Saint-Christophe, Aosta, Italy

In the last 10 years, several research projects funded by E. Noussan regional natural Science Museum in Aosta Valley Autonomous Region, within the Interreg Alcotra Italy-France project, analysed and monitored the presence of several rare bryophytes, some recorded in the Habitat Directive and some found during field research carried out in the last 20 years. Aosta Valley hold an important bryological biodiversity, with 46% of the Italian bryological flora, and a high number of species respect his small territorial extension. The discovery of numerous rare species has highlighted this important connotation even further.
Among the most interesting species there are: *Buxbaumia viridis* (Moug.) Moug. & Nestl., *Drepanocladus vernicosus* (Mitt.) Warnst., *Riccia breidleri* Jur., *Scapania carinthiaca* J.B.Jack ex Lindb. *Arnellia fennica* (Gottsche) Lindb., *Voitia nivalis* Hornsch., *Hygrohypnum polare* (Lindb.) Loeske, *Anastrophyllum assimile* (Mitt.) Steph. and many species within the *Sphagnum* genus.
The species analysed show a very particular ecology, many are arctic-alpine species linked to cold climates, while other species are linked to wetlands. Almost all of surveying habitat types are targeted for conservation by the Habitats Directive. These species are very sensible to the drying up of the soils, the variations of water-table's chemism, and climate changes. They are, therefore, valid bioindicators to monitoring the scale of climate changes and the conservation of these habitats.
Assessments on the rarity of these species have been presented through the analysis of new locations where species have been found, the confirmation of locations already known, but also the lack of species in some other locations.
Given the impossibility to take action on the climate changes very quickly, it is essential to intervene on appropriate environmental management to identify and protect the micro-environments where these species live and prevent the fragmentation of these habitats.

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Strategies of heavy metal tolerance in metallicolous and non-metallicolous populations of mosses: the role of photoprotective mechanisms

Antonio De Agostini[1], Annalena Cogoni[1], Pierluigi Cortis[1], Andrea Vacca[2], José María Becerril[3], Antonio Hernández[3], Raquel Esteban[3]
[1]Department of Life and Environmental Sciences, University of Cagliari, Via Sant'Ignazio 13, 09123, Cagliari (CA), Italy; [2]Department of Chemical and Geological Sciences, University of Cagliari, Cittadella Universitaria (Blocco D) – S.S. 554, 09042, Monserrato (CA), Italy; [3]Department of Plant Biology and Ecology. University of the Basque Country (UPV/EHU), PO Box 644, 48080, Bilbao, Spain

Plants are intimately linked to water, atmosphere, and soil, so that any contamination of these matrices immediately echoes on them, plants have been consequently widely used to study environmental pollution both as biomonitors and as biological models. Bryophytes are an important group abundant in diverse biomes worldwide. They led the colonization of land and still nowadays present the capacity to colonize a wide range of habitats, including extreme environments (e.g. mine areas). Nevertheless, bryophytes also appear to be somehow overlooked as it regards the study of their adaptation to heavy metals (HM hereafter) polluted ecosystems, as we found in a data compilation analysis from published works (1999 to 2019). This evidenced that the majority of the studies addressed the relationships between bryophytes and HM on non-preadapted species or individuals suddenly exposed to HM treatments under laboratory conditions. To deepen the relationship between bryophytes and HM by an adaptation-focused perspective rather than by a more acclimation-oriented view, we characterized four populations of mosses (*Lewinskya rupestris*, *Polytrichum commune*, *Ptychostomum compactum* and *Rhynchostegium confertum*) as regards their photoprotective response to environmental HM. Two sites were selected, an abandoned Zn-Pb mining site and a non-contaminated site, both sites being spontaneously colonized by the above-mentioned bryophyte species. Both sites were located in the municipality of Carranza (Basque Country, Spain) and present similar edaphoclimatic conditions, except for the presence of HM in the soil. Both metallicolous and non-metallicolous populations of mosses were characterized by the analysis of their physiological performance (photosynthetic efficiency) and biochemical parameters (chlorophylls, carotenoids, tocopherols, total antioxidant capacity, and anthocyanins content, as well as foliar pH). The results marked species-specific tolerance patterns: *i*) high pollutant uptake and marked dissipative and antioxidative strategies characterized less tolerant species; *ii*) medium accumulation of pollutants and less marked photoprotective mechanisms characterized intermediate tolerant species and *iii*) most tolerant species exclude metal uptake and consequently evolved few changes in their physiological properties. A key role in these differential tolerance patterns was linked to the different bryological attributes of the species. More precisely some bryological attributes (i.e. epilithic growth habit and tolerance to soil acidity) appear to favour the colonization of the metalliferous study site. Among the studied species, *R. confertum* due to its high pollutant uptake rates and the high physiological sensitivity to them appeared to be a good sentinel species of environmental HM. A remarkable response was the accumulation of the lipophilic antioxidant γ+β-tocopherol in the studied metallicolous populations. This trend also observed in vascular plants under HM, emerged in this study as a functional responsive trait also in mosses copying with environmental HM stress.

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Insight into the use of chlorinated oxidants for the removal of cyanobacterial toxins produced by *Microcystis aeruginosa*: good practice for drinking water plants

Mara Simonazzi[1], Laura Pezzolesi[1], Franca Guerrini[1], Carmela Dell'Aversano[2], Luciana Tartaglione[2], Rossella Pistocchi[1]
[1]Department of Biological Geological and Environmental Sciences (BiGeA), University of Bologna, Via Sant'Alberto, 163, 48123 Ravenna, Italy; [2]Department of Pharmacy, University of Napoli Federico II, Via D. Montesano 49, 80131 Naples, Italy

The ever-increasing occurrence of cyanobacterial blooms in freshwaters has become an emerging environmental issue; these blooming events may occur in natural and drinking water bodies, altering the physicochemical characteristics of water column, and subsequently affecting freshwater ecosystems. Some cyanobacteria are able to produce toxins (i.e. cyanotoxins), which are reported as harmful to both animals and humans after ingestion and/or skin contact; thus, the presence of cyanotoxins in water intended for drinking purpose represents a serious health threat. Moreover, undesirable blooms may affect the efficiency of water treatments for the removal of both cyanobacterial cells and toxins, possibly preventing the achievement of high quality standards for drinking water. In Italian drinking water plants, different strategies may be employed for the removal of cyanobacteria and their toxins; among them, one of the most common is the oxidation with chlorine-based compounds, which are usually added directly to raw water (pre-oxidation) to kill or inactivate harmful organisms such as cyanobacteria. Nonetheless, some concerns arise from the use of these oxidants, in terms of both efficiency and safety.
In the present study, two commonly used chlorine-based oxidants (i.e. sodium hypochlorite, NaClO and chlorine dioxide, ClO_2) were tested on the toxic cyanobacterium *Microcystis aeruginosa* grown in culture, evaluating their effectiveness in removing cells and/or cyanotoxins at different concentrations (0.5-2.0 mg L^{-1}), in combination with increasing exposure time (1-3 h). To assess *M. aeruginosa* cell viability, photosynthetic efficiency (%) was evaluated after each test with respect to the control. Prior to toxins analysis, cyanobacterial biomass and water were separated through centrifugation, thus extra- and intra-cellular toxins were determined.
After each treatment, photosynthetic efficiency markedly decreased, showing that cyanobacterial cells were affected after only 1 h. Toxins' removal seemed to be more effective at higher doses of oxidant, while no major differences were observed when increasing time exposure from 1 to 3 h. Overall, both NaClO and ClO_2 performed better on toxins in the biomass than on those in the water, as intracellular toxins were efficiently removed, regardless of oxidant, concentration, and time exposure. Nonetheless, a complete degradation could be excluded, as a simultaneous increase in the extracellular fraction was observed, especially when using oxidants at the lowest concentration; thus, a possible release of the toxins from the cells directly to the water has been suggested. Finally, no major differences were observed in the relative distribution of toxins among the tested treatments.
Further in-depth investigations on the possible formation and release of disinfection by-products (DBPs) following each treatment will clarify the observed trends, helping to understand which one would perform better. Conclusively, some considerations will be suggested for treatment optimization to ensure safety and quality standards for drinking water.

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Progress on compiling a DNA barcode inventory of the genus *Ulva* Linnaeus in the Mediterranean Sea

Simona Armeli Minicante[1], Nathan Beda[2], James T. Melton[3], Damiano Spagnuolo[4], Antonio Manghisi[4], Giuseppa Genovese[4], Marina Morabito[4], Juan Lopez-Bautista[2]
[1]Institute of Marine Sciences, National Research Council, Arsenale 101-104, Castello 2737F, 30122 Venice, Italy; [2]Department of Biological Sciences, University of Alabama, 500 Hackberry Lane, Tuscaloosa, Alabama, USA; [3]Department of Biology, Spelman College, 350 Spelman Lane Southwest, Atlanta GA 30314, USA; [4]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Salita Sperone 31, 98166 Messina, Italy

The genus *Ulva* Linnaeus is well known for its global distribution and for containing many controversial and taxonomically debated species.
Several taxa show a rapid prolific growth and have the ability to tolerate a wide range of environmental conditions, including those characterizing marine, brackish and freshwater environments. Furthermore, *Ulva* species are known for their morphological plasticity and cryptic nature that overrepresents and underrepresents their biodiversity, respectively, and makes their morphological identification indefensible, so that many synonyms have been generated over time.
An accurate biodiversity assessment is essential for monitoring biological introductions, and also critical for providing a baseline to detect evidence for short- and long-term trends in biodiversity and human induced environmental changes.
Research conducted by The University of Alabama, the University of Messina and the Institute of Marine Science (CNR-ISMAR) of Venice, allows us to progress on compiling a DNA barcode inventory of most critical *Ulva* taxa. Using the plastid marker *tuf*A, we present molecular data for Ulvaceaen samples adding information to the list of *Ulva* species of the Mediterranean basin.

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Bioactive compounds from brown algae inhabiting the north-western Mediterranean Sea

Anna Maria Mannino
Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, Via Archirafi 28, 90123 Palermo, Italy

Marine algae produce a great amount of secondary metabolites (e.g. terpenoids, fatty acids, steroids, polysaccharides and polyphenols) to face environmental stressors they are exposed to, but also to defend themselves from biological pressures such as competitors, pathogens, grazers and epiphytes. Over the last decade, secondary metabolites isolated from brown algae and phenolic compounds, in particular, are receiving increasing attention due to their putative ecological roles, and several biological properties. Brown fucoid algae of the genus *Cystoseira sensu latu* (Sargassaceae) and Dictyotaceae produce a wide variety of secondary metabolites which have been investigated mainly for their defensive properties and biological activities. We present here the results of a study on a) the polar metabolites of the extracts of marine brown algae belonging to the genera *Cystoseira sensu lato* (Sargassaceae), *Dictyopteris* and *Padina* (Dictyotaceae) collected along the coast of Sicily (north-western Mediterranean Sea) and b) the antibacterial activity of these extracts. Differences in the number of polar compounds and biological activity were observed among genera and species. The genus *Cystoseira sensu lato* showed a higher number of polar compounds (29) than *Dictyopteris polypodioides* (17) and *Padina pavonica* (11). Within the genus *Cystoseira sensu lato*, the more superficial species, *Ericaria amentacea* and *Cystoseira compressa*, living in the infralittoral fringe (0–0.5m) showed the highest number of compounds and these were quite similar. Among secondary metabolites, the phenol profiles displayed differences among the genera but also within the genus *Cystoseira sensu lato*, with *Ericaria amentacea* showing the highest number of phenols. Even though in a previous study *D. polypodioides* showed a higher total phenolic content than *Ericaria amentacea* and *Cystoseira compressa*, its phenolic diversity was quite lower. Indeed, taxa of the genus *Cystoseira sensu lato* presented a higher number of phenolic compounds than Dictyotaceae. Within the Dictyotaceae, the extracts of *D. polypodioides* showed a higher richness in phenolic compounds than those of *P. pavonica*.
With respect to biological activities, the extracts from all the analysed genera (*Cystoseira sensu lato*, *Dictyopteris* and *Padina*) showed cytotoxic activity. Conversely, only extracts from Sargassaceae showed antibacterial activity, inhibiting the growth of Gram-positive strains. *Cystoseira compressa* and *Ericaria amentacea* showed the highest antibacterial activity. None of the analysed extracts inhibited the growth of the Gram-negative bacterium *E. coli*. A small group of polar compounds, exclusively present in the active extracts, could be considered as responsible for the antibacterial activity, acting alone or in synergy with other compounds. Nevertheless, it is noteworthy that on seaweed surfaces lives a rich microbial community and bacteria, interacting with their host, constituting a rich source of bioactive compounds. Currently, it is still unclear whether the antibacterial activity of the analysed extracts is determined by the macroalgae themselves or by the epiphytic bacteria. Therefore, additional studies are required in order to fully characterize the metabolic profile of the analysed algae and to test individual compounds or mixtures in order to identify the compound(s) actually responsible for the antibacterial activity. Regarding the biological activities, studies to test the potential of secondary metabolites present in the extracts of the genus *Cystoseira sensu lato* on the fitness of sea urchins, important grazers of these algae, are also being carried out.

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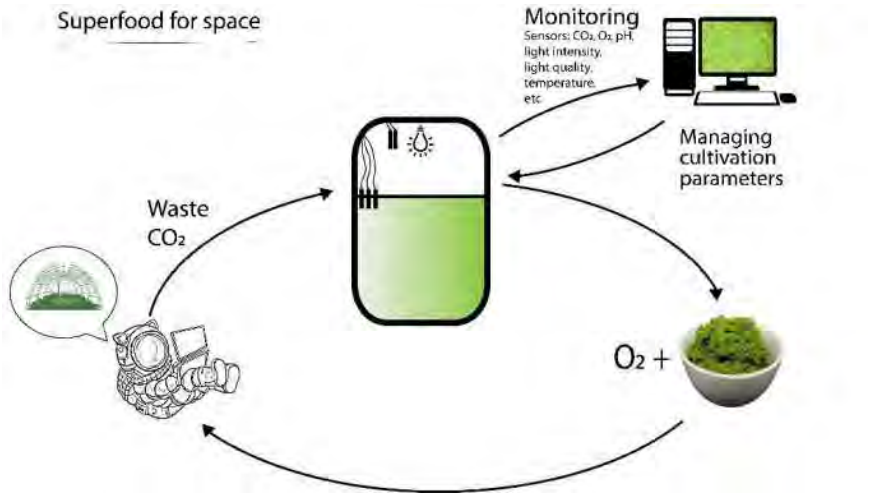
The key role of Duckweed (Lemnaceae) in human space flight

Leone Ermes Romano, Giovanna Aronne
Department of Agriculture, University of Naples Federico II, Via Università 100, 8100 Portici (NA), Italy

At present, to support life during long interplanetary missions, self-sufficiency of life support systems is mandatory; closed life support systems such as the bioregenerative life support system (BLSS) can regenerate resources from waste, lowering substantially initial payload weight. Part of the resources regenerated is used to produce a stable food supply for the cosmonauts, closing the carbon loop. The photosynthetic compartment of a BLSS ensures food and oxygen for the astronauts. This compartment relies on the photosynthetic activities of higher plants, algae, or photoautotrophic bacteria. In the framework of plant-based BLSS, it is mandatory to identify plant species that can merge and adapt to these systems and the extreme condition of space agriculture. This system must guarantee a reliable source of food, recycles carbon dioxide, produces oxygen, and recycles waste and water.
Even if crop species are a reliable source of food on earth, their use in space agriculture is not foregone, due to volume limitation, low efficiency in the biomass production (small edible percentage compared to the whole plant), need for high levels of radiation, absence of pollinators (animals, insects, and wind), reduced or absence of gravity and long life cycles. All the previously mentioned downsides of plants in BLSS could be solved with the adoption of duckweed-based BLSS.
The macrophytes family of Lemnacea is composed of 5 genera and 36 accepted species. These plants are usually known as the smallest flowering plant and the fastest growing angiosperm (doubling time of 1,5 days). However, they are less known for the incredible nutritional qualities: protein content (up to 40%), fat content (3-4%) represented by 60% polyunsaturated acids, low fiber content (<5%), high starch content (up to 40%) in its turion form. These properties are earning interest for their cultivation as a superfood on Earth. Likewise, considering the outstanding performances, Duckweeds are suggested as favorable candidates for space cultivation. Duckweed-based BLSS would maximize the edible biomass (due to the 100% harvest index) and improve the growth efficiency (due to the possibility of submerged cultivation and multi-layered vertical farming). In addition, the high nutrient uptake of these plants would maximize water purification procedures. Specifically, the ammonia uptake as the primary source of nitrogen, makes Lemnaceae interesting for nutrient recovery of human waste.
The European Space Agency has financed “Superfood for Space”, a research project aimed to define the requirements of a novel automated cultivation system for species of the Lemnaceae family. In this contribution, we describe details of the project and how the system could maximize the performances and nutritional values of the plants within the constraints of the space environment. In particular, we focus on the cultivation protocols that could boost the growth and nutritional performances of Lemnaceae in space.



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Waste-to-value approach: the production of lipids from the microalga *Tisochrysis lutea* using a reject brine

Michele Maglie[1], María José Ibáñez González[2], Elvira Navarro López[2], Simonetta Pancaldi[1], Emilio Molina-Grima[2], Tania Mazzuca-Sobczuk[2]
[1]Department of Life Sciences and Biotechnology, University of Ferrara, C.so E. I d'Este, 32, 44100, Ferrara, Italy; [2]Department of Chemical Engineering, University of Almería, Carretera Sacramento s/n 04120 La Cañada de San Urbano, Almería, Spain

Population growth together with changing climate are leading to a continuous demand for water and to impoverishment of water reserves, exacerbating the problem of water scarcity in many regions of the world (1). To satisfy the water demand, the use of desalination plants is increasing with the consequent brine production, a by-product of water desalination characterized by extreme salinity (2). Brine disposal represents an economic and environmental challenge. In fact, brine may include minute concentrations of contaminants; moreover, its disposal from multiple plants operating close to each other for extended periods could negatively affect the marine environment (2; 3). In a "waste-to-value" approach, the production of microalgae using brine may represent a solution (4). In fact, microalgae can provide the removal of possible nutrients from wastewaters and produce biomass enriched in high added value biomolecules (lipids, proteins, pigments, etc.) (5; 6). In this study, we tested the growth and the lipids production of *Tisochrysis lutea* in brine from Almería desalination plant. Cultures in SWES medium in artificial seawater ESAW were used as control. The brine used in this study had a salinity of 80‰, about twice the normal salinity of seawater and synthetic medium used. After a period of adaptation to different dilutions of brine with different salinity levels, a solution of brine and distilled water (8:2) with about 64‰ of salinity was selected for the growth of *T. lutea* (equivalent to a solution of brine: sea water: 6:4). Cultures were grown in 2 L photobioreactors, exposed to 100 µmol photons m^{-2} s^{-1} photosynthetically active radiation (PAR) (12:12 h light-dark photoperiod), in semi-continuous mode. During the first 6 days of cultivation the cell density of brine cultures was comparable to that of control cultures. After this period, the cell density remained lower than in the controls, reaching a stationary state at 10 days of cultivation. The accumulation of lipids during growth was evaluated by fluorimetric analysis on cells stained with Nile Red. Cells grown in brine showed higher fluorescence than controls. Lipid accumulation in cells grown at higher salinity resulted between 35 and 55% higher than in the control. Fatty acid determination by gas chromatography highlighted increased accumulation of oleic acid (C18:1n9) and stearidonic acid (SDA; C18:4n3) in brine grown cells, with an increase of 51% and 26% respectively. Docosahexaenoic acid (DHA; 22:6n3) levels, on the other hand, were comparable to those of the control.
The cultivation of *T. lutea* in brine has still some limitations, but it is noteworthy that the algal biomass obtained showed increased accumulation of lipids and a promising blend of polyunsaturated fatty acids (PUFAs) with the added advantage of a higher environmental sustainability linked to a lesser consumption of freshwater and valorisation and reuse of brine.

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Biodeterioration of surfaces: the development of an experimental protocol involving *Nostoc commune* as pioneer organism

Giulia Santunione[1], Alberto Muscio[2], Elisabetta Sgarbi[1]
[1]Department of Life Sciences, University of Modena and Reggio Emilia, Via Amendola, 2, 42124 Reggio Emilia, Italy; [2]Department of Engineering "Enzo Ferrari", University of Modena and Reggio Emilia, Via Vivarelli, 10, 41125 Modena. Italy

Biodeterioration phenomena - damages and corrosion induced by the growth of different type of organisms - changes the aesthetical appearance of the materials and can even compromise their durability. Phototrophic organisms as algae and cyanobacteria, identified as the first ones to colonize surfaces, allow the formation of following biocenosis, where also heterotrophic bacteria and fungi are involved. Both cultural heritage and building materials are affected by biological colonization, which depends mainly on climate and microclimate conditions over and all around surfaces, mainly moisture and light availability. The study of the bio-fouling is becoming more and more interesting for the scientific community because of its consequences on building structures and materials properties. However, mainly due to the wide variability of environmental conditions and by the geographical position of the building, bio-deterioration phenomenon has not yet quantitatively defined. Thus, this study was firstly aimed to develop a laboratory procedure able to reproduce biological colonization on new materials in a repeatable way, in order to quantify the loss of energy performance due to the biological growth. Another goal was to reduce the bio-ageing test duration, which normally requires many years through natural exposition. In the present study, *Nostoc commune* (Cyanophyceae) has been selected to induce biological colonization on building surfaces, as it is a freshwater/terrestrial species growing throughout the world. The experimental setup included a bioreactor - the Temporary Immersion System (TIS) Platform and three different types of high-efficiency building material samples (cool materials), precisely asphalt shingle (AS), single ply white membrane (WM) and white paint (WP). Bioreactors contained 500 ml of Blue Green-11 (BG-11) liquid culture medium, which was inoculated with 20 ml of *N. commune* cultured in the same medium. At the beginning, TIS bioreactors were maintained in a growth chamber at 25°C, under LED lights and a 14-10h photoperiod, with the purpose of optimizing the growth of cyanobacteria. Material samples were put on the internal basket of the bioreactor and submitted to immersion cycles (6 per day, each one for 15 minutes). This "flood and dry" system was able to promote a homogeneous contact between the materials surfaces and cyanobacteria through a temporary and complete immersion of samples into the liquid medium, followed by air-drying cycles. Both biological growth and physical parameters of the samples surface were measured and monitored within 3 weeks: every 3 days a sample was picked up to measure solar reflectance (ρ_{sol}), colorimetry and the fraction of colonization area, this last through image analysis. Moreover, a sample of liquid medium was taken to quantify biological growth during the test. *N. commune* biomass was collected, weighted, and submitted to an extraction protocol to measure total pigments content. This quantification was correlated both to cyanobacteria growth and to colonization areas on samples. High attention was paid to the repeatability of the tests, in order to define a reproducible setup protocol.

The variation of the solar reflectance between new samples (t0) and after 3 weeks of bio-ageing exposure (t1) was found not significant in white membrane (WM) and in cool paint (WP) samples; however solar reflectance variation was higher (p-value <0.05) for AS. Moreover, solar reflectance loss was higher over the time, particularly until the 15th day of exposure, when cyanobacteria growth seems to reach a plateau. Pigments extraction and quantification through a spectrophotometric method, resulted in an increased amount of total pigments (chlorophylls and total carotenoids) per unit of growth medium during the three weeks of experimental trials. The amount of extracted pigments was correlated with cyanobacteria biomass, finding out a linear correlation between pigments production and biomass growth (R^2 always >0.7). Colorimetry results highlighted high repeatability on WP samples through the repeated trials (p-value was always >0.05). On the contrary, this did not always occur in WM and AS samples, in which the 2nd and 3th trials resulted statistically different from the first one.

This work allowed to obtain a preliminary protocol to study bio-ageing processes on different materials, involving *N. commune* as a pioneer organism. Solar reflectance, microorganism growth parameters and colonized area data on bio-aged samples seem to keep a similar behavior through repeated trials; however, more trials are requested to improve and confirm the repeatability detected in this study.

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Spatial distribution and conservation status of species-rich Nardus grasslands in Lombardy region (Northern Italy)

Michele Dalle Fratte[1], Stefano Armiraglio[2], Marco Caccianiga[3], Bruno Enrico Leone Cerabolini[1]
[1]Department of Theoretical and Applied Sciences, University of Insubria, Via J.H. Dunant 3, I-21100 Varese, Italy; [2]Museo Civico di Scienze Naturali, Via Ozanam 4, 25128 Brescia, Italy; [3] Department of Biosciences, University of Milano, Via G. Celoria 26, 20133 Milano, Italy

The Habitats Directive (92/43/EEC) represents the cornerstone for the conservation of biodiversity and, together with the Birds Directive, establishes the Natura 2000 Network of protected areas at EU level. One of the objectives of the Directive is to maintain a satisfactory conservation status of natural habitat types, by evaluating various criteria, such as distribution, structure and functions, and future prospects for survival, for which favourable reference values must be determined. The assessment of habitat types with respect to environmental factors is therefore of crucial importance for identifying the optimal range of distribution and any anomalies that do not correspond to the definition of the habitat itself.
The present study aims to evaluate the conservation status of the priority habitat type 6230* " Species-rich Nardus grasslands, on siliceous substrates in mountain areas (and submountain areas, in Continental Europe)" in Lombardy region (Northern Italy), in relation to the distribution along environmental gradients and to the floristic composition. The distribution of the habitat was analysed for the whole territory of Lombardy with respect to the main environmental variables, including the treeline, land use, and the contacts with adjacent habitat types. We calculated the favourable reference values for the indicators of structure, functions, and future prospects of the three habitat subtypes recognized for Lombardy, using the Lombardy databases of floristic vegetation surveys referred to habitat types. These thresholds were used to evaluate the conservation status of specific groups of reliefs referred to Nardus grasslands already present within the database.
Habitat 6230* was partially present in environmental conditions that were inconsistent with its definition, in particular several polygons were located on carbonate substrates and in the extra-sylvatic belt. The analysis of the conservation status indicators identified for habitat 6230* applied to extra-sylvatic Nardus grasslands resulted in a moderate state of conservation (U1, Unfavourable inadequate), while applied to those of carbonate substrates it indicates a bad state of conservation (U2, Unfavourable bad). Therefore, a more accurate evaluation will be necessary for the polygons on carbonate substrate, while those mapped in the extra-sylvatic belt should no longer be referred to the habitat type 6230* itself, as they probably correspond to degradation stages from overgrazing of the adjacent natural grasslands at high altitudes. Finally, based on the intersection with the land use map and the contacts with adjacent habitat types, most of the polygons already mapped within the Natura 2000 network corresponded to shrub cover classes, indicating that the habitat is seriously threatened by the abandonment of traditional agro-forestry-pastoral practices and the consequent advance of woody species also from nearby habitat types.

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Neglected habitats in central Apennines

Simona Casavecchia[1], Giampiero Ciaschetti[2], Daniela Gigante[3], Daniele Viciani[4]
[1]Department of Agriculture, Food and Environmental science, Polytechnic University of Marche; [2]Majella National Park; [3]Department of Agriculture, Food and Environmental science, University of Perugia; [4]Department of Biology, University of Florence

Several national and European sets of rules, above all the 92/43/EEC "Habitats" Directive, protect natural and semi-natural habitats of conservation interest. Many of these habitats are well represented in the Italian peninsula and particularly in the Apennines, as shown in the Natura 2000 Data Forms and, last in a chronological sense, in the 4th Monitoring Report. Nevertheless, several natural and semi-natural Apennine vegetation types are not considered in any of these lists of concern, even if they play an important role in representing remarkable sections of biodiversity. Many of these plant communities contain, or are even dominated by, rare and/or steno-endemic plant species, or represent important elements of the landscape.
A first list of these neglected habitats was produced in addition to the Italian interpretation manual of the 92/43 Habitats Directive and sent to the Italian Environment Ministry to propose their insertion in the list of habitats of European interest. This list is here updated with reference to the central Italian peninsula, with particular attention to the Apennines. For each of the proposed plant communities, a technical form that has the same layout as the European and Italian interpretation manuals is presented.

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Relevant but neglected habitats by the Directive 92/43 EEC in southern Italy

Giovanni Spampinato[1], Valeria Tomaselli[2], Luigi Forte[2], Sandro Strumia[3], Adriano Stinca[3], Croce Antonio[3], Simonetta Fascetti[4], Leonardo Rosati[4], Romeo Di Pietro[5], Valentina Lucia Astrid Laface[1], Carmelo Maria Musarella[1]

[1]Dipartimento di Agraria, Università Mediterranea di Reggio Calabria; [2]Dipartimento di Biologia, Università di Bari; [3]DiSTABiF, Università della Campania Luigi Vanvitelli, Caserta; [4]Dipartimento di Biologia, Difesa e Biotecnologie Agricole e Forestali, Università della Basilicata, Università degli Studi della Basilicata, Potenza; [5]Dipartimento di Pianificazione, design, tecnologia dell'architettura, Università Sapienza, Roma

The “Habitats” Directive (Dir. 92/43/EEC, DH) is the main European Union legal instrument concerning nature conservation. It defines plant and animal species and natural habitat types of Community importance, listed in the Directive's annexes. The habitat types of Community importance, listed in the Annex I of the DH, are all phytosociology-based, mostly distinguished by their floristic and geographical characteristics. The phytosociological analysis is the fundamental tool for their characterization and for defining and monitoring their conservation status. The list of habitats included in the Annex I to the DH has been later implemented, from time to time, by the accession of new Member States, with the latest in 2013 (Croatia). However, in spite of the significant production of vegetation studies in the last 30 years throughout the whole of Europe, and notwithstanding the awareness of gaps in protection of several vegetation types and syntaxa, no amendments or additions have been adopted for the Member States initially included in the first DH version. During the drawing up the “Italian Interpretation Manual of the Dir. 92/43/EEC”, the need to propose new habitat types of Community interest, relevant for nature conservation in the Mediterranean bioregion and consisting of several types not included in the DH, has clearly emerged. In this contribution, on the basis of bibliographic investigations and field surveys carried out in various regions of southern Italy, new habitat types are proposed and described, in order to implement the list of habitat types of Community interest in the DH. These are important habitats for the biodiversity protection in southern Italy as they are characterized by phytocoenoses which host plant species of conservation interest. They are briefly reported below.

“Stream environments of the southern Apennines and Sicily” - Macrocategory 32 (Running water). Stream environments of the southern Apennines and Sicily, located in correspondence of springs, depressions with flowing and well oxygenated waters or along small watercourses with permanent regime, flowing into mesophilic, deciduous broad-leaved forest of the mountain belt characterized by hygrophilous herbaceous communities that host various species of phytogeographic importance.

“Mediterranean dripping cliffs (*Adiantion*)” - Macrocategory 72 (Calcareous fens). Cliffs with water percolation in Mediterranean environment, colonized by hygrophilous vegetation characterized by carpets of mosses on which various species of pteridophytes and spermatophytes grow.

“Mediterranean helophytic sub-halophilous meadows” - Macrocategory 14 (Mediterranean and thermo-Atlantic saltmarshes and salt meadows). Mediterranean helophytic sub-halophilous plant communities belonging to the *Scirpion maritimi* and partly to the *Phragmition australis*, growing in coastal areas, usually in correspondence of humid areas behind the dunes and of river mouths, on soils flooded by brackish waters for medium-long periods. Three sub-types can be distinguished: a) communities dominated by tall rushes and sedges; b) communities dominated by small rushes and sedges; c) subalophilous reedbeds of the mouth of Mediterranean rivers and of the back-dune lagoons.

“Helophytic communities of flowing and well oxygenated waters” - Macrocategory 32 (Running water). Herbaceous communities, mainly helophytic, of flowing and well oxygenated fresh waters of the streams of the temperate and Mediterranean regions, which fall within the *Glycerio-Sparganion* and *Apion nodiflori*.

“Mediterranean and sub-Mediterranean dwarf garrigues with presence of rare and/or endemic species” - Macrocategory 54 (Phrygana). Thermo-meso-Mediterranean and sub-Mediterranean, primary and secondary shrubs, growing on poorly developed substrates, dominated by chamaephytes, and characterized by species of particular conservation value, that characterize large part of the Mediterranean landscape.

“Mofettes and mud volcanoes” - Macrocategory 83 (Other rocky habitats). Phenomena of secondary volcanism characterized by cold emissions of carbon dioxide, various other gases, water or mud. This habitat differs from fumaroles because set on sedimentary sequences and characterized by low temperatures.

“Acidophilous oak woods with *Quercus petraea* ssp. *austrotyrrhenica* of the southern Apennines and Sicily” - Macrocategory 92 (Mediterranean deciduous forests). Forests dominated by southern sessile oak, of supra-mediterranean and mesotemperate bioclimate, on acid soils of submontane and montane belts.

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Relevant habitats neglected by the Directive 92/43 EEC: the contribution of Vegetation Science for their reappraisal and protection in Sicily

Guarino Riccardo[1], Bazan Giuseppe[1], Crisafulli Alessandro[2], Caldarella Orazio[3], Giusso Del Galdo Gianpietro[4], Gristina Alessandro Silvestre[5], La Mantia Antonino[6], Marcenò Corrado[7], Minissale Pietro[4], Pasta Salvatore[5], Sciandrello Saverio[4], Scuderi Leonardo[4], Spampinato Giovanni[8], Troìa Angelo[1], Gianguzzi Lorenzo[9]
[1]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo, Italy; [2]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences (CHIBIOFARAM), University of Messina, Italy; [3]Via Maria SS. Mediatrice 38, Palermo, Italy; [4]Department of Biological, Geological and Environmental Sciences (BIGEA), University of Catania, Italy; [5]Institute of Biosciences and BioResources (IBBR), Italian National Research Council (CNR), Unit of Palermo, Italy; [6]Via Giotto 64, Palermo, Italy; [7]Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno, Czech Republic; [8]Department of Agricultural Sciences, Mediterranean University of Reggio Calabria, Italy; [9]Department of Agricultural, Food and Forest Sciences (SAAF), University of Palermo

Even if originating from a species-specific concept (Kirk et al. 2016), the term "habitat" is frequently used to designate an integral unit, supporting ecological functions and species assemblages (Morrison & Mathewson, 2015). This happens both in the EUNIS habitat classification (Rodwell et al. 2018) and in the Directive 92/43 EEC, the latter representing the most important regulatory instrument for the conservation of natural and semi-natural ecosystems and plant biodiversity in Europe. Along with the lists of plant and animal species to be protected, the census of natural habitats "of community interest" from the same Directive has given new impetus to basic taxonomic, syndynamic and ecological research, involving different disciplines. Among these, Vegetation Science offered a significant contribution, considering that the habitats codified in Annex I of the Directive often refer to the species composition and the structure of plant communities and sometimes explicitly refer to phytosociological units.
The mention of some syntaxa by the Directive 92/43 EEC is important, also because this implicitly recognizes one fundamental postulate of phytosociology, that is: each phytocoenosis corresponds to a specific range of variability of environmental drivers. However, phytosociological studies carried out across the entire Sicilian territory during the last three decades pointed out the biogeographical importance of several habitats that are not included in the Annex I of the same Directive. Among the 'neglected' habitats deserving greater attention in order to ensure their conservation, it is worth mentioning:
a) relict communities dominated by plants at the edge of a wider distribution range, such as: the birch woods of *Betula pendula* subsp. *aetnensis* (*Cephalanthero longifoliae-Betuletum aetnensis*), restricted to the supramediterranean vegetation belt of the north-eastern slopes of Mt. Etna (Brullo et al. 2012); the forest nuclei dominated by *Celtis tournefort*i subsp. *aetnensis* (*Pistacio terebinthi-Celtidetum aetnensis*), restricted to Sicani Mts., Nebrodi Mts. and Mt. Etna (Gianguzzi et al. 2014) or by *Ostrya carpinifolia*, scattered on the main mountain ranges of the island (Brullo et al. 2012); the broomfields with *Cytisus scoparius*, *Genista monspessulana* and *Adenocarpus* spp. occurring under cool and humid climatic conditions on the acidic soils of Nebrodi and Peloritani Mts.; the vegetation of dripping and shady rocky faces with Woodwardia radicans, occurring in few gorges of the Peloritani Mountains (Crisafulli 2007). The latter has been proposed to be included within the habitat 7250 (not yet ratified by the Directive) but deserves to be treated as a separate habitat, together with similar moss- and fern-rich communities, linked to warm and wet microhabitats scattered throughout the Mediterranean region;
b) some outstanding habitats barely taken into account by the Directive and not adequately protected by national and regional laws, linked to localized and peculiar geomorphological units and hosting several vegetation units. This is the case of the annual swards and perennial grasslands, the sedge communities and the brackish temporary ponds occurring near the mud volcanoes and on the badlands of Macalube di Aragona, Terrapilata, Vulcanelli di Adrano, etc. (Pasta 2001; Brullo et al. 2010, 2013); the vegetation of the gypsum outcrops of southern and inner Sicily (Guarino & Pasta 2017). Such places should be considered as a whole (like Habitat 8320 "volcanic outcrops and lava fields") and should be all included in the regional network of Natura 2000 sites and protected areas. This would ensure better protection also to the vegetation dominated by the endemic *Tripolium sorrentinoi* (*Asteretum sorrentini*, see Brullo et al. 2010), currently lumped into the omni-comprehensive and ubiquitous habitat 6220;
c) traditional landscapes shaped by century-long agro-forestry practices, such as the dehesa-like communities dominated by *Ceratonia siliqua* on the Hyblean Plateau (SE-Sicily). This would strengthen the unit 63 of the Directive, i.e. sclerophyllous grazed forests (dehesas), currently recognizing only the habitat 6310 ("Dehesas with evergreen *Quercus* spp.").

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Discovering neglected vegetation in Sardinia: from knowledge to conservation

Mauro Fois[1], Gianluigi Bacchetta[1], Maria Carmela Caria[2], Donatella Cogoni[1], Emmanuele Farris[3], Giuseppe Fenu[1], Manuela Manca[4], Silvia Pinna[1], Stefania Pisanu[3], Giovanni Rivieccio[3], Simonetta Bagella[2,3]
[1]Department of Life and Environmental Sciences, University of Cagliari, Viale Sant'Ignazio da Laconi 13, 09123 Cagliari, Italy; [2]Desertification Research Center, University of Sassari, Viale Italia, Sassari, Italy; [3]Department of Chemistry and Pharmacy, University of Sassari, Via Piandanna 4, 07100 Sassari, Italy; [4]Agenzia Regionale FoReSTAS, Viale Merello 86, 09123 Cagliari, Italy

The "Habitats Directive" (92/43/EEC) was the first EU policy document to provide a list of habitats of conservation concern (Annex I). The habitat types of community interest fall into at least one of these criteria: are in danger of disappearance in their natural range; have a small natural range following their regression or because of their intrinsically restricted area; present outstanding examples of typical characteristics of one or more of the nine European biogeographical regions. However, methodological criteria adopted for their identification have mainly remained unclear, and many relevant vegetation types surprisingly out. The leading causes of this gap could be knowledge lack due to the difficulty of access to the places of presence, the seasonality of the blooms or their small range.
This research aims to present a preliminary list of valuable vegetation types occurring in Sardinia, currently not included in the Annex I, to bring them to the attention of the scientific community and managers to promote actions for their conservation. We highlighted the plant communities that can be considered of potential conservation interest basing on expert knowledge, supported by phytosociological relevés already published or unpublished. So far, we have identified more than 30 types of vegetation with different ecological and physiognomic-structural characteristics, as summarized below.
Halophytic habitats: glasswort swards with dwarf plants, hosting rare endemics such as *Nananthea perpusilla* and *Filago tyrrhenica*, depending on a delicate soil balance of humidity and salinity; alo-nitrophilous vegetation with *Malva lusitanica* subsp. *lusitanica*.
Psammophilous habitats: semi-natural vegetation with *Echium arenarium* and *E. sabulicola* hosting rare species such as *Anchusa sardoa*; *Ephedra distachya* mantles.
Watercourses: communities of the endemic alliance *Caricion microcarpae* hosting rare species such as *Carex panormitana*.
Garrigues: mountain garrigues with *Euphorbia spinosa* and other endemic *Lamiaceae* such as *Nepeta foliosa*; coastal garrigues with localized species such as *Helianthemum caput-felis* or *Viola arborescens*, often hosting other relevant species such as *Polygala sinisica, P. rupestris* and *Coris monspeliensis*.
Scrubs: *Pruno-Rubion* communities with endemic species such as *Ribes sardoum* and *R. sandalioticum*; *Bupleurum fruticosum* maquis; hygrophilous formations with *Myrtus communis* and *Oenanthe pimpinelloides* surrounding Mediterranean temporary ponds.
Grasslands: hygrophilous communities with *Orchis laxiflora*; submediterranean grasslands with the endemic *Festuca morisiana*; localized formations with *Stipa offneri (= S. juncea), Astragalus verrucosus* or *Sesleria* gr. *insularis*; wooded grasslands with deciduous *Quercus* spp., with high botanical and faunistic value.
Calaminarian habitats: chamaephytic vegetation of the alliance *Ptilostemo-Euphorbion cupanii*, hosting several endemic species such as *Limonium merxmuelleri* and *Iberis integerrima*.
Bushes: edapho-hygrophilous vegetation with *Rhamnus persicifolia*.
Woods: *Quercus calliprinos* woodlands; *Ostrya carpinifolia* Mediterranean forests.
As expected, given the high number of endemic species that characterize Sardinian flora, most neglected vegetation types have a small natural range due to the intrinsically restricted area of the characteristic species.
These narrow ranges often provide buffered conditions that can be compromised by changes in land use or environmental conditions, including climate change, so they need appropriate attention to ensure conservation.
During the research, several issues emerged that deserve further investigation to obtain a list of plant communities in Sardinia as complete as possible.

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"Ecosystems under fire": assessment of anthropogenic fire threat for compiling the Red List of Ecosystems in Italy

Giulia Capotorti[1], Laura Zavattero[1], Riccardo Copiz[2], Eva Del Vico[1], Laura Facioni[1], Sandro Bonacquisti[3], Marco Di Fonzo, Renato Sciunnach[4], Carlo Blasi[3]
[1]Department of Environmental Biology, University of Rome 'La Sapienza', P.le Aldo Moro 5, 00185 Roma, Italy; [2]Free consultant, Via Casilina sud, 22, 03100 Frosinone, Italy; [3]Interuniversity Research Center "Biodiversity, Ecosystem Services and Sustainability" (CIRBISES), c/o University of Rome 'La Sapienza', P.le Aldo Moro 5, 00185 Roma, Italy; [4]Comando Carabinieri Tutela Forestale-Nucleo Informativo Antincendio Boschivo, Via G. Carducci 5, 00100 Roma

"Ecosystems under fire" is an effective statement posted at the IUCN website, drawing attention not just to the natural but also to the anthropogenic origins of fire disturbance. As a matter of fact, the effects of the threat, especially when unnatural, are being recognized as potentially very serious on ecosystems and associated biodiversity, as in the case of tropical forests.
In the strictly Mediterranean but also in the Temperate Italy, the phenomenon is particularly pronounced as well and must be necessarily taken into account for properly assessing the status of ecosystems, as much as other main pressures (namely, land cover and land use change, ecosystem fragmentation, coastal erosion, intensive agriculture, environmental pollution, biological invasions). For this reason, forest fires have been considered in the compilation of the Red List of Ecosystems of Italy according to the IUCN criteria, a project commissioned to the SBI by the Ministry of the Ecological Transition and recently concluded.
Specifically, thanks to the data made available by the *Comando Carabinieri per la Tutela Forestale*, fire has been counted among the threatening processes able to tamper the characteristic native biota and/or ecological processes of forest ecosystems. Together with the *Nucleo Informativo Antincendio Boschivo* of the Command, the original data were processed in order to be included among the functional symptoms of collapse joined to the degradation of the abiotic environment (IUCN evaluating criterion C), both in the recent past and for present (sub-criteria C1 and C2).
First, the information available at the level of administrative province (NUTS 3) as regards mean burnt forest areas and mean number of events per year was extracted as an indicator of incidence and frequency of the pressure, respectively. Second, in order to implement the IUCN assessment model, the incidence and frequency per province were combined and converted into relative severity by an expert-based rescaling of the observed values. Third, in keeping with the same model, the extent of native forest and Mediterranean maquis ecosystems subject to degradation was estimated in terms of the fraction of the total distribution of the ecosystem type affected by the decline. For such an estimate, the forest ecosystems complexes that are typical of each administrative province have been defined and then combined with the data on broad forest categories mostly affected by fire events in the recent observation period. Although based on different boundaries (i.e. administrative vs ecoregional), the method allowed the interaction between the spatial extent of the threat and the spatial distribution of ecosystem occurrences to be integrated.
Results returned that forest fire represents a considerable pressure for many of the most threatened ecosystems in Italy, along with intensive agriculture, soil consumption and biological invasions. As expected, it is especially diffuse in the Mediterranean ecoregions (Tyrrhenian and Adriatic ecoregional provinces), but new evidence has been provided on the specific ecosystems involved, on the temporal trends of the phenomenon and on its conjunction with other co-occurring threatening processes.
The outcomes can be also combined with the conservation status of ecosystems, complementarily assessed according to the difference between actual and potential extent and to landscape configuration. Together, these assessments represent an important knowledge base for the design and pursuing of biodiversity targets at the national and sub-national level, such as the facilitation and protection of old-growth forest ecosystems especially among the most vulnerable typologies.

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Post-fire management and forest type affect species diversity, composition and resprouting during early recovery stages in a Mediterranean vegetation area

Elisa Carrari[1], Patrizio Biagini[2], Federico Selvi[1]
[1]Department of Agriculture, Food, Environment and Forestry, Laboratories of Applied and Environmental Botany, University of Florence, P. le Cascine 28, I-50144 Firenze, Italy; [2]Bandite di Scarlino, Via Martiri D'Istia 1, 58020 Scarlino, Grosseto, Italy

The risk of wildfires in the Mediterranean region is expected to increase with climate change. Fire is one of the main driver of plant diversity and composition, as it triggers succession processes that vary according to the fire characteristics and species regeneration traits. Human management modulates these processes through the application of practices such as salvage logging, to promote the recovery of the burned soil-vegetation system. Effects of these practices have been studied mostly for coniferous forests, with contrasting results, while little is known about the responses of evergreen broadleaved vegetation in terms of diversity, composition and plant fire-related traits. In this work, we analysed the three-years response of a Mediterranean maquis and a pine stand in central Italy, destroyed by fire in 2017. Three management practices were applied after fire: 1) no intervention (NT), 2) salvage logging + mulching (SM and SMP for the pine stand), 3): salvage logging + mulching + erosion control (e.g. fascines for hill slope stabilization; EC). Overall, the increase of post-fire vegetation cover was negatively affected by both SM and EC treatments, while their effects on ɣ- and α-diversity were positive. Species diversity was significantly lower in pine than in maquis plots, and compositional differences were significant. Abundance of woody species was higher in the unsalvaged sites, excepted for a few species (e.g. *Quercus ilex* L.), while the other plant growth forms were not affected by management practices. Species composition in EC plots was different from the other plots in the first and second year, while it tended to converge three years after fire. Effects of management practices on the frequency of resprouters were minor, as well as on the proportion of species with persistent vs. transient soil seed bank on; such traits were mainly driven by forest type. Our findings support that non-treatment is the best post-fire management for a fast recovery of woody species in the typical Mediterranean broadleaved sclerophyllous maquis. However, the combination of salvage logging with soil erosion control practices has no negative impacts on diversity and reduce species composition changes, allowing to combine biomass recovery with a higher ecological sustainability.

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Plant mortality on ultramafic soils after an intense heat and drought event

Andrea Coppi[1], Lorenzo Lazzaro[1], Federico Selvi[2]
[1]Department of Biology, University of Firenze, Via P.A. Micheli 1, I-50121 Firenze, Italy; [2]Department of Agriculture, Food, Environment and Forestry, Laboratories of Botany, University of Firenze, Piazzale delle Cascine 28, I-50144 Florence, Italy

Plant mortality associated with growing frequency, duration and intensity of heat waves and drought spells is increasingly occurring in forest ecosystems across the world. However, the impacts of these extreme climate events on non-forest plant communities that are specialized for ultramafic soils are still poorly known. Here, we describe a first case of plant mortality on serpentine outcrops in central Italy following the extreme heat and drought event occurred from autumn 2016 to end of summer 2017. Mortality of perennial plants was assessed in early autumn 2017 in thirty plots located in ten major serpentine areas along a SW to NE gradient, with the typically sparse vegetation of ultramafic soils. Statistical modelling was based on temperature and precipitation data of the ten areas and local site conditions potentially affecting plant persistence.
We found that mortality increased with decreasing number of rainy days and duration of the heat wave. Moreover, total mortality decreased with increasing distance from the Tyrrhenian coastline, reaching 60% in the southern and western areas with a typical Mediterranean climate. Local site conditions such as increasing soil depth and north-facing slope aspect significantly increased plant survivorship. Responses of single species were largely different and not related to their taxonomic position, growth form or degree of edaphic specialization for serpentine soils. Notably, obligate serpentine endemics were not less impacted that non-endemics. Remarkably, mortality decreased with increasing species chromosome number, pointing to higher tolerance and adaptive capacity in taxa of likely polyploid origin.
The present findings support that extreme drought and heat can have a previously unrealized impact on plant communities of ultramafic soil in the short term, reducing population size and, most likely, genetic variability of a number of species. However, further studies should examine the recovery capacity and resilience of serpentine plants, together with the effects of such events in the long term.

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Monitoring vegetation dynamics after wildfire events in Mediterranean mountain using satellite data

Michele Di Musciano[1], Walter De Simone[1], Valter Di Cecco[1,2], Giorgia Ferella[1], Anna Rita Frattaroli[1]
[1]Department of Life Health and Environmental Science., University of L'Aquila Via Vetoio, 67100 L'Aquila, Italy; [2]Maiella seed bank, Maiella National Park, loc. Colle Madonna, 66010 Lama Dei Peligni, Italy

Wildfires are currently one of the most important environmental problems, as they cause disturbance in ecosystems generating environmental, economic, and social costs. Due to the fires, over 1 million hectares of forest were lost in Europe in 2017, of which 140000 in Italy. In the same year, due to the extreme drought summer in the central Apennines, many wildfires' events occurred also in the Abruzzo region. One of the largest wildfire events occurred in Campo Imperatore within the Gran Sasso – Monti della Laga National Park (central Italy) affecting different habitat types (e.g alpine grasslands, shrublands, beech and coniferous forest). We have focused on this area to highlight the potentiality of Sentinel-2 for post-fire vegetation monitoring. The main objective of this study is to evaluate the dynamics of vegetation in each habitat after the fire event. This was done by measuring and identifying burnt areas and monitoring the short-term response of the vegetation. Relativized Burn Ratio (RBR) was calculated to identify 'burn scar' and discriminate the 'burn severity' classes (Fig. 1). A 2-year monitoring was carried out using Normalized Difference Vegetation Index (NDVI) to investigate the short-term vegetation dynamics of the burned habitats (habitats refer to Annex I of the European Directive 92/43/EEC). The first important result was the identification and quantification of the area affected by fire. The RBR allowed us to identify even the less damaged habitats with high accuracy. The main results have shown that the most affected habitats, considering the burned area, are grasslands (6170, 6210, 6230, 6430) and shrublands (4060) whereas forests vegetation are the least resilient habitats (Fig. 2). As far as the former are concerned, the analysis lead to interesting results, showing that grassland habitat, even in mountain and sub-alpine areas, can rapidly recover, similarly to the Mediterranean communities. On the other hands, in forest vegetation we obtain the slowest recovery both for beech and coniferous, suggesting as these habitats require many years to be restored.
The survey highlighted the importance of these open-source tools for qualitative and quantitative evaluation of fires and the short-term assessment of vegetation recovery dynamics. The information gathered by this type of monitoring can be used by decision-makers both for emergency management and for possible environmental restoration of the burned areas.

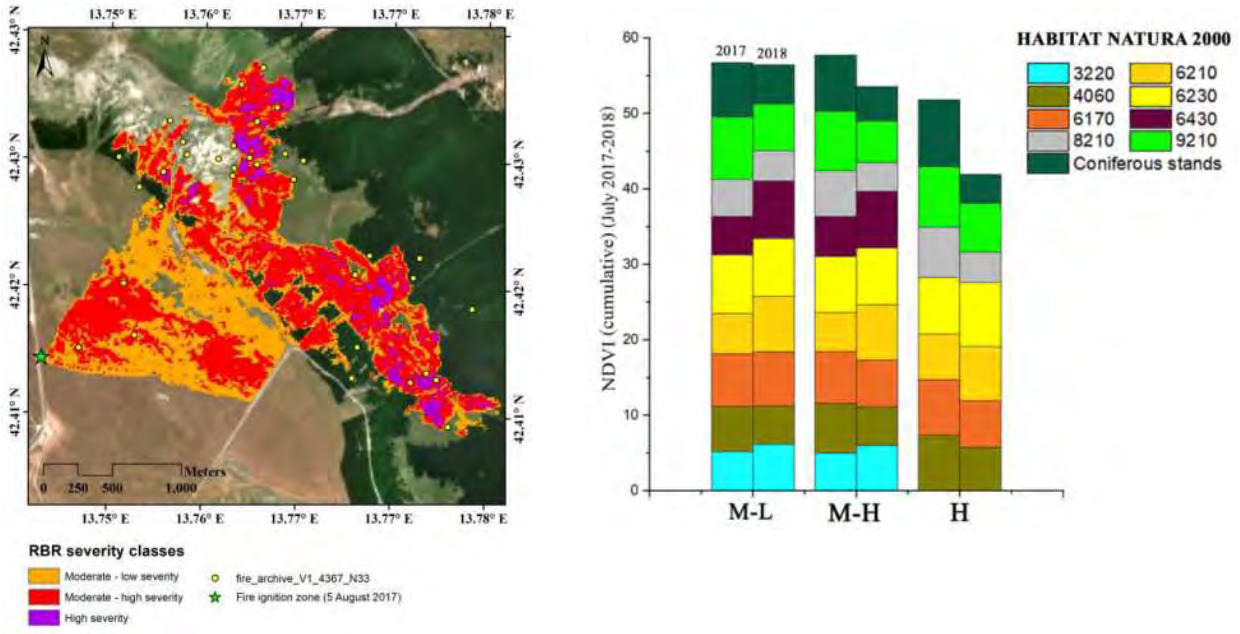

Fig. 1. Relativized Burn Ratio (RBR) for the study area. The index has been discretized in three 'burn severity' classes (Moderate-Low, Moderate-High and High severity).
Fig. 2. Monthly comparison in two years of monitoring (July 2017 and July 2018).

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Understorey changes after an extreme drought event are modulated by overstorey tree species mixtures in thermophilous deciduous forests

Giovanni Iacopetti[1], Filippo Bussotti[1], Elisa Carrari[1], Federico Selvi[1]
[1]Department of Agriculture, Food, Environment and Forestry, University of Firenze, P.le Cascine 28, 50144, Firenze, Italy

Understorey vegetation is a key component in temperate forests, on average representing over 80% of the total vascular plant diversity. It plays a number of ecosystem functions and provides relevant benefits. Overall, however, it is strongly affected by global change. In particular, events of extreme drought are a potential major driver of this layer. Although these events are predicted to increase in frequency and duration, their impacts on woodland understorey communities are still largely unknown.
In this paper, we adopted an observational approach by re-surveying a set of 36 plots in mature thermophilous deciduous forests of central Tuscany, eight months after the end of an extremely dry period in the years 2016-2017. The plots are part of a European network representing a gradient of overstorey richness from 1- to 5-tree species mixtures in comparable site conditions, with variable proportions of five species, *Quercus cerris*, *Q. petraea*, *Q. ilex*, *Castanea sativa* and *Ostrya carpinifolia*.
We took advantage of this model system to test the hypothesis that species richness and identity of dominant trees in the overstorey may modulate the impact of drought on understorey cover, diversity and composition. In spring 2018, a ca. 50% reduction of understorey ground cover occurred in both mono-specific and mixed plots, in line with a similar previous study on pine forests of C Europe. Moreover, mixed models showed that species evenness and Shannon α-diversity increased in the monospecific plots, due to a reduction of abundance of tree species at juvenile stage and the appearance of non-forest weeds in genera such as *Erigeron*, *Senecio* and *Sonchus*, which did not occur in the mixtures. Overstorey species identity effects on understorey diversity were negligible. Overall compositional changes (temporal ß-diversity) were moderate, though larger in the mixed plots and in those with the less drought-adapted tree species in the overstorey, such as *Q. petraea*, *C. sativa* and *O. carpinifolia*. Ellenberg ecological profiles of the communities did not change significantly but light values tended to increase in the monospecific plots, contrary to the mixtures. Overall, our findings provide circumstantial support to a higher diversity stability in the understorey of mixed plots shortly after extreme drought, despite variations in species composition. More studies and monitoring programs are needed to assess understorey recovery capacity and resilience after such events in the long-term.

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Different ways to success: plant community trajectories along time and a soil moisture gradient in restored wetlands

Davide Mosanghini[1,2], Giuseppe Oriolo[2], Francesco Boscutti[1]
[1]Department of Agricultural, Food, Environmental and Animal Sciences, University of Udine, Via delle Scienze 91, 33100 Udine, Italy; [2]FOR NATURE SRL, Via T. Ciconi 26, 33100 Udine, Italy

Ecological restoration is one of the most promising strategies to contrast historical wetland losses caused by land use changes. Restored areas represent ideal sites to study plant succession and changes in ecosystem functions. In northeastern Italy, the agricultural drainages occurred during 1900 caused a dramatic contraction of wetlands of Friuli springs. In the early 2000s, the concern for the threatened status of these ecosystems led to important restoration projects (i.e., "Life Friuli Fens"), involving three Special Areas of Conservation, namely: "Risorgive dello Stella" (IT3320026), "Palude Selvote" (IT3320028), and "Paludi di Gonars" (IT3320031).
Relying on a long-term vegetation monitoring, we studied the plant community succession of four target plant communities along the restored waterlogging gradient: (from free water to dry soils) (i) *Cladium* fens, (ii) low alkaline fens, (iii) *Molina* wet meadows, and (iv) dry meadows. We analyzed twenty-two permanent plots of 4 m^2 surface (2x2 m) equally distributed along the waterlogging gradient, and four plot of vegetation target reference (one for each considered habitat), representing a chronosequence time span of about twenty years. We used generalized linear mixed effects models to analyze the trajectories of plant community in terms of functional traits (i.e., exotic status, life forms, indicator values and leaf traits), biodiversity (i.e., species richness and evenness) and species composition changes (i.e., beta-diversity). Most of the traits were affected by the age of succession independently by the habitat type. Number of exotic and annual species, nutrient indicator value, and leaf size decreased in mature stages of restoration. In contrast, hemicryptophytes and geophytes, moisture indicator value, and leaf dry matter content increased in old stands. Moreover, we found a significant interaction between habitat type and age for changes in biodiversity. Along the succession, species richness decreased in flooded habitats (i.e., *Cladium* fen, low alkaline fen) and increased in meadows, showing a stronger relationship in dry than in wet meadows (Fig 1). For each habitat type, the trajectories moved toward the value measured in the target vegetation plots.
Changes in species composition were assessed in term of species turnover and nestedness using regression on distance matrices. Results confirmed contrasting dynamics between different restored areas, according to their vegetation target. Considering *Cladium* fens, age since restoration did not affect neither species turnover nor nestedness. Low alkaline fens presented both species turnover and nestedness to drive changes in plant community, whereas meadows dynamics were driven only by turnover.
Our findings showed that waterlogging stress acts during succession as main abiotic filter, triggering contrasting trajectories of plant communities. In this light, our contribution provides useful insight into understanding of plant ecological succession in restored wetlands and consequently into assessing progress of restored sites when compared with natural reference sites.

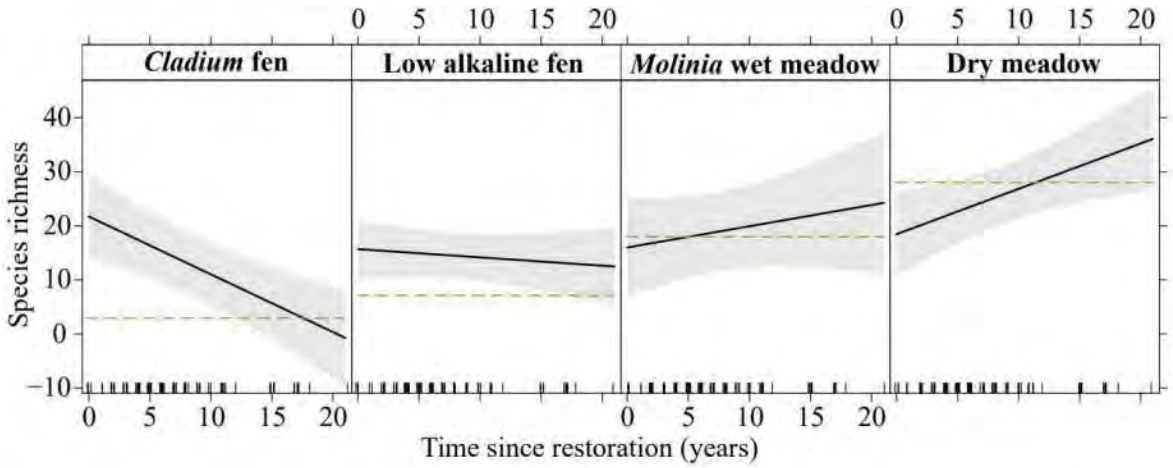

Fig 1. Relationships between species richness and time since restoration in four habitats along a waterlogging gradient. Shaded areas indicate confidence intervals (95%). Dashed green lines indicate the natural reference sites' values.

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European larch growth and CO_2 flux responses to heat and drought

Ludovica Oddi[1], Edoardo Cremonese[2], Mirco Migliavacca[3], Consolata Siniscalco[1], Giorgio Vacchiano[4], Marta Galvagno[2]

[1]Department of Life Sciences and Systems Biology, University of Torino, viale Mattioli 25, 10125 Torino, Italy; [2]Agenzia Regionale per la Protezione Ambientale della Valle d'Aosta (ARPA VdA), Località Grande Charrière 44, 11020 Saint-Christophe (AO), Italy; [3]Department Biogeochemical Integration, Max Planck Institute for Biogeochemistry, Jena, Germany; [4]Dipartimento di Scienze Agrarie e Ambientali (DISAA), Università degli studi di Milano, via Celoria 2, 20133 Milano, Italy

Climate extremes, such as drought and heat waves, were predicted to become more frequent in the future, due to modifications of the water cycle and increasing temperatures. Drought and heat waves have the potential to alter forest functioning, that plays a fundamental role in the regulation of the atmospheric CO_2 concentration and, consequently, in the stabilization of the global climate system. Indeed, climate extremes may limit CO_2 fixation by trees, inducing plant stomatal closure to prevent water loss by transpiration, and consequently causing the interruption of carbon assimilation. Therefore, the occurrence of severe climate extremes, limiting plant carbon assimilation, has the potential to transform forests from carbon sinks to carbon sources, with important consequences for climate, biodiversity and ecosystem services.

This study was carried out in a subalpine *Larix decidua* Mill. forest, located in the western Italian Alps at 2100 m asl. In the study area, we monitored microclimatic conditions (i.e. air temperature, precipitation, soil temperature and water content, solar radiation, and vapor pressure deficit) with a meteorological station, and H_2O and CO_2 exchanges (i.e. evapotranspiration, gross primary production and net ecosystem exchange), using the eddy covariance technique, from 2013 to 2018. Moreover, we measured larch sap flow, using the thermal dissipation method, and larch stem growth, using dendrometers and performing tree ring measurements, from 2015. Finally, stem radial increment values were used to calculate the tree water deficit.

Based on microclimatic data, we identified 2015 and 2017 as 'anomalous' years, due to particularly high air temperatures and vapor pressure deficit, as well as very low soil water availability. Furthermore, these two years showed different microclimatic conditions, being 2015 very hot, but not as dry as 2017. We compared anomalous years with all the other years, that were grouped as 'normal' years and averaged, in order to investigate the larch forest responses to the climatic fluctuations occurred during the study.

The results about evapotranspiration (ET), gross primary production (GPP) and net ecosystem exchange (NEE) showed that ET and, consequently, carbon assimilation were not negatively affected by climate extremes that occurred in 2015 and 2017. On the contrary, we observed an increase of the total annual GPP and NEE, due to a shift forward of the growing season onset, as the consequence of higher spring temperatures. Data about larch sap flow did not show any decrease during the anomalous years, confirming that *L. decidua*, behaving as anisohydric species, was not negatively affected by climate extremes at the study site. Nevertheless, the results about tree water deficit and stem growth showed that during 2017, the driest year, soil water availability became limiting for larches, which maintained their sap flow at the expense of their own water content, and showed a shorter growing period and a lower growth compared to normal years.

In conclusion, this study highlighted the ecological importance of larch forests for their ability to counteract the effects of climatic fluctuations, thanks to the response of *L. decidua*, which, being adapted to live in dry inner Alpine valleys, was able to maintain its optimal sap flow regardless of high temperatures and drought. On the other hand, we found early signals of tree water deficit, under severe drought conditions. The consequences of severe drought events require further investigations to improve the prediction efficiency of future scenarios.

Effects of flooding stress on the saltmarsh halophyte *Salicornia fruticosa* (L.) L.: upscaling perspectives

Marco Vuerich[1], Paolo Cingano[1], Elisa Petrussa[1], Enrico Braidot[1], Elisa Pellegrini[2], Michele Mestroni[3], Francesco Boscutti[1]
[1]Department of Agriculture, Food, Environmental and Animal Sciences, University of Udine, Via delle Scienze 91, 33100 Udine, Italy; [2]Department of Biology, University of Copenhagen, Universitetsparken 4, 3rd floor, 2100 København Ø, Denmark; [3]Agricoltura Innovativa Mestroni, Via Divisione Julia 18, Mereto di Tomba, Udine, Italy

One of the major aftermaths of global warming is the ongoing global sea level rise, which is expected to seriously treat the worldwide coasts. Among coastal environments, saltmarshes are particularly sensitive to the increase of flooding and frequency of storm surges. Indeed, although saltmarshes respond with a sediment accretion and coastal niche shifting, sea rise rates and the coastal squeeze phenomenon jeopardize the acclimation capacity of saltmarsh species and plant communities. For these reasons, monitoring and studying the response of saltmarshes to flooding is crucial to understand the fate of these fragile ecosystems. For a comprehensive understanding of these processes, it is necessary to link different ecological scales using an ecological upscaling approach. We analyzed main traits of plant community and the growth of the key species *Salicornia fruticosa* (L.) L. in 9 saltmarshes along the flooding gradient (Marano and Grado lagoon, northern Adriatic Sea). In particular, we considered community (*i.e.* species richness, dry biomass, dry matter content) and individual traits (*i.e.* shoot annual growth, dry biomass, dry matter content, plant height) analyzing the effect of flooding. Simultaneously we carried out a UAV (Unnamed Aerial Vehicle) multispectral survey, in order to obtain remote sensing-derived vegetation indices (*e.g.* NDVI Normalized Difference Vegetation Index) for the upscaling of plant responses. We found that the flooding gradient produced a significant decrease of plant biomass and growth, affecting both plant traits and plant community features. We also found NDVI effective to predict flooding effect only on the dry biomass of the community. In contrast, flooding intensity shaped the relationship between NDVI and the individual plant growth. In particular, the annual growth was satisfactorily explained by NDVI in the areas less subjected to flooding, whereas lack of correlation between these two parameters was observed for sites frequently flooded. Our findings shed new light on the potential use of the remote sensing tool for the understanding of the response of saltmarshes vegetation to the future increase of the sea level. Further attention should be paid to species morphological traits and additional ecophysiological responses (*e.g.* secondary metabolites, soil redox status) in order to fully understand such crucial processes at the plant community scale.

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Identification of bioplastic degrading fungi

Ilaria Filippi[1], Federica Spina[1], Viktoria Ilieva[2], Pierangiola Bracco[2], Marco Zanetti[2], Giovanna Cristina Varese[1]
[1]Department of DBIOS, University of Turin, Viale Mattioli 25, 10125, Turin, Italy; [2]Department of Chemistry, University of Turin, Via Pietro Giuria 7, 10125 Turin, Italy

Since the middle of the last century, the demand for plastic materials has continuously increased reaching a global production of 368 million tons in 2019. Because of the large use of conventional plastics, their dispersion and accumulation into the environment have increased, causing the pollution of aquatic and terrestrial ecosystems and the release of micro- and nano- plastics. The persistence of plastics in the environment and the effects on flora and fauna have led researchers to focus on the development of new materials with a lower environmental impact: in this perspective, biodegradable plastics are emerging as an alternative to the traditional ones. The worldwide bioplastics production exceeded 2 million tons in 2019. However, although biodegradable and compostable plastics are designed to be mineralized by microorganisms in environments suitable for their disposal, if they are not properly collected or treated, they can accumulate in the ecosystems and cause harmful effects on the environment as well as non-biodegradable plastics. Therefore, it is necessary to investigate the mechanisms involved in their degradation by microorganisms, identifying the released by-products to evaluate the actual harm they may cause both in natural (soil and water) and in controlled environments, such as composting plants.
In this context, the identification of fungal strains able to degrade different types of polymers may help strengthening the process. Indeed, in literature, several microorganisms are already known to be promising biopolymer degraders, fungi included. To this purpose, in this work, several fungi were isolated from a plastic-polluted landfill soil and their degradation ability against biodegradable polymers was explored.
About one hundred of fungi, preserved at *Mycotheca Universitatis Taurinensis,* were grown in presence of a biodegradable aliphatic polyester, the poly-butylene succinate (PBS), as a representative of the biodegradable plastics. PBS was the sole carbon source present in the growth medium, and it was used in the form of an electrospinnated membrane that allowed a primary rapid selection of organisms able to use PBS as a source of nourishment. Fungal growth was monitored by measuring the colony diameter and identifying possible halo zones on the PBS membrane. About forty fungal strains (belonging to the genera *Alternaria, Aspergillus, Cladosporium, Fusarium, Penicillium* and *Purpureocillium*) showed the capability to degrade the polymer and were assayed by a more selective screening, using PBS films as sole carbon source. The most performing fungi were observed under the low vacuum scanning electron microscope in order to assess the physical changes on the PBS surface due to the attack by microorganisms.
One fungus, *Purpureocillium lilacinum,* with high capacity for colonization and degradation of PBS, was inoculated in liquid culture to study the polymer degradation mechanism: the HPLC-analysis studied the released monomers, revealing the presence of 1,4-butanediol whereas succinic acid was not detected. No oligomers were observed, suggesting that the fungus leads to the progressive hydrolysis of the ester bonds of PBS and to a fast utilization of succinic acid.
Moreover, one of the main goals is to identify the enzymes involved in the process, in particular the presence of lipase, cutinase and esterase, primarily responsible for the degradation. The identification of the enzymatic pathway involved in the degradation process is under study since it would be useful to build up innovative enzymatic treatments to enhance the bioplastic transformation processes and to help the development of a plastic-based circular economy. Plastic waste may indeed become a resource of monomer recycling, ultimately enhancing the sustainability of the entire life cycle of bioplastic.

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The potential of fungi to enhance the yields and sustainability of anaerobic digestion

Andrea Zanellati[1], Federica Spina[1], Luca Rollé[2], Elio Dinuccio[2], Giovanna Cristina Varese[1]
[1]Department of Life Sciences and Systems Biology, University of Torino, Viale Mattioli 25, 10125, Torino, Italia; [2]Department of Agriculture, Forestry and Food Science, University of Torino, Largo Paolo Braccini 2, 10095, Grugliasco, Italia

Anaerobic digestion (AD) is a biological process that foresees the conversion of organic feedstocks into biogas (methane), which can be exploited to obtain heat, steam, electricity, fuel and chemicals. Moreover, AD has the potential to produce various by-products and particular attention has been given to the digestate, which mainly contains water, inorganic compounds, and undigested organic matter. AD is among the most efficient technologies for renewable energy production, but there is still room for some significant improvements. For instance, the increased use of dedicated crops for energy purposes has led to competition with food-feed production and land use. The enhancement of the process efficiency and/or the use of alternative and sustainable feedstocks, such as agro-industrial wastes and by-products, may contribute to improve the environmental and economic sustainability of AD. In this context, previous studies have reported the possibility of exploiting the solid fraction of digestate (SFD) as a feedstock for a further AD step, in order to recover economically attractive amounts of methane and to reduce greenhouse gas emissions during the storage. Unfortunately, the proposed use is not generally a feasible option as SFD is largely composed of recalcitrant lignocellulose. Nevertheless, many fungi are known for the ability to produce lignocellulolytic enzymes capable of effectively degrading the structural components of plant cells and they can be exploited to develop biological pretreatments aimed at increasing the anaerobic digestibility of lignocellulosic biomasses.
In the present study, the fungal biodiversity was investigated to develop processes aimed at improving the biogas yields, and consequently the valorisation, of the SFD. Following a preliminary screening, 3 strains belonging to the species *Coprinopsis cinerea* (MUT6385), *Cyclocybe aegerita* (MUT5639), *Cephalotrichum stemonitis* (MUT6326) were selected to carry out the pretreatment in solid-state fermentation and non-sterile conditions. The main physicochemical characteristics and the content of plant cell wall polymers (cellulose, hemicellulose, lignin) of untreated and fungal-pretreated SFD samples were analysed. The effects of fungal pretreatments on subsequent AD were studied by means of biochemical methane potential (BMP) tests in batch systems. The fungi showed the ability to degrade the lignocellulosic fractions, but different degradation profiles were observed depending on the species and the pretreatment duration. All the fungal pretreatments improved the digestibility of the SFD, leading to a significant ($p < 0.05$) increase in the daily and cumulative production of biogas and methane in the samples pretreated with fungi compared to the untreated controls (Fig. 1). In detail, the most effective pretreatment was that carried out with *C. stemonitis* MUT6326, which led to approximately three-fold higher biogas and methane yields (+182% and +214%, respectively) than the untreated SFD (Fig. 1). The increase in AD yields was ascribable both to the addition of fungal biomass, which acted as organic feedstock, and to the lignocellulose transformation due to fungal activity during pretreatments.
In conclusion, the developed fungal pretreatments have the potential to enhance the polysaccharides accessibility of SFD and untap its biogas potential for a further digestion step, thus allowing a potential improvement in the total biogas plant yields and contributing to the development of a by-product management strategy, in accordance with a circular economy and biorefinery perspective.

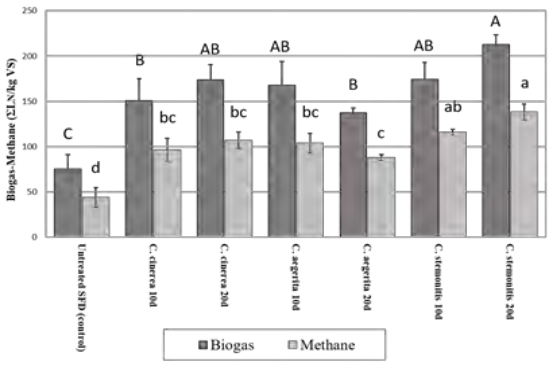

Fig. 1. Cumulative biogas-methane yields of SFD, untreated (control) and pretreated with fungi for 10 and 20 days.

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Myco-booms application in Port waters for bioremediation of heavy metals: the GEREMIA and QUALIPORTI projects experience

Grazia Cecchi[1], Simone Di Piazza[1], Laura Cutroneo[2], Marco Capello[2], Mirca Zotti[1]
[1]Laboratory of Mycology, Department of Earth, Environment and Life Sciences, University of Genoa, Corso Europa 26, 16132 Genoa, Italy; [2]Laboratory of Oceanography, Department of Earth, Environment and Life Sciences, University of Genoa, Corso Europa 26, 16132 Genoa, Italy

Contamination of marine Port waters by inorganic compounds represents one of the most critical and important problems of the marine environments, affecting not only biodiversity but also ecosystems. The scientific community and European Commission have recently evidenced the marine environments and ecosystems protection and restoration by sustainable green technologies among the main objectives of their scientific programs. As a result of this complex scenario, several research and application projects have been funded by the European Community to find efficient processes for the management and remediation of Port seawaters. Some of these projects have been set up under the European Project Interreg ITA-FR Marittime 2014-2020, such as GEREMIA and QUALIPORTI. The first one aims at developing integrated management tools and innovative methods (such as mycoremediation) in the treatment of port waters for an improvement of their quality. The second one aims at identifying, reducing, and controlling the sources of wastewater drains and pollution in tourist ports inside commercial ports.
One of the main primary goals of sustainable restoration and remediation of Port environments is the research of new biotechnologies employable in the decontamination of marine waters. In this context, microorganisms and in particular fungi can play a central and crucial role as best tools of sustainable and green remediation processes. Two Ports involved in the Interreg Maritime projects were selected for a pilot study application of mycoremediation: Genoa harbor (Liguria, Italy; GEREMIA) and Cavo harbor (Elba Island, Tuscany, Italy; QUALIPORTI). After the mycological characterization of the port waters, among the isolates some autochthonous strains of microfungi known for their high metals tolerance and bioaccumulation capability were selected for the pilot study. At the same time, some tests were carried out to study the possibility to employ in the pilot experiment the macrofungus *Pleurotus ostreatus* P. Kumm., Führer Pilzk. Waters from both Ports are analyzed about their metals content. Finally, myco-booms were built. Some commercial booms consisting of absorbent plastic material were compared to myco-booms consisting of straw and sawdust inoculated with fungi. After fungal inocula, the myco-booms were incubated in the dark at 24°C up to the complete growth of mycelia, and after, they were positioned semi-submerged in the Ports. As control, booms made only of mixture of straw and sawdust were also prepared. All the booms and myco-booms were collected after 15 and 30 days, to verify the metals accumulation potential over the time. Preliminary results showed that myco-booms can concentrate metals and trace metals diluted in the seawaters. Moreover, *P. ostreatus* also showed high salinity tolerance and to be a good candidate for this application. This technology does not employ opportunistic or pathogenic fungi and helps to sustainably improve the water quality of the Ports. This is also reflected in the best public opinion of port environments generally seen as very polluted and polluting environments, favoring the dissemination of good practices and acting as an example for the community.

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Metabolic responses of soil fungi to the presence of hexachlorocyclohexane isomers

Andrea Ceci[1], Flavia Pinzari[2, 3], Anna Maria Persiani[1]
[1]Department of Environmental Biology, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy; [2]Institute for Biological Systems, National Research Council of Italy (CNR), Rome, Italy; [3]Life Sciences Department, Natural History Museum, London, UK

Contamination represents one of the main threats to the stability and functioning of soils worldwide. The halogenated organic compound hexachlorocyclohexane (HCH), widely used as the insecticide Lindane (γ-HCH), is toxic and carcinogenic for humans and can cause severe damage to natural ecosystems in the short and long term. Due to its chemical properties, HCH can persist for a long time in soils and bioaccumulate along the food chain. Three isomers, α-HCH, β-HCH and γ-HCH, are persistent organic pollutants, included in the Stockholm Convention since 2009. Fungal bioremediation (mycoremediation) can be an environmentally friendly, feasible and cost-effective solution for HCH contamination of soils since fungi are versatile microorganisms that can adapt to extreme and heterogeneous environmental conditions of soils. They possess efficient extracellular degradative enzymes with relatively non-specific activities that can transform organic pollutants into less toxic forms or completely degrade them. The study of interactions between HCH isomers and fungal species can help to find potential candidates for soil bioremediation and better understand the biotransformation mechanisms involved.
Four fungal strains, isolated from highly HCH-contaminated soils, were selected and their phenotypes compared in control and test conditions. The Biolog® Phenotype Microarray™ system (PM) was used to test their metabolic feedbacks in the presence of β-HCH or a mixture of HCH isomers (α-HCH, β-HCH, γ-HCH, δ-HCH) against many different carbon sources. The response of the fungi to toluene, used as a solvent to prepare the stock solutions, was also tested. Phenotypic profiles were analysed through the *opm* R package. Results highlight the tolerance of the selected fungal isolates to HCH isomers (β-HCH or HCH mixture) in the presence of specific carbon sources. Some Biolog substrates triggered significant differences between the strains in their metabolism at the presence of the xenobiotic compounds (toluene, β-HCH or HCH mixture), thus suggesting the activation of differential pathways.

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Screening methods to detect potential bioremediation fungi

Marta Elisabetta Eleonora Temporiti[1], Lidia Nicola[1], Chiara Daccò[1], Solveig Tosi[1]
[1]Department of Earth and Environmental Sciences, University of Pavia, via Sant'Epifanio 14, 27100 Pavia, Italy

Environmental pollution is an increasingly alarming problem, and identifying microorganisms involved in the bioremediation process is an essential task. This work aimed at developing fast and effective screening methods to select fungal strains with high bioremediation potential.
In order to identify fungal strains able to use difficult-to-degrade organic matter as nutrients, different growth tests were developed using mineral culture media supplemented with recalcitrant substances as the sole carbon source (Figure 1). For this purpose, both natural and artificial substances were used, such as humic acids, complex mixtures of hydrocarbons (vaseline and used motor oil), and powders of different plastic polymers (PET, PP, PS, PUR, PVC, Plasmix). Qualitative enzymatic colorimetric tests were associated with the growth tests to investigate the production of esterase, laccase, peroxidase, and protease (Figure 2). These enzymes are involved in the main degradative processes of recalcitrant material, and their constitutive secretion by the examined strains could be convenient in the perspective of bioremediation.
More than 200 fungal strains belonging to our mycological collection were tested. The most common genera were *Actinomucor*, *Alternaria*, *Arthrobotrys*, *Aspergillus*, *Botrytis*, *Chaetomium*, *Chrysosporium*, *Cladosporium*, *Cunninghamella*, *Fusarium*, *Geotrichum*, *Melanospora*, *Paecilomyces*, *Penicillium*, *Pochonia*, *Purpureocillum*, *Rhizopus*, *Scedosporium*, *Staphylotrichum*, *Trametes* e *Trichoderma*.
The main result of our work was the individuation of several fungal strains with good bioremediation potential. Interestingly, different strains, even belonging to the same species, gave different responses to the performed tests, probably due to adaptations to their isolation habitat. As confirmation of the efficacy of these screening tests, additional experiments were performed (e.g. gas chromatography and Raman spectroscopy tests) and they demonstrated the bioremediation potential of the selected strains.
In conclusion, the described screening tests are an easy and low-cost method to identify fungal strains with high bioremediation potential. In addition, among all the developed methods, it is possible to select the most suitable ones, depending on the target pollutant substance to be degraded.

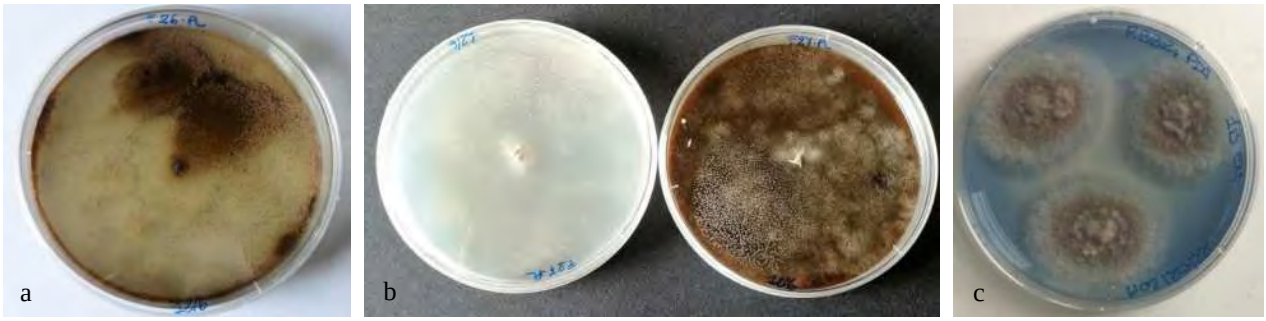

Fig. 1. Growing tests: *Paecilomyces* sp.1 on used engine oil (a), *Paecilomyces* sp.2 on humic acid (b), *Alternaria alternata* on Remazol Brilliant Blue R (c).

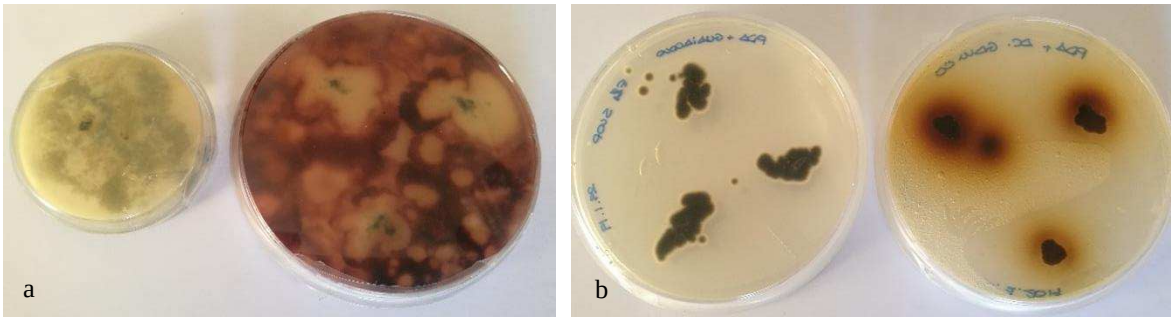

Fig. 2. Enzymatic tests for laccases (a) and peroxidases production (b).

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Fungal mining of precious elements from urban mine: the MYRAEE project experience

Simone Di Piazza[1], Michela Mazzoccoli[2], Grazia Cecchi[1], Ester Rosa[1], Mirca Zotti[1], Anna Maria Cardinale[3]
[1]Department of Earth, Environment and Life Sciences, University of Genoa, C.so Europa 26, 16136 Genoa, Italy; [2]Department of Civil, Chemical and Environmental Engineering, University of Genoa, via Montallegro 1, 16145 Genova, Italy; [3]Department of Chemistry and Industrial Chemistry, University of Genoa, Via Dodecaneso 31, 16146 Genoa, Italy

Precious elements such as rare earths and the strategic elements exploited for the production of electronic devices will soon become a limiting factor of future technological development in several areas worldwide, EU included. According to the European Union Report, the level of recovery of REEs is very limited (1%) [Tsamis et al. 2015].
The countries that still have natural deposits of these products, in the coming years, will certainly find themselves advantageous compared to those countries that are instead forced to import them. The only viable solution in areas where these elements are not naturally present is to treat electronic waste with a circular approach through the urban mining. Urban mining is the process of recovering these rare metals from the urban disposal AKA urban mine. The methods used to carry out this process were mainly mechanical or chemical and very similar to the traditional extraction methods. The most commonly used methods for separation, purification and preconcentration of the REE are solvent extraction, ion exchange, coprecipitation, crystallization and adsorption [Krishnamurthy and Gupta 2015]. Unfortunately, these techniques are often expensive and harmful for the environment [Duman et al. 2020].
The MYRAEE (MYco Recupero di Apparecchiature Elettriche ed Elettroniche) project aims to scale up a patented technology [Di Piazza et al. 2017] to recover REEs from WEEE-based (waste electrical and electronic equipment) exploiting fungal strains.
During the project three strains belonging to genera *Aspergillus, Penicillium* and *Trichoderma* were tested. All strains tested have shown that they can grow on a medium enriched with e-waste dust. In particular, two strains identified as *Penicillium glandicola* and *Aspergillus tubingensis* showed up to 100-fold bioconcentration factor for some elements, in particular, Cu and Y. These results, even if obtained on a laboratory scale, open excellent prospects for the use of these strains in bioreactors for the extraction of these precious elements.

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The genus *Laccaria*: is it possible to get the correct identification of the different specimens? *Laccaria macrocystidiata*, a case study

Francesco Dovana[1], Gabriel Moreno[2], Roberto Para[3], Carmine Lavorato[4] and Marco Mucciarelli[1]
[1]Dipartimento di Scienze della Vita e Biologia dei Sistemi, Università di Torino, Viale P.A. Mattioli 25, 10125 Torino, Italia; [2]Department of Life Sciences (Botany), Biology Building, University of Alcalá, 28805 Alcalá de Henares, Madrid, Spain; [3]Via Martiri di via Fani 22, I-61024 Mombaroccio, Italia; [4]Contrada Calamia, 10, I-87069 San Demetrio Corone, Italia

Laccaria Berkeley & Broome is a large and widely distributed genus able to form mutualistic symbiosis with numerous species of plants spread in both hemispheres. Several studies have shown that this type of symbiosis induces a faster growth of plants and protects them from fungal pathogens. Despite *Laccaria bicolor* being one of the first organisms to be completely sequenced, confirming the interest in this genus of symbiotic fungi, for many European species (including *L. bicolor*) there are no sequences of the holotypes or neotypes. The absence of reference sequences is even more disappointing to consider that the morphological approach, due to the monotony of the features of the different species of this genus, does not allow certain identifications. To fill the lack of molecular data on the original material, a molecular study of the holotype of *Laccaria macrocystidiata*, a taxon originally described as *Laccaria affinis* f. *macrocystidiata* from Central Italy, was performed but without success. For this reason, in this study, samples morphologically corresponding to the original description of L. *macrocystidiata* were analyzed to select an appropriate collection within which to choose the epitype of *L. macrocystidiata*. The ITS and LSU regions were analyzed, and thirteen collections were studied. Our results showed that all *L. macrocystidiata* specimens studied grouped in the same clade, confirming the morphological identification.

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Fomitiporia punctata missing: ITS-LSU analysis in Lombardia detects the polyphagous pathogen *F. mediterranea* only

Carolina Elena Girometta[1], Rebecca Michela Baiguera[1], Francesco Bracco[1], Simone Buratti[1], Marco Cartabia[1,2], Dario Poggi[1], Laura Garzoli[3], Elena Savino[1]
[1]Department of Earth and Environmental Sciences, University of Pavia, Via Sant'Epifanio 14, 27100 Pavia, Italy; [2]MOGU s.r.l., Via S. Francesco 62, 21020 Inarzo (VA), Italy; [3]CNR-Water Research Institute (IRSA), 28922 Verbania, Italy

Fomitiporia punctata (P. Karst.) Murrill is a wood decay fungus on broadleaves. It has only recently been re-evaluated as a distinct species with respect to *Phellinus punctatus* (P. Karst.) Pilát *sensu lato* species complex, a group taxonomically complex to discriminate. In turn, *F. mediterranea* M. Fisch. was recognized as an independent species in 2002 based on strains isolated from *Vitis vinifera* L. in Germany. Since that moment, the species has been increasingly reported in Europe and Southern regions particularly, with special focus on its pathogenicity for some cultivated woody plants. Nevertheless, it has not been clarified yet whether *F. punctata* and *F. mediterranea* are sympatric in south Europe and South of Alps in particular.
Aim of this work was to investigate whether *F. punctata* and *F. mediterranea* are both present in Lombardia by considering different provinces and hosts.
Samplings in different provinces and environments in Lombardia provided the culture collection of DSTA-Unipv (MicUNIPV) with over 60 dikaryon strains in pure culture morphologically recognized as belonging to *P. punctatus sensu lato*. ITS analysis on a subset including strains from Pavia, Varese, Como and Brescia provinces revealed the presence of *F. mediterranea* only, whereas no strains were attributed to *F. punctata*.
For a strain subset, LSU sequences were obtained as well. Interspecific discrimination was furtherly explored by both ITS and ITS-LSU approach with respect to all available sequences in Genbank belonging to *P. punctatus sensu lato* and including sequences specifically referred to *F. mediterranea*. The cladograms clearly show that all the MicUNIPV isolates lie in *F. mediterranea* clade, as well as other available Italian strains examined by different authors in previous years. On the other hand, all the European strains lying in putative *F. punctata* clade come from areas lying North of Alps. Notwithstanding, *F. mediterranea* clades also include strains from German and Swiss vineyards (based on Fischer's results).
Despite the wide majority of currently available *F. mediterranea* sequences is thus referred to Mediterranean regions, the Northern front of this species is likely to be placed in correspondence of Alps, except for accidental introduction further North as a parasite of cultivated plants. The role of cultivated and wild host plants as carriers for *F. mediterranea* is discussed with respect to the sister species *F. punctata* (Fig. 1a-1b). Adaptation of *F. mediterranea* to other allochthonous species is discussed as well.
In conclusion, this study confirmed that *F. punctata* and *F. mediterranea* are not sympatric in Lombardia; data thus suggest *F. mediterranea* only is present South of Alps. Since *F. mediterranea* is an important pathogen for several plants (unlikely *F. punctata*), it is of major concern to discriminate the two species, as well as to define the native ranges and whether climate change may favour *F. mediterranea* emergence.

Fig. 1a-1b. Perennial basidiomes of *F. mediterranea* on different hosts in Lombardia.

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Metabolomic profiling and biological activities of *Pleurotus columbinus* Quél. cultivated on different agri-food by-products

Paola Angelini[1], Roberto Maria Pellegrino[1], Giancarlo Angeles Flores[1], Husam B.R. Alabed[1], Federica Ianni[2], Francesca Blasi[2], Lina Cossignani[2,3], Giustino Orlando[4], Luigi Menghini[4], Claudio Ferrante[4], Roberto Venanzoni[1]

[1]Department of Chemistry, Biology and Biotechnology, University of Perugia, Via del Giochetto 6, 06122 Perugia, Italy; [2]Department of Pharmaceutical Sciences, University of Perugia, Via San Costanzo, 06126 Perugia, Italy; [3]Center for Perinatal and Reproductive Medicine, Santa Maria della Misericordia University Hospital, University of Perugia, Sant'Andrea delle Fratte, 06132 Perugia, Italy, [4]Department of Pharmacy, 'Gabriele d'Annunzio' University, via dei Vestini 31, 66100 Chieti, Italy

Pleurotus spp., commonly known as oyster mushrooms, are classified as white-rot fungi and after *Agaricus bisporus* (J.E. Lange) Imbach and *Lentinula edodes* (Berk.) Pegler, they represent the third largest group of cultivated edible mushrooms worldwide. At present, *Pleurotus* spp., including *P. citrinopileatus* Singer, *P. djamor* (Rumph. ex Fr.) Boedijn, *P. eryngii* (DC.) Quél., *P. flabellatus* Sacc., *P. florida* Singer, and *P. ostreatus* (Jacq.) P. Kumm., have achieved wide acclaim as nutraceuticals due to their exceptional nutritional and medicinal properties and their ability to grow on various sources of agricultural waste. Chemical analyses have shown that many of the biologically active compounds isolated from *Pleurotus* mushrooms belong to hemicelluloses, polysaccharides, lipopolysaccharides, peptides, proteins, glycoproteins, nucleosides, triterpenoids, complex starches, lectins and lipids.

Pleurotus spp. also have high ability to use a wide variety of lignocellulosic waste such as sawdust (i.e., *Populus* spp., *Quercus* spp., *Fagus sylvatica* L.), wheat straw (*Triticum aestivum* L.), rice straw (*Oryza sativa* L.), corn stover (*Zea mays* L.), grass residues [*Cynodon dactylon* (L.) Pers.], sunflower residues (*Helianthus annuus* L.), etc.

The studies of the influence of substrate formulations used for mushroom cultivation, on the bioactive chemical profile of different species of *Pleurotus* (Fr.) P. Kumm. are reported by various researchers. Chopped wheat straw is one of the main components of the growth medium used for the cultivation of *Pleurotus* spp., but often, in order to increase the yield, it is supplemented with materials rich in proteins.

Although there are many studies that have been published regarding the phytochemical composition of *Pleurotus* spp., there are very few studies dealing with the phytochemistry, antioxidant and antimicrobial activities of *P. columbinus* Quél.

Metabolomics is a new discipline which is defined as the monitoring of metabolite concentration in fungi, bacteria and plants. Nowadays, the metabolomics-based approach has been gradually applied in the field of edible and medicinal mushrooms to gain insight into the chemical compositions of biological processes and the understanding response of mushrooms to certain environmental conditions.

In this study, an ultra-performance liquid chromatography mass spectrometry (UHPLC)-QTOF method, coupled with different multivariate data analyses, such as principal component analysis (PCA), was applied to the *P. columbinus* metabolome in order to investigate the influence of four different agri-food residues as growth substrates for *P. columbinus* cultivation, on the bioactive chemical profile of fruiting bodies and to evaluate their potential as antioxidants and antimicrobials.

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Mycoprotein: a way for a more sustainable food system

Elisa Moscato[1], Federica Spina[2], Iolanda Perugini[2], Maria Maddalena Cavalluzzi[5], Giuseppe Mannino[2], Cinzia Margherita Bertea[2], Giovanni Lentini[5], Giuseppe Venturella[3], Maria Letizia Gargano[4], Laura Gasco[1], Giovanna Cristina Varese[2]

[1]Dipartimento di Scienze Agrarie Forestali ed Alimentari, Università degli Studi di Torino, Largo P. Braccini 2, Città Grugliasco; [2]Dipartimento di Scienze della Vita e Biologia dei Sistemi, Università degli Studi di Torino, Via Accademia Albertina 13, Città di Torino; [3]Dipartimento Scienze Agrarie, Alimentari e Forestali, Università degli Studi di Palermo, Viale delle Scienze 4, Città di Palermo; [4]Dipartimento di Scienze Agro Ambientali e Territoriali, Università degli Studi di Bari "Aldo Moro", Via Giovanni Amendola 165, Città di Bari; [5]Dipartimento di Farmacia - Scienze del Farmaco, Università degli Studi di Bari "Aldo Moro", Via E. Orabona 4, Città di Bari

The seventeen Sustainable Development Goals (SDG) are the blueprint to achieve a better and more sustainable future for all. One way to reach this goal is to look and be inspired by nature biodiversity. Microbial biodiversity could be indeed a key factor to achieve several SDG, as environmental degradation, climate change, and sustainable food system. The transition to more sustainable food systems will require many changes, including the search for a new source of food or feed with a lower impact of intensive agriculture and farming. Fungal biomass, known as mycoprotein, could be an alternative source of protein. Why mycoprotein could be a sustainable novel food? First, fungal fermentation is independent of land exploitation, avoiding ethical issues by not competing with food and farm industries. Moreover, fungal growth could be handled in an economic and energetic sustainable way, as required by the principle of the green economy. Indeed, fungal fermentation may be carried out using agro-industrial by-products (AIBPs), valorizing them as valuable feedstocks for a new production system.

This project was aimed to investigate the production of high-value fungal biomass with low economic and environmental impact. Medicinal mushrooms were chosen because they are known to metabolize many kinds of substrates and to produce high-value compounds. *Pleurotus ostreatus*, *Ganoderma lucidum*, *Cordyceps militaris* and *Pleurotus eryngii* were grown in different cultural conditions. Twenty-five cultural lines were set up in submerged fermentation using different by-products including AIBPs. The first goal was to assess the effects of different AIBPs, C, and N sources and ratios on the biomass production yields: results indicated the optimal growth medium was often strain-dependent. More in detail, the best yields were obtained in the presence of insect breeding and AIBPs, where the biomass recovery of some strains was comparable to the control (rich synthetic medium).

Once the optimal growth condition was set for each fungus, the nutritional quality of the biomass was evaluated. It should be indeed noted that great variability in the nutritional composition may be found among fungi, due to species, strains, type of substrate used, fermentation time, type of storage, and conservation process. In this project, the chemical composition of the fungal biomasses was investigated using centesimal composition analysis. Fungal biomass was extracted using different methodologies and the extracts were analyzed through spectrophotometric analysis (Folin-Ciocalteu assay, FRAP, and DPPH assays) to assess the presence of bioactive molecules that could have a positive outcome on animal and human health.

Data confirmed the major role of culture media on the nutritional value of the produced biomass. Moreover, different strains shown different biomass quality as well. *G. lucidum* grown on insect breeding by-product had a very high protein content, while higher lipid content was measured in *C. militaris* grown on cereal molasses by-products.

Results are indeed very promising but, in the future, additional effort should be done to optimize fermentation conditions, in order to achieve good nutritional value and to set up the optimal conditions for biomass production scale-up.

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The marine mycobiota as a possible bioresources: the fungal biodiversity associated with microplastics

M. Florio Furno[1], D. Ferrero[1], A. Poli[1], V. Prigione[1], F. Spina[1], I. Perugini[1], C. Pretti[2], G. C. Varese[1]
[1]DBIOS, Università di Torino, Viale Mattioli 25, 10125 Torino, Italy; [2] CIBM, Viale Nazario Sauro 4, 57128 Livorno Italy

The environment that is most affected by the accumulation and permanence of microplastics (MP) is certainly the marine one: it is estimated that 92% of the plastic present in the sea is represented by MP. MPs accumulate in sediments and in the water column causing several problems: they can be ingested by marine organisms and go up the food chain, also carrying chemical pollutants, or they can constitute a new ecological niche for microorganisms, including pathogens. Among the different microorganisms present on MPs there are also fungi. They are found in all ecosystems, including the marine one, where they play a fundamental role in the decomposition of organic matter and in the recycling of elements.
Nowadays, marine fungi also represent an essential resource for the development of various biotechnological applications: in fact, they are considered among the most promising sources of secondary metabolites useful in many industrial and pharmaceutical processes. In addition, in nature the production of non-specific extracellular enzymes (such as hydrolases and oxidoreductases) allows numerous fungi to degrade very recalcitrant molecules such as lignin. This enzymatic characteristic allows to attack very complex and degradation-resistant xenobiotic molecules such as plastics. Despite the exceptional results obtained in this field, the potential of these microorganisms still remains largely unexplored. The study of marine fungal biodiversity, including that associated with MPs, is therefore of fundamental importance for expanding knowledge and developing new technologies also useful for reducing the environmental impact caused by the increase in MP.
This work, carried out at the Mycotheca Universitatis Taurinensis (MUT), is part of a large project born from the collaboration between different research institutes which has as its main objectives: the quali-quantitative evaluation of MPs present both in sediments and in the water column , along a transept between the harbor of Livorno and the Marine Protected Area "Secche della Meloria", and the study of the marine microbiota associated with MP, combining both a metagenomic and a culturomic approach.
More in detail, in this work an analysis of the mycobiota associated with the MPs of the sediments of three sites characterized by a different degree of anthropic impact (harbor of Livorno, the Secche della Meloria and an intermediate area of the transept) was conducted and compared with the mycobiota present in sediment and sediment water. In order to maximizing fungal biodiversity, the isolation phase was carried out in solid, through the use of three different cultivation media with the addition of sea salt (ss - 2% w/v) and antibiotics to prevent bacterial growth: Corn Meal Agar (CMAss), Malt Extract Agar (MEAss) and Syntetic Nutrient-Poor Agar (SNAss). The samples were incubated at two temperatures (15 °C and 25 °C) for 21 days, in succession. As regards the MPs, after sonication in sterile sea water, aimed at detaching the biofilm, the supernatant was plated in replicates. The fungal isolation of the sediment water was carried out by filtering it and positioning the filters on the different isolation grounds; while the sediment, on the other hand, was treated using the soil dilution plate technique. A polyphasic approach was used to identify the isolated strains, which involves the combination of molecular analysis with specific markers (e.g. β-tubulins, Actins, ITS) and the evaluation of morpho-physiological characteristics.
The results show that the mycobiota of all three matrices (MPs, sediment and sediment water) is dominated by Phylum Ascomycota: *Penicillium spp.* and *Cladosporium spp.* followed by the Basidiomycota yeast-like *Sporobolomyces spp.* are among the most abundant genera. The sediments and the water sediments showed a greater specific richness than MPs, in fact there are species belonging to Phylum Mucoromycota and genera present exclusively in them, including *Rizhopus spp.*, *Trichoderma spp.*, *Aspergillus spp. Fusarium spp.*.
Focusing on the MPs-isolated fungi, a total of 60 fungal strains were isolated, attributable to 20 taxa. Analyzing these strains for their origin, 34 out of 58 come from the harbor, 16 from Meloria and 8 from the Intermedia. Furthermore, the mycoflora present on microplastics shows a high specificity for the sampling site: respectively 41.2% and 17.6% of the taxa were found exclusively in the Porto and Meloria sites; the Intermediate, on the other hand, has a high sharing of taxa found, especially in sharing with the Port (17.6%).
The nature and biodiversity of filamentous fungi associated with microplastics in the marine environment is still poorly understood; this study therefore contributes to a greater understanding of the marine mycobiota associated with them, opening new scenarios on their possible exploitation in different biotechnological areas, including bioremediation.

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New species from the New World: early archaeobotanical and archaeozoological evidence from the Santi Quattro Coronati complex in Rome (Italy)

Claudia Moricca[1], Francesca Alhaique[2], Lia Barelli[3], Alessia Masi[1], Raffaele Pugliese[4], Laura Sadori[1]
[1]Department of Environmental Biology, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy; [2]Bioarchaeology Service, Museum of Civilizations, Piazza Guglielmo Marconi 14, 00144 Rome, Italy; [3]Department of History, Representation and Restoration of Architecture, Sapienza University of Rome, Piazza Borghese 9, 00186 Rome, Italy; [4]Independent researcher, Via del Forte Tiburtino 120d, 00159 Rome, Italy

The European colonization of the Americas at the end of 15th century AD has brought with it a series of revolutions, including the introduction of numerous new plant and animal species, many of them of great economic value and currently at the base of European diets. Plants include *Solanum lycopersicum* L. (tomato), *Solanum tuberosum* L. (potato), *Capsicum* spp. (pepper and hot chili), *Theobroma cacao* L. (cocoa), *Cucurbita* spp. (gourds and pumpkins), *Phaselous* spp. (beans), *Zea mays* L. (corn), *Helianthus* spp. (sunflower), and the inedible, but important plant from an economic perspective, *Nicotiana* spp. (tobacco). Domestic animals are much fewer: *Meleagris gallopavo* L. (turkey), *Cavia porcellus* Pallas (guinea pig), and *Cairina moschata* L. (Muscovy duck). Their introduction occurred at different times and rates, due to numerous factors, such as the specific area of origin and the similarity to certain Old-World plants; for example, potatoes and tomatoes were initially associated to the roots and fruits of the poisonous mandrake.
These phenomena can be observed through the study of iconographic evidence, ancient texts and recipes, but also rare archaeobotanical and archaeozoological findings.
An interesting case study is represented by the Santi Quattro Coronati Complex, located in the center of Rome (Italy), between the Colosseum and St. John's Basilica. For this research, two specific contexts are taken into consideration. The first one is a disposal pit excavated in 1996, which was obtained by the closure of a dismissed staircase. Such pit contained, among the others, well preserved plant and animal remains. Based on ceramic evidence, its use for discarding refuses can be framed between the end of the 15th century and the mid-16th century AD, when the Santi Quattro Coronati complex served as the residence of the cardinal. The second context was investigated during more recent excavations, carried out in 2011–2012 in a former porch located in the west side of the garden of the complex. Here, a surface made of mortar (Layer 521) dated to the beginning of the 17th century AD provided a small faunal assemblage, but no plant remains.
The study of the disposal pit yielded a rich archaeobotanical assemblage in both qualitative and quantitative terms. More interestingly, it also included seeds of two New World taxa: *Cucurbita maxima*/*moschata* and *Cucurbita pepo* L. (Fig. 1a). From the faunal point of view, a reference to the Americas came from the mortar layer, where a *Cavia porcellus* pelvis (Fig. 1b) was found.
Both attestations represent one of the oldest evidences of such *taxa* in Europe. These early introductions are an indicator of wealth of the inhabitants of the complex, which is further enhanced by the richness of the plant assemblage and the presence of prestigious plants such as *Punica granatum* L. (pomegranate) and spices such as *Coriandrum sativum* L. (coriander). The possible explanation for such early presence of New World species could be that the Cardinal's palace, a very prestigious and wealthy landmark, was one of the first stops along the trading routes that emanated from the New World, leading to the rapid availability of exotic plant and animal species. This may also be related to the role of Cardinal Lorenzo Pucci, titular of the Santi Quattro between 1513 and 1524, as supervisor for the Church of the Indies in the Consistory.

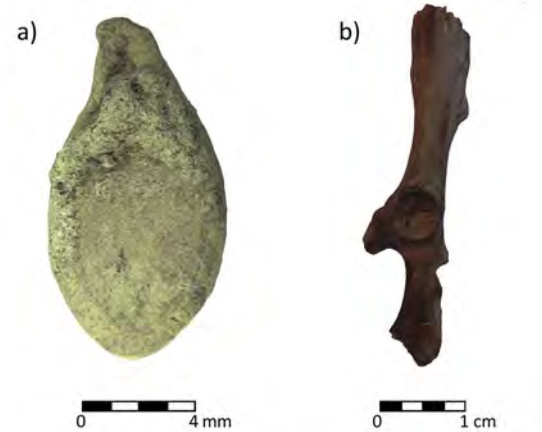

Fig. 1a Seed of *Cucurbita pepo* L.; 1b Pelvis of *Cavia porcellus* Pallas

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Beech and stone Pine, the Italian landscape modelled by valuable ritual trees

Alessia Masi[1,2], Fanny Gaveriaux[2], Lucrezia Masci[3,2], Marta Mariotti Lippi[4], Claudia Moricca[1], Laura Motta[5], Jordan Palli[6], Cristiano Vignola[1,2], Laura Sadori[1]

[1]Department of Environmental Biology, Sapienza University of Rome, Piazzale A. Moro 5, 00185, Roma Italy; [2]Palaeo-Science and History Group, Max Planck Institute for the Science of Human History, Kahlaische Str. 10, 07745 Jena, Germany; [3]Department of Earth Science, Sapienza University of Rome, Piazzale A. Moro 5, 00185, Roma Italy; [4]Biology Department**,** University of Florence, G. La Pira, 4, 50121, Firenze, Italy; [5]Kelsey Museum of Archaeology, University of Michigan, Ann Arbor, 48109 MI, USA; [6]Dendrologylab, Department of Agriculture and Forest Sciences (Dafne), University of Tuscia, Via Santa Maria in Gradi, 4, 01100 Viterbo, Italy

Beech (*Fagus sylvatica* L.) and stone pine (*Pinus pinea* L.) are quite different trees, at present widespread in Italy, the first often in primeval forests, the second in monospecific artificial coastal woods. The modern Italian beech population can be distinguished genetically from the north European one, and spread from few refugia located in central and southern Italy (Magri et al., 2006, New Phytologist, 199-221). In contrast, the genetic variability of stone pine is extremely low, making the reconstruction of its spread in the Mediterranean region quite complicated (Viñas et al., 2016, European Atlas of Forest Tree Species, 130-131). The archaeobotanical record indicates the arrival of stone pine early in the Iron Age, while beech seems to have been preserved outside its natural distribution area. Besides the economic importance of the two trees, the ritual meaning of both plants must be taken into account.

According to available data, the oldest recovery of stone pine is at the Phoenician site of Motya (Sicily) where macroremains date back to the period between the mid-8th cent. and the mid-6th cent. BC (Moricca et al., 2021, Vegetation History and Archaeobotany, 1-15). The hypothesis that Phoenicians and Punics played a major role in the spread of stone pine is confirmed by the recovery of pine cones and shells at Santa Giusta (Sardinia) during the 6th–2nd cent. BC, and later on in 3rd–2nd cent. BC (Sabato et al., 2019, Vegetation History and Archaeobotany, 9-16). Pine pollen probably ascribable to stone pine is found since Roman times at Pompeii (Vignola et al., 2021, Vegetation History and Archaeobotany, 1-16) from the 1st half of the 1st cent. BC, and in the city of Rome at the *Horti Lamiani* since the end of the 1st cent. BC (Masi and Vignola, unpublished data), and at the Roman villa in via De Lollis (Sadori and Masci, Palladium, 2019) in the 4th cent. BC and in the 3rd cent. AD. Stone pines are well known to have been important as ritual, votive and funerary plants.

The presence of beech in the Italian peninsula is widespread, being this tree a component of the altitudinal forest. In Tuscany, pollen and macroremain records indicate a spread of Fagus at low altitude, possibly from the late Bronze to early Iron Age (Sadori et al., 2015, Review of Palaeobotany and Palynology, 217-230). Coeval attestations arrive from Umbria, Marche and Latium regions with recoveries from Lago Trasimeno (Angelini et al., 2014, Plant Biosystems, 713-722) Monte Croce-Guardia (Arcevia) and Casale Rocchi (Rome; Vignola et al., unpublished data). At Gabii between the end of the 8th and the end of the 6th cent. BC fragments of beech charcoal have also been recovered. In all these sites, beech timber was found outside its present distribution area. Quite interesting, is the finding of both pollen and macroremains in the city of Rome. Charcoals have been recognized in the open area of the Horti Lamiani since the end of the 1st cent. BC. The tree was probably present among other taxa planted in the gardens. A single beech pollen grain was recovered at the Roman villa in via de Lollis in Rome, dated to the 4th century BC, in an area sacred to Jupiter. At the moment this is the oldest evidence of beech pollen in Rome and a confirmation of written sources that reported the presence of sacred beech groves, named by Latins *Lucus Fagutalis*.

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Cannabis pollen records in Italy: when the *taxon* actually entered the Mediterranean?

Jordan Palli[1], Assunta Florenzano[2], Alessia Masi[3,4], Scott Mensing[5], Gianluca Piovesan[1], Laura Sadori[3], Anna Maria Mercuri[2]

[1]Department of Agricultural and Forest sciences (DAFNE), University of Tuscia, via san Camillo de Lellis, snc, 01100 Viterbo, Italy; [2]Laboratory of Palynology and Palaeobotany, Department of Life Sciences (DSV), University of Modena and Reggio Emilia, Via Università 4, 41121 Modena, Italy; [3]Department of Environmental Biology, Sapienza University of Rome, Piazzale A. Moro 5, 00185 Roma, Italy;[4]Palaeo-Science and History Group, Max Planck Institute for the Science of Human History, Kahlaische Str. 10, 07745 Jena, Germany; [5]Department of Geography, University of Nevada, Reno, N. Virginia Street 1664, 89557 Reno, NV, USA

The history of the migration and spread of *Cannabis*, a genus of the Cannabaceae family, from its center of origin is still uncertain. While its sister-genus *Humulus* is acknowledged to have naturally dispersed from Asia to Europe prior to human agency, *Cannabis* is commonly thought to have been spread by humans when cultures began to enjoy the multiple uses for which this species is suitable – most importantly fibre. Many authors ascribe the center of origin of *Cannabis* to Mongolia, Northern Cina, Persia, eastern India, southeastern Russia or eastern Europe and argue that the spreading of the species would have been driven toward the Mediterranean by ancient nomad cultures during the Bronze-Iron ages, e.g. the Scythians (see literature cited in McPartland et al., 2018. Vegetation History and Archaeobotany, 27: 635–64). Today, *Cannabis sativa* L. is defined as an archaeophyte alien species for Italy (Galasso et. al, 2018. Plant Biosystems 152: 556–592). Modern occurrences are due to individuals escaped from cultivation; populations are casual, non-naturalized and distinct from the wild oriental type (Galasso et. al, 2018). However, palynological and archaeological surveys carried out in Italy and other Mediterranean countries, suggest the presence of Cannabis (and/or Cannabaceae in sensu lato) in the area since the Late Glacial – long before the origins of agriculture in the western Eurasia (Mercuri et al., 2002. Vegetation History and Archaeobotany 11: 263–276).

The pollen morphology of *Cannabis* and *Humulus* is very similar. It is possible to discern *Cannabis* from *Humulus* pollen by the size, the wall thickness and the pore morphology, but these morphological features often overlap. Thus, many authors prefer to identify these *taxa* at the family level, making the understanding of which species has contributed most, impossible.

Here we sum up pollen occurrence of *Cannabis* from Italian pollen records spanning from the Late Glacial to the Roman time, when its cultivation and use were widespread (Mercuri at al., 2002). We include published and unpublished pollen data to support the debate on the origin of *Cannabis* use and cultivation in the Mediterranean.

Cannabis was identified from the Late Glacial-early Holocene onwards, mostly in central Italy. Pollen grains of *Cannabis* became more frequent in the sediment samples at the transition between the Neolithic and the Copper Age. However, only since the Bronze Age concentrations became higher and the occurrence more continuous. The available evidence calls into question the assumption that *Cannabis* was first introduced into the Mediterranean during the Bronze Age. The genus might have been introduced earlier (early Holocene) in Mediterranean or there may have been indigenous population before the domestication occurred during the Bronze Age. An interdisciplinary approach is needed to address the unresolved issue of the arrival of *Cannabis* in the Mediterranean.

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Environmental implications and evidence of natural products from dental calculi of a Neolithic–Chalcolithic community (central Italy)

Alessia D'Agostino[1], Gabriele Di Marco[1], Mauro Rubini[2,3,4], Silvia Marvelli[5], Elisabetta Rizzoli[5], Antonella Canini[1], Angelo Gismondi[1]

[1]Department of Biology, University of Rome "Tor Vergata", Via della Ricerca Scientifica 1, 00133 Rome, Italy; [2]Department of Archaeology, University of Foggia, Via A. Gramsci 89/91, 0881 Foggia, Italy; [3]Anthropological Service of SABAP-LAZ (Ministry of Culture Italy), Via Pompeo Magno 2, 00133 Rome, Italy; [4]Anthropological Laboratory of VA.VE. (Villa Adriana and Villa d'Este), Via degli Stabilimenti 5, Tivoli, 00019 Rome, Italy; [5]Laboratorio di Palinologia e Archeobotanica-C.A.A. Giorgio Nicoli, Via Marzocchi 17, 40017 San Giovanni in Persiceto, Bologna, Italy

Ethnobotanical data from early Neolithic to Copper Age are quite absent in central Italy, since only a few plant macrofossils were found in the relative settlements. Nowadays, many research groups have exploited the great informative potential contained in ancient human dental calculus to open a window on past lifeways. The recovery of microparticles trapped inside this matrix has been employed to infer new awareness about the impact of plants on different prehistoric and historical communities. In this contribution, we investigated the role of plants in the prehistoric community of Casale del Dolce (Anagni, FR, central Italy), through plant microdebris recovered from dental calculus. The finding of a great amount of pollen types, even in form of compact lumps, could indicate use of natural substances, such as honeybee products and/or conifer resins. This plant-microremain record also suggested environmental implications relative to the Neolithic and Chalcolithic period. Additionally, the stability of the tartar microenvironment had preserved starches and other microparticles, such as one epidermal trichome, a sporangium, and fragments of plant tissue, rarely detected in ancient dental calculus. The detection of secondary metabolites by gas-chromatography mass-spectrometry in this matrix confirmed the familiarity of the community with plant resources. The simultaneous presence of traces of Poaceae and Fabaceae outlined a very important combination from a nutritional point of view. Although restricted to a limited series of samples, dental calculus analysis allowed us to confirm that these prehistoric individuals were comfortable with plants and able to exploit natural matrixes, offering the opportunity to maximize the biographical detail of this ancient population. All these data supply various interesting information and expand the knowledge about the potential of dental calculus in archaeological and archaeobotanical fields with a special focus on palaeoecology.

Fig. 1. Skeletal remains of the individual no.7 from Casale del Dolce community.

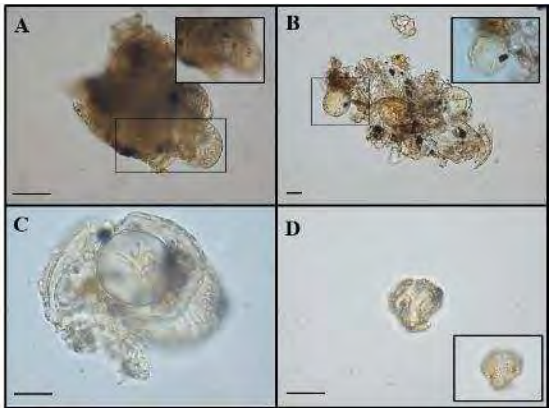

Fig. 2. Plant microremains detected by microscopy analysis from ancient dental calculus. Aggregates of pollen and spores (A,B); Pinaceae and Cupressaceae pollen grains (C); Brassicaceae pollen grain (D). The scale bars indicates 15 µm.

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VIDEO ABSTRACTS

1 = A comprehensive aequorin-based toolset to investigate intracellular calcium changes in *Lotus japonicus* in response to pathogenic and symbiotic molecules

Filippo Binci[1], Enrico Cortese[1], Ute C. Vothknecht[2], Marco Giovannetti[1], Lorella Navazio[1]
[1]Department of Biology, University of Padova, Via U. Bassi 58/B, 35131 Padova, Italy; [2]Plant Cell Biology, Institute of Cellular and Molecular Botany, University of Bonn, Kirschallee 1, D-53115 Bonn, Germany

During their lifecycle plants encounter myriads of microorganisms in the soil, either harmful or beneficial. While hosting few of them up to the inner root layers to establish mutualistic interactions, such as the ancient and well-spread arbuscular mycorrhizal (AM) symbiosis, plants must also defend from pathogens, which in some cases can hijack the plant endosymbiotic hosting machinery to invade roots. Whereas few components of the symbiotic and pathogen-related signalling cascade are known, some of them overlap and the mechanisms underlying the trade-off between these two opposing phenomena are an area of intense research. The perception of different microbe-associated molecular patterns (MAMPs) is mediated by a plethora of transmembrane receptors. Concerning the fungal kingdom, cell wall-derived chito-oligosaccharides (COs) play different roles as MAMPs according to their length. Short-chain COs (tetrameric and pentameric) are supposed to be symbiotic, while longer ones (mainly octameric) are pathogenic. However, a clear distinction between a set of symbiotic and pathogenic COs is still missing. In fact, MAMPs perception is usually transduced into distinct changes of intracellular calcium concentration ($[Ca^{2+}]$) that lead to specific transcriptional readouts. A classic hallmark of endosymbiotic signals is the induction of nuclear and perinuclear high-frequency Ca^{2+} spiking. However, recent evidence suggests that long-chain COs, likewise short-chain COs, can induce nuclear Ca^{2+} spiking. Moreover, there is still a lack of knowledge about how symbiotic and pathogenic signals are discriminated by early Ca^{2+}-mediated events occurring in the cytosol.
To disentangle this question, we exploited the modular GreenGate cloning system to create a set of genetically encoded Ca^{2+} reporters based on aequorin chimeras specifically targeted to different intracellular compartments and with the possibility of using cell-type specific promoters. By using *Agrobacterium rhizogenes*-mediated hairy root transformation of *Lotus japonicus*, we showed that both tetra-COs and octa-COs trigger dose-dependent cytosolic Ca^{2+} signals with specific signatures. To uncouple cytosolic and nuclear Ca^{2+} signalling, we expressed the aequorin-based Ca^{2+} reporters in different Lotus mutants, impaired in different steps of the common symbiotic signalling pathway. At the same time, we used aequorin-expressing lines of *Arabidopsis thaliana* as a non-symbiotic plant to further discriminate between symbiotic and pathogenic signals.
Altogether this work will provide a comprehensive picture of the differentially located intracellular Ca^{2+} changes in response to pathogenic and symbiotic molecules in a number of Lotus genetic backgrounds.

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1 = Photosynthetic phenotyping of mature chlorophyll-deficient wheat mutant lines cultivated under fluctuating natural light

Andrea Colpo[1], Marian Brestič[2], Alessandra Sabia[1], Simonetta Pancaldi[1], Lorenzo Ferroni[1]
[1]Department of Life Sciences and Biotechnology, University of Ferrara, Corso Ercole I d'Este 32, 44121 Ferrara, Italy;
[2]Department of Plant Physiology, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 94976 Nitra, Slovakia

According to a diffuse concept, chlorophyll-deficient crops could be more productive than the corresponding WT genotypes because of a better use of light through the canopy. *Chlorina* lines of wheat are affected by mutations leading to lower chlorophyll contents and a selective loss of chlorophyll *b*. Breeding programs introduced the mutation into vigorous cultivars, obtaining early yellow-green phenotypes that tend to progressively revert to green. However, the expectations for high productivity were not met in the open field, which was attributed to a disturbance of the photosynthetic electron flow regulation emerging as a side effect of the chlorophyll deficiency. A phenotyping experiment in controlled conditions tested the response of the mutants under continuous or fluctuating irradiance provided by LED lights, confirming that the mutants were subjected to electron bursts potentially mining the photosynthetic membrane integrity. The retarded growth of the mutants was ascribed to both the impaired electron flow regulation and the compensatory mechanisms that subtract metabolic energy. In particular, two mechanisms were found to probably relieve over-reduced states of the photosynthetic membrane: the accumulation of inactive Q_B-non reducing photosystem II (PSII) and an increased capacity of electron transport between PSII and PSI, especially under fluctuating light. Because these appeared as unprecedented regulatory mechanisms in *chlorina* wheat and were evidenced in a growth chamber simulating light fluctuations, we wondered whether they could also be relevant in a natural context.
Seeds of a collection of wheat lines (*Triticum aestivum*: ANBW4A, ANBW4B, ANK32A and the WT, NS67; *Triticum durum*: ANDW7A, ANDW7B, ANDW8 and the WT, LD222) were sown in the autumn 2020 in the Botanical Garden of the University of Ferrara, in continuation with the experiments conducted in growth chambers at the Slovak University of Agriculture in Nitra (Slovakia). The site was under the canopy of deciduous tree species, so after tillering the plants were reached by either direct sunlight for some hours per day, or by the lightflecks filtering through the canopy. At the heading stage (late spring 2021), the flag leaves were analysed with respect to their photosynthetic pigment content, light-response curves with pulse amplitude chlorophyll *a* fluorescence, and prompt chlorophyll *a* fluorescence.
The chlorophyll content and the chlorophyll *a*/*b* ratio were consistent with previous results obtained in mature leaves of the same lines in the open field and confirmed the severity series of the mutants, which were characterized by an increased chlorophyll *a/b* ratio. However, all mutants developed a good control of electron transport. Q_B-non reducing centres accumulation was invariable between *T. durum* lines, while all the mutants of *T. aestivum* tended to accumulate a slightly higher amount of such inactive reaction centres. The mutants were characterized by a higher intersystem electron transport capacity proportional to the severity of the phenotype, especially evident in ANK32A and ANDW7B. Surprisingly, the marked growth retardation affecting the mutant ANBW4B under the fluctuating light provided by LEDs was not observed in a natural fluctuating light regime.
Overall, in a condition of natural light fluctuations the enhancement of the intersystem electron transport capacity plays a fundamental role in compensating the regulatory defects in the mutant lines. Conversely, the accumulation of inactive PSII reaction centres is marginally involved in the compensation process.

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1 = Morpho-physiological classification of Italian tomato varieties (*Solanum lycopersicum* L.) according to water stress tolerance during vegetative and reproductive growth

Veronica Conti[1], Marco Romi[1], Sara Parri[1], Iris Aloisi[2], Giovanni Marino[3], Giampiero Cai[1], Claudio Cantini[4]
[1]Department of Life Sciences, University of Siena, Siena, SI 53100, Italy; [2]Dipartimento di Scienze Biologiche, Geologiche e Ambientali, Università degli Studi di Bologna, via Irnerio, Bologna 40126, Italy; [3]National Research Council of Italy, Institute of Sustainable Plant Protection (CNR-IPSP), Via Madonna del Piano 10, 50019 Sesto Fiorentino, FI, Italy; [4]CNR-IBE (Consiglio Nazionale delle Ricerche-Istituto per la Bioeconomia), Follonica, GR 58022, Italy

Global warming is causing the world to face various obstacles, one of which is increasing drought. Irrigation is fundamental for agriculture but, as climate change becomes more persistent, there is a need to conserve water and use it more efficiently. It is therefore crucial to identify crop varieties that can tolerate drought. For economically relevant crops, such as tomatoes, this purpose takes on an even more incisive role and local agrobiodiversity is a large genetic reservoir of promising varieties. In this study, nine Italian varieties of tomatoes were considered. These experienced about 20 days of drought stress, both in vegetative and reproductive phases. Various parameters were monitored, such as stomatal conductance (g_s), photosynthesis (A), water use efficiency (WUE), growth (GI) and soil water content (SWC). For comparison, four commercial varieties, subjected to the same conditions, were also considered. The different responses and behaviors allowed to divide the varieties into three groups: resistant, susceptible, and intermediate. The classification was also confirmed by a principal component analysis (PCA). The study, in addition to deepening the knowledge of local Italian tomato varieties, reveals how some varieties perform better under stress condition than commercial ones. Moreover, the different behavior is dependent on the genotype and on the growth phase of plants. The results suggest that an accurate selection of varieties could lead to a more sustainable agriculture and less wasteful irrigation plans.

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1 = The VACUOLAR SORTING PROTEIN 13 (VPS13) affects female germline establishment and progression in *Arabidopsis thaliana*

Mara Cucinotta[1], Rosanna Petrella[1], Flavio Gabrieli[1], Letizia Cornaro[1] and Lucia Colombo[1]
[1]Università Degli Studi di Milano, Dipartimento di Bioscienze. Via Celoria 26, 20133, Milan, Italy

Ovules are the precursors of seeds and arise from the placenta, a meristematic tissue inside the ovary. In the primordium of the ovule, one of the subepidermal cells of the nucellus, the archespore cell, differentiates into the megaspore mother cell (MMC), that undergoes meiosis to form four spores, in a process named megasporogenesis. The three most apical spores degenerate, while the remaining one, the functional megaspore (FM), enters megagametogenesis to ultimately form the mature female gametophyte, or embryo sac. Recent findings suggested that several molecular pathways are involved in the correct establishment and progression of the female germline. Here we reported a novel role for *VACUOLAR SORTING PROTEIN 13 (VPS13)* in ovule development. Using different marker lines, we showed that alteration of *VPS13* expression affects MMC identity, thus affecting the correct progression of megasporogenesis. Our results provide insights into the molecular mechanisms that determine the correct differentiation and development of the female germline and pave the way for a better understanding of the hub factors determining this process.

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1 = Multidisciplinary approach to unravel the relations between vine hydraulics, pedo-climatic conditions and yield in Falanghina grapevine

Nicola Damiano[1], Giovanna Battipaglia[2], Paolo Cherubini[3], Antonello Bonfante[4], Chiara Amitrano[1], Chiara Cirillo[1], Arturo Erbaggio[5], Veronica De Micco[1]
[1]Dipartimento di Agraria, Università degli Studi di Napoli Federico II, Via Università 100-80055, Portici; [2]Dipartimento di Scienze e Tecnologie Ambientali, Biologiche e Farmaceutiche, Università della Campania Luigi Vanvitelli Via Vivaldi, 43- 81100, Caserta; [3]Swiss Federal Research Institute for Forest, Snow and Landscape Research, Zürcherstrasse 111, CH-8903 Birmensdorf (ZH); [4]Dipartimento di Scienze Biologiche ed Agroalimentari DiSBA, Istituto per i sistemi agricoli e forestali del mediterraneo, CNR, Piazzale Enrico Fermi 1 - Loc, Porto del Granatello, 80055, Portici; [5]Libero professionista

In a climate change scenario, with increasing temperature and frequency of extreme events, such as prolonged periods of drought, there is need to improve the resource use efficiency of crops (e.g. water use efficiency) and to increase yield, quality and stability of productions, especially in high profitability and vulnerable crops as grapevine, traditionally cultivated in Mediterranean regions. Therefore, the improvement of knowledge about the plasticity of morpho-functional traits in vines and their responses to environmental constraints will be necessary to achieve a correct management of cultivation factors towards sustainability.
The objective of this study is to reconstruct past vine hydraulic behaviour by analysing tree-ring series from the stemwood of vines in four vineyards of *Vitis vinifera* L. subsp. *vinifera* 'Falanghina'. The vineyards were located in southern Italy (La Guardiense farm, Benevento, Campania region) and were characterised by different pedoclimatic conditions. Wood cores were extracted by the vine trunk and prepared for microscopy and stable isotope analyses to quantify functional wood anatomical traits and $\delta^{13}C$ to assess plant water use efficiency. The relations between tree-ring traits, both anatomical and isotopic, and climatic parameters were analysed in order to evaluate the plasticity of Falanghina with regards to changing environmental conditions.
In-vivo measurements based on morphological and eco-physiological parameters as well as UAV techniques were also applied in 2019, in order to check the relations among wood biomass production, hydraulic traits, physiological parameters, yield and must traits.
All parameters linked with vine hydraulics, resource use and growth efficiency showed a site-specific precise coordination linked with different water and resource availability as influenced by pedo-climatic conditions. The different vines hydraulic behaviour at the four sites, derived from the analysis of the tree-ring series (Fig. 1) and confirmed by *in-vivo* plant monitoring, contributed to different vines productivity and quality of musts. The isotopic signal of wood and must showed a similar trend, suggesting that they both record the same ecophysiological information which was in line with evidence derived from quantitative wood anatomy. These innovative results suggest the possibility to use must as a good matrix to perform carbon isotope analysis and derive information on plant water use in response to pedo-climatic factors (OIV-VITI 517-2015). Therefore, there is evidence that $\delta^{13}C$ assessment in must can be used profitably in zoning processes.
The overall information gained through the proposed methodological approach seem to be promising to better understand plant-environment relations in the continuum soil/plant/atmosphere, also to forecast future vine behaviour and developing a wine quality functional irrigation strategy.

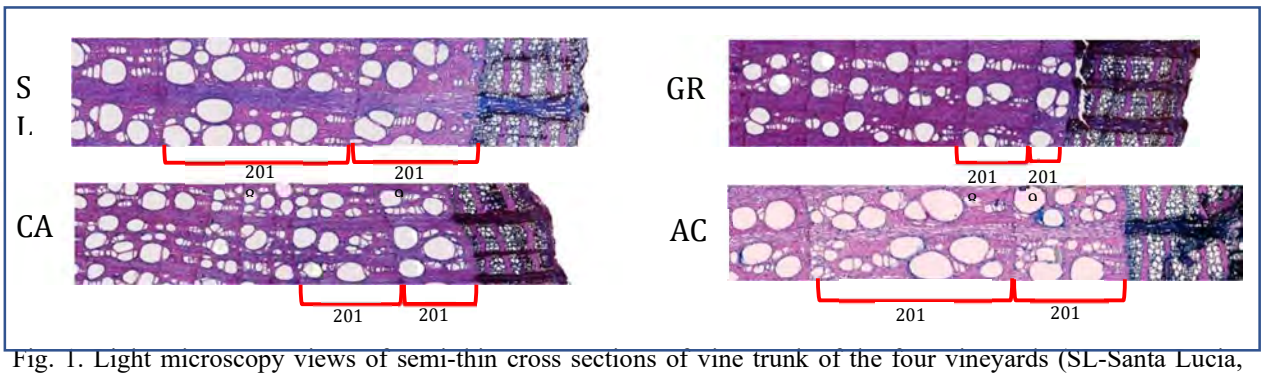

Fig. 1. Light microscopy views of semi-thin cross sections of vine trunk of the four vineyards (SL-Santa Lucia, CA-Calvese, GR-Grottole, AC-Acquefredde), showing different biomass accumulation and wood traits as affected by different pedoclimatic conditions.

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1 = The role of pollination in controlling *Ginkgo biloba* ovule development

Greta D'Apice*[1,2], Silvia Moschin*[1,2], Fabrizio Araniti[3], Sebastiano Nigris[1,2], Maurizio di Marzo[4], Antonella Muto[5], Camilla Banfi[4], Leonardo Bruno[5], Lucia Colombo[4], Barbara Baldan[1,2]
[1]Botanical Garden, University of Padova, 35123 Padova, Italy; [2]Department of Biology, University of Padova, 35121 Padova, Italy; [3]Department of Agricultural and Environmental Sciences, University of Milan, 20133 Milan, Italy; [4]Department of Biosciences, University of Milan, 20133 Milan, Italy; [5]Department of Biology, Ecology and Earth Sciences (DiBEST), University of Calabria, 87036 Arcavacata of Rende (CS), Italy

In angiosperms the fertilization of the central cell of the female gametophyte triggers the activation of the seed coat developmental processes: the post-fertilization production of auxin in the endosperm downregulates the Polycomb Repressing Complex 2 (PRC2) gene expression, activating the downstream pathways, such as gibberellin (GA) biosynthesis and the accumulation of flavonoids needed for seed coat differentiation. Differently, in gymnosperms pollination and fertilization events are temporally separated and the developmental processes leading the switch from ovule integument into seed coat are still unknown. The species under investigation is *Ginkgo biloba*, a gymnosperm characterised by a single ovule integument that differentiates into three layers upon maturity. The single ovule integument of *Ginkgo* acquires the typical characteristics of the seed coat long before the fertilization event, differentiating an outer and fleshy *sarcotesta* rich of fatty acids, a middle and lignified *sclerotesta*, and an inner and papery *endotesta*.
In this study we investigated whether pollination is the triggering event that leads to the transformation of the ovule integument into the seed coat in *Ginkgo*, since from the pollination till the fertilization about four months pass, and meanwhile ovules undergo several morphological modifications. During this time frame ovules differentiate the three seed coat layers and prepare themselves for the fertilization.
We produced a morphological atlas describing the developmental stages of *Ginkgo* ovule development from ovule initiation until embryo formation, and we performed transcriptomics and metabolomics analyses on ovules just prior and after pollination in order to test our hypothesis. Omics analyses allowed an accurate description of the main changes that occur in *Ginkgo* ovules during the pollination timeframe, highlighting that the metabolic pathways involved in the lignin biosynthesis and in the production of fatty acids are activated upon pollination. This suggests that seed coat formation begins prior to fertilization, right after the pollination has happened, supporting the hypothesis of the crucial role of the pollen arrival on the progression of ovule development in *Ginkgo biloba*.

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1 = Radiation-induced morpho-anatomical and nutritional responses of *Brassica rapa* L. microgreens: is there a dependence on the target of irradiation?

Sara De Francesco[1], Giulia Costanzo[1], Chiara Amitrano[1], Ermenegilda Vitale[2], Mariagabriella Pugliese[3], Cecilia Arrichiello[4], Gianluca Ametrano[4], Paolo Muto[4], Carmen Arena[2], Veronica De Micco[1]
[1]Department of of Agricultural Science, University of Naples Federico II, Via Università 100, 80055, Portici, Italy; [2]Department of Biology, University of Naples Federico II, Via Cinthia 21, 80126, Naples, Italy; [3]Department of Physics, University of Naples Federico II, Via Cinthia 21, 80126, Naples, Italy; [4]Radiotherapy Unit, Istituto Nazionale Tumori - IRCCS - Fondazione G. Pascale, Via Semmola 53, 80131, Naples, Italy.

The permanence of man in Space will increase in sight of future Space exploration, thus creating a need for the production of food onboard to guarantee the astronauts an adequate and balanced diet, with a minimum supply from Earth. In this context, higher plants could play a key role in Space nutrition, providing fresh food rich in bioactive compounds useful to counteract the adverse effects of Space environmental factors on human health. However, cosmic radiation in Space represents a significant issue not only for humans, but also for plants, with potential critical outcomes on morpho-anatomical, eco-physiological and biochemical aspects depending on plant species, cultivar and development stage at the time of radiation.
In this study, we analyzed the effect of different doses of X-rays (0-control, 0.3, 1,10, 20, and 30 Gy) on morpho-anatomical and nutritional traits of microgreens of *Brassica rapa* L. subsp. *sylvestris* var. *esculenta*. Irradiation was performed on two different developmental stages, namely dry and germinated seeds. After the irradiation treatments, both seed types were incubated, and microgreens were cultivated in controlled conditions. At harvest, morpho-biometric traits such as stem elongation (Fig. 1), fresh and dry biomass, and total leaf area were measured. Leaf functional anatomical traits (e.g., lamina thickness, localization of phenolics, stomatal frequency) were quantified through light and epi-fluorescence microscopy and digital image analysis (Fig. 2). The nutritional value of the above-ground biomass was evaluated in terms of antioxidants and chlorophyll content (Fig. 3). The results showed that the outcomes of radiation are dose-specific and dependent on the irradiated target stage. The information gained from this study is valuable for: a) understanding the mechanisms of radioresistance; b) assessing the impact of radiation on plant growth at early stages of development, critical for the establishment of cultivations in Bioregenerative Life Support Systems (BLSS); c) evaluating the possibility to produce plant-derived fresh food rich in antioxidants directly onboard.

Fig. 1a-1b. Morpho-biometric measures of *Brassica* microgreens.

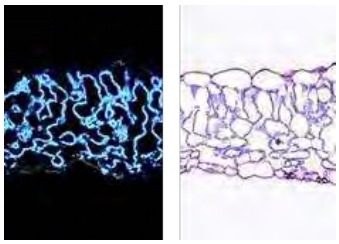

Fig. 2. Light and epi-fluorescence microscopy view of leaf lamina of *Brassica* microgreens.

Fig. 3. Extraction of photosynthetic pigments from *Brassica* microgreens.

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1 = Green Harmonic Solution technology: A preliminary study on the effects of acoustic stimuli on morpho-functional and biochemical attributes of Solanaceae

Marcello Salvatore Lenucci[1], Riccardo Tornese[1], Simone Gatto[2], Anna Montefusco[1]
[1]Department of Biological and Environmental Sciences and Technologies, University of Salento, Via Prov.le Lecce-Monteroni - Campus Ecotekne, 73100 Lecce, Italy; [2]Study of music therapy and creative sciences "Qui ed Ora", Via Ardigò 1, 73048 Nardò, Lecce, Italy

It is widely accepted that hearing only pertains to the kingdom Animalia. However, one might step back to a more general belief where the sense of hearing is simply regarded as the response to acoustic (i.e. vibrational) stimuli by a set of mechanoreceptors triggering specific behaviors. Under this broad view, the hypothesis that other kingdoms might have the capability of "hearing" cannot be ruled out a priori.
Plants, as sessile organisms, must constantly adapt growth and development to the dynamic and complex conditions of the environment they live in. This plasticity rests on the ability they evolved to perceive, integrate and react adequately to a myriad of exogenous stimuli, which synchronize physiological and morphogenetic processes with seasonal changes and minimize the effects of environmental perturbations, ultimately guaranteeing survival. Over the past centuries, research has shown that plants respond to several environmental factors including light, temperature, humidity and mechanical perturbations, however responses to sound vibrations, a subset of mechanical stimuli, have received attention only recently. Indeed, good evidence supports the idea that plants do respond to periodic mechanical signals transmitted through air, as those produced by the wingbeat of pollinator insects (1). Besides, growing evidence supports the idea that certain frequencies can affect, positively or negatively, the fitness and productivity of plants (2).
In this research a preliminary study was carried out to evaluate the effects of a prototypal technology, known as Green Harmonic Solution (GHS), for the acoustic treatment of plants on some morpho-functional and biochemical attributes of two important horticultural crops for the Italian agri-food sector: tomato (*Solanum lycopersicum* L., cultivar Tiburon) and aubergine (*Solanum melongena* L., cultivar Fantastic rz f1). The system, developed by the music therapist Simone Gatto, provides a continuous treatment of "sound irrigation", which spreads by air a combination of vibrational stimuli (melodic scales and sequences, harmonic interferences, isochronic tones, ambient sounds to attract predators and parasites, etc.) through suitable loudspeakers. Differently from other systems [e.g. the Plant Acoustic Frequency Technology (PAFT; 3) developed at the Brawijaya University, Indonesia], the GHS combines the air sound diffusion with the ground propagation of specific harmonic intervals ranging from 32 to 7758 Hz (Circle of fifths) through electromagnetic transducers, disposed according to a precise geometry (Pythagorean lambdoma), that sink 40 cm in the soil. Plants were grown in a greenhouse at the Falconieri farm (Nardò, Lecce, Italy) during the spring-summer season 2019 (tomato) and the winter-spring season 2020 (aubergine). Controls were simultaneously cultivated at a distance from the system that guarantees a negligible sound pressure.
Our results, although preliminary, show a significant increase in plant height (34% and 7%), average leaf weight (54% and 89%) and area (26% and 25%), fruit weight and size (112% and 45%) in both tomatoes and eggplants, respectively. The contents of chlorophylls, carotenoids and vitamin C of ripe tomato and aubergine ripe fruits, as well as their TEAC (Trolox Equivalent Antioxidant Capacity) values were also positively affected by the GSH treatment. Although specific acoustic frequencies have been reported to positively influence the phenol and flavonoid contents of some plants (4), GSH treatment did not change the phenolic metabolism in both crops.
In conclusion, the GSH technology deserves further investigation to evaluate the effects it causes on plants. Indeed, the large-scale application of these innovative technologies may reduce the need for chemical fertilizers and pesticides, with immediate benefit for the agricultural sector by increasing the productivity and quality attributes of crops in a sustainable and eco-compatible way and helping to mitigate the impact of agriculture on environmental pollution and ensure greater well-being of plants, animals and humans.

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1 = Effect of plant apocarotenoids on plant-microbe interactions

Teresa Mazzarella[1], Jian You Wang[2], Cristina Votta[1], Salim Al- Babili[2] Luisa Lanfranco[1], Valentina Fiorilli[1]
[1]Department of Life Sciences and Systems Biology, University of Torino, Viale Mattioli 25, 10125 Torino, Italy; [2]King Abdullah University of Science and Technology, Division of Biological and Environmental Science and Engineering, the BioActives Lab, Thuwal, 23955-6900, Saudi Arabia

Carotenoids are important for all clades of life; however, their synthesis is restricted to photosynthetic organisms and some non-photosynthetic fungi and bacteria. In plants, carotenoid biosynthesis is a vital metabolic pathway, as it provides the pigments that enable photosynthesis. Carotenoid cleavage leads to products called apocarotenoids that include diverse set of metabolites from volatiles, colorants to signaling/regulatory molecules and hormones. Among apocarotenoid phytohormones, strigolactones (SLs), play a key role in shaping plant architecture and in promoting the establishment of arbuscular mycorrhizal (AM) symbiosis. Recently, we have identified zaxinone, an apocarotenoid required for a correct growth and mycorrhization of rice (1). In addition, zaxinone treatment suppresses transcript level of SLs biosynthesis genes leading to reduced SLs content in both root tissues and exudates.
Several plant pathogenic fungi were shown to be sensitive to the synthetic SLs analog (GR24) and a fungal growth inhibition was often observed upon treatment. To gain further insights into the effect of plant apocarotenoids on the growth of pathogenic fungi we exposed *Botrytis cinerea* and *Cryphonectria parasitica* to zaxinone, zaxinone analogs (Mizax3; Mizax5; 2), carlactonoic acid (a SLs precursor), TIS108 (a SLs biosynthesis inhibitor), β-ionone (a volatile compound), and GR24, as a positive control. We evaluated the *in vitro* growth, in terms of colony diameter, in a time point experiment where each fungus was exposed to a single compound embedded in the solid medium. GR24 and TIS108 reduced the radial growth of both fungi while Mizax3 only affected *C. parasitica*. Zaxinone and β-ionone treatment led to an increase of radial growth in *B. cinerea*, while, by contrast, it induced a growth inhibition of *C. parasitica*. In addition, to verify whether zaxinone, Mizax3, GR24 and TIS108 could affect the plant-pathogen interaction, we performed a pathogenicity test using the model system of *B. cinerea*-infected tomato leaves in the presence/absence of the specific apocarotenoid. GR24-treated plants showed a reduced percentage of infected leaves and reduced necrotic areas, in line with the direct inhibitory effect observed on the free-living mycelium. An opposite trend was detected on zaxinone-treated plants where both disease parameters showed higher values compared to non-treated plants. In their whole, these data highlight the potentials of some apocarotenoids as anti-fungal compounds and suggest complex mechanisms of action of these compounds within the host plant.

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https://drive.google.com/file/d/1982u9a1gptp1c8aM38nFxy4zpvz6htgp/view?usp=sharing

1 = Effects of compost amendment on glycophyte and halophyte crops grown on saline soils: isolation and characterization of rhizobacteria with plant growth promoting features and high salt resistance

Gianmaria Oliva[1], Francesco Guarino[1], Giovanni Vigliotta[1], Stefano Castiglione[1], Angela Cicatelli[1]
[1]Department of Chemistry and Biology "A. Zambelli", University of Salerno, Fisciano (SA), Italy

Climate change is already well-established, as well as the consequent loss of biodiversity, and they are the greatest challenge that our planet has ever faced with. The increase in global average temperatures is, in fact, compromising Earth climate and its effects, if not counteracted with concrete actions, are bound to worsen in the coming years. The climate change occurs as variations in average weather conditions (*i.e.*, start date wet season at the tropics), or in the frequency of extreme weather events (such as floods, storms, hurricanes, etc.). Due to long periods of drought, the excessive exploitation of water resources and the irrigation of agricultural fields with scarce quality waters, and rich of salts has led to the problem of soil salinization, and it is, therefore, one of the most critical environmental factors limiting crop productivity, since many of them are glycophyte (*i.e.*, plants sensitive to salinity). Thus, it is desirable to search for biotechnologies suitable not only for improving crop productivity, but also for recovering arid and saline unproductive lands. A good solution for this problem could be related to the use of compost for soil amendment, and/or to that of plant growth promoting rhizobacteria (PGPR). The goals of our research were to: i) evaluate the benefits of soil compost amendment on different crops (quinoa, tomato, maize, sunflower) irrigated either with 150 or 300 mM NaCl solutions; ii) select halotolerant bacteria from plant rhizospheres. Our results indicated that soil compost amendment not only was able to promote crop growth and biomass, but also their resilience to the salt stress caused by very high soil electrical conductivity (EC; up to 20 dS m^{-1}). Moreover, since compost is an important source of plant beneficial microorganism, we were able to select and isolated 13 strains halotolerant and potentially growth promoter. Among the isolated ones, two strains showed some biochemical PGP features [*e.g.*, phosphate solubilization capacity, ammonia, IAA (Indole Acetic Acid) and siderophore production], but even resistance to high salt concentrations (4.0 M NaCl) and high temperatures (55 °C). The use of compost and/or PGPR could be a promising strategy for a sustainable agriculture, which would lead to a reduction of soil fertilization costs for farmers, but also to multiple benefits for crops and agricultural environments, such as an increased productivity, resistance to biotic and abiotic stresses, reduction in the use of mineral fertilizers in the perspective of probable and coming climate change.

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1 = Photosynthesis-related processes of two Italian varieties of olive tree (*Olea europaea* L.) are differently affected by UV-B radiation

Chiara Piccini[1,2], Giampiero Cai[2], Maria Celeste Dias[3], Marco Romi[1], Claudio Cantini[2]
[1]Department of Life Sciences, University of Siena, Via Mattioli 4, 53100 Siena, Italy; [2]Institute for BioEconomy, National Research Council of Italy, Azienda Agraria Santa Paolina Via Aurelia 49, 58022 Follonica, Italy; [3]Department of Life Sciences, University of Coimbra, Via Calçada Martim de Freitas, 3000-456 Coimbra, Portugal

Given the extreme importance of olive tree in our country in terms of economic, territorial, social and landscape resources, it is important to study its responses to stress agents such as excessive UV-B radiation. The aim is double: understanding the molecular basis of defense mechanisms are the varieties that mostly implement those mechanisms to cope with stress. In the light of the analysis carried out in this study, we argue that UV-B radiation is a dangerous source of stress for olive trees, especially in the current increasingly changing environmental condition. There are few studies in the literature on the relationship between olive trees and UV-B radiations; therefore, the extent of damage caused by this stress and, above all, the actions that plants implement to counter it and develop resistance are not yet known. Both varieties under study (Giarraffa and Olivastra Seggianese), although capable of tolerate the UV-B treatment, showed evident effects, albeit in different ways and at different times. The photosynthetic mechanism is the target of UV-B stress. Under optimal conditions, the light radiation reaches the pigments and then the photosystems, producing ATP and NADPH necessary for the Calvin cycle. This cycle of reactions, catalyzed primarily by the Rubisco enzyme, generates glyceraldehyde 3-phosphate which will subsequently give rise to all the sugars that plants need. Under conditions of excess UV radiation, a series of alterations occurs at the level of this mechanism. The data collected in this study indicate that the fluorescence value of chlorophyll does not undergo significant changes, but the performance index of the photosynthetic apparatus significantly decreases. This suggests that pigments are likely to be preserved but that the damage still affects the photosystems. Therefore, where the energy dissipation mechanisms are not sufficient to dissipate excess UV radiation, plants face photo-oxidative stress and ROS production which cause damage to DNA, proteins and lipids. To deal with, plants have implemented a complex defense system consisting of antioxidants such as polyphenols and flavonoids. Consequently, olive trees can guarantee the production of sugars essential for growth, such as sucrose. Additional glucose could be produced by degradation of starch; this would allow plants to activate alternative metabolic response pathways, for example the production of mannitol, a protective alcohol-sugar, as found in the case of the varieties under study. Results indicate that plants of both Olivastra Seggianese and Giarraffa subjected to UV-B treatment show clear signs of stress, albeit in a different way. Data showed that the two varieties have different response times and the Giarraffa variety seems better suited to prolonged UV-B stress. This is likely due to a more efficient and rapid activation of the antioxidant response (e.g., flavonoids) and because of its capacity to maintain the photosynthetic efficiency as well as a relatively higher content of mannitol. Moreover, lower levels of pigments after an extended period of UV-B exposure can also be an adaptation mechanism triggered by Giarraffa to reduce energy absorption under UV-B stress. Olivastra Seggianese seems less suited to UV-B stress for a prolonged period (e.g., higher reduction of Fv/Fm) and has a higher requirement for sugars (e.g., glucose) possible to counteract stress and to restore adequate energy levels.

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1 = Exploration of the leaf surface: discovering functional traits useful in PMs and PAHs air phytoremediation

Antonello Prigioniero[1], Daniela Zuzolo[1], Alessia Postiglione[1], Mariano Mercurio[1], Francesco Izzo[1], Marco Trifuoggi[2], Maria Toscanesi[2], Pierpaolo Scarano[1], Maria Tartaglia[1], Rosaria Sciarrillo[1], Carmine Guarino[1]
[1]Department of Science and Technology, University of Sannio, Via de Sanctis snc, 82100, Benevento, Italy; [2]Department of Chemical Sciences, University of Naples Federico II, Via Cintia, 80126 Naples, Italy

Particulate matter (PM) and polycyclic aromatic hydrocarbons (PAHs) are typical air contaminant in urban areas which may cause serious problems due to their harmful effects on health. In this study, we aimed at exploring the potential of plant species as air filters to improve air quality in these areas. Four plant species were chosen among the most frequent in the urban forest of the city of Naples: *Chamaerops humilis* L., *Citrus* × *aurantium* L., *Magnolia grandiflora* L., and *Quercus ilex* L. We focused on the relationships between particulate matter (PM) and polycyclic aromatic hydrocarbons (PAHs) leaf accumulation rates and the main species-specific leaf surface functional traits. We quantified PM_{10} and $PM_{2.5}$ deposited on leaf surfaces and trapped in cuticles by a double-step extraction, filtration and weighing. PAHs congeners extracted from entire and dewaxed leaves and analyzed by gas chromatography. For each plant species, we also assessed the morphological and chemical features of leaf surface. Cuticle thickness was measured by optical microscope, in epifluorescence and bright field. Stomata density, trichomes coverage and morphology were determined by optical microscope. Infrared analyses were carried out to investigate the functional group compositions of the leaves surfaces and assess the esterification index (ER). We found significant differences between the accumulations rates of both particulate fraction of the different species. It was found that $PM_{2.5}$ accumulation over the experimental period mainly involved *C. humilis*. The spectral features of both *Q. ilex* and *M. grandiflora*, highlighted the highest ER. Multivariate analyses allowed us to identify the principal directions, called principal components, in which the data varied. We found that solid phase pollutants (PMs and HMW PAHs) retention is mainly due to roughness of the leaf surface (influenced by trichome morphological complexity and coverage) and cuticle thickness. We also highlighted that the chemical composition of the cuticle influences this accumulation, finding that a lower esterification degree may be associated with the translocation of LMW and MMW PAHs in subcuticular tissues. We observed that variation in both morphological and chemical traits of leaf may influence the interception of particulate matter of different sizes, as well as various PAHs molecular weights. This study unveils the role of some plant functional traits in air phytoremediation and highlights distinctive features that may improve knowledge about Nature Based Solutions approaches.

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1 = Moss greening systems for building envelope

Enrica Roccotiello[1*], Dario Gisotti[1], Katia Perini[2], Paola Castellari[2], Andrea Giachetta[2], Claudia Turcato[1]
[1]Department of Earth, Environment and Life Sciences, University of Genoa, Corso Europa 26, 16132 Genoa, Italy; [2]Department of Architecture and Design, University of Genoa, Stradone di Sant'Agostino, 37, 16123 Genoa, Italy
*Corresponding author: enrica.roccotiello@unige.it

Vertical greening has been widely proposed as a valuable tool for the reduction of the urban heat island (1), the stormwater management (2), the contribution to the local biodiversity (3) and the health and wellbeing of the urban population (4).
Nevertheless, the high costs of installation and maintenance, the considerable weight and high water demand of most of these systems is a liability to the wide-scale application of traditional vertical greening for a sensible improvement of the life quality in urban areas. The use of mosses as the biotic component of vertical greening systems has been proposed as a viable solution to these issues (5).
The aim of the present study is to develop a low-cost and low-maintenance greening system based on mosses and to verify its applicability and performances in cities of the Mediterranean area.
We tested the growing capacity of 15 moss species on different building materials under controlled conditions in a plant growth chamber. These *taxa* were selected based on ecological, physical, and aesthetical features, to obtain a pool of species able to resist the abiotic stresses of urban environments, to grow quickly on the selected materials and efficient for pollution absorption and heat reduction.
The testers were prepared applying on the materials a patented moss paste (6). Testers of three species *(Barbula unguiculata*, *Grimmia pulvinata* and *Homalothecium sericeum)* were exposed outdoor in an intensely urbanized area to evaluate their capacity of retaining fine dust, in relation to the data from a nearby air quality monitoring station of ARPAL (Agenzia Regionale per la Protezione dell'Ambiente Ligure).
The best of the tested materials was selected to elaborate a modular panel with built-in irrigation that was tested in different setups to evaluate the water-holding capacity. The panels were subsequently covered with the moss paste of six different moss species, selected from the pool for their growth speed, tuft thickness and drought resistance.
The results obtained so far show that the use of mosses in built environments could represent an interesting and affordable solution for both horizontal and vertical surfaces.
This interdisciplinary study between plant biology and architecture provides a more comprehensive way to identify new perspectives for greening urban surfaces.

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1 = Gas exchanges and root morphology of lettuce plants grown with insect-treated bio-compost

Adriano Sofo[1], Antonino Zumbo[2], Giulio Scarpino[2], Benito Antonino D'Ambrosio[2], Antonio Gelsomino[2], Agostino Sorgonà[2]
[1]Department of European and Mediterranean Cultures: Architecture, Environment, Cultural Heritage, University of Basilicata, Via Lanera 20, 75100 Matera, Italy; [2]Department of Agriculture, University "Mediterranean" of Reggio Calabria, località Feo di Vito, snc, 89122, Reggio Calabria, Italy

Composted organic material is usually applied onto agricultural fields for soil fertility restoration, but in a broader perspective it represents an environmentally friendly strategy for the ecosystem management and the integrated *in situ/ex situ* conservation practices. Indeed, compost enhances the natural revegetation of degraded soils, increases the carbon storage and the plant diversity in grassland ecosystems, and is used in peat-free growing media for the *ex situ* conservation of rare, endangered and medicinal plant species. As an objective of a regional project, a bio-compost was obtained by an insect-assisted composting of different agro-industrial biowastes using black soldier fly (*Hermetia illucens* L.) (BSF) larvae (Fig. 1). According to the Italian Legislation (D. Lgs 75/2010), this bio-compost meets the limit values of several chemical, physical and microbiological parameters so that it can be used in agriculture as an organic amendment. However, agronomic tests are also needed before its use as a plant-conditioner. In this respect, the morpho-physiological responses of *Lactuca sativa* L. plants to bio-compost derived from an insect-based bioconversion of dry olive cake (DOC) mixed with poultry manure (CM) and olive leaves (OL) (CM:DOC:OL, 45%:45%:10%, w/w) were evaluated. The lettuce plants were cultivated in pots filled with soil:sand ratio (50/50, v/v) and fertilized with different bio-compost rates (1, 2 and 3 kg m^{-2}) (Fig. 2). The control plants were not treated with bio-compost fertilizer. Biometric parameters were evaluated as plant performance. The gas exchanges (photosynthetic and transpiration rates, stomatal conductance and water use efficiency intrinsic) were measured by LICOR 6400XT. The root morphological parameters (length, surface area, root length of different diameter classes, root length ratio, root mass ratio, root fineness and root tissue density) were analyzed by WINRHIZO system.
The results pointed out 1) an increase varying between 13.3-16.5% of plant fresh weight in all compost treatments, 2) an enhancement of the root length, surface area and fine root length at 2 kg m^{-2} of compost rate and 3) the improvement of the root length was determined by an increase of the biomass allocation, 4) these morphological performances were sustained by higher levels of photosynthetic rates although an increase of water losses by higher transpiration rates was observed.

Fig. 1. Larvae of *Hermetia illucens* L. in action on agro-industrial biowastes.

Fig. 2. Lettuce plants treated with 1, 2 and 3 kg m^{-2} of biocompost or not treated (C).

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1 = Treatment of landfill leachate with an experimental constructed wetland system of the hybrid type

Ivano Spiniello[1], Angela Cicatelli[1], Dario Tangredi[1], Stefano Castiglione[1], Francesco Guarino[1]
[1]Department of Chemistry and Biology "A. Zambelli", University of Salerno, Via Giovanni Paolo II, 132, 84084 Fisciano (SA), Italy

This study concerns the treatment of landfill leachates, from municipal solid waste, by means of innovative phytotechnology as alternative solutions to traditional wastewater treatments. The main issue in landfill leachate treatment is the extremely high concentrations of ammonia, organic nitrogen and carbon. The scientific guidelines, provided by the decision of the European Commission of 10 August 2018, n.2018/1147/EU, established a list of best available technologies (BAT) suitable for landfill leachate treatment. Therefore, the most used ones, on the basis of the organic matter biodegradability degree, are: biological oxidation to active sludge, Advanced chemical oxidation (*e.g.,* Fenton processes), advanced chemical oxidation using ozone and Membrane BioReactor (MBR). An alternative green and landscape friendly technology exploitable for landfill leachate treatment is the phytoremediation by means of constructed wetlands (CWs), which are artificial wetlands areas mimicking the natural ones where the combined action of natural processes and ecological networks, such as plants (hydrophytes), microorganisms and aggregate materials contribute to pollutant degradation and/or removal. At present, the engineer literature suggests that the ratio of the needed area per equivalent inhabitants (m^2 PE^{-1}) and this has represented one of the main limiting factors for their large uses. Different ratios are used from country to country (4 to 10 m^2 PE^{-1}) for this reason the main goal of our research was to reduce this ratio. To this purpose, we designed an innovative CW pilot plant, with multiple layers of stratification (*e.g.*, using compost as source of beneficial microorganisms). The CW pilot plant scheme is reported in the figure below. The phases of the cycle treatment are: 1-Neutralization, 2-Oxidation-Nitrification, 3-HSSF filter bed (Sub-surface horizontal flow), 4-VF filter bed (vertical flow), 5-Discharge-Recirculation. The configuration process of the CW is, from hydraulic point of view, a hybrid plant where a HSSF (phase 3) is combined with a VF (phase 4). The pilot plant, filled with sand of different granulometry, carrier with high surface-volume ratio and compost, was conditioned with compost tea as additional source of plant beneficial microorganisms. The plant species used in the pilot plant were *Phragmites australis* (Cav.) Trin. ex Steud., *Arundo donax* L., and *Arundo plinii* Turra. Then, different landfill leachates (young and old) underwent the treatment, and the removal of their main pollutants (organic carbon, total nitrogen, ammonia, chlorides and metals) were assayed during the experiment as well the effect of the leachate on the different used plant species. The results showed that, after ten days of leachate recirculation in the pilot plant, the pollutant removal/degradation percentage ranged between 75 – 100% and, therefore, the outlet respected the final effluent quality target foreseen by Italian legislation (D.Lgs. 152/06 All. 5 P. Terza, Tab. 3). In the light of these results, the CW pilot plant has proved to be a suitable alternative to the traditional water treatment systems for both biological and chemical-physical processes. It is also noteworthy that the reduction of the CW surface area per PE reached 1.0 m^2. For this reason, the CWs may be, in the next future, the most viable solution for *in-situ* treatment of landfill leachate with important implications for economic and environmental perspective.

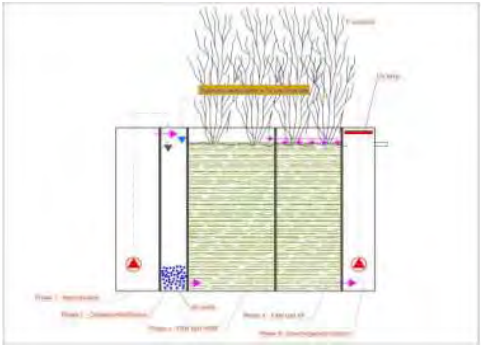

Fig. 1. Scheme of the hydraulic flow of the pilot plant.

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1 = Bioremediation of pluricontaminated soils: insights from a tripartite metatranscriptomics

Maria Tartaglia[1], Rosaria Sciarrillo[1], Daniela Zuzolo[1], Alessia Postiglione[1], Antonello Prigioniero[1], Pierpaolo Scarano[1], Maria Antonietta Ranauda[1], Valentino Ruggieri[2], Carmine Guarino[1*]
[1]Department of Science and Technologies, University of Sannio, Via de Sanctis snc, 82100 Benevento, Italy; [2]Biomeets Consulting, Carrer d'Àlaba, 61, Barcelona, Spain
[*]Corresponding author. Tel.: +39 824305156; fax: +39 824305151 E-mail address: guarino@unisannio.it (C. Guarino).

Phytoremediation involving the use of microorganisms with accumulating plant species represent a new frontier for on-site remediation of pluricontaminated soils. In this study the effectiveness of a biotechnological strategy, involving the use of Festuca arundinacea and a pool of microorganisms, was assessed by a mesocosm experiment and an in-deep rhizospheric metatranscriptomic analysis. The chemical profile of mesocosm soil at the end of the experiment (240 days) showed that the reduction of trace elements such as Cd, Hg, Pb, Sn, Tl, V and Zn in the soil was enhanced by our biological combination. In addition, also the organic pollutants (PAHs and PCBs) were strongly reduced up to 40.5 %. About two million transcritpts were identified and used for taxonomic and functional profiling. Transcripts read counts, tripartite among plant, bacteria and fungi were identified and quantified to provide an overview of the complex soil community composition. We observed that Actinobacteria and fungi abundance might be involved in remediation success. Functional analyses showed that trehalose biosynthesis as well as the antioxidant activity might have played a key-role in metaorganism effective interactions. The biotechnological approach remodelled the transcriptional profile towards organic pollutants degradation and heavy metal stress response.

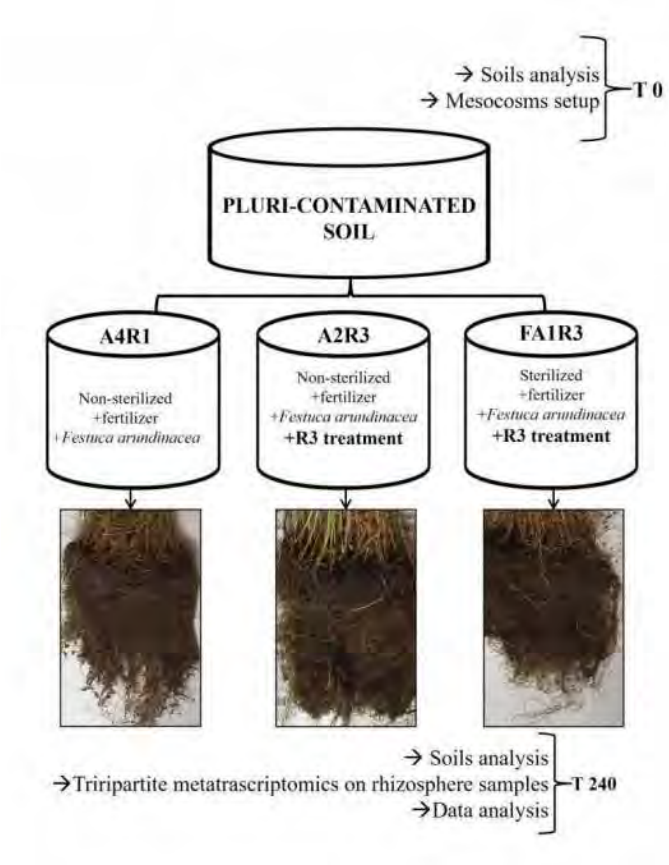

Fig. 1. Experimental design.

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1 = Impact of exogenous short-chain chitin oligomers application on *Medicago truncatula* root transcriptome

Veronica Volpe[1], Teresa Mazzarella[1], Andrea Crosino[1], Matteo Chialva[1], Andrea Genre[1]
[1]Dipartimento di Scienze della Vita e Biologia dei Sistemi, Viale Mattioli 25, 10125, Torino
veronica.volpe@unito.it

Arbuscular mycorrhizal (AM) association is an ancient symbiosis providing mineral nutrients and water to most crop plants. Symbiosis establishment is anticipated by a reciprocal exchange of chemical signals between fungi and host plants. The fungal perception of root exuded strigolactones, a class of carotenoid-derived molecules, boosts the release of plant-directed signals, called Myc-factors. Among them, tetra/penta-chitoligosaccharides (CO) activate symbiotic signalling in plant hosts, including Ca^{2+} spiking in the nuclei of root epidermal cells.
Here we applied exogenous CO, derived from crustacean exoskeleton, to pot-grown *Medicago truncatula* inoculated with the AM fungus *Funneliformis mosseae* during a time-course (10, 14, 21, 28 days) to investigate early and late root transcriptional responses using an RNA-seq approach. Transcriptome analysis was performed on inoculated and non-inoculated plants, with or without CO treatments.
A first global analysis revealed a few general trends: an increase in gene expression correlated with the progressive development of the symbiosis; a time-dependent decrease in the impact of CO treatment; a stronger effect of CO application at 10 days, suggesting an impact of the exogenous molecules on early symbiotic signaling. Furthermore, our analyses indicate an anticipated activation of intracellular accommodation processes in mycorrhizal CO treated plants, in line with the more extensive colonization observed at later time points. Alongside symbiosis promotion, CO treatment caused a general upregulation of the strigolactone biosynthetic pathway and induced a partial repression of plant defense. A strong downregulation was also recorded in several genes belonging to secondary metabolism, which can also be related to defense responses.

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2 = Phenolic content and potential health promoting properties of *Brassica incana* subsp. *raimondoi* (Brassicaceae) extract

Rosaria Acquaviva[1], Natalizia Miceli[2], Maria Fernanda Taviano[2], Federica Davì[2,3], Emilia Cavò[2,3], Barbara Tomasello[1], Irina Naletova[1], Anahi Elena Ada Bucchini[4], Vivienne Spadaro[5], Salvatore Ragusa[6], Giuseppe Antonio Malfa[1]

[1]Department of Drug and Health Sciences, Section of Biochemistry, University of Catania, Viale Andrea Doria 6, Italy; [2]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Viale Palatucci, 98168 Messina, Italy; [3]Foundation "Prof. Antonio Imbesi", University of Messina, Piazza Pugliatti 1, 98122 Messina, Italy; [4]Orto Botanico Center, Department of Biomolecular Sciences, University of Urbino "Carlo Bo", Via Donato Bramante 28, 61029, Urbino; [5]Department STEBICEF/Sect. of Botany, Anthropology and Zoology, University of Palermo, Via Archirafi 38, 90123, Palermo, Italy; [6]Department of Health Sciences, University "Magna Graecia" of Catanzaro, Viale Europa, Località Germaneto, 88100, Catanzaro, Italy

Several species of *Brassica* sect. *Brassica* show nutritional, pharmaceutical and industrial importance. Endemic *Brassica* species have been an important source of food in the diet of Sicilian populations for centuries as a part of the Mediterranean diet and several studies documented the nutritional and the medicinal properties of the edible wild plants respect to the cultivated crops.

Despite the large numerous biological activities reported for several species and cultivars belonging to *Brassicaceae*, not many studies have been conducted on the wild Sicilian taxa.

Brassica incana subsp. *raimondoi* (Sciandr., C. Brullo, Brullo, Giusso, Miniss. & Salmeri) Raimondo & Spadaro, belongs to *Brassica* sect. *Brassica* (Brassicaceae). It is a peculiar taxon, present in a very small population on the limestone cliffs facing north or east, at an altitude of 400–500 m a.s.l. of Castelmola, a little town close to Taormina (Sicily).

Due to the set of discriminating characters of the taxon described by Sciandrello & al., this taxon - according to our interpretation already expressed – can retain its autonomy in the subspecific rank; this is also based on the systematic approach followed in our studies on Sicilian wild brassicas. It is a suffruticose plant, 100–150 cm tall, easily distinguished by the white color of the flowers and by the hairy robust main stem often branching from the base. The slightly hairy leaves present a long petiole (5-18 cm) with an-ovate to ovate-laceolate shape, the rounded lobes show dentate margin.

The aim of the present research was to evaluate the qualitative and quantitative phytochemical profile and some biological effects of *B. incana* subsp. *raimondoi* leaves hydroalcoholic extract.

The plant leaves were collected, dried, and extracted for 1h at cold temperature with methanol-water (8:2) under stirring, for twice.

The total phenolic, flavonoid and condensed tannin contents of the extract were determined spectrophotometrically, resulting equal to 38.12 ± 0.50 mg GAE/g extract, 8.45 ± 0.60 mg QE/g extract and 4.70 ± 0.07 mg CE/g extract, respectively. Preliminary qualitative HPLC analysis confirmed the presence of kaempferol and derivatives and of isorhamnetin and derivatives.

The antioxidant properties were explored by cell free *in vitro* methods, to evaluate the different mechanisms by which the diverse antioxidant compounds contained in the phytocomplex could exert their effect: DPPH, reducing power and ferrous ions chelating activity assays. The extract exhibited radical scavenging activity in the DPPH test (IC_{50} = 1.33 ± 0.02 mg/ml), whereas it showed mild reducing power and no chelating properties.

The cytotoxic effects of the extract (10-50-100-200-400 µg/ml) were investigated on two different human cell lines, hepatocytes (HepG2) and fibroblasts (HFF-1). Moreover, the antioxidant activity was also evaluated in an *in vitro* cell system of oxidative stress assessing cellular ROS levels and non proteic total thiols (RSH) groups amounts. Data obtained showed that *B. raimondoi* leaves extract did not exert cytotoxicity on human cell lines and was able to counteract oxidative stress induced by H_2O_2 treatment on HepG2 cells, significantly decreasing ROS levels in a concentration-depending manner. These results were confirmed by increased amount of intracellular RSH levels.

Finally, no toxicity against brine shrimp larvae (*Artemia salina* Leach) was found for the extract, which indicated its potential safety.

These results suggest the health promoting properties of *B. incana* subsp. *raimondoi* and its potential as nutraceutical source.

Acknowledgements: The authors wish to thank the Foundation "Prof. Antonio Imbesi" for financial support, and *PLANTA*/Center for Research, Documentation and Training (Palermo).

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2 = *Calluna vulgaris* (L.) Hull: investigation of the immunomodulatory mechanism in human mononuclear cells

Marco Biagi[1], Giulia Baini[1], Anna Rosa Magnano[1], Elisabetta Miraldi[1], Giorgio Cappellucci[1]
[1]Department of Physical Sciences, Earth and Environment, University of Siena, Via Laterina 8, 53100 Siena, Italy

Calluna vulgaris (L.) Hull is a species that belongs to the family of Ericaceae. It is a small evergreen shrub which grows in uplands, spread all over the Europe and in other countries of the world. Its high content of phenols and the biological activity of these compounds provided the basis for its beneficial effect as a medicinal plant: in ethnobotany, *C. vulgaris* aerial parts decoction has been used for urinary complaints and as antimicrobial.
In this work, first we characterized and compared the polyphenolic profile of water extracts of *C. vulgaris* collected in Tuscany, Piedmont, and South Tyrol (Italy), then we tested a chemically defined and optimized *C. vulgaris* water extract (CWE) for its immunomodulatory activity in an *in vitro* model on human Peripheral Blood Mononuclear Cells (PBMC).
Italian *C. vulgaris* aerial parts were found to mainly contain chlorogenic acid and its derivatives, and flavonoids; *C. vulgaris* samples collected in Tuscany, Piedmont, and South Tyrol were found to have a similar quali-quantitative phytochemical profile.
CWE (>10% polysaccharides, >4% total flavan-3-ols, >0.5% chlorogenic acid, >0.5% flavonoids as hyperoside) clearly modulated cytokines release in PBMC at 1-5-10 μg/ml at different treatment times; 5 μg/ml was found the most effective concentration after 24 hours of treatment. In these conditions, CWE markedly upregulated the cytokines IL-1β, IL-6, IL-8 and regulated IL-10 production to a lesser extent. In inflammatory conditions obtained with the stimulation of PBMC with bacterial lipopolysaccharide (LPS), CWE was able to revert the production of the cytokines IL-1β, IL-6 and IL-8. The investigation of the intracellular mechanism underlying CWE immune activity highlighted the involvement of the signaling pathway ERK1/2 MAPKs (Mitogen-Activated Protein Kinase).
We could conclude that *C. vulgaris* revealed a very interesting and sounding *in vitro* immunomodulatory effect clearly associated with specific molecular pathways worthy to be better investigated both in animal models and in human trials.

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2 = Lignans from the roots of *Daphne mucronata* Royle subsp. *linearifolia* (Hart) Halda (Thymelaeaceae) and their effects on angiogenesis

Anna Cacciola[1,2], Giuliana Donadio[3], Valentina Parisi[3], Valeria D'Angelo[2], Ammar Bader[4], Maria Paola Germanò[2]

[1]Foundation Prof. Antonio Imbesi, University of Messina, P.zza Pugliatti 1, 98122 Messina, Italy; [2]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences (Polo Annunziata), University of Messina, SS. Annunziata 98168 Messina, Italy; [3]Dipartimento di Farmacia, Università di Salerno, Via Giovanni Paolo II 132, 84084 Salerno, Italy; [4]Department of Pharmacognosy, Umm Al-Qura University, 21955 Makkah, Saudi Arabia

Angiogenesis, the growth of new blood vessels, is involved in several pathological conditions. Antiangiogenic therapy has become established in recent years as a promising strategy for cancer prevention and numerous efforts have been conducted on antiangiogenic drugs. Presently, the inhibition of angiogenesis is considered an important strategy for cancer treatment and related diseases. Accordingly, the research of new natural compounds which may inhibit angiogenesis could represent an important tool. In our continuous search for novel antiangiogenic agents from plant extracts, 10 lignans were isolated from the root of *Daphne mucronata* Royle subsp. *linearifolia* (Hart) Halda (Thymelaeaceae), a well-known species in Jordanian ethnomedicine to treat inflammation and rheumatism. The dried and powdered plant material was sequentially extracted with *n*-hexane, $CHCl_3$, $CHCl_3$:MeOH (9:1) and MeOH and then preliminarily evaluated on angiogenesis using *in vivo* chicken chorioallantoic membrane, a simple model that allows screening of a large number of samples in a short time. Chromatographic and spectroscopic analyses of active $CHCl_3$:MeOH extract afforded 15 polyphenols of which 10 lignans. These isolates were tested on the chorioallantoic membranes (CAMs). Results showed that pinoresinol-β-D-glucopyranoside, siringin and 3"-hydroxydaphnodorin J exhibited the best antiangiogenic response inducing a marked reduction of the microvasculature of the CAMs (from 44.61% to 55.75% of inhibition at 20 μM compared to control). In addition, the decrease of the hemoglobin content, as an index of vascular density in the treated CAMs, also supported the antiangiogenic activity of pinoresinol-β-D-glucopyranoside, siringin and 3"-hydroxydapnodorin J. Furthermore, as siringin and 3"-hydroxydapnodorin J showed to be powerful scavengers of DPPH radical (IC_{50} 0.11 mM and 0.32 mM, respectively), a potential beneficial role against the formation of dangerous and reactive species involved in many oxidative stress-related diseases may be not excluded.

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2 = Essential oils bearing specialized metabolites with potential acetylcholinesterase inhibitory activity

Francesca Capetti[1], Antonio Corgiat-Mecio[1], Cecilia Cagliero[1], Arianna Marengo[1], Giulia Mastellone[1], Carlo Bicchi[1], Patrizia Rubiolo[1], Barbara Sgorbini[1]
[1]Dipartimento di Scienza e Tecnologia del Farmaco, Università degli Studi di Torino, Via Pietro Giuria 9,10125 Turin, Italy

Alzheimer's disease (AD) is one of the most widespread neurodegenerative diseases involving dementia and mainly affecting people over 65 years of age. The therapy of early and moderate stages of AD is mainly based on acetylcholinesterase (AchE) inhibitors such as donepezil and galanthamine. The aim of this study is to explore new AChE inhibitors from plant volatile specialized metabolites. Seventy-one essential oils (EOs) from different plant species and botanical families (Annonaceae, Apiaceae, Asteraceae, Betulaceae, Burseraceae, Caryophyllaceae, Cupressaceae, Ericaceae, Geraniaceae, Lamiaceae, Lauraceae, Mirtaceae, Oleaceae, Pinacee, Piperaceae, Poaceae, Rosaceae, Rutaceae, Santalaceae, Stiracaceae, Verbenaceae, Zingiberaceae) were tested *in vitro* using a colorimetric assay based on Ellman's method. Each EO was chemically characterized using gas chromatography coupled to mass spectrometry (GC-MS). Promising AChE inhibitory activities were observed for the OEs of *Laurus nobilis* L., *Salvia officinalis* L., *Hyssopus officinalis* L., *Lavandula angustifolia* Mill., *Lavandula latifolia* Medik., *Origanum vulgare* L., *Rosmarinus officinalis* L. and *Chamaemelum nobile* L. According to literature data, the potentially bioactive constituents responsible for the AChE inhibition, of the screened EOs, are 1,8-cineole, α-pinene, carvacrol, linalool, linalyl acetate and camphor. Further experiments are currently being carried out to verify the biological activity of the above mentioned compounds and to investigate on potential antagonist, additive, or synergistic interactions among them. Furthermore, a bio-guided fractionation approach will be adopted to isolate and identify the active fractions/compounds of *Chamaemelum nobile* EO, which to the best of authors' knowledge have been poorly investigated as potential tyrosinase inhibitors. Despite further experiments are still required, these preliminary results suggest that EOs are a promising source of potential AChE inhibitors.

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2 = *Lavandula angustifolia* and *Coriandrum sativum* essential oils and their main constituent linalool protective effect against amyloid-β neurotoxicity

Lucia Caputo[1], Ilaria Piccialli[2], Roselia Ciccone[2], Vincenzo De Feo[1], Giuseppe Amato[1], Laura De Martino[1], Anna Pannaccione[2]
[1]Department of Pharmacy, University of Salerno, via Giovanni Paolo II,132, 84084 Fisciano, Italy; [2]Department of Neuroscience, Division of Pharmacology, Reproductive and Odontostomatological Sciences, School of Medicine, "Federico II" University of Naples, 80131 Naples, Italy

Alzheimer's disease (AD) is a neurodegenerative disorder leading to cognitive deficits and cognitive decline. Since no cure or preventing therapy is currently available to counteract AD, natural-derived compounds are investigated to find new potential neuroprotective agents for its treatment. In the present study, we tested the neuroprotective effect of *L. angustifolia* L. and *C. sativum* L. essential oils (EOs) and their main active constituent linalool, against the neurotoxicity elicited by $A\beta_{1-42}$ oligomers, a key molecular factor in the neurodegeneration of AD; in an *in vitro* model of AD consisting of neuronal growth factor (NGF)-differentiated rat pheochromocytoma (PC12) cells exposed to $A\beta_{1-42}$. Importantly, our findings on neuronally differentiated PC12 cells exposed to $A\beta_{1-42}$ oligomers are in accordance with previous *in vivo* studies reporting the neuroprotective potential of lavender and coriander EOs and linalool. The results of our *in vitro* experiments suggest that both lavender and coriander EOs at the concentration of 10 μg/mL are able to protect cells from the molecular damages induced by $A\beta_{1-42}$ oligomers, such as the activation of caspase-3, a key enzyme in the apoptotic cascade, and the increase of intracellular ROS production. Moreover, as it is the main component of both lavender and coriander EOs, was also tested the possible neuroprotective effect of linalool. Interestingly, also this compound at the concentration of 10 μg/mL was able to counteract $A\beta_{1-42}$ neurotoxicity.
These results suggest that linalool could be partially responsible for neuroprotective activities of EOs, despite further experiments are needed to investigate this aspect. In fact, it cannot be excluded that other chemical components of lavender and coriander EOs may exert different beneficial effects or act synergistically with linalool in preventing the $A\beta_{1-42}$ neurotoxic effects. In this context, linalool as well as lavender and coriander EOs emerge as natural products of potential interest in the treatment of AD. Subsequent biochemical studies along with functional analyses on neuronal cultures and animal models of AD will be performed to further understand the beneficial effects of these substances.

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2 = Phenolic constituents, antioxidant activity and toxicity assessment of the aerial part extracts from the infraspecific taxa of *Matthiola fruticulosa* (*Brassicaceae*) endemic to Sicily

Emilia Cavò[1,2], Natalizia Miceli[1], Federica Davì[1,2], Vivienne Spadaro[3], Francesco M. Raimondo[4], Salvatore Ragusa[5], Francesco Cacciola[6], Yassine Oulad El Majdoub[1], Luigi Mondello[1,7,8,9], Maria Fernanda Taviano[1]
[1]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Viale Palatucci, 98168 Messina, Italy; [2]Foundation "Prof. Antonio Imbesi", University of Messina, Piazza Pugliatti 1, 98122 Messina, Italy; [3]Department STEBICEF/Section of Botany, Anthropology and Zoology, University of Palermo, Via Archirafi 38, 90123 Palermo, Italy; [4]*PLANTA*/Research, Documentation and Training Center, Via Serraglio Vecchio 28, 90123 Palermo, Italy; [5]Department of Health Sciences, University "Magna Graecia" of Catanzaro, V. Europa, Località Germaneto, 88100 Catanzaro, Italy; [6]Department of Biomedical, Dental, Morphological and Functional Imaging Sciences, University of Messina, Via Consolare Valeria, 98125 Messina, Italy; [7]Chromaleont s.r.l., c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Viale Palatucci, 98168 Messina, Italy; [8]BeSep s.r.l., c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Viale Palatucci, 98168 Messina, Italy; [9]Department of Sciences and Technologies for Human and Environment, University Campus Bio-Medico of Rome, via Àlvaro del Portillo 21, 00128 Rome, Italy

Brassicaceae plants have been an attractive research topic for years, due the presence of a variety of bioactive metabolites with valuable potential applications in health improvement. In recent years, our research team has focused on the study of taxa that grow spontaneously in Sicily (Italy) included in the *Brassicaceae* family, with the aim of discovering new sources of bioactive compounds for their possible use in the pharmaceutical, nutraceutical and cosmetic fields. Currently, our research is addressed to the specific and infraspecific taxa comprised in the genus *Matthiola* R. Br.. In this work, the infraspecific taxa of *Matthiola fruticulosa* (L.) Maire have been selected, of which very limited information is available to date.
Matthiola fruticulosa (L.) Maire is a perennial species native to Southern Europe, Northern Africa and Western Asia. In the "Flora Europaea" and "Flora Hellenica", as well as in taxonomic database "The Plant List", three different subspecies are indicated under *M. fruticulosa*, i.e. the nominal subspecies, the subsp. *valesiaca* (Gay ex Gaudin) P. W. Ball, and the subsp. *perennis* (P. Conti) P. W. Ball. Instead, in the latest edition of the "Flora d'Italia" only two infraspecific subdivisions are recognized for this taxon, the nominal subspecies and the subsp. *coronopifolia* (Sm.) Giardina & Raimondo; both the subspecies occur in Sicily, as reported by Raimondo et al. (2010) in the "Checklist of the vascular flora of Sicily". The use of *M. fruticulosa* in the traditional medicine of Libya for the treatment of kidney stones and piles is reported.
This study aimed to define and compare the phenolic profile and the antioxidant properties of the hydroalcoholic extracts obtained from the aerial parts of both the *M. fruticulosa* infraspecific taxa endemic to Sicily. A comprehensive insight into the qualitative–quantitative profile of the polyphenolic constituents contained in the extracts was achieved by HPLC-PDA/ESI-MS analysis. A total of twenty polyphenols were identified in *M. fruticulosa* subsp. *fruticulosa*, eleven flavonoids and nine phenolic acids; on the other hand, eleven compounds were characterized in the subsp. *coronopifolia*, six flavonoids and five phenolic acids. The quantity of polyphenols detected in the extract of *M. fruticulosa* subsp. *fruticulosa* was found to be approximately 3-fold higher than that of the subsp. *coronopifolia* (151.6 mg/g extract and 51.8 mg/g extract), accounting the phenolic acids about one third of the total polyphenols for both extracts. Three *in vitro* methods, which rely on different mechanisms, were used for assessing the antioxidant activity: 1,1-diphenyl-2-picrylhydrazil (DPPH), reducing power and Fe^{2+} chelating activity assays. Butylated hydroxytoluene (BHT) and ethylenediaminetetraacetic acid (EDTA) were utilized as reference standards. The results of the antioxidant tests showed that the extracts possess different antioxidant ability: it is interesting to note, in fact, that the extract of *M. fruticulosa* subsp. *fruticulosa* exhibited much higher radical scavenging activity than that of the subsp. *coronopifolia* (IC_{50} = 1.25 ± 0.02 mg/mL and 2.86 ± 0.05 mg/mL), which in turn has been shown to be a better source of metal chelating antioxidants (IC_{50} = 1.49 ± 0.01 mg/mL and 0.63 ± 0.01 mg/mL). At last, *Artemia salina* lethality bioassay was performed for toxicity assessment. The results of the bioassay showed the absence of toxicity against brine shrimp larvae for both the extracts.
Overall, the obtained results improve the knowledge of the *Matthiola* taxa endemic to Sicily, also indicating the infraspecific taxa of *M. fruticulosa* as new and safe sources of antioxidant compounds included in the *Brassicaceae* family.

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2 = Giffonins, antioxidant diarylheptanoids from *Corylus avellana*, and their ability to prevent oxidative changes in human plasma proteins

Antonietta Cerulli[1], Milena Masullo[1], Gianluigi Lauro[1], Bogdan Kontek[2], Beata Olas[2], Giuseppe Bifulco[1], Sonia Piacente[1]
[1]Department of Pharmacia, University of Salerno, Via Giovanni Paolo II n. 132, 84084 Fisciano (SA), Italy; [2] Faculty of Biology and Environmental 510 Protection, Department of General Biochemistry, University of 511 Lodz, 90-236 Lodz, Poland

Corylus avellana L. (Betulaceae) (hazelnut tree) is one of the most popular nut trees on a worldwide basis. Hazelnut is a nutritious food with a high content of healthy lipids, used by the confectionary industry and consumed raw (with the skin) or roasted (without the skin) (1).
Italy is the second largest hazelnut producer in the world after Turkey, and 98% of its production is due to four regions: Latium, Piedmont, Sicily, and Campania.
In Italy, the cultivars "Tonda di Giffoni" and "Nocciola Piemonte" are very appreciated for their processing quality and their nut which received the Protected Geographical Indication (PGI) label by the European Union. Our previous investigations on the different parts (leaves, leafy covers, flowers and nuts) of *Corylus avellana* cultivar "Tonda di Giffoni" led to the discovery of giffonins A-V, unusual diarylheptanoids never reported before. Some giffonins showed the ability to prevent oxidative damage of human plasma lipids, induced by H_2O_2 and H_2O_2/Fe^{2+}, being more active than the antioxidant curcumin.
As part of our ongoing research on the antioxidant constituents of *C. avellana* polar extracts, a phytochemical investigation of the MeOH extracts of *C. avellana* cv. "Tonda di Giffoni" and "Nocciola Piemonte" leaves was performed. Analysis by LC-ESI/LTQOrbitrap/MS/MS of the MeOH extracts of the leaves allowed the isolation and identification of the new giffonins W (**1**) and X (**2**) along with 22 known related compounds. The structures of **1** and **2** were established by the extensive use of 1D- and 2D-NMR experiments along with ESIMS and HRMS analysis (Fig. 1). The relative configurations of the new compounds were assigned by a combined QM/NMR approach, comparing the experimental $^{13}C/^{1}H$ NMR chemical shift data and the related predicted values. The absolute configurations of **1** and **2** were established by analysis of electronic circular dichroism (ECD) spectra compared with the TDDFT simulated curves. New (**1** and **2**) and known giffonins (**3**-**11**) were evaluated as inhibitors of plasma lipid peroxidation induced by H_2O_2 and H_2O_2/Fe^{2+}. Giffonins with the highest inhibitory activity were tested for their ability to reduce oxidation of thiol groups and carbonylation in plasma proteins, and some of them exhibited higher antioxidant activity than curcumin, used as reference compound.

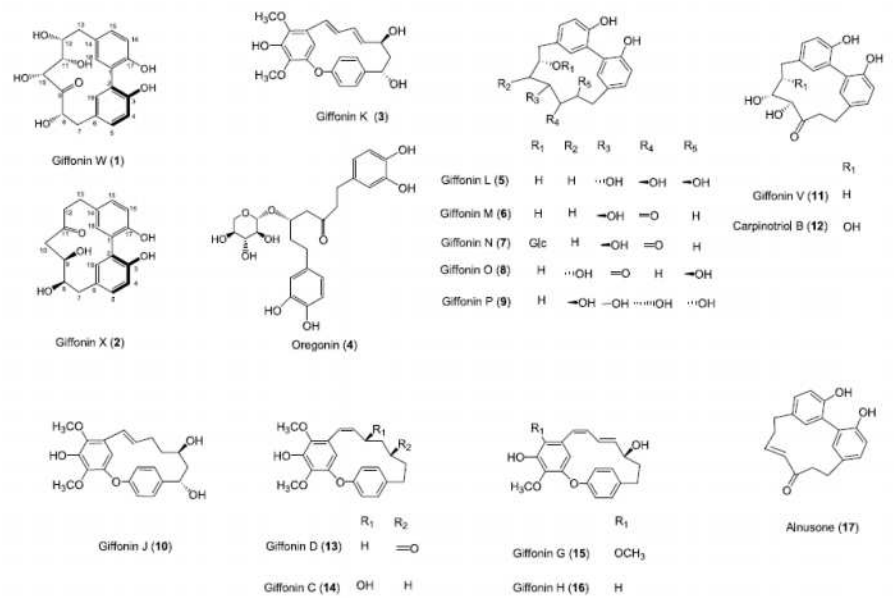

Fig. 1. Diarylheptanoids isolated from *C. avellana* leaves tested for their antioxidant activity.

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2 = ^{1}H NMR-based metabolomics of *Cynara cardunculus* L.: seasonal variation, flavonoid and phenolic content and antioxidant activity

Valentina Maria Daniele[1], Elisabetta Grillini[1], Ilaria Chiocchio[1], Manuela Mandrone[1], Cinzia Sanna[2], Ferruccio Poli[1].
[1]Department of Pharmacy and Biotechnology (FaBit), University of Bologna, Via Irnerio 42, 40126, Bologna, Italy; [2]Department of Life and Environmental Sciences, University of Cagliari, Via Sant'Ignazio da Laconi 13, 09123, Cagliari, Italy

Cynara cardunculus L. (Asteraceae) is a perennial plant native to the Mediterranean basin. It comprises three varieties: wild cardoon (var. *sylvestris*), domesticated cardoon (var. *altilis*) and globe artichoke (var. *scolymus*). This species is a perennial herb with an annual development cycle: by late spring the plant develops a leaf-branched floral scape bearing several heads and its reproductive cycle is completed by the summer.
It has been historically used as food, ornamental and medicine in Mediterranean tradition. In particular, in Sardinia (Italy), *C. cardunculus* aerial parts decoction is used as hepatoprotective, blood depurative, hypocholesterolemic, digestive and intestinal spasmolytic agent. Besides its traditional use, this plant is also considered a functional food for its numerous bioactive properties. In fact, *C. cardunculus* extracts were found active as anti-inflammatory, antioxidant, antimicrobial, hepatoprotective, anticarcinogenic and bile-expelling agent. This plant is enriched in several bioactive secondary metabolites such as phenolic acids, flavonoids and guaianolide type terpenes (i.e. cynaropricrin).
This work was aimed at studying the metabolomic profile of *C. cardunculus* (aerial parts) during spring and summer. In addition, total phenolic and flavonoid content and *in vitro* antioxidant activity were also evaluated in the two seasons. Thus, samples were harvested in five different Sardinian areas, the ^{1}H NMR-based metabolomic profile was analyzed, and data were treated through multivariate data analyses (PCA and OPLS-DA). According to these analyses, samples were highly differentiated in the two seasons (Fig. 1), in particular cynaropricrin (one of the most representative terpenes), malic acid and succinic acid were higher in summer, while spring samples are more enriched in flavone-types flavonoids. Moreover, during the spring season the harvesting site was also slightly affecting the metabolomic profile.
Both spring and summer samples were enriched in flavonoids and phenolics, in three areas (CyD, CyS, CyG) flavonoids were higher in spring (Fig. 2). This trend was reflected in the antioxidant potential, since the same three samples resulted more active in spring than in summer (Fig. 3). Moreover, it was found a positive statistical correlation between total phenolic and flavonoid content and antioxidant activity (p= 0.84 r^2= 0.7014). In conclusion, based on these results, if the highest level of cynaropricrin is expected *C. cardunculus* must be harvested in summer. Moreover, as shown by NMR-profiling, the composition and chemical structure of phenolic compounds and flavonoids vary considerably from summer to spring, and further studies are ongoing in order to chemically characterize these compounds.

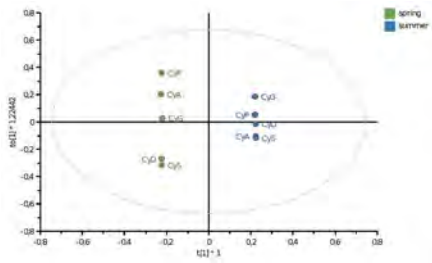

Fig. 1. OPLS-DA of *C. cardunculus* samples collected in spring and summer.

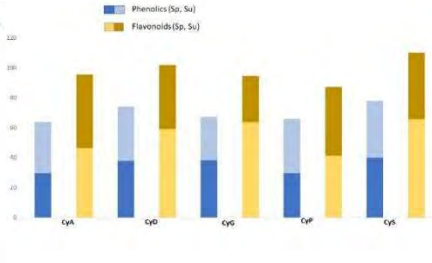

Fig. 2. Phenolic (mg gallic acid eq./g DW) and flavonoid (mg rutin eq./g DW) content of *C. cardunculus* harvested in five different Sardinian areas in summer and spring. Sp=spring; Su=summer; CyA=Sant'Antonio; CyD=Domusnovas; CyG=Gairo Taquisara; CyP=Porcu e Ludu; CyS=Siliqua.

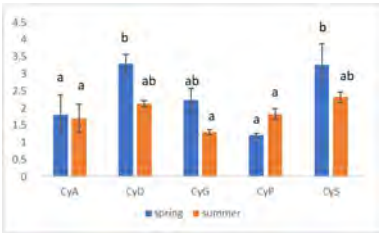

Fig. 3. Antioxidant activity of *C. cardunculus* harvested in five different areas in summer and spring.

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2 = Antioxidant and anti-inflammatory properties of *Imperatoria ostruthium* L. leaf extract

Cristina Danna[1], Miriam Bazzicalupo[1], Antonella Smeriglio[2], Marcella Denaro[2], Domenico Trombetta[2], Bruno Burlando[3], Laura Cornara[1]
[1]Department of DISTAV, University of Genoa, Corso Europa, 16132, Genoa, Italy; [2]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Via G. Palatucci, 98168 Messina, Italy; [3]Department of Pharmacy, University of Genoa, Corso Europa, 16132, Genoa, Italy

Imperatoria ostruthium L. (syn *Peucedanum ostruthium* (L.) W.D.J. Koch) is a rhizomatous perennial species belonging to the Apiaceae family. Native to the mountains of Central-Southern Europe, it is widespread around the world and generally grows in rivers banks (Fig. 1), wet grassy and anthropic areas. The rhizome has a long tradition of medicinal use and therefore the plant has been known as '*Divinum remedium*' since the 18th century and considered a panacea for all ailments. The rhizome is included in the BELFRIT list (2019) for the regulation of the digestive function, the fluidity of bronchial secretions and as a tonic, relieving physical and mental fatigue.
Our study aims at enhancing the supply chain of medicinal plants, following the principles of circular economy. We have focused our research on the leaf of *I. ostruthium*, whose traditional uses have been documented in ethnobotanical researches. In the Aosta Valley (Italy), leaves are claimed to treat skin diseases (e.g. wounds, infections, insect bites), muscular inflammations, and haematoma. The scientific validation of these traditional uses is essential for the inclusion of the plant in herbal medicinal products.
Plant material was collected in the Gran Paradiso National Park, Aosta Valley, (Fig. 2) and the leaf was investigated from the micromorphological, phytochemical, and pharmacological points of view. Anatomical features were elucidated by light and electron scanning microscopy (Fig. 3). The hydroalcoholic leaf extract, used in cell-free assays, showed strong and dose-dependent antioxidant (IC_{50}, 1.03-24.11 µg/ml) and anti-inflammatory activities (IC_{50}, 24.78-57.06 µg/ml), well correlated with the high content of total phenols (10.67 g GAE/100 g DE) and flavonoids (50.88.g RE/100 g DE). Tests on proinflammatory enzymes showed that, at 170 and 300 µg/ml, the extract inhibits cyclooxygenase (COX) at 67.3% and 43.8%, and lipoxygenase (LOX) at 51.97% and 78.74%, respectively. The extract showed no cytotoxicity on human HaCat keratinocytes and low cytotoxicity on L929 murine fibroblasts. Wound healing effects were observed at doses of 15, 70, and 170 µg/ml on L929 fibroblasts; the effect was dose-dependent and comparable to that of positive control allantoin at 170 µg/ml.
The results suggest a possible use of *I. ostruthium* leaves in phytoterapic products. Further assays on Hacat cells are needed to confirm the wound healing effects on human cells, leading to the validation of traditional uses and new pharmaceutical applications.

Figs. 1-2-3. *Imperatoria ostruthium* L.: (1) plants in their habitat; (2) plant collection; (3) leaf surface detail observed by scanning electron microscopy (SEM).

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2 = *Juniperus horizontalis* Moench: a possible natural-based herbicide

Laura De Martino[1*], Daniela Gruľová[2], Beáta Baranová[2], Vincent Sedlák[3], Valtcho D. Zheljazkov[4], Mária Konečná[3], Janka Poráčová[3], Giuseppe Amato[1], Lucia Caputo[1], Vincenzo De Feo[1]

[1]Department of Pharmacy, University of Salerno, Via Giovanni Paolo II, 132, 84084 Fisciano, Italy;[2]Department of Ecology, Faculty of Humanities and Natural Sciences, University of Presov (UNIPO), Ul. 17. Novembra 1, 08001 Prešov, Slovakia; [3]Department of Biology, Faculty of Humanities and Natural Sciences, University of Presov, Ul. 17. Novembra 1, 08001 Prešov, Slovakia; [4]Department of Crop and Soil Science, Oregon State University, Corvallis, OR, USA

Approximately 50 to 67 species of junipers, coniferous trees and shrubs, belonging to *Juniperus* genus (Cupressaceae), are widely distributed throughout the Northern Hemisphere, from the Arctic to the south of tropical Africa and from west Europe to eastern Tibet in the Old World. Previous and recent studies focused on ecological, morphological, chemical, medicinal and molecular characteristics of several *Juniperus* species.

Juniperus horizontalis Moench, creeping juniper or creeping cedar, is a low-growing shrubby native of North America: it grows in Canada, from the Yukon to Newfoundland, in the United States, in Alaska and locally from Montana to Maine. It is 10 to 30 cm tall and spreads few meters wide. This species is closely related to *J. virginiana* L., and often hybridizes with it in southern Canada or with *J. scopulorum* Sarg. Several varieties were bred and cultivated in gardens and around the houses to cover the ground.

Few papers report the essential oil (EO) composition in relation to chemotaxonomic studies. Other recent publications refer to its biological activity. The EO of *J. horizontalis* was reported as a potential phytotoxic agent on lettuce.

The aims of this study were: i) to characterize the EO of *J. horizontalis* from Bighorn Mountains (Wyoming); and ii) to evaluate its possible phytotoxic effects on two monocots and two dicot species.

Twenty-nine components have been identified, accounting for 93.4% of all components. Sabinene (38.7%) was the dominant compound identified, followed by α-pinene (10.0%), elemol (8.6%), γ-terpinene (8.3%), limonene (7.8%) and α-thujene (5.3%). The potential phytotoxic effect of 11 different doses (0.0625 to 100 µg/mL) of the EO was evaluated on the seeds of two dicotyledonous (*Sinapis alba* L. and *Lepidium sativum* L.) and two monocotyledonous species (*Hordeum vulgare* L. and *Triticum aestivum* L.).

Seeds of *Triticum aestivum* were the most susceptible to the EO: the seed germination (Tab. 1) decreased with all doses of EO except one (0.25 µg/mL). Statistical significance ($p \leq 0.05$) was confirmed only at dose 5 µg/mL. As for as radicle elongation (Tab. 2), the root growth was stimulated by application of *J. horizontalis* EO in three species, *S. alba, L. sativum* and *H. vulgare*. The root growth of *T. aestivum* after application of higher doses of EO (100, 50, 25, 10 and 5µg/mL) was visibly but not statistically suppressed. The present study also evaluated the possible effect of *J. horizontalis* EO on the number of the roots in the tested monocot species: *H. vulgare* responded increasing the number of roots after application of the EO (except at the highest dose 100 µg/mL), while the number of roots of *T. aestivum* decreased (except with the two doses 25 and 10 µg/mL). Cluster analysis clearly pointed up differences in the activity between the tested monocots and dicots.

	S. alba % ±SD	*L. sativum* %±SD	*H. vulgare* %±SD	*T. aestivum* %±SD
control	70±10	100±0	60±10	60±10
100 µg/mL	80+7.3	100±0	56.7±11.5	53.3±5.8
50 µg/mL	80±10	100±0	80±10	46.7±25.2
25 µg/mL	76.7±5.8	90±10	26.7±15.3	40±20
10 µg/mL	63.3±5 .8	80±10	63.3±5.8	40±10
5 µg/mL	56.7±5.8	96.7±5.8	73.3±11.5	20±17.3*
2.5 µg/mL	73.3±5 .8	93.3±5.8	60±17.3	46.7±15.3
1.25 µg/mL	76.7±20.8	100±0	43.3±5.8	53.3±25.2
0.625 µg/mL	80±17.3	100±0	36.7±15.3	56.7±11.5
0.25 µg/mL	80±10	100±0	50±10	60±10
0.125 µg/mL	86.7±23.1	100±0	63.3±30.5	50±20
0.0625 µg/mL	76.7±5.8	100±0	53.3±23.1	56.7±23.1

Tab. 1. Effects of different doses of *J. horizontalis* essential oil on germination of different seeds. *p<0.05 *vs* control.

	S. alba % ±SD	*L. sativum* %±SD	*H. vulgare* %±SD	*T. aestivum* %±SD
control	2.8±2.0	6.3±2.9	2.6±1.7	1.1±1.0
100 µg/mL	3.4±2.3	7.1±2.9	1.7±1.3	0.8±0.7
50 µg/mL	4.0±2.8	7.4±2.1	3.1±1.6	1.0±0.8
25 µg/mL	3.1±2.7	6.5±2.6	2.6±1.4	0.9±0.8
10 µg/mL	4.7±2.5**	8.0±2.8*	2.8±1.3	0.7±0.5
5 µg/mL	4.2±1.8*	6.3±3.2	2.9±1.8	0.5±0.5
2.5 µg/mL	3.5±2.7	7.6±3.0	3.4±1.7	1.8±1.5
1.25 µg/mL	3.6±1.9	7.4±2.6	3.0±1.8	2.7±1.2
0.625 µg/mL	3.3±2.1	8.2±2.1*	3.8±1.5	2.8±1.3****
0.25 µg/mL	3.8±2.3	9.5±2.4**	3.2±1.5	2.7±1.3****
0.125 µg/mL	2.7±2.2	7.6±2.8	2.6±1.1	3.2±0.9****
0.0625 µg/mL	2.9±2.5	8.9±2.8**	3.1±1.3	2.8±1.4****

Tab. 2. Effects of different doses of *J. horizontalis* essential oil on radical elongation of different seeds. *p<0.05; **p<0.01;****p<0.0001 *vs* control.

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2 = *Hura crepitans* L. extract: chemical characterization, nanoformulation and bioactivity in cell model

Immacolata Faraone[1,2], Daniela Russo[1,2], Sileo Annalisa[1], Antonio Vassallo[1], Maria Francesca Armentano[1,2], Carla Caddeo[3], Maria Josefina Gualtieri[4], Angela Ostuni[1,2], Faustino Bisaccia[1,2], Luigi Milella[1]
[1]Department of Scienze, University of Basilicata, Viale dell'Ateneo Lucano 10, 85100 Potenza, Italy; [2]Spinoff BioActiPlant s.r.l., Viale dell'Ateneo Lucano 10, 85100 Potenza, Italy; [3]Department of Scienze della Vita e dell'Ambiente, Sezione di Scienze del Farmaco, University of Cagliari, Via Ospedale 72, 09124 Cagliari, Italy; [4]Department of Pharmacognosy and Organic Medicaments, University of Los Andes, 5101 Mérida, Venezuela

Hura crepitans L. (HC) belonging the Euphorbiaceae family, is commonly known as "*Monkey-no-climb*". It is toxic, but usually used in ethnomedicinal applications for rheumatism, treatment of skin diseases, intestinal worms in leprosy.
The aim of the present work was the evaluation of nanoformulation for improving the biological activity in cells. The leaves of *H. crepitans* were collected in Venezuela in 2018, dried, powdered, and subjected to different extraction methods. Preliminary data obtained by different *in vitro* antioxidant in tube test and cell assays, as well as the chemical profile, allowed the selection of the macerated extract (HC-M) as the most performing to be encapsulated into liposomes in order to promote cell interaction and enhance the biological activity.
The ability of liposomes to maintain and accommodate the stability of encapsulated compounds is the major advantage of this formulation. For this reason, the liposome technology is employed in manufacturing of functional food, nutraceutical, cosmetic, and pharmaceutical products.
Liposomes were prepared by a simple method involving the sonication of the phospholipid (soy lecithin) and HC-M extract in water. Empty liposomes were also prepared and characterized to evaluate the effect of the incorporation of the extract on the vesicle arrangement.
The characterization of empty liposomes displayed small size (73 nm), good homogeneity (P.I. 0.25), and highly negative zeta potential (~ −50 mV). When the HC-M extract was incorporated, there was a slight increase in size (84 nm) with an improvement in the homogeneity (P.I. 0.20).
Then, HC-M extract and extract-loaded liposomes (LHC-M) were subjected to analysis of bioactivity on human hepatoma cells (HepG2) cells.
In particular, the dye 3-(4,5-dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) and the 20,70-dichlorodihydrofluorescein diacetate (DCFH-DA) were used to evaluate the cell viability for 24 h and the intracellular reactive oxygen species (ROS) level, respectively.
The cytotoxicity of liposomal formulation is dose-dependent with an IC_{50} value approximately of 30 µg/mL.
Therefore, concentrations lower than the IC_{50} value were used assessing the antioxidant activity of the liposomal formulation of LHC-M.
Interestingly, the liposomes were able to maintain ROS levels close to endogenous ones, preventing the oxidative stress caused by ROS already at the lower concentration (3.125 µg/mL), without statistical difference between the tested concentrations.
Moreover, in order to identify the compounds involved in these effects, the macerated extract was subjected to liquid chromatography–mass spectrometry analyses.
Fourteen compounds were identified and the most abundant were the phenols caffeic acid, gallic acid, and quercetin.
These results suggest the use of *H. crepitans* as antioxidant source, especially when incorporated into liposomes. In fact, the extract incorporates in liposomes is an advantage to facilitate the interaction with cells and allow the release of the payload in the cytoplasm, where the antioxidant activity is exerted.

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2 = Preliminary studies on flavonoidic content and antioxidant activities of *Hedera helix* L. honey and its characterisation

Laura Giamperi[1*], Anahi Elena Ada Bucchini[1*], BrunoTirillini[1]
[1]Botanical Garden Centre, University of Urbino, Department of Biomolecular Science, University of Urbino, Via Bramante 28, 61029 Urbino, Italy
*Equal contributors

The term 'honey' refers to the natural and sweet substance that bees (*Apis mellifera*) produce from nectar or the secretions of parasitic insects through a long and complex process involving the collection, processing, storage and dehydration phases.
Nectar contains water, glucose, fructose and sucrose and various other sugars present in smaller quantities. There are also traces of vitamins and amino acids, organic acids, minerals, enzymes and pigments. The nectar quali-quantitative composition has a decisive influence on that of the honey, also determining its physical characteristics which can be detected by organoleptic tests.
Single-flower honey from *Hedera helix* L. is a very rare and little-studied food product. For this reason, we thought it would be interesting to focus our studies on different samples of *Hedera helix* L. honey (comparing them with multi-flower honey) and to assess the regulatory parameters necessary for its production and marketing.
The parameters studied were dry weight, conductivity, pH determination, antioxidant capacity, flavonoids assay, DPPH, ß-Carotene/Linoleic Acid Assay.
The preliminary results showed that the dry weight values are homogeneous and that the honey (*Hedera helix* L. and multi-flower honey) do not differ in moisture content. Ivy and multi-flower honey do not differ significantly in conductivity detected at temperatures ranging from 20 to 26°C.
The average pH value determined among the ivy honeys was 3.85; as reported in the literature, this value was in the optimal pH range (pH 2.5 - 5.5) for preserving the honey and avoiding deterioration.
The flavonoids assay showed significant differences between honey samples. The free radical scavenging activity, detected by DPPH test, is comparable in honeys of the same origin and also in samples produced in different years. Values obtained from the ß-carotene/linoleic acid test showed significant differences between the various kinds of honey.

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2 = Metabolomic study of *Dactylis glomerata* L. growing on different islands of Aeolian archipelago (Italy)

Elisabetta Grillini [1], Lorenzo Marincich[1,2], Ilaria Chiocchio[1], Valentina Maria Daniele[1], Manuela Mandrone[1] Alessandro Chiarucci[3], Piero Zannini[3], Guarino Riccardo[4], Ferruccio Poli[1]
[1]Department of Pharmacy and Biotechnology (FaBit), University of Bologna, Via Irnerio 42, 40126, Bologna, Italy. [2]Department for Life Quality Studies, University of Bologna, Corso D'Augusto 237, 47921, Rimini, Italy. [3]Department of Biological, Geological, and Environmental Sciences, University of Bologna, Via Irnerio 42, 40126, Bologna, Italy. [4]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, Via Archirafi 38, 90123 Palermo, Italy

Dactylis glomerata L. (Poaceae), known as orchardgrass or cocksfoot, is a perennial cool season bunchgrass. Although in some areas it has become an invasive species, it is widely grown as pasture for grazing in North America, Europe, and Oceania.
The aim of this work was to compare the phytochemical profiles of *D. glomerata* leaves harvested in June 2019 in a vegetative state at different altitudes on four islands of the Aeolian archipelago, a volcanic archipelago in the Tyrrhenian Sea, namely: Lipari, Vulcano, Stromboli and Panarea (Fig.1). The peculiar geological features, including the presence of the volcanoes, and the wide variety of environments and soils, have important impact on native plants. In particular, *D. glomerata* exhibits a remarkable phenotypic variability in the Aeolian archipelago, which was the subject of biometric and enzymatic polymorphism studies, carried out in the 1980s by a team coordinated by R.E. Scossiroli (1991).
Different environmental conditions strongly affect plants metabolome, driving them to increase and diversify the metabolites production in response to biotic and abiotic stimuli. In order to compare a high number of plant phytochemical profiles, metabolomic approach proved a successful strategy, which generally relies on untargeted analysis protocols, whose results are handled with chemometrics (multivariate data treatment).
Samples of *D. glomerata* were analyzed by ^{1}H NMR profiling and data were handled developing proper multivariate analysis models. A first overview of the results obtained was acquired by performing Principal Components Analysis (PCA), using ^{1}H NMR bucketed spectra as *x* variables (Fig.2a). In this model samples collected on Stromboli showed peculiar differences among them and compared to the samples collected on the other islands, which were not significantly diversified. Then an Orthogonal partial least squares (OPLS) model was developed analyzing each sample collected on Stromboli island, using as *y* variable the altitude (Fig.2b). This model highlighted that samples growing at increasing altitude on Vulcano island have an increasing concentration of fumaric acid, aspartic acid, phenylalanine compared to the samples growing at low altitude. Conversely, the concentration of fatty acids, valine, proline, glutamic acid, malic acid, glycine betaine, sucrose, glucose, and a cinnamoyl derivative is higher in the samples growing at lowest altitude. In addition to the altitude, these metabolomic features might be determined also by the presence of the volcano.

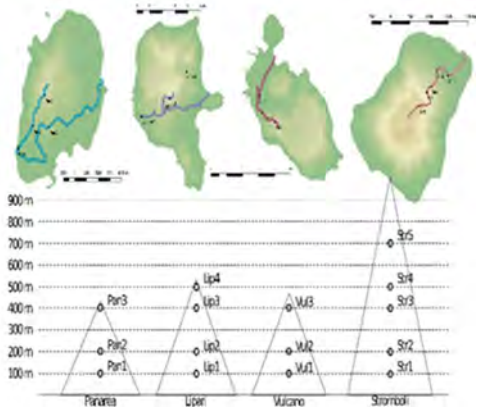

Fig.1. Collection sites.

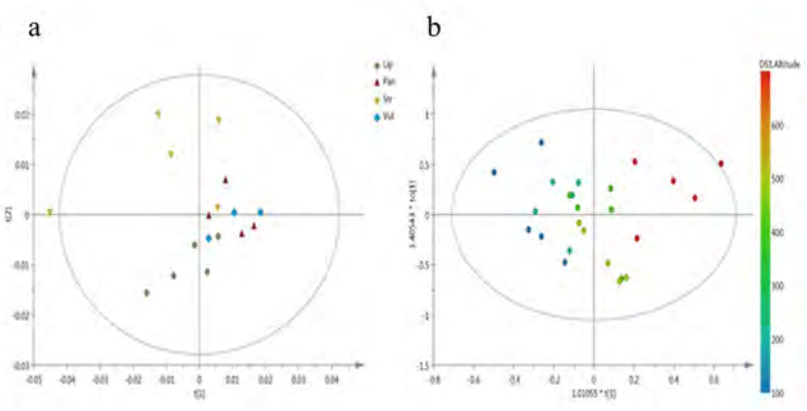

Fig. 2a-2b. a) PCA based on ^{1}H-NMR profile of the Aeolian islands sampled station. b) OPLS based on ^{1}H-NMR profile of Stromboli samples (y=altitude).

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2 = Phenylethanoid glycosides profile and anti-inflammatory effects of *Verbascum thapsus* L. leaves

Alfonsina La Mantia[1], Giovanna Calabrese[2], Francesco Pappalardo[1], Rosaria Acquaviva[1], Claudia Di Giacomo[1], Barbara Tomasello[1], Giuseppe Antonio Malfa[1]
[1]Department of Drug and Health Sciences, Section of Biochemistry, University of Catania, Viale Andrea Doria 6, Italy; [2]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, Polo Annunziata, University of Messina, 98168 Messina, Italy

Verbascum thapsus L., commonly known as Common Mullein, is a Eurasian plant belonging to the Scrophulariaceae family. It is a monocarpic and biennial herb which largely grows in cliffs, meadows, fields and ridges on dry, sandy and rocky soils.
In the first year the plant forms a low vegetative rosette characterized by several hairy elliptic-lanceolate cauline and alternate leaves. During the second growing season, from early spring to late summer the plant forms a raceme inflorescence composed by small yellow sessile flowers densely grouped on an indeterminate spike up to 2 m tall. The fruit is a bi-septate brown capsule that contains numerous seeds.
In Sicily, it can be easily found, on cultivated lands, along roads and in rocky areas over the altitude of 1000 m a.s.l..
V. thapsus is widely used in traditional medicine as a medicinal herb since the year dot. Leaves and flower extracts have been used as a domestic medication against inflammatory diseases, asthma, pulmonary disorders, migraine, fever, congestion, allergies and colic. In fact, plants from genus Verbascum L. are a source of a wide variety of chemical constituents such as iridoids, saponins, flavonoids, phenolic acids and phenylethanoid glycosides (PhEG) a class of water-soluble compounds of which the most known is verbascoside. Verbascoside has extensively been characterized as an effective scavenger of biologically active free radicals and an anti-inflammatory and wound healing agent both *in vitro* and *in vivo*.
Recently, interest in PhGs has been growing and several studies described various beneficial biological effects involved in the prevention and treatment of various human diseases.
In this scenario we utilized an aqueous leafs extract of *V. thapsus* in a cell model of osteoarthritis. The PhGs profile of the extract was performed by HPLC-DAD and the total phenolic content by Folin-Ciocalteu.
Subsequently, the possible anti-inflammatory and anti-osteoarthritic activities of the extract was assessed by gene expression of specific inflammatory mediators and compared in the same conditions to a standardized extract of *Harpagophytum procumbens* (Burch.) DC. ex Meisn., a well-known anti-osteoarthritic phytotherapeutic agent.
The total phenolic contents determined spectrophotometrically by the Folin–Ciocalteu method was 124,0 ± 0,7 mg gallic acid equivalent (GAE)/g of extract and six phenylethanoid glycosides were identified which represent 9.92 % (W/W) of total extract. Moreover, the treatments with the different concentration of extract (50, 100 µg/ml) were able to reduce iNOS, COX 2, IL1β and IL6 gene expression without affecting cell viability. Obtained data will support further in-depth studies for the possible therapeutic usage of *V. thapsus* L. leaves in the treatment of the early stages of inflammation and osteoarthritis.

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2 = Comparing wild and cultivated *Arnica montana* L. from the Italian Alps to explore the possibility of a sustainable production using local seeds

Valeria Leoni[1], Gigliola Borgonovo[1,2], Luca Giupponi[1,3], Angela Bassoli[1,2], Davide Pedrali[1], Marco Zuccolo[1], Alessia Rodari[1], Annamaria Giorgi[1,3]

[1]Centre of Applied Studies for the Sustainable Management and Protection of Mountain Areas (CRC Ge.S.Di.Mont.), University of Milan, Via Morino 8, 25048 Edolo, Italy; [2] Department of Food, Environmental and Nutritional Sciences (DEFENS), University of Milan, Via Celoria 2, 20133 Milan, Italy; [3] Department of Agricultural and Environmental Sciences—Production, Landscape and Agroenergy (DISAA), University of Milan, Via Celoria 2, 20133 Milan, Italy

Mountain arnica (*Arnica montana* L.) is an herbaceous long-lived plant of nutrient-poor grasslands of European mountains. It is a widely used medicinal plant for the treatment of injuries and accidents, the secondary metabolites that mediate the anti-inflammatory effects of arnica flowerheads being mainly sesquiterpene lactones (SLs, Fig. 1). Habitat fragmentation, abandonment of pasturing and collection for herbal use led to the rapid decline of this species, for this reason, the sustainable production instead of the collection of wild material, should be incentivized. A wild accession of arnica inflorescences *versus* a commercial from Valsaviore (Italian Alps) were confronted in the germination performances and the phytochemical characterization through High Performance Liquid Chromatography (HPLC), Nuclear Magnetic Resonance (NMR) and Gas Chromatography–Mass Spectrometry techniques (GC-MS). Germination percentage (GRP) was high (>75%) both for commercial and wild seeds. ^{1}H NMR spectrum of arnica extracts were very similar and demonstrated a signal richness confirming the presence of sesquiterpene compounds, esters of helenaline and dehydroelenaline. The main compounds identified were 6-O-(2-methylbutyryl)-helenalin (HMB) 6-O-isobutyryl-11α,13-dihydrohelenalin (DHIB), 6-O-(2-methylbutyryl)-helenalin (HIB), 6-O-(2-methylbutyryl)-11α,13-dihydrohelenalin (DHMB) and 6-O-metthacryloylhelenalin (HM). The major compounds in *A. montana* volatiles composition were germacrene D (found in a quantity of 26 µg/g in wild arnica and 10 µg/g in the cultivated one), α-Bergamotene (18 µg/g in the wild cultivar and 11 µg/g in the cultivated flos), cymene (14 µg/g) limonene (11 µg/g) and α-fellandrene (15 µg/g) in the cultivated arnica and δ-cadinene (13 µg/g) in the wild one. A significantly high percentage of acetic acid methyl ester (38 µg/g) and 2-methyl-methyl ester of propanoic acid (31 µg/g) were found for cultivated arnica and were probably associated with fermentation processes linked to the traditional method of air drying on trellis. In applied botany, the possibility to grow *A. montana* in marginal territories is of interest for farmers in alpine regions looking for alternative crops in high mountain farming. In our case the wild germplasm was eligible for cultivated cropping both for chemical composition (Fig. 2) and germination performance. The possibility of growing *A. montana* and a controlled local first transformation are important to incentivize local, good quality and sustainable production (Fig. 3). The growing of seedlings "in loco" could be of great interest both for farmers and for natural conservation purposes.

1)

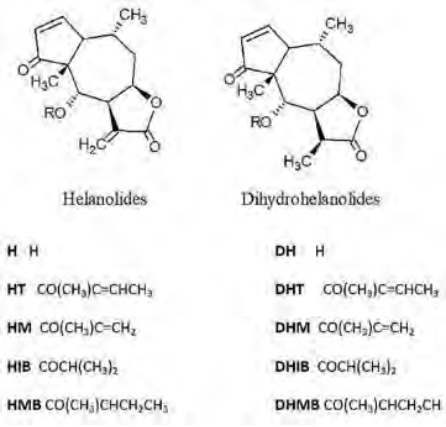

Fig. 1. Sesquiterpene lactones found in *Arnica Montana* L. analyzed.

2)

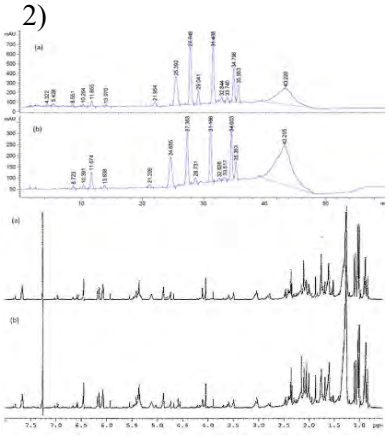

Fig. 2. 1H NMR chromatograms of wild and cultivated arnica. It is possible to see the great similarity.

3)

Fig. 3. Cultivated arnica field in Shanty Mae Farm in Valsaviore.

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2 = Safe and poisonous garden plants: pharmacognostic comparison between leaves of *Laurus nobilis* L. and *Prunus laurocerasus* L.

Paola Malaspina[1], Federica Betuzzi[1]*, Cristina Danna[1], Antonella Smeriglio[2], Domenico Trombetta[2], Laura Cornara[1]
[1]Department of Earth, Environment and Life Sciences (DISTAV), University of Genoa, Corso Europa 26, Italy; [2]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Via G. Palatucci, 98168 Messina, Italy
*Corresponding author: federica_betuzzi@libero.it

Many plant species produce toxic compounds that are very dangerous for both humans and animals. The misidentification between edible and poisonous plants is a growing problem nowadays, due to the current trend of being environmentally friendly, leading people to collect wild plants without knowing their possible toxicity. Moreover, morphologically similar species are related to accidental contamination or intentional adulteration of products for human and animal consumption. Since the toxic potential of plants is frequently underestimated, another potential risk is the presence of poisonous species in public and private gardens or pastures, threatening children's and animals' health.
Laurus nobilis L. and *Prunus laurocerasus* L. are two plants commonly cultivated as ornamental trees in garden and urban green areas in the Mediterranean region. The first species is widely used in flavour foods or to treat ailments, while the second one to realize hedges. *L. nobilis* is considered a non-toxic plant, although it is rich in essential oil whose excessive uptake can cause neurological problems, especially in children. In contrast, *P. laurocerasus* leaves, which can be confused with those of *L. nobilis*, contain from 1 to 2.5% cyanogenic glycosides that release by hydrolysis hydrogen cyanide, which is dangerous for humans at high concentrations and extremely toxic for ruminants already at low doses.
In this study, a pharmacognostic evaluation and comparison between *L. nobilis* and *P. laurocerasus* leaves collected from the Genoa Botanical Garden (Liguria, Italy) was carried out. Light microscopy revealed distinguishing features in each species, which were highlighted and confirmed by Scanning Electron Microscopy. A paracytic stomatal apparatus (Fig. 1c) and secretory idioblasts containing essential oil responsible for the typical aroma (Fig. 1a) can be observed in *L. nobilis*. On the other hand, *P. laurocerasus* leaves show extrafloral nectaries at the leaf base near the petiole, an anomocytic/anisocytic stomatal apparatus (Fig. 1d), many calcium oxalate prismatic crystals (Fig. 1b) and, in the upper surface, a collenchymatous protrusion situated above the vein.
In conclusion, a macro- and micro-morphological characterization with phytochemical colorimetric methods, combined with High Performance Liquid Chromatographic (HPLC) and Gas Chromatographic (GC) methods has allowed to easily discriminate these two species, even in case of plant fragments found in biological samples or pruning residues. These results can be useful for plant identification in quality control tests, intoxication cases and crimes.

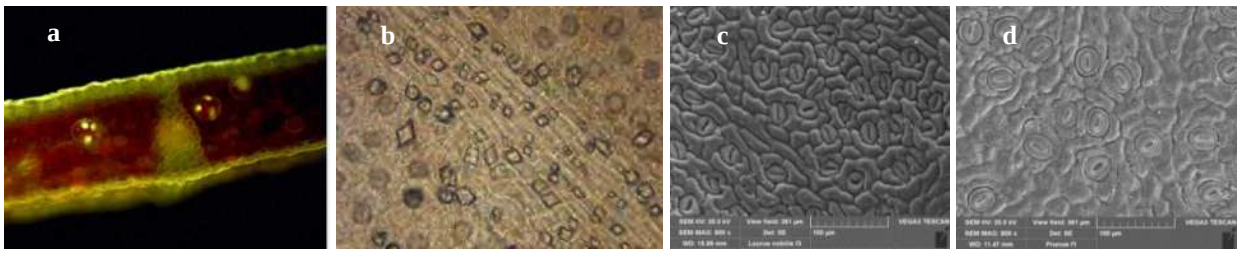

Fig. 1. LM micrograph (a-b). Cross-section of *L. nobilis* (a): fluorol yellow shows secretory idioblasts scattered between the mesophyll. Leaf epidermal peel of *P. laurocerasus* (b): prismatic crystals are visible along a vein. SEM micrograph (c-d). Abaxial surface: paracytic stomata with sunken guard cells in *L. nobilis* (c); anomocytic stomata in *P. laurocerasus* (d).

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2 = Biological investigation and chemical study of *Brassica villosa* subsp. *drepanensis* (Brassicaeae)

Giuseppe Antonio Malfa[1], Alessandra Braca[2], Rosa Tundis[3], Marinella De Leo[2], Monica Rosa Loizzo[3], Barbara Tomasello[1], Vivienne Spadaro[4], Francesco Maria Raimondo[5], Vincenzo Musolino[6], Rosaria Acquaviva[1]

[1]Department of Drug and Health Sciences, Section of Biochemistry, University of Catania, Viale Andrea Doria 6, Italy; [2]Department of Pharmacy, University of Pisa, Via Bonanno 33, 56126 Pisa, Italy; [3]Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, Via P. Bucci, 87036 Rende (CS), Italy; [4]Department STEBICEF/ Sect. of Botany, Anthropology and Zoology, University of Palermo, Via Archirafi 38, 90123 Palermo, Italy; [5]*PLANTA*/Center for Research Documentation and Training, Via Serraglio Vecchio 28, Palermo, Italy; [6]Department of Health Sciences, University "Magna Graecia" of Catanzaro, Viale Europa, Località Germaneto, 88100, Catanzaro, Italy

Many studies carried out in the last decades have focused on particular plants capable of effectively dealing with the fight against the development of numerous diseases and presumably able to prevent their onset.
Several experimental evidence, has attributed beneficial effects on human health to Brassicaceae species and their horticultural varieties due to the presence of secondary metabolites such as nitrogen-sulfur derivatives (glucosinolates, isothiocyanates) and polyphenols, including hydroxycinnamic acids and flavonoids.
Brassica villosa subsp. *drepanensis* (Caruel) Raimondo & Mazzola is a suffruticosa plant, 30-60 cm tall, like the other four subspecies. It is a Sicily endemic plant, present in the "drepano-panormita" district areas between Trapani and Palermo. The leaves are covered with a thick tomentum. The stem, woody at the base, bears lower lyrate-lobed leaves with very large terminal lobe and toothed margins, while the upper leaves are lanceolate and with an entire margin. The rich racemose inflorescence, present from February to April, consists of flowers with pale yellow petals. The fruit is a quadrangular section of siliqua. Based on molecular analysis, it was proposed to consider *B. villosa* subsp. *drepanensis* as a distinct species.
In this study, the quali-quantitative composition, the polyphenol content, the anti-inflammatory and antioxidant properties of *B. villosa* subsp. *drepanensis* extract were analyzed for the first time.
The plant leaves were collected, dried, and extracted at room temperature with ethanol-water 7:3. The total amount of polyphenols (18.0 ± 0.2 µM of gallic acid) and flavonoids (9.0 ± 0.1 µM of catechin) in the whole extract was calculated using the Folin-Ciocalteu method and its antioxidant potential was performed by DPPH, ABTS, FRAP assays, β-carotene bleaching test and the SOD-like activity assays, reporting the following IC_{50} values: µg/mL 368 ± 1.7, 3.32 ± 0.88, 10.5 ± 1.2, 38.3 ± 2.5 and 66.0 ± 1.1, respectively. Subsequently, cytotoxicity was assessed on both human and murine normal cell lines (HFF-1 human fibroblasts, RAW 264.7 murine macrophages), by the MTT test, treated for 24 h with different concentrations of alcoholic extract. Results showed that leaves extract of *B. villosa* subsp. *drepanensis* showed no toxicity in both cell lines. To simplify the chemical analysis and separation, the total ethanol-water extract (10.9 g) was partitioned between ethyl acetate, *n*-butanol, and water to obtain three fractions of 0.73, 0.30, and 8.77 g, respectively. The phytochemical profile of the three fractions was performed by LC-MS analysis and the *n*-butanol extract was also subjected to chromatographic separation to obtain two flavonol glycosides, one phenolic glycoside, two aminoacids, one purine and one pyrimidine bases. The presence of glucosinolates was detected in the water extract. In addition, in an *in vitro* model of inflammation LPS induced, the total extract showed anti-inflammatory properties inducing an inhibitory effect on NO release in a dose-dependent manner. Conversely the treatment with the three different fractions did not exert any anti-inflammatory activities. This result allows us to affirm that the anti-inflammatory activity shown by the total extract is due to the synergistic effect of the phytocomplex rather than by a single fraction or by their additive effects.
To further confirm the correlation between the antioxidant properties and the anti-inflammatory effect, the ROS levels in the same cellular model of inflammation LPS induced were determined. The total extract caused a significant decrease in ROS levels at all concentrations tested confirming that the extract acts primarily as an antioxidant agent. Data obtained in the present study confirmed the health-promoting properties of *B. villosa* subsp. *drepanensis*.

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2 = Characterization of non-psychotomimetic cannabinoids and phenolic compounds in fiber-type *Cannabis sativa* L. aerial parts at different vegetative stages as a potential source of antioxidants

Giulia Mastellone[1], Arianna Marengo[1], Francesca Capetti[1], Barbara Sgorbini[1], Patrizia Rubiolo[1], Cecilia Cagliero[1]
[1]Department of Scienza e Tecnologia del Farmaco, University of Turin, Via Giuria 9, 10125 Turin, Italy

Cannabis sativa L. is a native plant from Central Asia, and it is one of the main members of the Cannabaceae family. Different classifications of *Cannabis* varieties were proposed, the most important refers to drug-type (richer in Δ^9-THC) and fiber-type (richer in CBD or related compounds). Although the benefits of this plant have been known since ancient times, nowadays there is a renewed interest for this species, thanks to the rich phytocomplex of the plant, together with its fiber and agricultural features, opening the way to its exploitation in different fields. Besides the well-known Δ^9-THC and CBD cannabinoids, hemp produces other numerous chemicals, including terpenes, phenolic compounds and non-psychotomimetic cannabinoids that have been associated with health-promoting properties.
The current study aimed to chemically characterize different samples of fiber-type *C. sativa*, grown in the Western Po Valley, and collected at four progressive vegetative stages, from mid vegetative to early flower stage. An ultrasound-assisted solid-liquid extraction (UA-SLE) method, coupled to UHPLC-UV-MS/MS analysis, was first optimized through an experimental design approach, in order to obtain a fingerprinting of the investigated phytocomplex. The results clearly highlighted that a methanolic extraction allows to obtain a more complete profile, with flavonoids and non-psychotomimetic cannabinoids as main classes of compounds, while an acetone extraction is more effective in selectively isolating cannabinoid compounds (see Figure 1C). The phenolic pattern is chiefly composed of flavone glycosides (i.e. apigenin, luteolin, diosmetin, acacetin *O*-glycosides) while the phytocannabinoids are mainly propyl derivatives (varinic compounds). 24 samples of fiber-type *C. sativa* aerial parts (mainly stems and leaves), which differed according to the vegetative stage (mid vegetative, late vegetative, shooting, early flower), location of the land plot (2×12 m^2 plots with 3 replicates: A, B and C) and the method of drying (forced-draft oven or freeze-drying) were then analyzed with the aim of investigating potential differences in the phytochemical profiles. A multivariate statistical elaboration of the results (hierarchical clustering, principal component analysis, partial least squares discriminant) was carried out showing that the main differences between the samples are related to the method of drying (Figure 1D). In particular, the oven drying samples presented a lower amount of cannabinoids and flavonoid glycosides while the corresponding aglycones were in higher quantity compared to the freeze-drying samples. The freeze-drying method seems therefore to better preserve the chemical composition of the plant, while the higher temperature of the oven (65°C) for prolonged time, may have led to a partial degradation of the glycosides in the corresponding aglycones. The same degradation probably occurred for the acid form of cannabinoids which are thermally unstable. The location of the land plot and the vegetative stage of the plants did not influence their chemical pattern showing that the phytochemical composition of *C. sativa* aerial parts is independent from the plant harvesting period, provided that it is collected before the flowering stage.
Finally, based on the well-known antioxidant properties of the phenolic compounds, preliminary antioxidant assays were carried out, both on methanolic and acetone extracts of the hemp samples, to investigate the potential synergistic contribution of cannabinoids to the antioxidant power of the samples.

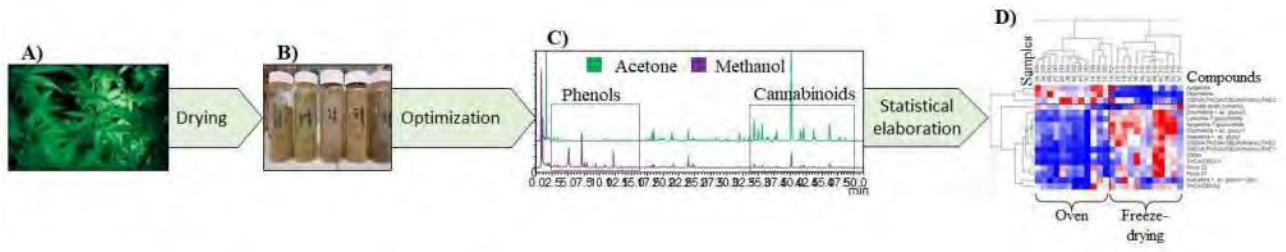

Fig. 1. Flowchart of the main steps of the characterization of fiber-type *Cannabis sativa* L. aerial parts.

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2 = Optimization of the microwave-assisted extraction of *Trachyspermum ammi* essential oil using a design of experiment methodology

Eugenia Mazzara[1], Serena Scortichini[2], Dennis Fiorini[2], Filippo Maggi[1], Riccardo Petrelli[1], Loredana Cappellacci[1], Giuseppe Morgese[1], Mohammad Reza Morshedloo[3], Giovanni Filippo Palmieri[4], Marco Cespi[4]
[1]Department of Chemical Sciences, University of Camerino, Via Sant'Agostino 1, 62032 Camerino, Italy; [2]Chemistry unit, University of Camerino, Via Sant'Agostino 1, 62032 Camerino, Italy; [3]Department of Horticultural Science, University of Maragheh, 55181 83111 Maragheh, Iran; [4]Pharmaceutical Technology unit, University of Camerino, Via Gentile III da Varano, 62032 Camerino, Italy

Trachyspermum ammi (L.) Sprague is a plant belonging to the Apiaceae family, traditionally used all over the world, especially in the Middle East medicine, for its several pharmacological properties, including the expectorant, anti-inflammatory, antimicrobial and insecticidal ones. These beneficial effects are ascribed to the essential oil, obtained by the fruits of this Iranian spice (Fig. 1), and, in particular, to its main constituent thymol. On this basis, the aim of the present work was the optimization of the microwave-assisted extraction (MAE) (Fig. 2) of *T. ammi* essential oil (Fig. 3), through a two-steps design of experiment (DoE) approach. After a preliminary screening design, to identify the experimental parameters that can influence the MAE process, a response surface methodology (RSM) design was employed, to obtain mathematical models able to understand the relationship between factors (extraction conditions) and responses (essential oil yield and composition). Hydrodistillation (HD) was performed for comparative purposes and the chemical constituents of the essential oils obtained from MAE and HD were evaluated through GC-MS and GC-FID analysis. The most abundant identified compound was represented by thymol, which was also isolated and characterized as pure by NMR analysis, followed by *p*-cymene and γ-terpinene. Especially the extraction time (min), and then the microwave irradiation power (W/g), were identified as the most important parameters to enhance the essential oil yield and chemical profile. The composite desirability function allowed us to find the best conditions to maximize the yield and thymol content at the same time. MAE treatment led to comparable oil yield values and higher concentrations of thymol, when compared with HD. In conclusion, the microwave-assisted extraction represents a sustainable and eco-friendly method to obtain high yields of *T. ammi* essential oil, enriched in valuable active compounds. So, it may be exploited to manufacture innovative products, due to its interesting potentialities from a pharmaceutical, nutraceutical and cosmeceutical point of view.

Fig. 1. *T. ammi* dry fruits.

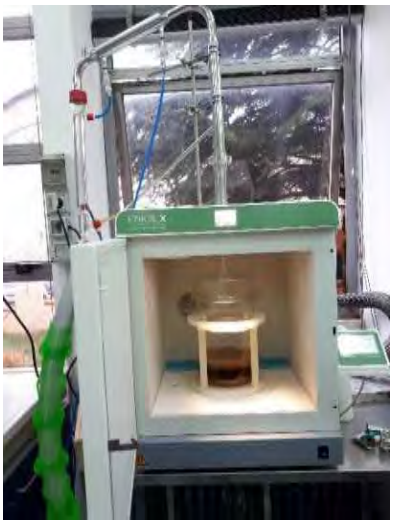

Fig. 2. MAE device.

Fig. 3. *T. ammi* essential oil.

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2 = A dermo-functional ingredient from chestnut (*Castanea sativa* Mill.) wastes: development of a topical formulation and clinical investigation of skin tolerability and efficacy

Mencherini Teresa [1,2,3], Esposito Tiziana[1,2,3], Francesca Sansone[1,2,3], Puca Rosa Valentina [4], Iandoli Raffaele [4], Rita P. Aquino [1,2,3]
[1]Department of Pharmacy, University of Salerno, Via Giovanni Paolo II, 132, 84084, Fisciano, SA, Italy; [2] Unesco Chair Salerno, Plantae Medicinales Mediterraneae, University of Salerno, Italy; [3]COSM-HI lab,"San Giuseppe Moscati" National Hospital (AORN), Contrada Amoretta, 83100 Avellino (AV), ItalyAvellino (AV), Italy; [4]Dermatology and Dermosurgery, "San Giuseppe Moscati" National Hospital (AORN), Contrada Amoretta, 83100 Avellino (AV), Italy

Spiny burs are widely generated as wastes during the chestnut harvesting. They are usually burned or left to rot in the woods by the farmers. However, it is proven that agro-food wastes yet contain bioactive molecules with potential application in human health and wellness products. Furthermore, nutraceutical and skin care industries have a great interest in antioxidant polyphenols-rich natural extracts obtained from renewable and cheap sources. If topically applied the green ingredients can reduce oxidative stress and inflammatory phenomena exerting protection from damaging free radicals produced when skin is exposed to ultraviolet light or allowed to age naturally. However, the incorporation of vegetable ingredients is often a critical point of the formulative phase because it can negatively affect the organoleptic characteristics and the stability of the final product. On the other hand, a not optimal formulation could reduce the functionality or prevent the release of the active at the target site.
In the present study, a *C. sativa* bur extract was used as an active ingredient, in a topical delivery system (O/W cream) evaluating its chemical-physical stability, also proving *in vitro* skin permeation profile, and *in vivo* tolerability and efficacy by clinical trials. In our previous study, the hydroalcoholic extract, obtained with aqueous ethanol 50% v/v (CSE), was found to be rich in polyphenols (mainly tannins and flavonoids) and with a strong *in vitro* free radical scavenging activity. Based on this promising antioxidant activity and no cytotoxicity effect in spontaneously immortalized keratinocytes (HaCaT) cell line at active concentration, 0.1% w/w of CSE extract was added to an O/W cream (B). A blank cream (A) was prepared as a control. pH and viscosity values were acceptable for cutaneous application. The CSE-loaded cream microstructure resulted homogeneous and stable over time. A and B creams showed a typical pseudoplastic non-Newtonian behavior. The presence of CSE affected the B viscosity value by reducing it, giving a desirable feature for easy skin application of the cream. The B cream was able to enhance the *in vitro* CSE skin permeation also preserving its functionality. It resulted non-irritating, with a zero value of total skin irritation and edema index. B had a significant moisturizing effect on 20 Caucasian female volunteers, increasing the skin barrier function. The formulation unchanged the sebum, pH, and erythema values. The results of the sensory evaluation showed that B cream met consumer appeal and acceptance requirements also with great sustainability profiles that consumers value very much.

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2 = Unravelling the phytochemical composition and the pharmacological properties of the fruit from *Prunus mahaleb* L.: From traditional liqueur market to the pharmacy shelf

Luigi Menghini[1*], Annalisa Chiavaroli[1], Alessandra Acquaviva[1,2], Simonetta Cristina Di Simone[1], Francesca Di Corpo[1] and Claudio Ferrante[1]
[1]Department of Pharmacy, Botanic Garden "Giardino dei Semplici", Università degli Studi "Gabriele d'Annunzio", via dei Vestini 31, 66100 Chieti, Italy; [2]Veridia Italia Srl, Via Raiale 285, 65100 Pescara, Italy

Prunus mahaleb L. is a deciduous tree belonging to the Rosaceae family, subfamily Prunoideae. The plant material is constituted by the fruit that is used in the production of traditional fragrances, lotions and liqueurs. Intriguingly, the traditional liquor called "Mirinello di Torremaggiore" (Apulia, Italy) and prepared via traditional hydroalcoholic maceration of the whole fruit from *P. mahaleb* displayed an appreciable amount of phenolic compounds, particularly in the solid residues of liqueur production. The kernels of the ground seeds have a characteristic bitter taste and therefore are used as flavoring agents in bagels, cakes, muffins and in folk medicine as diuretic, antidiabetic, tonic, aphrodisiac and expectorant agents. The whole fruit also displayed scavenging/reducing activity, *in vitro,* whereas protective effects were described in a preclinical model of ulcerative colitis. However, there is still lack in scientific literature about phytochemical composition and pharmacological potential of this plant. Therefore, in the present multidirectional study focused on unravelling peripheral and central protective effects, antimicrobial but also anti-COVID-19 properties by the water extract of *P. mahaleb.* Anti-inflammatory effects, evaluated through the gene expression of COX-2, TNFα, HIF1α and VEGFA, were studied in isolated mouse colon exposed to lipopolysaccharide; neuroprotection, measured as blunting effect on hydrogen-peroxide induced dopamine turnover, was investigated in hypothalamic HypoE22 cells; antimicrobial effects were tested against different Gram+ and Gram- pathogen bacterial strains; whereas anti-COVID 19 activity was studied in human lung adenocarcinoma H1299 cells, where the gene expression of angiotensin-converting enzyme 2 (ACE2) and transmembrane protease serine 2 (TMPRSS2) was measured after treatment with the extract. The bacteriostatic effects induced on Gram+ and Gram- strains, together with the inhibition of COX-2, TNFα, HIF1α and VEGFA suggest the potential of *P. mahaleb* water extract in contrasting the clinical symptoms related to ulcerative colitis. The inhibition of hydrogen peroxide DOPAC/DA ratio indicates promising neuroprotective effects. Finally, the downregulation of the gene expression of ACE2 and TMPRSS2, in H1299 cells, suggests the potential to inhibit SARS-CoV-2 virus entry in the human host. Overall, the results of this multidirectional research point to the valorization of the local cultivation of *P. mahaleb*, an ancient botanical resource with promising health perspectives.

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2 = The traditional uses of wild and cultivated plants: ethnobotanical and floristic novelties from Southern Calabria (S-Italy)

Carmelo Maria Musarella[1], Serena Maria Postiglione[1], Valentina Lucia Astrid Laface[1], Giovanni Spampinato[1]
[1]Department of Agriculture, Mediterranean University of Reggio Calabria, Località Feo di Vito snc, 89122, Reggio Calabria, Italy

This paper presents an ethnobotanical survey of the traditional uses of plants in San Ferdinando area, in the Gioia Tauro plain area, including the hamlet Villaggio Praia (Reggio Calabria province, Calabria region, Southern Italy). San Ferdinando is characterized by a wild and uncontaminated beach and a wide pine forest of *Pinus pinea* L. The aim of the study was to record local knowledge about plants used for food, medicine and other purposes, based on the information provided by local inhabitants, in accordance with the Ethical Code by International Society of Ethnobiology (ISE). Sixteen people, including 9 women and 7 men, aged between 25 and 81 years, were interviewed. The survey was carried out directly in the field, so it was possible to observe closely all the recorded species with the interviewees. The plant specimens, photographed and picked up, were carefully dried and collected at the Herbarium of the Department of Agriculture of the Mediterranean University of Reggio Calabria (REGGIO). A semi-structured interview was used to ask everyone the same questions about local plants, so as to collect data to be entered in an electronic database created with Microsoft ® Access®.
The total interviews were 156 and 86 taxa, belonging to 40 different families, have been recorded; *Asteraceae* family (14 species) was the most cited. Alimentary (57 citations), medicinal (50) and handicraft (15) uses were the most cited: for the others, there was a low number of interviews.
Thirty-eight species for food have been identified, mainly from the Asteraceae family. The most used parts of these plants are the leaves, mainly boiled or raw for salads, and some of these are still used for local dishes. Some examples of food plants found are: *Helminthotheca echioides* (L.) Holub, *Raphanus raphanistrum* subsp. *maritimus* (Sm.) Thell., *Oxalis latifolia* Kunth, *Opuntia ficus-indica* (L.) Mill., *Borago officinalis* L.
Thirty-one species for medicinal uses have been collected, mainly from Apiaceae (7) and Lamiaceae (7) families. Also in this case boiled leaves are used and some traditional methods made by a mix of plants are still used nowadays. Some examples of medicinal species recorded are: *Cynodon dactylon* (L.) Pers., *Malva sylvestris* L., *Ficus carica* L., *Laurus nobilis* L., *Plantago lanceolata* L. Twelve species for handicraft uses have been identified, mainly from the Poaceae (3) family. It is still possible to see some tools made with *Arundo donax* L., the *cannizza* (Fig. 1a), or *Castanea sativa* Mill. for *farlazza* (Fig. 1b), by local inhabitants for example to dry foods as figs in August. Other examples of cited uses are: cosmetic, agricultural, ornamental. For the latter two important species have been found: *Convolvolus soldanella* L. and *Retama raetam* subsp. *gussonei* (Webb) Greuter (Fig. 2). The presence of some species found during this study in the San Ferdinando area have never been reported for the Calabria region from the floristic and, then, ethnobotanical point of view: for this reason, they are currently the subject of further studies.
The achieved results were also compared with data from ethnobotanical literature of other territories and allowed to bring several novelties and confirmations.
In conclusion, this study shows how in the San Ferdinando area the traditions related to the local flora are still present and thanks to studies like this it is possible to preserve this important knowledge, also for viewing its rediscovery and enhancement.


Fig. 1a-1b. Manufacts to dry foods (*cannizza* and *farlazza*).


Fig. 2. *Retama raetam* subsp. *gussonei* (Webb) Greuter.

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2 = *In vitro* evaluation of *Isatis tinctoria* (Brassicaceae) phenolic-rich fraction-(ItJ-EAF) effects against fatty acid accumulation

Vincenzo Musolino[1], Roberta Macrì[1], Natalizia Miceli[2], Maria Fernanda Taviano[2], Salvatore Ragusa[1]
[1]Department of Health Sciences, University "Magna Graecia" of Catanzaro, Viale Europa, Località Germaneto, 88100, Catanzaro, Italy; [2]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Viale Palatucci, 98168, Messina, Italy

Isatis tinctoria L. is a species belonging to the Brassicaceae family. The *Isatis* genus includes about 80 species distributed in the Middle East and central Asia and extending to the Mediterranean region. *I. tinctoria* is widespread from the sea level up to the maximum altitude of 1900 meters. It usually prefers hot spots and nitrogen-rich limestone soils. *I. tinctoria* is an herbaceous annual to perennial species or short-lived perennial species more or less hairless to hairy, greyish with an erect stem, up to 120 cm in height, simple below, and branched above. Basal leaves are oblong-lanceolate, with an entire toothed margin, and long-petioled. Cauline leaves are simple, entire, sagittate, usually amplexicaul, with acute auricles. The flowers are gathered in a racemose inflorescence, with yellow petals.
I. tinctoria represents a source of bioactive compounds such as alkaloids, polyphenols, polysaccharides, glucosinolates, carotenoids, volatile constituents, and fatty acids, which exhibit several activities ranging from antioxidant, anti-inflammatory, antiviral to anti-tumour.
The phenolic-rich fraction (ItJ-EAF), obtained from the cauline leaves of *I. tinctoria* growing wild in Sicily (Italy), has been reported to have good radical scavenging potential and anti-proliferative effects against human anaplastic thyroid carcinoma cell lines.
Since evidence suggests that polyphenols are able to improve fatty acid accumulation, through the modulation of lipid metabolism and mitochondrial activity, the aim of this study was to determine the effect of ItJ-EAF on the fatty acid accumulation *in vitro.* Fatty acid accumulation was induced in MCArh-7777 cells, a rat hepatoma line, through the administration of oleic acid 100 µM – BSA 33.3 µM complex in DMEM 1% of FBS for 24 hours. The MCArh-7777 cells were co-treated with oleic acid 100 µM and increasing concentration of ItJ-EAF.
The cell metabolic activity was evaluated through MTT assay, to assess the non-toxic concentration of ItJ-EAF. The phytocomplex at concentrations of 0.01 mg/ml, 0.05 mg/ml, 0.075 mg/ml, and 0.1 mg/ml did not show significant alterations of metabolic activity; however, at 0.25mg/ml and 0.5 mg/ml it significantly reduced the percentage of cells metabolically active.
The potential protective effect of ItJ-EAF in reducing triglycerides was assessed by adipored assay, whereas total fatty acid accumulation was assessed by oil red O staining under the stimulus of oleic acid 100 µM.
Our results showed that ItJ-EAF at concentration of 0.1 mg/ml was able to significantly reduce the fatty acid accumulation induced by oleic acid 100 µM, restoring the conditions of the control cells.

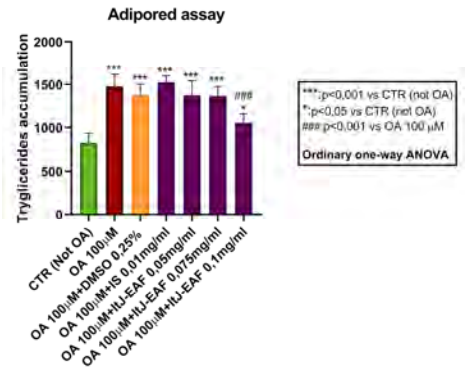

Fig.1. Adipored Assay.

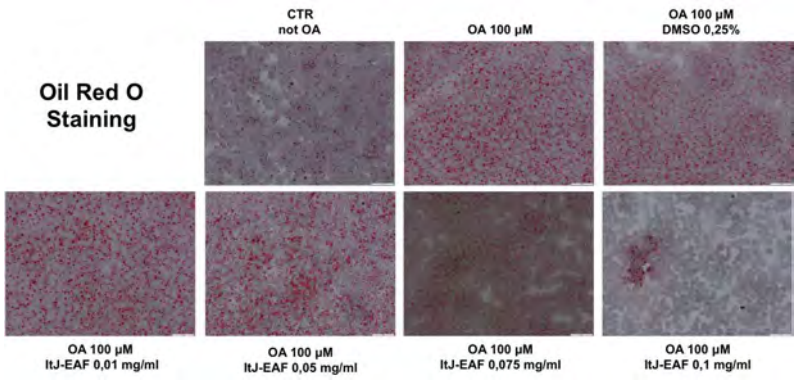

Fig.2. Oil Red O Staining.

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2 = Lavender essential oil: chemical characterization and biological activity assays on environmental bacteria and HL60 human leukemia cells

Elisa Ovidi[1], Valentina Laghezza Masci[1], Giovanni Turchetti[1], Anna Rita Taddei[2], Antonio Tiezzi[1], Stefania Garzoli[3]

[1]Dipartimento per la Innovazione nei Sistemi Biologici Agroalimentari e Forestali, Università degli Studi della Tuscia, L. go dell'Università, Viterbo; [2]Centro Grandi Attrezzature, Università degli Studi della Tuscia, L. go dell'Università, Viterbo; [3]Dipartimento di Chimica e Tecnologie del Farmaco, Università di Roma "La Sapienza", P. le Aldo Moro 5, Roma

Essential oils of the *Lavandula* genus and its hybrids are widely used for the production of perfumes and soaps as well as for their biological properties. Both liquid and vapor phase of the essential oil of *Lavandula* × *intermedia* "Grosso" (LEO) grown in the Lazio Region (Italy) were analyzed by the autosampler headspace coupled to a gas chromatograph/mass spectrometer (HS-GC/MS). The obtained results showed that the most abundant components were linalool (41.6%, 35.8%), linalyl acetate (23.0%, 7.5%), 1,8-cineol (5.2%, 19.8%) and terpinen-4-ol (4.8%, 2.9%) followed by lavandulyl acetate (3.2% 0.8%) and borneol (2.8%, 1.2%) as minor compounds detected in liquid and vapor phase, respectively.
The assessment of biological activity was carried out by testing both LEO liquid and vapor phase on environmental bacteria and human leukemia cells. Assays on gram-negative bacteria (*Escherichia coli*, *Acinetobacter bohemicus* and *Pseudomonas fluorescens*) and gram-positive bacteria (*Bacillus cereus* and *Kocuria marina*) showed good antibacterial activity.
LEO showed cytotoxic activity on HL60 human leukemia cells and it was further evaluated whether cell death induced by LEO treatment was related to the apoptotic process. By means of flow cytometry, expression of annexin V and labeling with propidium iodide were observed in cells treated for 24 hours with LEO. The percentage of apoptotic cells was 70.22% ± 6.93% and the activation of the apoptotic process was confirmed by the expression of caspase-3 in western blot and immunofluorescence. Furthermore, an apoptotic staining was carried out. Scanning and transmission electron microscopy investigations highlighted the morphological alterations typical of the apoptotic process.
The most abundant molecules present in LEO were subsequently tested to determine their ability to induce apoptosis and, among these, terpinen-4-ol and linalyl acetate were able to induce apoptosis to a greater extent.
Further studies will be carried out to test the single or combined activity of the main constituents and their possible uses in the food and pharmaceutical fields.

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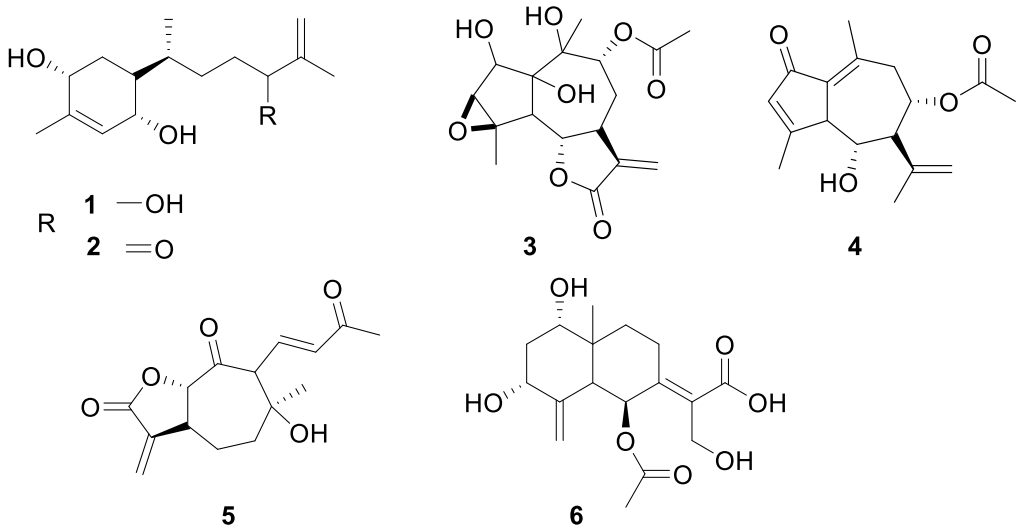
2 = Sesquiterpenes from *Ammoides atlantica* (Coss. & Durieu) H.Wolff

Valentina Parisi[1,4], Sihem Boudermine[2], Massimiliano D'Ambola[1], Alessandra Braca[3], Maria Laura Bellone[1,4], Nunziatina De Tommasi[1]

[1]Department of Pharmacy, University of Salerno, Via Giovanni Paolo II 132, 84084 Fisciano, Italy; [2]Department of Chemistry, University of Constantine, Research unit, Development of Natural Resources, Bioactive Molecules and Physiochemical and Biological Analysis, 20100 Constantine, Algeria; [3]Department of Pharmacy, University of Pisa, Via Bonanno Pisano 33, 56126 Pisa, [4]PhD program in Drug Discovery and Development

Ammoides altantica (Coss. & Durieu) H.Wolff is a plant belonging to Apiaceae family and together with *A. arabica* (T.Anderson) M.Hiroe and *A. pusilla* (Brot.) Breistr. represent the only three species of *Ammoides* genus. *A. atlantica*, a perennial or biennial species, presents slightly branched stems and white umbrella flowering. The plant is endemic in Algeria where it is traditionally used, as infusion, for the treatment of headache, fever, diarrhea and for mental debility in children. Previous investigations reported the antioxidant activity of aqueous extract, the chemical composition and the antibacterial activity of essential oils of *A. atlantica*. The present phytochemical study is focused on the study of the aerial parts of *A. atlantica*, collected in Jijel Province in the north-east of Algeria, to deepen the sesquiterpene components typical of the Apiaceae family. Dried leaves and young stems were subjected to extraction with solvent of increasing polarity, n-hexane, $CHCl_3$, $CHCl_3$-MeOH (9:1) and MeOH. Part of chloroform extract was subjected to column chromatographic separation using silica gel as stationary phase and eluting with $CHCl_3$ followed by increasing concentrations of MeOH in $CHCl_3$. Moreover $CHCl_3$-MeOH (9:1) extract was fractionated through column chromatographic separation using Sephadex® LH20 as stationary phase and eluting with MeOH. The simplified fractions obtained from the extracts were successively separated by an HPLC equipped with a reversed-phase column. Phytochemical investigation of fractions led to isolation of five pure sesquiterpenes (**1-5**) never reported in literature before. The structures were elucidated by 1D-and 2D-NMR spectroscopy (^1H, ^{13}C, DQF-COSY, HSQC, HMBC, ROESY) and confirmed by high resolution mass spectrometry.



https://drive.google.com/file/d/1WpZR4Mu1Uae50GnwukYFXs_bLt1-T6JP/view?usp=sharing

2 = Phytochemical characterization of *Arbutus unedo* L. leaves and fruit extracts at various ripening stages

Pierpaolo Scarano, Antonello Prigioniero, Daniela Zuzolo, Maria Tartaglia, Alessia Postiglione, Maria Maisto, Maria Antonietta Ranauda, Carmine Guarino and Rosaria Sciarrillo
Department of Science and Technology, University of Sannio, Via F. de Sanctis, s.n.c., 82100 Benevento, Italy

In this work, the *Arbutus unedo* L. leaves and fruits have been characterized, the second ones at different levels of ripeness. The strawberry tree leaves and fruits, that ripen through four different colours (Fig. 1), have been extracted through two different techniques, maceration and sonic waves, comparing them in the same conditions (solvent, pH, temperature). The results showed that the extracts obtained by different techniques had different characteristics in terms of total phenolic contents, antioxidant activity, scavenging activity and bioactive components. The use of the extraction technique of sonic waves has proved more performing than conventional extraction with maceration. The presence of phenolic, antioxidant and scavenging compounds was determined using the Folin-Ciocâlteu reagent and the DPPH method, respectively.
The data obtained showed different and interesting results depending on the ripeness of the fruits. Extracts in the ethanol-water mixture showed a more positive effect than the use of the single aqueous solvent in terms of the total phenolic compounds content. In addition, the bioactive compounds and amino acids contents of *Arbutus unedo* L. extracts were characterized by the use of liquid chromatography coupled with mass spectrometry. Anthocyanins, carotenoids, catechins, phenolic acids and polyphenols in general have been found: some molecules have been characterized for the first time in extracts from *A. unedo*.
Finally, the results suggest that fruits of *A. unedo* may be of great interest as a natural source of many bioactive compounds for food and nutraceutical applications.

Fig. 1. *Arbutus unedo* L. leaves and fruits.

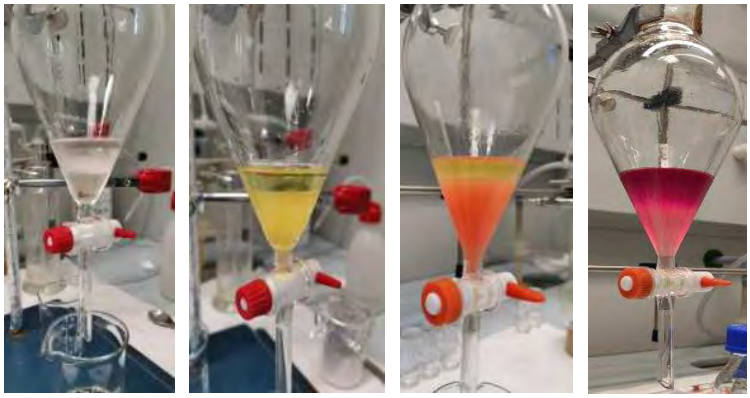


Fig. 2a-2b-2c-2d. Liquid-liquid extraction of *A. unedo* L. fruits.

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2 = Assessment of antioxidant and anti-inflammatory activity of flowering tops of *Mentha pulegium* L. (Lamiaceae) on human primary cells

Federica Sgrò[1,2], Marcella Denaro[1], Francesco M. Raimondo[3], Laura Cornara[4], Domenico Trombetta[1], Antonella Smeriglio[1]

[1]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Via G. Palatucci, 98168 Messina, Italy; [2]Foundation Prof. Antonio Imbesi, University of Messina, Piazza Pugliatti 1, 98122, Messina, Italy; [3]*PLANTA*/Autonomous Center for Research, Documentation and Training, Via Serraglio Vecchio, 28, 90123 Palermo, Italy; [4]Department of Earth, Environment and Life Sciences (DISTAV), University of Genova, Corso Europa, 26, 16132 Genova, Italy

Mentha pulegium L., belonging to the Lamiaceae family, is an aromatic and tomentose perennial herb widespread in Europe, Middle East, and North Africa, which grows in alluvial plains, riparian habitats, and freshwater wetlands. *M. pulegium* is well-known since ancient times for its medicinal properties such as emmenagogue and abortifacient effects as well as for the treatment of gastrointestinal ailments and skin itching. In this study, the flowering tops (flowers and leaves) of three *Mentha* specimens grown in three different Sicilian (Italy) areas (namely from here on I-III) with peculiar pedo-climatic conditions, were investigated.

Phytochemical studies allow to identify three different chemotypes: (I) Kampferide/Rosmarinic acid, (II) Rosmarinic acid, and (III) Jaceidin Isomer A. Preliminary investigations carried out on *in vitro* cell-free models showed interesting antioxidant and anti-inflammatory properties of all hydroalcoholic extracts of flowering tops of *M. pulegium* investigated, with flower extracts generally showing the best biological activity. The chemotype II, coming from Castronovo di Sicilia (PA), is the most interesting because in all the tests carried out showed the higher antioxidant and anti-inflammatory activity. These preliminary results have been corroborated by new experiments carried out on two *in vitro* cell-based models with human primary cells. Human erythrocytes have been used to evaluate the ability of the *M. pulegium* extracts to counteract the heat-induced haemolysis, as well as to investigate the free-radical scavenging activity preventing the formation of intracellular ROS induced by treating the cells with hydrogen peroxide. On the contrary, the anti-inflammatory activity of *M. pulegium* extracts was evaluated on LPS-treated human lymphocytes, by monitoring the release of some key inflammatory markers such as TNF-α, IL-6 and NO.

In conclusion, the results of this study experimentally confirmed that the pedo-climatic features influence the chemotype and the biological activity of *M. pulegium* and that the use of the flowering tops of this plant species finds a rationale in enhancing the biological activity of the plant complex.

Fig. 1. Flowering tops of *Mentha pulegium* L.

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2 = Secondary metabolite content of a commercial strain of *Lentinula edodes* (Berk.) Pegler and effect of medium enrichment with agro-industrial waste extracts on *in vitro* mycelium growth

Massimo Tacchini, Alessandro Grandini, Guglielmo Paganetto, Gianni Sacchetti, Alessandra Guerrini
Department of Life Sciences and Biotechnology, University of Ferrara, Piazzale Luciano Chiappini, 3, 44123, Malborghetto di Boara, Ferrara, Italy

Fungi are great producers of biomolecules due to their rich enzymatic pool that allows them to thrive in very different habitats, exploiting the most diverse sources to obtain nourishment and energy, and to synthesize molecules important for their biological activity. We focused on the edible and medicinal species of mushroom *Lentinula edodes* (Berk.) Pegler. Since ancient times, this mushroom has been used as a delicate ingredient due to its pleasant taste and considered one of the most important medicinal mushrooms in traditional Asian medicines. Due to its strong biological activities, such as reduction of platelet aggregation, antiviral, antibacterial, hypolipidemic and immuno-modulatory action and as a source of protein, fiber and lipids, vitamin D, B vitamins and minerals, it also became a popular ingredient of health supplements in the European market and in the mass retail. In this study sporophores from *L. edodes* were grown in a controlled environment, extracted through two eco-friendly strategies, ultrasound assisted extractions (UAE) and supercritical fluids extractions (SFE), and analyzed for the content of glucans (26,30±0,75 mg of total glucan/g of fresh mushroom), erythadenine (0,036±0,001 mg/g of fresh mushroom) and ergosterol (0,61±0,03 mg/g of fresh mushroom). In addition, the antioxidant activity (DPPH and ABTS test) of the various extracts was evaluated in parallel with their cytotoxic capacity (MTT test). Considering the food and health applications of *L. edodes*, the target of this project is to improve its market quality employing growth enhancers and promoting the secondary metabolites production. In order to follow the direction of the European community towards a circular economy, this research work was oriented towards a sustainable use of raw/secondary materials. In fact, the first phase of this study, focused on improving the in vitro growth of *L. edodes* mycelium, used several wastes from the agri-food chain to be valorized as growth stimulators and thus as secondary raw materials. Extracts obtained from grape and olive pomace gave the best results by inducing an increase in mycelium growth speed (mycelium arrived at confluence in 10 days instead of 12 days) and a thickening of the mycelial texture (fig. 1 and 2). In a second step, modified substrates were formulated for fruiting body growth and they will be analyzed to verify the ability of the extracts to modify growth and content of secondary metabolites.

Fig. 1. *L. edodes* mycelia grown in a simple medium.

Fig. 2. *L. edodes* mycelia grown in enriched medium.

https://drive.google.com/file/d/1hhaE-mWNEEqrE86I8dwPrtQ30V4jjF8j/view?usp=sharing

2 = In vitro cultured red carrot cells as effective, stable and safe ingredient for skin care, nutraceutical and food application

Giovanna Pressi[1], Oriana Bertaiola[1], Chiara Guarnerio[1], Raffaella Rizzi[1], Elisa Barbieri[1], Flavia Guzzo[2]
[1]Ætherabiotech Srl, Via dell'Innovazione 1, 36043 Camisano Vicentino, Vicenza, Italy; [2]Department of Biotechnology, University of Verona, Cà Vignal 1, Strada Le Grazie 15, 37134 Verona, Italy

Ætherabiotech Srl, an Italian green biotech company, has developed CROP® (Controlled Release of Optimized Plants), a proprietary and innovative biotech platform based on in vitro plant cell cultures.
Through this technology, non-GMO and alternative to traditional cultivation methods, we obtain a highly standardized phytocomplex with primary as well as secondary metabolites.
Without potential quantity limits, CROP® allows the production of those active ingredients that are difficult to find in nature or difficult to produce by chemical synthesis. The process allows to produce stable and standardized products at high title using multidisciplinary approaches to assure batch to batch highest reproducibility in composition & efficacy. By process, we guarantee the absence of solvents, pesticides, fertilizers and herbicides, pyrrolizidine derivatives, aflatoxins, heavy metals and GMO.
We combine extraordinary production capacity with a process that guarantees complete release from seasonal and availability variations and geographical limits. This platform, which has one of the largest production capacities in Europe, is also the shortest supply chain ever automated and managed with computerized processes to ensure the quality of product and accurate traceability. Ætherabiotech Srl, with the collaboration of the University of Verona, has produced and studied a phytocomplex of *Daucus carota (L.)* from in vitro cell cultures.
The carrot cell line R4G was selected from an anthocyanin-accumulating cell line, particularly stable at the light and at the temperature, generated from wild carrot.
Several families of plant secondary metabolites, such as anthocyanins, exhibit both antioxidant and anti-inflammatory activities and represent a great potential for the cosmetic and nutraceutical industries thanks to their hypothesized role in the prevention, respectively, of skin aging and of several diseases.
The carrot cell phytocomplex (DC-P) proved to have antioxidant and anti-inflammatory activities in J774 monocyte/macrophage cell cultures, and it was able to reduce nuclear translocation of NFkB jointly to an increase in the expression of VEGF-A on in vitro reconstructed human skin models.
These results guarantee a potential strategy against skin aging by blocking all aging processes associated to NF-kB nuclear translocation and an angiogenic effect that can reactivate microcirculation by increasing the capillary density.
Since these activities make the DC-P attractive for the development of ingredients for topical application, nutraceutical supplementation, as well as functional color in food (due to the stability at light and temperature of the anthocyanins of this specific product), we investigated its equivalence with natural red carrot extracts available in the market (by UPLC-MS analysis and untargeted metabolomics approaches followed by multivariate analysis).

Fig. 1. Microscopic image of Daucus carota cells.

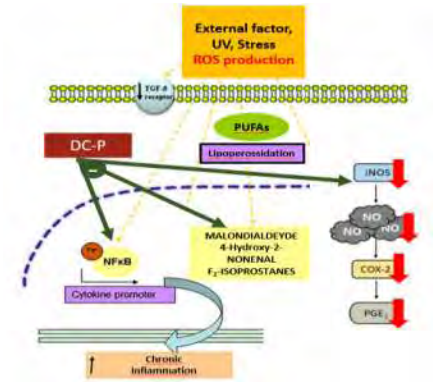

Fig. 2. Protective effects of DC-P on the skin.

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3 = Mt Etna: non-native species are also a problem of yours

Giuseppe Bonanno[1,2]
[1]Department of Agriculture, Food and Environment, University of Catania, Via Santa Sofia 100, 95123 Catania, Italy;
[2]Department of Biological, Geological and Environmental Sciences, Via Antonino Longo 19, 95125 Catania, Italy.

Mt Etna is the largest and most active volcano in Europe, and rises to its full grandeur in north-eastern Sicily, by occupying a surface of c. 1200 km^2, and reaching the height of c. 3300 m a.s.l. Mt Etna hosts also the oldest regional park of Sicily, established in 1981 but in fact created in 1987, which covers a total surface of c. 58000 ha. However, despite the presence of this huge protected area, Mt Etna is not immune to the presence and inevitable spread of non-native species.
Recent studies report that the flora of Mt Etna includes about 1100 taxa of which 94.5% are native (c. 1040 taxa) and 5.5% non-native (c. 60 taxa). The presence of non-native species on Mt Etna is quite remarkable considering that the total number of non-native taxa in Sicily are c. 440, thus implying that Mt Etna hosts 13.6% of the whole non-native flora of Sicily.
Despite the significant presence of a non-native flora, current actions of monitoring, control and prevention are not sufficient. Some critical issues include:
a) most of the non-native taxa are more than 10-year-old records;
b) lack of updated checklists;
c) scarce information on distribution and abundance of non-native species;
d) lack of information on the aggressiveness level of some potentially invasive non-native species, e.g., *Pennisetum setaceum*, *Centaurea diluta*, *Nicotiana glauca*, *Erigeron canadensis*, *Eclipta prostrata* (Figg. 1–5).
In particular, *E. prostrata* shows ever growing stands across uncultivated wet grounds, ditches and irrigation canals. This species has been relatively ignored because it is often mistaken for *Bidens* sp.pl.
As a first step, a widespread monitoring network should be set up to have a more exhaustive picture of the current presence and distribution of all non-native species across Mt Etna. Moreover, given the wide extent of Mt Etna and the presence of numerous nature lovers visiting periodically the Volcano, monitoring and control actions may be also supported by *citizen science* activities.
Dealing with non-native species is a complex issue that involves space-time variables but also social, cultural and economic factors. Given these multiple aspects, a constructive and close collaboration is necessary among the managing body of the Park of Mt Etna, neighboring municipalities, scientific and academic institutions, NGOs, local communities, and the private sector.

Fig. 1. *Pennisetum setaceum*.

Fig. 2. *Centaurea diluta.*

Fig. 3. *Nicotiana glauca*.

Fig. 4. *Erigeron canadensis*.

Fig. 5. *Eclipta prostrata*.

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3 = Using the Carta della Natura and distribution data of invasive alien plants of (European) Union concern in Sardinia for assessment of impacts in invaded habitats, definition of risk and invasion scenarios, and for drafting dedicated action plans

Manuela Manca[1], Giuseppe Brundu[2], Vanessa Lozano[2], Giovanni Piras[3], Roberta Capogrossi[4], Lucilla Laureti[4]
[1]FoReSTAS Agency, Via Deffenu 7, 08100 Nuoro, Italy; [2]Department of Agricultural Sciences, University of Sassari, Viale Italia 39, 07100 Sassari, Italy; [3]AGRIS Agency, Loc. Bonassai, S.S. 291 Sassari-Fertilia, km 18,600, 07100 Sassari Italy; [4]ISPRA-Istituto Superiore per la Protezione e la Ricerca Ambientale, Via V. Brancati 48, 00144 Roma, Italy

Article no. 3 of the Italian framework law on protected areas (L. no. 394/91) provides for the creation of a "Carta della Natura" (nature map, hereafter CdN), i.e., a map that "... *identifies the state of the natural environment in Italy, highlighting natural values and vulnerability profiles …* ". The national coordination of CdN was assigned to ISPRA which over time has defined methods and standards for the elaboration of this tool for the knowledge of the territory, based on the use of reliable basic data available for the national territory and IT/GIS procedures specifically tailored.
CdN for the Sardinia Region was created as part of a collaboration between ISPRA, the Sardinian regional Authority for the Environment and the University of Sassari. The works started with a first phase in 2006; subsequently it was completed in 2010. The present contribution aims to explore the potential of applying the CdN in the context of risk assessment and evaluation of negative impacts on habitats, as well as in the definition of strategies for tackling biological invasions, with special concern to the presence and distribution of 5 invasive alien species (IAS) of (European) Union concern pursuant to the Regulation (EU) no. 1143/2014 and subsequent amendments and updates. In fact, we focus on the following 5 species invasive in Sardinia: *Ailanthus altissima*, *Acacia saligna*, *Cenchrus setaceus*, *Hydrocotyle ranunculoides*, and *Pontederia crassipes*.
From an operational and methodological point of view, the geodatabase with the known distribution records of the 5 IAS in Sardinia was used for a topological overlay with the Sardinian CdN (Habitat map with a precision resolution of 1: 50,000). In this way, the polygons invaded by the different species and the different types of habitats involved were identified. The relationship among invaded habitats and type of invasive species, the expected or realized different types of impacts mechanism according to the EICAT / IUCN classification (e.g., competition, hybridization, disease transmission, changes in combustibility, interaction with other IAS), were assessed and on the basis of existing literature and of expert opinion. impacts on the habitats currently invaded were therefore quantified and spatially mapped. The analysis of the invaded habitats gives the opportunity to evaluate risk for those habitats of the same type that are not yet invaded, producing models for the assessment of the risk of invasion. These models take into concern additional predictors such as distance from areas already invaded, distance from roads, altitude, mode of propagation of the species, propagule pressure.
We are convinced that these preliminary results strongly support the usefulness of CdN in the management of plant invasions in Sardinia, with special concern to the invasive alien plants of Union concern.

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3 = Occurrence and distribution of alien plants along the sandy coastal ecosystems of the Italian peninsula

Simona Sarmati[1,2], Claudia Angiolini[2], Simona Maccherini[2], Bruno Foggi[4], Daniele Viciani[4], Matilde Gennai[4], Marta Gaia Sperandii[5,1], Silvia Cascone[1], Angela Stanisci[3], Alicia T.R. Acosta[1]
[1]Department di Science, University of Roma Tre, Viale G. Marconi 446, Roma, Italy; [2]Department of Life Sciences, University of Siena, Via P.A. Mattioli 4, Siena, Italy; [3]Department of Biosciences and Territory, University of Molise, Via Francesco De Sanctis, Campobasso, Italy; [4]Department of Biology, University of Florence Via G. La Pira 4, 50121, Florence, Italy; [5]Centro de Investigaciones sobre Desertificación (CSIC/UV/GV), Valencia, Spain

Although coastal ecosystems are transitional environments highly resilient to natural disturbance, they are strongly threatened by anthropogenic impact, especially due to invasion by alien plant species. Here we present a descriptive study on the occurrence and distribution of alien species along the Italian coasts, based on the analysis of data collected in sandy coastal ecosystems from 2002 to 2018. The data come from a standardized survey of 644 square ($4m^2$), georeferenced plots distributed along the Italian coasts into 8 macro-sites belonging to the Mediterranean bioclimatic region (Fig. 1), of which 152 (23.6%) are invaded. We counted a total of 244 species of which 13 were alien (5.3%), most of them neophyte-invasive (84.6%) and only two naturalized (*Avena sterilis* and *A. fatua*). The two species of the genus *Avena,* as well as *Arundo donax,* can be considered as archaeophytes. More than half of the exotic species observed (53.8%) originate from the American continent, probably as a consequence of the intense trade and migratory exchanges that for centuries have linked the New Continent to Europe and which have also involved the transport of plants between the two continents. Instead, only two species were African (genus *Carpobrotus*) and only one was Australian (*Acacia saligna*). The African alien species were found only along the Tyrrhenian coast, mainly in the Latium macro-sites (LA1, LA2 and LT1). On the contrary, the only Australian species (*A. saligna*) was observed principally along the Adriatic coast, but its presence was also recorded on the Tyrrhenian side (CA1, in Campania region), even though in a single plot. *Xanthium orientale* subsp. *italicum* was the only specie detected in all macro-sites. There was a clear disproportion between the number of plots invaded in the Adriatic (5.3%) and in the Tyrrhenian coasts (95%), probably due to the smaller extent of the former (respectively about 70 km and 167 km, Fig. 2). As to biological forms, most of the native species were Therophytes and Hemicryptophytes (respectively 39.4% and 25.8%), as expected in dune environments; alien species were instead mostly represented by Phanerophytes (38.4%, all of them invasive) and Therophytes (30.8%; Fig. 3). Compared to previous works where species classified as invasive were mainly herbaceous (Therophytes and Hemicryptophytes), we detected an increase in the presence of invasive Phanerophytes. The mean richness of native species in invaded sites was generally lower than in not-invaded sites, except for two sites (PU1 and TO1, respectively in Apulia and Tuscany regions) where it was similar. The highest values of mean species richness in Invaded vs. Not-Invaded sites were detected in TO1 (9.54) and in PU1 (9.45), respectively; the lowest value was recorded in CA1 (3.5 Invaded and 5.5 Not-Invaded, Fig. 4). According to the EUNIS classification, the habitat types with the greatest number of invaded plots were the embryonic and mobile dunes (B1.3, 27.6%), the coastal stable dune grassland (B1.4, 15.7%), and the coastal dune scrub (B1.6, 11.8%). Furthermore, the invasion phenomenon mainly characterized sites under intensive anthropic disturbance (D) to which it was not possible to associate a specific habitat type (34.2%, Fig. 2).

Fig. 1. Site map.

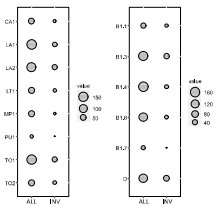

Fig. 2. Distribution of plots per site (on the left) and per habitat type (on the right).

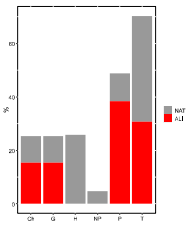

Fig. 3. Biological forms. All species (grey) vs. Alien species (red).

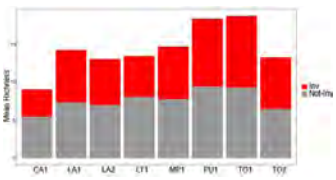

Fig. 4. Mean richness of native species per site. Invaded (red) vs. Not-Invaded plots (grey).

https://drive.google.com/file/d/1nv9-9t5Yve3__7Fz53AF5-dbGmElV5OS/view?usp=sharing

3 = Identification and removal of exotic fir trees for *in situ* preservation of *Abies nebrodensis*

Rosario Schicchi[1] Filippo Amato[1], Giuseppe Di Noto[1], Anna Geraci[2], Gaetano La Placa[1], Stefano Secci[3], Roberto Danti[3]
[1]Department of Agricultural, Food and Forest Sciences, University of Palermo, Viale delle Scienze, bldg. 4, I-90128 – Palermo, Italy; [2] Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, via Archirafi 38, I-90123 Palermo, Italy; [3]CNR - Institute for Sustainable Plant Protection, Via Madonna del Piano, 50019 Sesto Fiorentino, Firenze, Italy

The residual population of *Abies nebrodensis* (Lojac.) Mattei consists of only thirty adult trees distributed in a small area of the territory of the Madonie Regional Park, between 1375 and 1690 m elevation a.s.l. Among the various problems faced in the last twenty years for the protection of *A. nebrodensis*, those related to the safeguarding of the genetic integrity of the species have a considerable importance.
The LIFE00 NAT/IT/007228 Project recognized the presence of exotic fir trees, introduced through reforestation interventions at the beginning of the 80s' of the last century, as one of the main threats for the conservation of the genetic integrity of the natural population. In the aforementioned LIFE project, about 1000 individuals of non-native fir trees, such as *A. alba* Mill and *A. cephalonica* Loudon were removed or used as rootstocks for the propagation of *A. nebrodensis* between 2001 and 2005.
The reforestation site with prevailing *A. cephalonica* closest to the native range of *A. nebrodensis* is just over 3 km away and is located in the Comunello district in the municipality of Isnello, covering an area of about 5.5 hectares. Various 40 m^2 sampling areas were used to evaluate the amount and size of both the adult trees and the natural regeneration in this site and to plan the interventions for eradicating the exotic fir trees. To this end the natural regeneration was divided into plants younger than one year and seedlings older than one year, in turn divided into progressive 3 cm-height classes.
The data collected evidenced the occurrence of 1730 adult trees of Greek fir in 5.5 hectares, with a mean diameter of 35 cm at breast height and a mean height of 19 m.
The natural regeneration process has been remarkable, especially in the last five years. A mean of 4825 seedlings was found in the sampling areas: 133 seedlings had a height between 3 and 6 cm; 249 seedlings between 6.1 and 9 cm; 39 seedlings between 9.1 and 12 cm; 70 seedlings between 12.1 and 15 cm; 6 seedlings between 15.1 and 18 cm; 6 seedlings between 18.1 and 21 cm; 1 seedling was taller than 24 cm.
Based on the checklist of the alien vascular plant species reported in Italy by Galasso et al. (2018), *A. cephalonica* is an allochthonous species naturalized in Sardinia, Marche, Umbria and Friuli Venezia Giulia, while it is still allochthonous in Abruzzo and Sicily. However, according to recently acquired data here showed, *A. cephalonica* can be considered as a naturalized allochthonous species for Sicily too.
Results of this investigation evidenced that the prompt removal of the adult trees and the natural regeneration of *A. cephalonica* introduced in the Madonie Regional Park is of primary importance to safeguard the genetic integrity of *A. nebrodensis*.

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4 = A karyological study of *Dianthus virgineus* group (Caryophyllaceae) in the Central Mediterranean area

Giovanni Astuti[1], Gianluigi Bacchetta[2], Giulio Barone[3], Fabrizio Bartolucci[4], Liliana Bernardo[5], Angelino Carta[6], Fabio Conti[4], Gianniantonio Domina[3], Božo Frajman[7], Jacopo Franzoni[5], Gianpietro Giusso del Galdo[8], Duilio Iamonico[6], Mauro Iberite[9], Luigi Minuto[10], Marco Sarigu[2], Lucia Varaldo[10], Daniel Volgger[7], Lorenzo Peruzzi[6]

[1]Botanic Garden and Museum, University of Pisa, Via Luca Ghini 13, 56126 Pisa, Italy; [2]Centre for Conservation of Biodiversity (CCB), Department of Life and Environmental Sciences, University of Cagliari, Viale S. Ignazio da Laconi 13, 09123 Cagliari; [3]Department of Agricultural, Food and Forest Sciences, University of Palermo, Viale delle Scienze ed. 4, 90128 Palermo, Italy; [4]School of Biosciences and Veterinary Medicine, University of Camerino, Camerino, Italy; [5]Department of Biology, Ecology and Earth Sciences, University of Calabria, 87036 Arcavacata di Rende (Cosenza), Italy; [6]Department of Biology, University of Pisa, Via Derna 1, 56126 Pisa, Italy; [7]Department of Botany, University of Innsbruck, Sternwartestrasse 15, A-6020 Innsbruck, Austria; [8]Department of Biological, Geological and Environmental Sciences, University of Catania, Via A. Longo 19, I-95125 Catania, Italy; [9]Department of Environmental Biology, Sapienza University of Rome, P.le A. Moro 5, 00185 Rome, Italy; [10]Department for the Earth, Environment and Life Sciences (DISTAV), University of Genoa, Genova, Italy

Mediterranean region hosts a high number of *Dianthus* L. species, many of which included in the *D. virgineus* L. group. In the central Mediterranean area (Mediterranean France, Italian Peninsula, Sardinia, Sicily), this group is constituted by a high number of morphologically similar taxa, most of which are endemic to this area with narrow distribution ranges, especially in Sardinia and Sicily. In order to check for differences in karyological features that could serve as intrinsic reproductive barriers among these putatively different taxa, we characterized the genome size and/or chromosome number for 22 of them (51 populations in total).

For the estimation of relative genome size (RGS), we applied flow cytometric analyses of silica-dried leaf tissue from three individuals for each of 44 populations, chopped together with an appropriate amount of reference standard (*Bellis perennis* L.). For chromosome number characterization, we used root tips from germinating seeds collected in 29 populations, seven of which were not characterized for RGS; root tips were pre-treated with 0.4% colchicine and stained in leucobasic fuchsine after hydrolysis in 1N HCl at 60°C. For 22 populations both RGS and chromosome number have been characterized.

All the 29 populations surveyed are diploid with $2n = 30$ chromosomes and also additional 22 populations for which only RGS was estimated are RGS-diploid, confirming previous counts available in literature for the study area. The chromosome number was characterized for the first time in the following 15 taxa: *D. borbonicus* Brullo, C.Brullo, Colombo, Giusso, Ilardi & R.Perrone, *D. brachycalyx* A.Huet & É.Huet ex Bacch., Brullo, Casti & Giusso, *D. cyatophorus* Moris subsp. *minae* (Mazzola, Raimondo & Ilardi) Raimondo, *D. gasparrinii* Guss., *D. genargenteus* Bacch., Brullo, Casti & Giusso, *D. graminifolius* C.Presl, *D. ichnusae* Bacch., Brullo, Casti & Giusso subsp. *ichnusae*, *D. ichnusae* subsp. *toddei* Bacch., Brullo, Casti & Giusso, *D. insularis* Bacch., Brullo, Casti & Giusso, *D. oliastrae* Bacch., Brullo, Casti & Giusso, *D. sardous* Bacch., Brullo, Casti & Giusso, *D. saxicola* Jord., *D. siculus* C.Presl, *D. subacaulis* Vill. and *D. virgatus* Pasq. The homogeneity in ploidy level is contrasted by a slight variation in genome size. Despite a seemingly trend of increasing values from north-west towards south-east across the target area a however, we found no correlation between RGS and geographical distances (Mantel test, $r = 0.09$, $p = 0.08$). Nevertheless, ANOVA followed by post-hoc Tukey test returned that populations from Sardinia show significantly higher values with respect to all the other geographical groups, i.e. North-Western (South France and Liguria), Central (Tuscany and Latium), Southern (Apulia and Calabria), and Sicilian. No significant difference emerged among all other pairwise comparisons.

Therefore, the same chromosome number and the low RGS variation among 22 putatively different species occurring in Central Mediterranean seems not to provide relevant karyological discontinuities to allow complete divergence among lineages, with the exception of the seven taxa from Sardinia, which are collectively distinct and also geographically isolated from others.

Acknowledgements

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4 = New floristic and vegetation data on “Bosco di Santo Pietro”, a Site of Community Importance (S.C.I.) in southern Sicily (Italy)

Dario Azzaro[1], Salvatore Cambria[1], Gianpietro Giusso del Galdo[1]
[1]Department of Biological, Geological and Environmental Sciences University of Catania Via Antonino Longo 19, 95125 Catania, Italy

The S.C.I. "Bosco di Santo Pietro" (ITA 070005) is an area of remarkable naturalistic value, extending over an area of ca. 6,620 hectares within the municipality of Caltagirone (SW Catania). The flora of this territory has been investigated by several botanists (since 1845 by Taranto & Gerbino) and many sporadic data, chiefly related to rare or phytogeographically relevant species, are reported in literature. However, with the exception of an old and poorly known Flora by Zambrano dating back to 1889, preserved in the Caltagirone library, there is no specific study on the flora of Santo Pietro woodland. As regards the vegetation, there are only two contributions by Furnari (1965) and De Marco & Furnari (1976), mainly related to the woody vegetation belonging to the *Quercetea ilicis* Br.-Bl. ex A. & O. Bolòs 1950 and *Ononido-Rosmarinetea* Br.-Bl. in A. Bolòs y Vayreda 1950. Our investigations on the flora and vegetation of the Santo Pietro woodland started few years ago in order to have an in-depth knowledge on the vascular plant biodiversity of this important natural area, located within the severely anthropized landscape of south-eastern Sicily. In particular, our research allowed to identify about 500 taxa, including some rare species for Sicily and never reported for this area, such as *Astragalus caprinus* subsp. *huetii* (Bunge) Podlech, *Gagea apulica* Peruzzi & J.-M. Tison, *Isoëtes histrix* Bory, *Callitriche brutia* Petagna, *Romulea rollii* Parl., *Aphanes arvensis* L., *Lupinus gussoneanus* J. Agardh, *Juncus capitatus* Weigel, *Crassula alata* (Viv.) A. Berger, *Trifolium infamia-ponertii* Greuter, and *Trifolium suffocatum* L. At the same time, it was possible to confirm the occurrence of some very rare taxa known only for this area, such as *Gagea trinervia* (Viv.) Greuter, *Stachys arenaria* Vahl, *Echinophora tenuifolia* L., *Tuberaria villosissima* (Pomel) Grosser, and *Senecio glaucus* subsp. *hyblaeus* Brullo. As regards the vegetation, the phytosociological study was performed in order to have a complete syntaxonomic scheme including all the plant communities detected for the area. Further investigations are still on-going for the realization of an updated checklist of the vascular flora and plant communities of Santo Pietro woodland, including floristic and vegetation mapping, as well as assessment of the conservation status of the most peculiar and rare taxa found in the area.

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4 = *Foeniculum piperitum* and *F. vulgare*: a comparison between the two chemotypes

Natale Badalamenti[1], Maurizio Bruno[1,2], Angelo Troia[1], Vincenzo Ilardi[3]
E-mail: natale.badalamenti@unipa.it; maurizio.bruno@unipa.it; angelo.troia@unipa.it; vincenzo.ilardi@unipa.it
[1]Department of Biological, Chemical, Pharmaceutical Sciences and Technologies (STEBICEF), Università degli Studi di Palermo. Viale delle Scienze, ed. 17, 90128, Palermo, Italy; [2] Centro Interdipartimentale di Ricerca "Riutilizzo bio-based degli scarti da matrici agroalimentari" (RIVIVE), Università degli Studi di Palermo, Viale delle Scienze, 90128, Palermo, Italy; [3]Department of Earth and Marine Sciences (DISTeM), Università degli Studi di Palermo, Via Archirafi 22, 90123 Palermo, Italy

Foeniculum piperitum (Ucria) Sweet (Fig. 1a-b) was described as *Anethum piperitum* Ucria based on plants collected in Sicily, Italy. Currently it is treated either as a synonym of *F. vulgare* Mill., or as one of the two subspecies within that taxon.
Here we show that *F. vulgare* (Fig. 2a-b) and *F. piperitum* are two different, sometimes co-occurring, taxa and that given clear morphological and ecological separation, they should be treated as distinct species.
In the present study, indeed, the chemical compositions of the essential oils from roots, stems, leaves and fruits of *F. vulgare* subsp. *piperitum* collected in Sicily were evaluated by GC and GC-MS. The main components of the roots were terpinolene (33.15%), *γ*-terpinene (12.18%) and fenchyl acetate (11.23%). Stems and leaves were very rich in *α*-phellandrene (36.85% and 41.59%, respectively) and *β*-phellandrene (19.68% and 25.79%, respectively), whereas the main components of fruits were terpinolene (20.10%) and limonene (17.84%). These results were compared with those of the EOs of the same vegetative parts of *F. vulgare* subsp. *vulgare*, collected in the same station and in the same days. The oils of *F. vulgare* subsp. *vulgare* showed completely different compositions, with estragole, (*E*)-anethole and *α*-pinene as main compounds, clearly indicating the differentiation of the two subspecies.
Therefore, *F. vulgare* and *F. piperitum* are clearly different, not only from the morphological and ecological point of view, but also on the basis of phytochemical investigations; in this regard, unfortunately, many previous phytochemical data are scarcely usable due to this uncertain separation between the two taxa. In reality, as argued above, the difference is such that we cannot fail to consider it on a specific level. The phylogenetic relationships within the genus remain to be clarified, using molecular markers, as well as the definition of the ecology and chorology of *F. vulgare* and *F. piperitum*, due to the confusion linked to the aforementioned fusion of the two taxa. Owing to misapplication of names and wrong synonymizations, the ecology and chorology of *F. vulgare* and *F. piperitum* have to be better defined.

Fig. 1a-1b. *Foeniculum piperitum*.

Fig. 2a-2b. *Foeniculum vulgare*.

https://drive.google.com/file/d/1BfW3Qzcf8K_3s3hGCjJCJYHs7uJjy9-f/view?usp=sharing

4 = Centres of endemism in the vascular flora of Tuscany (Central Italy)

Gianni Bedini[1,2], Giovanni Astuti[3], Gianmaria Bonari[4], Lorenzo Pinzani[1], Giuliano Pacifico[5], Lorenzo Peruzzi[1,2], Francesco Roma-Marzio[3], Brunello Pierini[6], Angelino Carta[1,2], Daniela Ciccarelli[1,2]
[1]Department of Biology, University of Pisa, via Derna 1, 56126 Pisa, Italy; [2]CIRSEC, University of Pisa, via del Borghetto 80, 56124 Pisa, Italy; [3]Botanic Garden, University of Pisa, via Ghini 13, 56126 Pisa, Italy; [4]Faculty of Science and Technology, Free University of Bozen-Bolzano, Piazza Università 5, 39100, Bolzano, Italy; [5]Via Ponte del Vescovo 32/d, 54100 Massa-Carrara, Italy; [6]Via Zamenhof 2, 56126 Pisa, Italy

The Tuscan vascular flora features 79 exclusive endemic (hereafter EE) taxa. Several areas are known to host EE taxa. However, no comprehensive study has focused on the centres of endemism, i.e. areas with a high density of EE taxa, in the entire Tuscan territory. In this research, we identified such centres in a spatial dataset of georeferenced plant occurrence points.
We obtained the list of EE taxa from the Floritaly project (http://dryades.units.it/floritaly), then we sourced their georeferenced records from the Wikiplantbase #Toscana project (www.biologia.unipi.it/wpb/toscana/). Overlapping points of the same taxon were merged into a single record. We then scanned the dataset for areas of high record density, separated by areas of low density, with the OPTICS function of the DBSCAN R package. The function does not assume parametric distributions or variance properties, requires no prior knowledge on the number of clusters, and can find arbitrarily-shaped clusters of varying density and size. It only requires to set the minimum number of points to form a cluster (contiguous dense regions) and a starting radius; the latter is included mainly to reduce computation time and has little impact on the analysis.
To avoid arbitrary values of the first parameter, we started the analysis with a high value (150), proceeded in descending order until the first cluster was found, and iterated the process until the number of taxa in subsequently found clusters was less than four. The first identifiable cluster was centered on the Apuan Alps and contained 944-1,000 records of 33-36 species, depending on the settings; Elba Island came next, with 122-155 records of 9 species; the Mt. Amiata district and Capraia Island followed with lower values, then Montecristo, Mt. Argentario, and N-Apennine.
Regardless of the settings, the Apuan Alps and the Elba island are the most prominent centres of endemism. The former persists as a large, solid cluster at all settings. In contrast, the latter reveals an internal spatial structure at reduced density, which separates a western and an eastern cluster, matching the island geodiversity and phytogeography. Mt. Amiata, Capraia, Montecristo, Argentario, and the N-Apennine are smaller centres, linked to geological formations, geographical isolation, or high elevation (Table 1; Figure 1).
In conclusion, EE-taxa dense areas in Tuscany are linked to both geographical and ecological isolation. Geographical isolation acts mainly in the Tuscan Archipelago, while the continental centres are plausibly related to ecological isolation, mainly as a combination of geological substrate and elevation. As regards the number of taxa, the ecologically-isolated continental centres contribute most to EE taxa diversity in Tuscany.

Table 1. Clusters and associated number of taxa and records computed for different values of minimum number of points per cluster (MinPts) and minimum radius 5 km. Abbreviations: AMI = Mt. Amiata district; APP = N-Apennine; APU = Apuan Alps; ARG = Mt. Argentario; CAP = Capraia; EEL = E-Elba; ELB = Elba; MCR = Montecristo; WEL = W-Elba.

MinPts	Clusters	Taxa (min/mean/max)	Records (min/mean/max)
119	1: APU	33/33/33	944/944/944
42	2: APU, ELB	9/21.5/34	122/538/954
37	3: APU, ELB, CAP	7/16.66/34	41 /373.66/954
27	6: APU, WEL, EEL, CAP, AMI, MCR	4/10.83/34	35/207.33/954
18	8: APU, WEL, EEL, CAP, AMI, MCR, APP, ARG	4/10/34	26/166/957

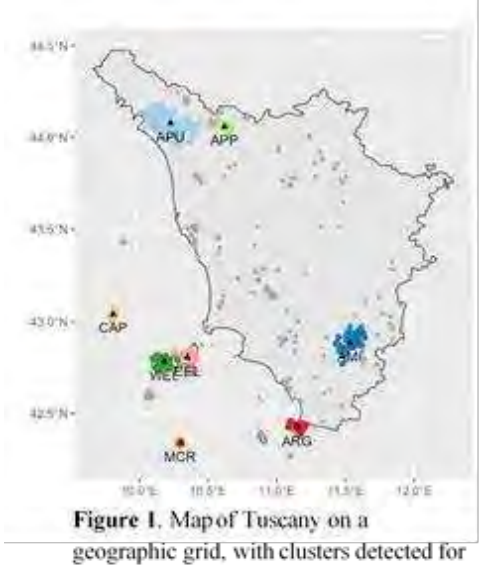

Figure 1. Map of Tuscany on a geographic grid, with clusters detected for MinPts=18. Abbreviations: see Tab. 1

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4 = Species richness in the vascular flora of Tuscany estimated through Species Distribution Model (SDM) approach

David Dolci[1], Angelino Carta[1], Brunello Pierini[2], Giulio Ferretti[3], Francesco Roma-Marzio[4], Giovanni Gestri[5], Gianmaria Bonari[6], Lorenzo Pinzani[1], Marco D'Antraccoli[5], Giuliano Pacifico[7], Jacopo Franzoni[1], Daniela Ciccarelli[1], Gianni Bedini[1], Lorenzo Peruzzi[1]
[1]Department of Biology, University of Pisa, Via Derna 1, 56127 Pisa, Italy; [2]Via Zamenhof 2, 56126 Pisa, Italy; [3]Department of Biology, University of Firenze, Via Giorgio La Pira 4, 50121 Firenze, Italy; [4]Botanic Garden and Museum, University of Pisa, Via Luca Ghini 13, 56126 Pisa, Italy; [5]Via Bartolomeo Bonfiglioli 30, 59100 Prato, Italy; [6]Faculty of Science and Technology, Free University of Bozen-Bolzano, Piazza Università 5, 39100, Bolzano, Italy; [7]Via Ponte del Vescovo 32/d, 54100 Massa-Carrara, Italy

Species richness is an important measurement of plant diversity. Despite species richness patterns on a global scale are relatively well known, local patterns are frequently less investigated. Different modelling methods have been proposed to estimate species richness of non-studied areas. A typical approach is modelling species-area relationship. An alternative method is based on cumulating species distribution models (SDMs) of those species known to occur in a region. SDMs are commonly used to forecast distributions of single species based on the relationship between species occurrence and environmental variables, but can be also used to generate community-level results and species richness estimations. To check for this potential application in the vascular flora of Tuscany and verify species richness patterns in the region, we used the data stored in the free online database Wikiplantbase #Toscana (http://bot.biologia.unipi.it/wpb/toscana/index.html) and the environmental variables from Wordclim (https://www.worldclim.org/) and Envirem (https://envirem.github.io/) databases. We selected 35 climatic and 2 topographic variables. We used a Principal Components Analysis (PCA) to reduce dimensionality and collinearity among environmental variables and retained the first three PCA axes, which accounted for 83.5% of variation. To generate species richness maps, we used the Maximum Entropy algorithm (Maxent) as modelling tool in the DISMO package.
Starting from the complete database, containing 266,440 records of occurrence for 4,352 species and subspecies (taxa hereafter), we removed all cultivated and casual alien taxa records. Then, we retained only those taxa with at least 5 records located in different grid cells (5' resolution), and generated potential distributions for all remaining taxa. We kept only the potential distributions derived from models with at least an Area under the curve (AUC) testing score > 0.6. This data filtering process led to a final dataset of 1,642 potential distributions referring to different taxa (about 44% of the established Tuscan flora), based on 196,998 occurrences. Finally, we summed all potential distributions obtained by a single-species modeling approach to obtain a potential species richness map for Tuscany.
The estimated mean potential number of taxa for Tuscany is equal to 935.96 per 10 × 10 km^2 cell, with a minimum of 842.59 taxa and a maximum of 1,078.49. There are significant differences in the estimated potential species richness among different areas. Coastal and near-coastal areas, including the area of Apuan Alps, as well as islands, are the richest in potential number of taxa. By contrast, inland areas show lower values of species richness, with a proportional decreasing with distance from the coastline. With respect to the mean value of potential species richness, the richest areas show an increase of 10-15%, the poorest areas show a decrease of 5-15%. Comparisons between our findings and previous investigation suggest that it is possible to generate reliable 'potential species richness' maps for the Tuscan flora using species distribution models.

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4 = Michele Lojacono-Pojero's specimens in the Herbarium Mediterraneum Panormitanum

Gianniantonio Domina[1], Emilio Di Gristina[1], Giulio Barone[1]
[1]Department of Agricultural, Food and Forest Sciences, University of Palermo, Viale delle Scienze, bldg. 4, I-90128 – Palermo, Italy

Michele Lojacono-Pojero (Palermo 1853 – Messina 1919), has been one of the botanists who most influenced floristic research in Sicily. He published 16 scientific papers on floristics and taxonomy of vascular and avascular flora, and on vegetation of the Island. His life's work, that includes the revision of the specimens of the herbarium of the Botanical Garden of Palermo, is summarized on the three volumes of his "Flora sicula".
Lojacono-Pojero published around 600 new scientific names; the search for the original material used by this scholar is of fundamental importance for the identification of nomenclatural types.
There is no news about the existence of a personal Lojacono-Pojero's herbarium. The largest part of his newly described taxa rests on the study of exsiccta housed in the *Herbarium Siculum* in Palermo (PAL) collected by others rather than Lojacono-Pojero himself (Todaro, Tineo, Reina, Citarda, etc.). The exsiccata collected by Lojacono-Pojero are partly preserved in Palermo, partly in the main Italian and European herbaria (e.g. B, BM, FI, G, K, L, P, RO, W).
Lojacono-Pojero had a strong personality that led him to come into conflict with both Agostino Todaro, his teacher and Director of the Botanical Garden of Palermo from 1856 to 1892, and with Antonino Borzì who succeeded Todaro in the direction of the Botanical Garden of Palermo.
In 1878 Lojacono-Pojero became temporary assistant at the Royal Botanical Garden of Palermo and in 1884 was appointed "Libero docente" (free professor) of Botany at the University of Palermo, but he will never be a salaried employee of the University. In 1913 Lojacono-Pojero, after 47 years of collaboration, in various capacities, with the Botanical Garden, definitively left Palermo to teach at the Royal Technical Institute of Messina.
We are aware of 11 Centuriae of plants sold by Lojacono-Pojero: seven of *Plantae Siculae Rariores* (1879 to 1884) and four of a second series, *Plantae Italicae Selectae* (1885 to 1888) that the botanist traded at reasonable prices to numerous correspondents to cover the expenses of the trips he made around Sicily and southern Italy to collect plants.
Among Lojacono-Pojero's collections in the herbarium of Palermo there are numerous samples collected in the early years of his collaboration at the Palermo institution.
We wanted to evaluate the consistency of these collections to provide a useful tool for nomenclatural and taxonomic studies on the taxa described by Lojacono-Pojero.
Altogether 1,868 samples were recorded; 714 of these have a date and were collected between 1870 and 1908. The exsiccatum dated 1870, if correctly labelled, suggests that Lojacono-Pojero developed a passion for botany at a very young age. At the age of twenty-three, in 1876, Lojacono-Pojero began collecting regularly for the Botanical Garden of Palermo. Most of the specimens date back to 1877 when Lojacono-Pojero was sent by Todaro on exploration on behalf of the botanical garden.
All the main Sicilian classical localities are represented, especially the Aeolian Islands, the Madonie Mountains and the surroundings of Palermo.
It is likely that when Lojacono-Pojero began selling centuries of exsiccata, in 1878, he destined the bulk of his collections to his correspondents rather than to the herbarium of Palermo, this aroused Todaro's anger.
Among the taxa collected by Lojacono-Pojero housed in PAL, the endemics and the species of biogeographical and taxonomic interest are scarcely represented. The plants published in the "Studies on rare or new critical plants of the Sicilian flora" and those cited in the "Report of a botanical excursion to Lampedusa" are missing. These gaps can be explained by Lojacono-Pojero's need to include rare and less known plants in the centuries he put up for sale.

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4 = The new prioritized list of wild plants of socioeconomic interest in Italy: a key step towards a conservation strategy for CWR and WHP

Domizia Donnini[1], Simona Ciancaleoni[1], Lorenzo Raggi[1], Giulio Barone[2], Daniela Gigante[1], Gianniantonio Domina[2], Valeria Negri[1]
[1]Department of Agricultural, Food and Environmental Sciences (DSA3), University of Perugia, Borgo XX Giugno 74, 06121 Perugia, Italy; [2]Department of Agricultural, Food and Forest Sciences, University of Palermo, Viale delle Scienze, Ed. 4, 90128 Palermo, Italy

It is well known that the Mediterranean basin is an important biodiversity hotspot, and the Italian Peninsula and its main Islands are among the areas with the highest number of endemics in Europe. Wild harvested plants (WHP) and crop wild relatives (CWR), respectively wild taxa that are genetically close to cultivated plants, and non-cultivated species which are collected from the wild, are part of that important segment of natural diversity known as 'Plant Genetic Resources' (PGR). They are widely used, directly or indirectly, by humans, thus have great socioeconomic importance. However, they have been largely neglected by the international policy tools for biodiversity conservation, such as the Bern Convention (Council of Europe 1979) and the Habitats Directive 92/43/EEC (European Commission 1992). With a view to enhancing PGR resources, the first and essential step is undoubtedly to develop and maintain updated lists and dedicated inventories of taxa, serving as the basis for analyzing their distribution, level of threat, current and desirable conservation actions. In this frame, in order to push towards the construction of appropriate management strategies, an updated annotated list of CWR and WHP has been prepared for the Italian territory (Italian peninsula, Sardinia, and Sicily) including information on known uses (see https://www.optima-bot.org/index.php/en/projects/8-category-en-gb/217-the-italian-cwr-whp-database). Following the approach in Landucci et al. (2014), who published a first version of the list, the included taxa were prioritized based on their value, native status, and need for protection/monitoring. The nomenclature was revised according to the most recent Italian checklists and updates and the regional distribution was updated. Additionally, information about the origin, endemic status, cultivation and uses, economic importance, gene pool, protection/monitoring needs, threat level at a national and global scale was provided. Recently identified taxa were added as well. Results show that 8,766 CWR/WHP taxa (belonging to 7,334 species) are recorded in Italy. Of these, 6,839 (5,516 species) are CWR only, 108 (108) WHP only, and 1,821 (1,710) both CWR and WHP. When we consider the Italian Peninsula, Sardinia and Sicily separately, taxa and species are distributed as follows: 7,916 (6,641), 2,745 (2,600), and 2,952 (2,738), respectively. The prioritization process allowed to identify 102 (82), 57 (50), and 735 (648) taxa belonging to the three defined protection priority categories ('A', 'B', and 'C', respectively). The taxa to be protected with highest priority in Italy ('A' category) belong to 36 different genera. Among them the most represented ones are: *Allium* L. (20 taxa), *Astragalus* L. (15), *Brassica* L. (12), *Vicia* L. (8), *Festuca* L. (7) and *Trifolium* L. (5).
The present study is intended to represent a starting point for developing *ex situ* and *in situ* conservation strategies at the national level, hoping to inspire a coordinated action by policy managers and the institutions.

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4 = Morphometric relationships among species of the *Santolina chamaecyparissus* complex (Asteraceae)

Antonio Giacò[1], Paola De Giorgi[1], Giovanni Astuti[2], Llorenç Sáez[3], Gianluigi Bacchetta[4], Lorenzo Peruzzi[1]
[1]Department of Biology, University of Pisa, Via Derna 1, 56126 Pisa, Italy; [2]Botanic Garden and Museum, University of Pisa, Via Luca Ghini 13, 56126 Pisa, Italy; [3]Department BABVE (Systematics and Evolution of Vascular Plants, associate unit to CSIC), Faculty of Biosciences, Autonomous University of Barcelona, 08193, Bellaterra Barcelona, Spain; [4]Centre for Conservation of Biodiversity (CCB), Department of Life and Environmental Sciences, University of Cagliari, V.le S. Ignazio da Laconi 13, 09123 Cagliari

The *Santolina chamaecyparissus* complex (Asteraceae, Anthemideae) includes 14 species of dwarf aromatic shrubs that are native to central-eastern Spain, southern France, Italy, Balearic Islands, Corsica, and Sardinia. Most species show an allopatric distribution and are endemic to relatively restricted areas. Some authors consider this species complex of recent evolution and hypothesize Spain as the centre of diversification for the whole genus. The systematics and taxonomy of the *S. chamaecyparissus* complex have been scarcely studied in the past, and several studies are ongoing in order to clarify the taxonomic relationships among species. A nomenclatural revision has been recently published and a cytogenetic study is currently under revision. All the continental species are diploid ($2n = 2x = 18$), except *S. villosa*, a species endemic to Spain, that shows two different cytotypes (tetraploid and hexaploid). *S. corsica*, endemic to Corsica and Sardinia is tetraploid, and *S. insularis*, endemic to Sardinia only, is hexaploid; *S. chamaecyparissus* is a pentaploid cultivated species of unknown (possibly anthropogenic) origin. In this contribution, preliminary results of a morphometric analysis of the whole complex are presented.
Twenty-three populations of 12 species have been sampled during summer 2020, and four more populations will be sampled during this summer, including the remaining species (*S. villosa* and *S. virens*). For each population, 20 specimens have been sampled (9 for *S. chamaecyparissus* and 13 for *S. vedranensis*), and 46 morphological characters (both quantitative and qualitative) have been recorded. A Principal Coordinate Analysis (PCoA) based on Gower distance has been carried out in order to visualize the multivariate distribution of samples. Classification methods have been applied to evaluate the correct classification of groups of populations according to the current taxonomic circumscription of species and/or other alternative groupings based on the results of the PCoA. Moreover, these analyses have been used to categorize morphological characters according to their discriminant power. Univariate analyses have been also carried out.
Preliminary analyses suggest that: a) *S. corsica* and *S. insularis* cannot be easily distinguished on their morphology; b) an isolated population of *S. decumbens*, a species endemic to Provence (Southern France), shows a morphology intermediate between the typical *S. decumbens* and *S. ericoides*, a species largely distributed in Spain and France, allopatric with *S. decumbens*; c) *S. chamaecyparissus* is strikingly similar to *S. corsica*/*S. insularis*.

Acknowledgements
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4 = The LIFE IMAGINE Project for the ex-situ conservation of the native flora of European concern in Umbria (central Italy)

Daniela Gigante[1], Federica Bonini[1], Laura Cencini[1,4], Maurizio Conticelli[2], Francesco Falcinelli[3], Valentina Ferri[1], Marco Fratoni[3], David Grohmann[1,4], Marco Maovaz[1,4], Maurizio Micucci[1,4], Antonella Montanucci[1,4], Lorenzo Raggi[1], Giuseppe Ratini[2], Valeria Negri[1]

[1]Department of Agricultural, Food and Environmental Sciences, University of Perugia, Borgo XX giugno 74, I-06121 Perugia, Italy; [2]GIROS Sezione Umbria, Via della Lepre, 11, 05100 Terni, Italy; [3]Carabinieri Biodiversity Department of Assisi, Viale Umberto I 5, I-06081 Assisi (PG), Italy; [4]C.A.M.S. and Botanical Garden, University of Perugia, Borgo XX giugno 74, I-06121 Perugia, Italy

The LIFE19 IPE/IT/000015 Project "IMAGINE" is an Integrated LIFE Project lasting seven years (2020-2027) aiming at supporting the development of an integrated, unified, coordinated, and participatory management strategy for the Natura 2000 network in the Umbria region (www.lifeimagine.eu). Among the different topics, action A11 is focused on the "Development of action plans and ex-situ conservation protocols for 5 Annex II-IV plant species" occurring in Umbria: *Adonis distorta* Ten., *Himantoglossum adriaticum* H.Baumann, *Iris marsica* I.Ricci & Colas., *Ionopsidium savianum* (Caruel) Ball ex Arcang., *Klasea lycopifolia* (Vill.) Á.Löve & D.Löve. The maintenance of Annex II-IV species in a good conservation status is a national duty for each EU country, directly deriving from the Council Directive 92/43/EEC. A correct approach to plant conservation implies a deep knowledge of their ecology, distribution, demography, biological habitats, and floristic-vegetational traits. Accordingly, in the first two years of the Project, Action A11 will be devoted to: i) demographic studies in the most representative sites of occurrence of the 5 target species; ii) development of specific Action Plans for the 5 target species; iii) additional selection of a list of "H-key" plant species with a critical role for grassland Annex I Habitats reinforcement; iv) development of a protocol for the ex-situ conservation of the populations of the 5 target species, including germplasm collection, conservation and analysis of genetic variability. Conservation of collected materials will be carried out at DSA3 Germplasm Bank (FAO ITA_363). A complementary conservation activity will be the design and construction of an Apennine flowerbed in the Botanical Garden of Perugia University, which will host typical species from the various types of Apenninic semi-natural grasslands (Annex I Habitats 6110*, 6210, 6220*, 6230, 6510) and, among them, the Annex II-IV target plant species occurring in Umbria. In this way, their ex-situ conservation will be performed. Thanks to the availability of living tissue, further analysis on population genetics will be possible for those species still in need of investigation (e.g., *Iris marsica*). The Project's activities will benefit from the support of valuable stakeholders, such as the Regional Section of the Orchidologists Association G.I.R.O.S., and the Carabinieri Biodiversity Department of Assisi (PG). Additionally, the involvement of citizens in species reporting is already providing relevant results.

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4 = "The Lichens of Italy" project on iNaturalist: a tool for citizen science and lichens

Deborah Isocrono[1], Cristiana Peano[1], Jurga Motiejūnaitė[2]
[1]Department of Agricultural, Forest and Food Sciences, University of Torino, Largo Paolo Braccini 10095 Grugliasco (TO) Italy [2]Laboratory of Mycology, Nature Research Centre, Žaliųjų ežerų Str. 49, 08406 Vilnius, Lithuania

Citizen science projects have proliferated over the last decade, making it possible to track the ecological and social impacts of large-scale environmental changes through different apps. iNaturalist (www.inaturalist.org) is a web-based and mobile-supported social platform where individuals can upload observations and identify organisms. Observations are media (images, videos, or audio files) of a single organism tagged with metadata such as taxonomic identification, date, geographic information. Aggregated data are available for download and scientists are already using them to estimate species distribution, develop checklists, document alien species. Lichens are still under-represented on this platform. As of 2021, iNaturalist contains over 69 million biological observations from users around the world; a search of lichen records returns less than 700.000 lichen observations worldwide. This is probably due to the difficulties in identifying lichens at species level looking only at images and the consequent lack of guidebooks suitable for amateurs. The need for chemical testing and microscopic observations often requires expert knowledge, making lichen identification particularly challenging for some species (e.g. members of the family Teloschistaceae, where you now have to know species in order to name its genus). Nevertheless, increasing understanding of lichens, which are frequently misunderstood organisms, through citizen science is considered a very important tool for opening up science to the public and raising awareness on endangered species, biological conservation, and response to climatic change.

"The Lichens of Italy" project was established in autumn 2020 to promote knowledge, conservation, and study of lichens. In this project, experts assess the taxonomic correctness of iNaturalist records. Until now, the project counts 2553 observations made by 494 participants and referred to 199 species. The most contributed lichens by iNaturalist volunteers were obviously the more common ones such as *Xanthoria parietina* (L.) Th.Fr. (265 records) and *Flavoparmelia caperata* (L.) Hale (100 records) and the easily recognizable *Lobaria pulmonaria* (L.) Hoffm. (53 records). From the lichen observations included in the project, the majority are "Needs ID" observations (69%), 768 (30%) are "research-grade" (i.e. the highest level in iNat when the community agrees with a level lower than family; RG observations are also included in GBIF database). Results showed that iNaturalist lichen records are widespread in the Italian territory (Fig. 1).

Nowadays the continuing improvement of communication technology, especially the advent of Web based and location-aware mobile technologies has considerably expanded people's ability to contribute to Citizen Science projects.

Participating in online citizen science initiatives has been shown to improve attitudes toward science, provide a better grasp of the nature of science, increase scientific knowledge, and provide more topic-specific understanding. Some initiatives may be carried out to strengthen these skills further, such as providing additional identification materials suitable also for non-specialists (keys, blogs, etc.) or organizing specific training events (like bioblitzes, training courses, etc.).

Fig. 1. Distribution of lichen records in the project "The lichens of Italy" on iNaturalist (accessed on 14 June 2021).

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4 = *Pimpinella anisoides* (Apiaceae): viability test and morphometric comparison with related species

Valentina Lucia Astrid Laface[1], Carmelo Maria Musarella[1], Gina Maruca[2], Giovanni Spampinato[1]
[1]Department of Agriculture, Mediterranean University of Reggio Calabria, Località Feo di Vito snc, 89122, Reggio Calabria, Italy; [2]Institute of Biosciences and Bioresources, National Research Council, Via G. Amendola 165/a, 70126 Bari, Italy

Pimpinella anisoides V.Brig. is an endemic species present only in southern Italy, in Latium, Campania, Basilicata, Apulia, Calabria and Sicily. In Calabria it can be found in several localities in the mountainous areas occupied by chestnut and oak mesophilic woods, mainly on the Reventino massif and in the Sila Catanzarese (central Calabria) between 700 and 900 m a.s.l. *P. anisoides* is known to be used in Calabria to flavour several baked products, such as "taralli", bread and biscuits, traditional liqueurs or as a seasoning. Although the species is currently considered LC (Least Concern) according to IUCN criteria, the indiscriminate fruits harvesting for commercial purposes is compromising the survival and size of the populations.
In this study, the viability and structure of fruits from three localities in the Sila Catanzarese, representing our three accessions under study, were examined. Fruits harvested at maturity in August 2018 by local farmers (who market the species) were subjected to Cut test and Tz test to preliminarily test their viability and quality.
The cut test involved transversely fruit cutting with a scalpel and observing the internal tissues: high quality seeds showed turgid, healthy tissues with a typical silvery-white colour and no pathogen or insect damage. Once observed, the seeds were placed in a matrix with three different categories: Vital; Non-vital; Vain. The % viability was then calculated. To perform the Tz test, the mericarps were immersed in water for 24 hours. The pericarp was then removed with the help of a scalpel. The prepared seeds were completely immersed in a 1% solution of 2,3,5 Triphenyl tetrazolium chloride in distilled water for 6 hours, at 30°C, in the dark.
Both tests were carried out on a total of 120 fruits (60 per test, 20 for accessions), selected from those that were found to be normal, i.e. without visible morphological damage, under the stereomicroscope. The Cut test showed that most of the seeds (90%) appear viable, present tissues corresponding to the above-mentioned characteristics and are free from damage caused by pathogens or insects. In particular, the first accession (Pa_1) had 100% of viable seeds, accession Pa_2 had 95% and accession Pa_3 had 75%. The percentage of total non-viable seeds is very low (5%); no vain seeds were found. The Tz test showed that 70% of the analysed seeds were viable, 28% were non-viable and 2% were considered doubtful (seeds not coloured properly or with irregular colour spots). In particular, accessions Pa_1 had 80% of viable seeds, 15% non-viable and 5% doubtful, accessions Pa_2 had 80% viable, accessions Pa_3 had 50% viable. Accessions Pa_2 and Pa_3 do not present doubtful seeds.
As the fruit is a fundamental character to distinguish the different species of *Pimpinella*, morphometric analyses were carried out in order to delineate the main characteristics of the monocarps of *P. anisoides*. In the populations examined, they are completely glabrous, with 5 grooves in the dorsal part, flanked in the ventral part, where the columella that joins the two monocarps is clearly visible, dark brown-black in colour and varying in shape from oblong-ovoid (length/width ratio 1.09) to oblong-cylindrical (length/width ratio 2.05), on average 2.77 (± 0.30) mm in length and 1.33 (± 0.14) mm in width; the length of the monocarp from the base to the maximum width is 1.12 (± 0.14) mm. The stylopodium is on average 0.45 (± 0.06) mm long and 0.33 (± 0.09) mm wide. The cross-section of the fruit has a smooth, thin cuticle. There are five small vascular bundles in the lower half of the dorsal and lateral ribs. The vittae in the mesocarp, containing essential oils, are divided into: 7 vallecular vittae between the central groove, the median groove and the median groove closest to the commissural side; 6 commissural vittae located on the commissural side. The endosperm occupies the largest part of the section.
The morphology of the fruits of *P. anisoides* was compared with that of two other related species used commercially: *P. anisetum* Boiss. & Balansa, which has ovoid, light brown, shaggy fruits with appressed hairs, and *P. anisum* L. which has white, bristly, ovoid-subglobose fruits with appressed hairs.
Trials should be initiated to cultivate endemic species of food interest, such as *P. anisoides*: this can certainly help to protect and safeguard wild populations of species subjected to indiscriminate harvesting for commercial purposes. Furthermore, knowing the distinctive characters of *P. anisoides* fruits is essential to avoid food frauds, such as those in which healthy fruits are mixed with others of lower quality or with fruits belonging to different species or genera.

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4 = Biodiversity of segetal flora in conventional and biologic globe artichoke fields in Sardinia

Vanessa Lozano[1], Maria Teresa Tiloca[1], Giuseppe Brundu[1], Luigi Ledda[2]
[1]Department of Agricultural Sciences, University of Sassari, Viale Italia 39, 07100 Sassari, Italy; [2]Department of Agricultural, Food and Environmental Sciences, Polytechnic University of Marche, Via Brecce Bianche 10, 60131 Ancona, Italy

Segetal flora refers to the set of spontaneous plant species that grow in agroecosystems, relates with crops, and whose composition and biomass depend on habitat conditions and management adopted. Plant communities in conventional cropping systems, often include a limited number of species, with dense covering, which may represent a limiting factor as their presence affect crop yield and quality. On the contrary, the presence of a more diverse segetal flora considerably increases the whole biodiversity of agroecosystems, supporting pollinators and other beneficial insects, with positive effects on the phytosanitary status of the crop and on the chemical-physical characteristics of the soil. The study was carried out in Sardinia (Italy) during 2018-2021 growing seasons within the framework of the CarBio project to evaluate the biodiversity of segetal flora in globe artichoke cropping system, thus providing support to farmers to maintain biodiversity and ecosystem services. The data was collected in five macro-areas representative of the Sardinian globe artichoke fields through georeferenced plots (1×1 m). Three different cropping systems were analyzed: conventional, transition (from conventional to organic) and organic. Based on 540 floristic surveys, it was possible to make an inventory of 115 plant species, including subspecies, that makes up the segetal flora. Not surprisingly, the most represented families were Asteraceae (18 species), Poaceae (16) and Fabaceae (10). The most frequent and often also the most abundant species (in terms of covering) were *Avena fatua* L. s.l., *Avena sterilis* L. s.l., *Calendula arvensis* (Vaill.) L., *Convolvulus arvensis* L., *Glebionis coronaria* (L.) Spach, *Lolium rigidum* Gaudin, *Oxalis pes-caprae* L., *Papaver hybridum* L., *Poa annua* L., *Ranunculus arvensis* L., *Ranunculus muricatus* L., *Stellaria media* (L.) Vill., and *Veronica persica* Poir.
The composition and biodiversity of the segetal flora of globe artichoke fields, analyzed in terms of α, β, γ diversity, significantly changes during the year and it is clearly influenced by the cropping system. Some species have been observed exclusively in organic cropping system (e.g., *Linaria reflexa* (L.) Desf.). In particularly *Calendula arvensis*, and *Oxalis pes-caprae*, in some cases with 100% coverage in the plots. While others, in particular *Glebionis coronaria* and *Stellaria media*, were more common in transition management, and in winter. *Lolium rigidum* has been observed with up to 100% cover of the plot managed in conventional management in winter. Other species such as *Amaranthus blitoides* S.Watson were more common in the organic management and *Beta vulgaris* L. subsp. *Maritima* (L.) Arcang., in transition system.
In general, the highest average coverage was observed in the organic management, where segetal vegetation often also occurs multilayered, with a greater number of species while in conventional management, the coverage of the segetal flora was lower. We now plan to investigate the relationships among α, β, γ diversity, crop performance and main ecosystem services.

Fig. 1. In the left and example of a Sardinian globe artichoke field, in the right the five macro-areas representative of the Sardinian artichoke fields.

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4 = Taxonomic and chorological remarks on *Clinopodium canescens* (Lamiaceae), a valid unrecognized species from Sicily

Vivienne Spadaro[1], Emilio Di Gristina[2], Giuseppe Venturella[2], Francesco M. Raimondo[3]
[1]Department STEBICEF/Sect. of Botany, Anthropology and Zoology, University of Palermo, Via Archirafi 38, 90123–Palermo, Italy; [2]Department of Agricultural, Food and Forest Sciences, University of Palermo, Viale delle Scienze, bldg. 4, 90128 – Palermo, Italy; [3]*PLANTA*/Center for Research, Documentation and Training, Via Serraglio Vecchio 28, I- 90123, Palermo, Italy

The taxonomic study of some critical plant populations of Lamiaceae in Sicily, traditionally referred to *Satureja* L. and *Calamintha* L. - all recently transferred to *Clinopodium* L. - led to the description of *Clinopodium raimondoi* Spadaro, Faqi & Mazzola, initially known from the western outskirts of the city of Palermo. Following the review of a group of critical European taxa, framed in the same genus, Melnikov [Novosti Sist. Vyssh. Rast. 47: 103 (2016)] recovers the ancient and forgotten taxon *Calamintha canescens* C. Presl and transfers it the genus *Clinopodium*, including *Cl. raimondoi*, considering the latter conspecific of *C. canescens* and synonymous with the Presl's taxon. This, however, without any explanatory comments. There is reason to believe that this author arrived at this determination based not only on the diagnostic characters published but also on the comparison of the published photo of the holotype with the original material by Presl preserved in Prague (PRC). *Calamintha canescens* is a taxon succinctly described in the "Flora Sicula" of K.B. Presl, published in Prague in 1826, following the visit made to Sicily a few years earlier (March-July 1817). In the protologue, the author of the species (C. Presl) does not report any data on the distribution in Sicily of the taxon that will be almost completely forgotten in later floristic works, which is why it will also escape to the authors of *Cl. raimondoi*. Even the recent checklist of the Italian flora does not mention *Cl. canescens* while it includes *Cl. raimondoi*. The new combination, *Clinopodium canescens* (C. Presl) Melnikov, will instead be incorporated only by Pignatti in the recent second edition of the "Flora d'Italia".
The study of the original material of *Calamintha canescens* allowed to verify the correspondence with *Clinopodium raimondoi,* a conclusion strengthened by the correspondence also of the localities and of the growth habitat - albeit generics - recorded in the author's handwritten label (*in pascuis apricis et ad vias prope Panormum*). In fact, the indications correspond well to the places and habitat where *Cl. raimondoi* was found. After the pubblication of *Cl. raimondoi,* new localites were found, particulary in the surroundings of the city of Palermo and in the territory of the same province: to the west in Marina di Cinisi and Terrasini, to the east near Termini Imerese, ca. 30 km from Palermo.
Material of *Cl. canescens* from the slopes of Monte Pellegrino, has been studied, sub *Cl. raimondoi*, for the knowledge of the composition of essential oils and their biological activity [Tuttolomondo & al., Acta Hortic. 1189. ISHS 2017. https://doi.org/10.17660/ActaHortic.2017.1189.102].

Clinopodium canescens in different habitats: *pascuis apricis* in Marina di Cinisi (Palermo), and roadside in the locality Addaura (Palermo).

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4 = Integrated taxonomy of Italian subspecies of *Armeria arenaria* (Plumbaginaceae), with a special focus on the putative subspecies endemic to Northern Apennine

Manuel Tiburtini[1], Giovanni Astuti[2], Fabrizio Bartolucci[3], Gabriele Casazza[4], Lucia Varaldo[4], Daniele De Luca[5], Maria Vittoria Bottigliero[5], Gianluigi Bacchetta[6], Marco Porceddu[6], Gianniantonio Domina[7], Simone Orsenigo[8], Lorenzo Peruzzi[1]

[1]Department of Biology, Unit of Botany, Pisa University, Via L. Ghini 13, 56126 Pisa, Italy; [2]Botanic Garden and Museum, University of Pisa, Via Luca Ghini 13, 56126 Pisa, Italy; [3]Scuola di Bioscienze e Medicina Veterinaria, Università di Camerino – Centro Ricerche Floristiche dell'Appennino, Parco Nazionale del Gran Sasso e Monti della Laga, San Colombo, 67021 Barisciano (L'Aquila), Italy; [4]Department for the Earth, Environment and Life Sciences (DISTAV), University of Genoa, Corso Europa 26, 16132 Genoa, Italy; [5]Department of Biology, University of Naples Federico II, Via Cinthia, 26, 80100 Naples, Italy; [6]Centre for Conservation of Biodiversity (CCB), Department of Life and Environmental Sciences, University of Cagliari, V.le S. Ignazio da Laconi 13, 09123 Cagliari; [7]Department of Agricultural, Food and Forest Sciences, University of Palermo, Viale delle Scienze ed. 4, 90128 Palermo, Italy; [8]Department of Earth and Environmental Science, University of Pavia, Via S. Epifanio 14, 27100 Pavia, Italy

Armeria arenaria (Pers.) Schult. is a highly polymorphic species that includes 13 subspecies in its whole western European range. Concerning the Italian flora, three subspecies are currently recorded: subsp. *arenaria* in the Alps, the Italian endemics subsp. *marginata* (Levier) Arrigoni and subsp. *apennina* Arrigoni in Northern Apennine. On the contrary, the occurrence of subsp. *praecox* (Jord.) Kerguélen in Italy is doubtful. These taxa have been recognised solely on qualitative morphological grounds. For this reason, we applied an integrative approach including morphometric, seed morpho-colorimetric, karyological, ecological and molecular analyses to test the current taxonomic scheme concerning these four subspecies. Twelve populations, including the *loci classici* of the four taxa, were sampled. We measured 57 qualitative and quantitative morphological characters, then subjected to morphometric analyses. Digital images of 100 seeds per accession were acquired, and 124 morpho-colorimetric features were extrapolated by using an ImageJ plugin. Chromosome number and karyotype asymmetry indices were calculated. Ecological niche overlap was measured by using a kernel smoothers to densities of species occurrences in gridded environmental space. The nuclear ITS region and four plastid DNA intergenic spacers (*psb*A-*trn*H, *trn*Q-*rps*16, *trn*F-*trn*L, and *trn*L-*rpl*32) were sequenced from tree individuals for each population.

Two major groups are apparent in the morphometric PCoA, and we informally named these groups "arenarioid" (merging all accessions of subsp. *arenaria* and subsp. *praecox*) and "marginatoid" (merging all accessions of subsp. *marginata* and subsp. *apennina*). Within the "arenarioid" group, subsp. *praecox* is only slightly recognisable. The population of Monte Prinzera, initially attributed to subsp. *apennina*, clearly falls within the variability of the "arenarioid" group. LDA performed to test the starting taxonomic hypothesis (4 subspecies) results in an 87% correct *a priori* classification. Hypothesizing, on the contrary, to merge subsp. *apennina* with subsp. *marginata* and to test 3 groups, we obtain 96% of correct *a priori* classification. Only slightly lower values (95.4%) are obtained by hypothesizing only the two groups ("arenarioid" and "marginatoid"). All the studied populations are diploid with $2n = 2x = 18$ medium-sized chromosomes. Karyotype asymmetry highly overlaps among subspecies, albeit "marginatoid" plants show higher M_{CA} (mean centromeric asymmetry) values. The niche of "arenarioid" and "marginatoid" groups are rather distinct, because they grow in regions experiencing different climatic conditions, as suggested by the similarity test. On the contrary, the niche overlap between subspecies is higher and each of them grows in climate as similar as possible to that of the other subspecies, given the available climatic conditions. Molecular markers show that variation is clearly geographically structured, with "arenarioid" French populations (*loci classici* of both subsp. *arenaria* and subsp. *praecox*) forming two separate groups. All the Italian accessions form a third group, further split in an "arenarioid" subgroup (including Monte Prinzera) and a "marginatoid" subgroup, which also includes a single alpine "arenarioid" accession (Piana di Salmezza). The results on seed morpho-colorimetric data are congruent with the molecular data.

We can conclude that the current taxonomic scheme is no longer advisable. There is no support for a distinction between subsp. *apennina* and subsp. *marginata*. On the contrary, there is a strong support to collectively distinguish "arenarioid" and "marginatoid" groups. Further research is needed to decide on the taxonomic value of subsp. *praecox* and its circumscription with respect to subsp. *arenaria*.

Acknowledgements

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6 = First evidence of fungistatic activity and inhibition of *Candida albicans* dimorphism by the green microalgae *Neochloris oleoabundans*

Costanza Baldisserotto, Shahrazad Aboulossoud, Simonetta Pancaldi
Department of Life Sciences and Biotechnology, University of Ferrara, C.so Ercole I d'Este, 32, 44121 Ferrara, Italy

Microalgae are photosynthetic microorganisms with great potential in a wide range of biotechnological applications, from bioenergy production, phytoremediation processes up to exploitation for human/animal wellbeing and health. As regards the latter, most literature studies refer to the benefits of microalgae thanks to the bioactive molecules that they can produce (vitamins, essential aminoacids, polysaccharides, phenolic compounds, polyunsaturated fatty acids, carotenoids, enzymes, etc.). Indeed, intense research is focused on testing microalgal biomass or extracts for possible use not only in the functional food industry, but also in the pharmaceutical one. Among microalgae, much attention is addressed to *Arthrospira platensis* (Cyanobacteria), or to the Chlorophyta *Dunaliella* sp., *Haematococcus* sp. or *Chlorella* sp., while less research is still available for other species. *Neochloris oleoabundans* is a green microalga highly studied due to the lipids it can accumulate and that can be exploited for biofuel production; furthermore, *N. oleoabundans* is used as an ingredient in some cosmetic products due to its moisturising power. Recently, polysaccharidic fractions extracted from this alga have been investigated for immunomodulatory effects on splenic lymphocyte proliferation, and preliminary tests point to interesting antioxidant properties of extracts obtained from the same algae. Thus, these findings point to a promising pharmaceutical capacity of this alga. Several pathologies that affect humans/animals are due to fungi activities, many of which are environmental fungi (e.g. *Aspergillus*, *Penicillium*, *Candida* and *Scedosporium*). One of the most common clinically important fungal pathogens is *Candida albicans* (Ascomycota, Saccharomycetaceae), characterized by dimorphism with three different morphologies: yeast-, pseudohyphal- and hyphal-form. In the yeast-form, it is a frequent commensal, non-pathogenic organism of humans but exhibits its negative effects when it switches to the pathogen hyphal-form, causing "candidosis". The possibility to develop new and natural tools to counteract the transition from the yeast- to the hyphal-form of *C. albicans* or to reduce hyphal proliferation of fungi in general represents an important advantage against candidosis or other fungal-induced pathogenesis, due to the increasing resistance to traditional antimycotics acquired by several pathogenic species.

Present research study, even if still at a preliminary stage, aimed to verify the antimycotic potential on the proliferation of environmental fungi by *N. oleoabundans*. In addition, the effectiveness of the algae, used in different forms, on the inhibition of *C. albicans* dimorphism from yeast- to pathogenic hyphal-form was tested. To determine the antimycotic effect on fungi proliferation, Petri dishes divided into two sectors, one containing culture medium, the other agarised *N. oleoabundans* cells, were inoculated with fragments of environmental hyphomycetes; then, tests were set up in liquid medium inoculated with the algae and fungal samples. In parallel, control cultures of algae or fungi were organized. Growth and photosynthetic aspects (pigments and PSII maximum quantum yield) of algae, and fungi proliferation were monitored during 21 days of cultivation in a glucose enriched medium. Light and transmission electron microscopy (LM and TEM, respectively) observations of samples were also performed. Subsequently, to verify the inhibition of *C. albicans* dimorphism by *N. oleoabundans*, in liquid cultivation trials of *C. albicans* induced to develop dimorphism (37°C incubation, addition of calf serum) and treated with algae *in toto*, or microalgal extracts or exhausted medium derived from algae cultivation, potentially enriched with bioactive molecules, were set up. Dimorphism inhibition was monitored through LM observations.

Results showed that the presence of *N. oleoabundans* inhibited hyphae proliferation in Petri dishes tests. Flasks cultures revealed that algae grew in similar ways (same kinetics, and same PSII maximum quantum yield and photosynthetic pigment content trends) both in controls (without fungi inoculation) and in co-culture with fungi. After 1 week-cultivation, algal cells contained translucent deposits, referred to starch after TEM observations, with different localization in control and co-cultured samples. Lipid accumulation occurred starting day 12 in both samples, while interesting cytoplasmic vesicles were evident only in co-cultured algae. The content of these vesicles was released nearby the co-cultured fungi. Differently, hyphae proliferation was inhibited, and their morphology was altered. Interestingly, in co-cultures the active gemmation of some yeast cells was observed parallel to algal growth, suggesting that *N. oleoabundans* has a fungistatic potential against hyphomycetes, but not against yeasts. Subsequent tests on the inhibition of dimorphism in *C. albicans* revealed that only *N. oleoabundans* extracts inhibited the transition from yeast- to hyphal- pathogen forms. *In toto* algae and their exhausted medium did not induce similar effects on *C. albicans*.

At the best of our knowledge, this is the first evidence for fungistatic and dimorphism inhibiting effects of *N. oleoabundans*.

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6 = Impacts of climate change on the developmental stages of *Cystoseira hyblaea* (Fucales) and its possible fate under a foreseen warming scenario

Giuliana Marletta[1], Marina Srijemsi[2], Gilda Savonitto[2], Luca Giuseppe Costanzo[1], Fabio Candotto[2], Mauro Tretiach[2], Giuseppina Alongi[1], Annalisa Falace[2]
[1]Department of Biological, Geological and Environmental Sciences, University of Catania, Via Empedocle 58, 95128 Catania, Italy; [2] Department of Life Sciences, University of Trieste, Via Giorgieri 10, 34127 Trieste, Italy

In the Mediterranean Sea, marine forests constituted by *Cystoseira sensu lato* (*s.l.*) species (Fucales, Phaeophyceae) support highly productive ecosystems, whose decline was ascribed to the interplay of several anthropogenic impacts. Recently, this decline has also been attributed to the increase of seawater temperatures and thermal anomalies, which can alter the reproductive phenology, germling growth and population viability of *Cystoseira s.l.* species. To manage the conservation of these communities is fundamental to evaluate how current and future foreseen warming scenarios will affect the thermal physiology of different life stages of these species. Moreover, a recognized strategy of management is the identification and protection of contemporary climatic refugia, which may safeguard the persistence of these habitat-forming species.
The present research focused on *Cystoseira hyblaea* Giaccone, which is endemic to the Sicily Channel (Central Mediterranean Sea) and has a narrow distributional range, being only reported in Punta D'Aliga (Sicily, Southern Italy), where it was no longer found, in Cap Bon (Northern Tunisian coast) and in Portopalo di Capo Passero (Sicily, Southern Italy). *Cystoseira hyblaea* lives in the intertidal and upper sublittoral zone on both semi-exposed and exposed rocky coasts, and its maximum vegetative and reproductive development occurs during winter. Considering the ongoing warming scenario, this intertidal species could be severely threatened being an intertidal species and due to its cold affinity, the winter reproductive phenology and the restricted distributional range.
Therefore, the aim of this study was to assess the thermal tolerance of both embryos and adults of *C. hyblaea*, in order to evaluate which stage might be the most vulnerable to climate change, and to foresee the possible fate and argue about the future conservation status of this species under a predicted global warming.
To perform this research, egg release, zygote settlement, embryo development and adult photosynthetic efficiency were studied, under five temperature treatments (12, 15, 18, 24, 28°C) in controlled mesocosms. In particular, ca. 1200 receptacles were cultivated on 6 Petri dishes per temperature treatment. At 0, 20, 44, 92 h after fertilization, 10 random subareas of 2x2 mm were examined in 3 Petri. Chlorophyll *a* fluorescence of adults was measured at 0, 24, 72, 120 h on 9 fronds in each of the 3 aquaria per treatment.
Through the trial, it was observed that the receptacles' release efficiency did not vary significantly among temperature treatments. However, the settlement efficiency of *C. hyblaea* increased from 12 to 18°C and decreased at 28°C. Indeed, the extremely low settlement efficiency was at 28 °C, due to the fact that zygotes had undergone lysis because of thermal stress. Regarding the early life stages, embryos showed a complete development only at 12 and 15°C, while yet at 18°C mortality increased sharply, being total at 28°C. In particular, the highest developmental rate was observed at 15°C (highest percentage of embryos with rhizoids already at 20 h AF), suggesting that this temperature constitutes the thermal optimum for reproduction and development of early developmental stages. On the contrary, adults of *C. hyblaea* showed a more plastic physiological response and thermal stress did not significantly affect photosynthetic efficiency.
The present study demonstrated that adults of *C. hyblaea* resulted not substantially affected by heat with an expected ability to acclimatise also to elevated temperatures. Contrarily, the early developmental stages resulted strictly stenotherm, requiring a very narrow range of low temperatures for an effective recruitment and development (12-15°C). Therefore, the recruitment could represent the most vulnerable process to the impacts of climate change in the population dynamics of *C. hyblaea*. Moreover, the results confirm the cold affinity of this species and suggest that the localities, in which *C. hyblaea* is confined, are acting as potential climatic refugia.
If failure in the recruitment process is reiterated for several years, it will lead to an impoverishment of *C. hyblaea* populations, ultimately hindering their long-term viability in its current range. Given the fragmented and restricted range and increased frequency of extreme climatic events, *C. hyblaea* may undergo local extinction. Considering this perspective, the areas in which *C. hyblaea* manages to survive should receive a higher level of protection. Moreover, this species should be included in the IUCN Red List of Species (IUCN, 2021), being classified as a "Critically endangered" or "Rare".

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6 = Structural modification of the green alga *Ulva lactuca* L. (Ulvaceae), grown in presence of high concentration of monoammonium phosphate

Alessio Papini[1], Sara Falsini[1], Emilio Corti[1], Diego Romei[1], Giovanni Stefano[1], Corrado Tani[1], Pietro Di Falco[1], Silvia Schiff[1], Luciana Renna[2], Stefano Mancuso[2], Ilaria Colzi[1], Cristina Gonnelli[1]
[1]Department of Biology, Università di Firenze, Via Micheli 3, 50121 Firenze, Italy; [2]Department DAGRI Università di Firenze, Sesto Fiorentino (FI), Italy

Ulva lactuca L. is a green alga, widely spread in the Mediterranean, belonging to the Ulvaceae family. This green alga is collected and used as food in many countries, and in some cases it is also cultivated. Another important application of *U. lactuca* is related to the wastewater treatment, due to its high growth rate and ability to take up large amounts of nitrogenous compounds. Consequently, it is usually used as biological filter to decontaminate the ponds used for fish farming (Fig. 1), which are usually enriched in nitrogen in the forms of ammonium, nitrate and organic nitrogen.
In this study we investigated the morpho-anatomical variations of *U. lactuca* under different concentration of $NH_4H_2PO_4$ trying to understand the algae response at these levels of nitrogen nutrients.
Ulva lactuca was cultivated for one month in presence of 50 µM, 500 µM and 5000 µM monoammonium phosphate inside a thermostatic chamber (25°C; 12-12 day/night). At the end of the treatment, the morphological and anatomical variations of *U. lactuca* were observed under Light Microscope (LM) and chlorophyll concentration was spectrophotometrically assessed. Besides, the pH variation of the solutions was weekly monitored.
The number of damaged cells observed under Light Microscope was similar in each treatment, showing an average of 2 damaged cell/0,01mm^2 (Fig.s 2 and 3).
In each treatment, excluding the 5000 µM condition, the pH of the growth solution changed back to the pH value observed in the control treatment, after the experimental time range.
Lastly, the spectrophotometer analysis highlighted higher chlorophylls amounts at the concentration of 500 and 5000 µM of monoammonium phosphate as compared to 50 µM and the control.
Altogether these findings demonstrate the ability of *U. lactuca* to grow at high concentration of $NH_4H_2PO_4$, to change back the pH of the culturing solutions to normal conditions and to increase the amount of chlorophyll proportionally to the amount of monoammonium phosphate in the growth medium.

Fig. 1. Orbetello lagoon; phytodepuration pool.

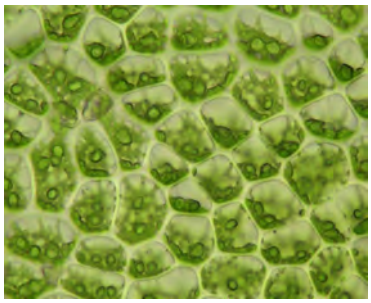

Fig. 2. Ulva parenchyma at 50 µM Monoammonium Phosphate.

Fig. 3. Ulva parenchyma at 500 µM Monoammonium Phosphate.

https://drive.google.com/file/d/1ZiocOFZptEY7BLNTSBwWUx4SlzTWH8sG/view?usp=sharing

6 = Metabolites production in algae exposed to UV-B stress

Elisabetta Sgarbi[1,2], Giulia Santunione[1], Giuseppe Montevecchi[2], Francesca Masino[1,2]
[1]Department of Life Sciences, University of Modena and Reggio Emilia, Via Amendola, 2, 42124 Reggio Emilia, Italy;
[2]Inter-Departmental Centre BIOGEST-SITEIA, P.le Europa, 1, 42124 Reggio Emilia, Italy

Some algae species show great adaptability towards abiotic stresses, which give them the capability to grow in different environmental conditions. In particular, limiting environmental conditions as lack of nutrients, extreme pH values, high/low temperatures, high light intensity, direct UV radiation, induce the production and accumulation of lipids, antioxidants and other bio-protective molecules, as part of the survival mechanisms. Nowadays, micro- and macroalgae represent a potential source of a wide range of bioactive molecules and nutrients as well as proteins, lipids, carbohydrates, vitamins, antioxidants and trace elements. In this regard, algae cultivation has garnered high interest for their potentials in food technology applications.
The present work was aimed to study algae growth and metabolites' production under UV-B irradiance as abiotic stress source. Two green algal species have been involved in this study: *Edaphochlorella mirabilis* (Chlorophyta) and *Klebsormidium flaccidum* (Charophyta). The experimental setup included a Temporary Immersion System (TIS) bioreactor, used as vessel for *in vitro* algal growth (Fig. 1). Both species were cultured in Bold's Basal Medium. TIS bioreactors were maintained under LED lamps for 14 h/day as photoperiod. The exposition to UV-B light was carried out for 2 hours a day using a UV-B lamp (Philips TL-20 W/01) with narrow waveband of between 305 and 315 nm, which peaks at 311 nm. The biomass growth within each bioreactor was monitored every 3 days to calculate growth rate. After two weeks of exposure, all samples were filtered to collect final algae biomass (Fig. 2) and to extract and analyze pigments and PUFA contents. Indeed, along with several compounds produced by algae, poly-unsaturated fatty acids (PUFAs) and pigments, specifically carotenoids, are getting increasing attention, due to their antioxidant potential. Carotenoids have a specific role in scavenging the reactive oxygen species (ROS) and dissipating excessive energy. Furthermore, PUFAs extracted from algae are essential dietary lipids as C18:3 (ω3) and C18:2 (ω6), which are the precursors for the biosynthesis of all the other essential ω 3 and ω 6 PUFAs. In this study, Folch's method was applied to lipid extraction, then PUFA's profile and amount were analyzed and identified through Gas Chromatography-Mass Spectrometry. Pigments were extracted from algae biomass through the application of three different organic solvents, then their profile was analyzed through High Pressure Liquid Chromatography (HPLC).
After three repeated trials different metabolites production has been outlined, depending on algal species. Specifically, C10, C12, and C21 produced by *E. mirabilis* and *K. flaccidum*, had a higher concentration in algae exposed to UV-B radiation if compared with the production in unexposed samples. Moreover, oleic acid (C18:1) and eicosatetraenoic acid (C20:5) increased their concentration in exposed samples of *E. mirabilis*, whereas poly-unsaturated fatty acids - as C16:2 -were found only in UV-B treated samples of this species. α-linoleic acid (C18:3) concentration significantly increased in treated *K. flaccidum* samples. Furthermore, total pigments increased after UV-B irradiation, particularly in *K. flaccidum*, α and β carotene increased in both species, while chlorophyll (*a* and *b*) concentration was higher only in *K. flaccidum* samples after UV-B irradiation. These results represent a valiant starting point to study metabolites production in *E. mirabilis* and *K. flaccidum* and their potential applications in food technology and innovation.

Fig. 1: TIS bioreactor with *E. mirabilis* in culture.

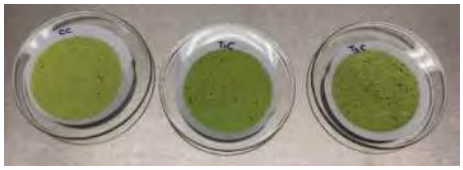

Fig. 2. Algae filtered and collected by 0.22 µm pores filters.

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6 = Evaluation of low environmental impact extraction from macroalgal species in transition environments

Damiano Spagnuolo[1], Simona Armeli Minicante[2], Antonio Manghisi[1], Marina Morabito[1], Giuseppa Genovese[1]
[1]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Salita Sperone 31, 98166 Messina, Italy; [2]Institute of Marine Sciences, National Research Council, Arsenale 101-104, Castello 2737F, 30122 Venice, Italy

One of the most used and remunerative applications of macroalgae is the extraction of phycocolloids, which have many uses from food to pharmaceutical application. In this context, many new species are often placed side by side to the conventional ones for extractions, especially invasive and thriving algal biomass in transitional environments. Conventional techniques for the extraction of bioactive compounds from macroalgae can be complex, as they require long times, a certain amount of solvents and energy consumption, which can be simplified through alternative techniques. The objective of this work concerns the comparison of different polysaccharide extraction techniques to evaluate yield, costs and potential environmental impact. In the present work, we evaluated the yield of the polysaccharides extracted from alien macroalgae, collected in Lake Ganzirri (Oriented Natural Reserve of Capo Peloro Lagoon, Messina) and in Venice Lagoon. The choice of the species is based on algae that produce large biomass and present invasive character. Conventional methods for the extraction of algal polysaccharides involve the use of dH_2O instead of HCl. In both cases, there are several disadvantages. Maintaining water temperature (70° C) for a long period involves a high expenditure of energy and consequently an increase in production costs, especially when transferred at industrial level. Another weakness of the extraction methods is represented by the use of chemicals (HCl), which can certainly be considered an environmental risk factor due to the potential toxicity. A new method is currently being evaluated that involves the use of microwaves. This technique appears to be promising due to its "green" inspiration, but the data currently available do not allow to fully evaluate its effectiveness. In this regard, we present data on the optimization of extraction using microwaves, testing the following parameters: extraction time. working temperature, HCl vs. water as an extraction solvent. Conventional and "green" extraction methods have been compared in order to evaluate them in terms of extract yield, time and cost reduction, and environmental impact.

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7 = An open question from Pantelleria Island (Sicily): which habitat type fits the best with a very rare and biogeographically important community?

Emanuela Carli[1], Laura Casella[1], Lorenzo Gianguzzi[2], Salvatore Pasta[3], Pierangela Angelini[1]
[1]ISPRA, Via Vitaliano Brancati 48 - 00144 Roma, Italy; [2]Department of Agricultural, Food and Forest Sciences, University of Palermo, Viale delle Scienze, I- 90128, Palermo, Italy; [3]Institute of Biosciences and BioResources (IBBR), Italian National Council of Research, Unit of Palermo, Corso Calatafimi 414, I-90129, Palermo, Italy

Bagno dell'Acqua (also known as ''Specchio di Venere"), located near the northern shores of the Island of Pantelleria (Sicily), represents a peculiar biotope at the European scale. This endorheic lake has a volcanic origin. The rainwater, drained downwards through the rocks of its watershed, is heated due to the residual thermal activity of local extinct volcano and rises up in form of vapour enriched with CO_2 and lime, inducing the surface precipitation of carbonates forming peculiar crusts around the small hot springs, located along the borders of the lake. The water body is located about 2 m a.s.l., with a maximum depth of around 12 m, and it has a surface of around 0.25 Km^2. The water regime of Bagno dell'Acqua is strongly influenced by summer drought, triggering the seasonal shrinkage of its volume and the reduction of the water level. The lake represents a key steppingstone for many migratory aquatic birds, and represents the only site of occurrence in Europe for several noteworthy plants like the narrow endemic *Limonium secundirameum*, *Cyperus laevigatus* subsp. *laevigatus* and *Schoenoplectus litoralis* subsp. *thermalis*. These latter two species form a peculiar hygrophilous community occurring only there within the European territory, recognised as *Cypero-Schoenoplectetum thermalis* and referred to the class *Phragmito-Magnocaricetea*, which does not match with any of the habitat types identified by the 92/43 EEC Directive. Considering that up to now this Directive appears to be the only effective tool to protect the European habitats and species and bearing in mind the paramount biogeographical importance of *Cypero-Schoenoplectetum*, we tried to identify the habitat type that fits the best with this community. We would like to address to all Italian botanists an open question: should we give priority to the physical and chemical characteristics of the lake and to local vegetation physiognomy, without considering the diagnostic plants species, or should habitat type selection be driven by taxonomic coherence, for instance looking for congeneric plant species occurring in similar habitat types?

Fig. 1. Vegetation of the *Cypero-Schoenoplectetum thermalis* at the Bagno dell'Acqua lake (Pantelleria Island).

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7 = Vegetation-plot based analysis: A first attempt for plot size standardization of Italian habitats (Habitat Directive)

Marco Cervellini[1], Raffaele Repoli[1], Simona Maccherini[2], Claudia Angiolini[2], Emanuela Carli[3], Pierangela Angelini[3], Laura Casella[3], Michele Di Musciano[1,4], Piero Zannini[1], Alessandro Chiarucci[1]
[1]BIOME lab, Department of Biological, Geological and Environmental Sciences, Alma Mater Studiorum, University of Bologna, via Irnerio 42, 40126 Bologna, Italy; [2]Department of Life Sciences Via P.A.Mattioli, 4 53100 Siena; [3]ISPRA, Italian Institute for Environmental Protection and Research, via Vitaliano Brancati 48 - 00144 Roma, Italy; [4]Department of Life, Health & Environmental Sciences, University of L'Aquila, Coppito, L'Aquila, AQ, Italy

Achieving a relevant and measurable improvement of the habitat conservation status inside and outside of Natura 2000 is one of the main targets of the Council Directive 92/43/EEC (Habitats Directive). According to the "Directive 92/43/EEC" this goal must be accomplished by each EU member state, by maintaining a favourable conservation status of the habitats. One of the criteria to monitor the habitat conservation status assessment is "Structure and Functions" parameter (SF). Floristic and vegetation field survey allows to evaluate SF parameter for most of the habitats listed in the Annex I.
In 2016 the Italian Institute for Environmental Protection and Research (ISPRA) provided for the 124 Italian Habitat Types a manual with methodological guidelines for data collection at site level (see Angelini et al. 2016). Among the priority variables described in the monitoring forms to perform the on-field survey, plot size surface and shape are still lacking for some habitats or, when they have been identified, there is no standardisation within the nine habitat macro-categories (i.e., 1xxx, 2xxx, etc.). Vegetation varies along different spatial and temporal scales, and pattern detection is strictly related to the plot size and shape selection.
Here we present a first attempt to fill the gap present in the national manual, by proposing standardized plot size and shape for each macro-category. We first have gathered all the information available for each habitat, according to the national manual, then through literature research we identified a vegetation type based criterion for the standardization of plot size surfaces and shape within each macro-category. Subsequently we used an "expert based" approach to analyse this first proposal, and to reduce the plot types, based on operational efficiency for the field work in our Country. Finally, we discuss the resulting standardization to the ISPRA working group on habitats, to obtain feedback on the compliance with the Habitat Directive reporting requirements.
We propose five square plot sizes and two rectangular ones, to standardise the plot categories according to Habitat Types dimensions and characteristics in Italy. Future on-field applications of the standardized plot sizes will be required to test their operational efficiency.

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7 = Structural gradients of submerged aquatic vegetation in deep lakes

Alice Dalla Vecchia[1-2], Martin Wilkes[3], Mattia Azzella[4], Alex Laini[1], Rossano Bolpagni[1-2]
[1]Department of Chemistry, Life Sciences, and Environmental Sustainability, University of Parma, 33/a Parco Area delle Scienze, 43124 Parma, Italy; [2]Institute for Electromagnetic Sensing of the Environment, National Research Council of Italy (IREA-CNR), 15 Via E.Bassini, I-20133 Milan, Italy; [3]Centre for Agroecology, Water and Resilience, Coventry University, Ryton-on-Dunsmore, UK; [4]via Piemonte 35, 00013 Fonte Nuova (Roma), Italy

The key roles of submerged aquatic vegetation (SAV) in guaranteeing freshwater diversity and functioning are well acknowledged, although SAV is facing a severe impairment worldwide mainly due to habitat destruction, water pollution and consequent reduced light availability. Vegetation structure is a highly explored topic in the context of shallow lakes, however, in deep lakes, the SAV structure and dynamics have been poorly investigated so far, especially for the communities dominated by charophytes. These communities belong to the EU habitat "3140 – Hard oligo-mesotrophic waters with benthic vegetation of *Chara* spp." that is listed as vulnerable in the recent Habitat Red List, and among those at major risk of decline in Europe. A main issue in this context is the difficult identification of pristine sites to be compared to impacted ones, which could give valuable insights into internal vegetation structuring processes happening within lakes. Our aim is to compare SAV composition along a pristine to impacted gradient of conditions in deep lakes. We hypothesized that most complex community structures would be found in pristine lakes, while this structure would break down in impacted lakes due to enhanced competition among species and habitat filtering due to the reduction of niches available for colonization. Submerged species abundance and environmental data (physical and chemical drivers of water and sediments) were collected in 5 deep volcanic lakes in central Italy, with transects that investigated a water depth gradient up to 20 m of depth. We employed multivariate analysis methods (mMCA, multivariate Multiscale Codependence Analysis), which implement species records and detailed environmental parameters with spatial information to describe the spatial structure of communities. Our results provide limited support to our hypothesis, suggesting that only the intermediate conditions lake is highly structured, despite the presence of well-developed charophyte meadows in near pristine lakes. Indeed, the essential role of charophytes in structuring SAV in deep lakes is clearly highlighted by the analysis, and no structure was identified when charophytes were absent. The progressive reduction in light availability is the predominant driver of *Chara* stands simplification and decline, as a consequence of increased trophic level. Our study confirms the key contribution of deep lakes in preserving the *Chara*-habitat, posing the urgency to not neglecting this peculiar SAV component in conservation actions for these environments, since *Chara* species have shown to drive the whole SAV structure.

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7 = The vegetation of the pioneer forest nuclei dominated by *Celtis tournefortii* subsp. *aetnensis* (cl. *Quercetea ilicis*) recently found in the submontane belt of Rocca Busambra (western Sicily)

Lorenzo Gianguzzi[1], Orazio Caldarella[2]
[1]Department of of Agricultural, Food and Forest Sciences (SAAF), University of Palermo, Viale delle Scienze 13, 90128 Palermo, Italy; [2] Via Maria SS. Mediatrice 38, Palermo, Italy

Celtis tournefortii Lam. is a widely distributed species, gravitating along the southern edge of the European continent, with main range present in the eastern Mediterranean region – between the southern part of the Caucasus and the Aegean-Balkan area – and some isolated subpopolations located in Sicily (Gianguzzi et al., 2014a). These disjoint stands, probably issue from the past (Miocene) connections between the Balkan area and the Tyrrhenian territories of the Italian Peninsula, where it probably became extinct, during the glacial and postglacial climatic upheavals of the Quaternary. As highlighted in a recent work (Gianguzzi et al., 2014b), the Sicilian populations – referred to subsp. *aetnensis* (Tornab.) Raimondo et Schicchi – occur in peculiar refugial sites; being located at the western limit of the species range, they have a significant phytogeographic value. The Sicilian micro-woods represent a relict vegetation feature and show a fragmented distribution pattern. *C. tournefortii* behaves as pionieer species, growing on shallow soils deriving from different rock outcrops such as volcanites (Mount Etna), quartz sandstones (Peloritani, Nebrodi and Madonie Mountains), marls, chalky-marls and limestones (Sicani Mountains) and dolomitic-limestones (Rocca Busambra). From the phytosociological point of view, these communities were referred to the *Pistacio terebinthi-Celtidetum aetnensis*, association belonging to the *Oleo-Ceratonion* alliance (cl. *Quercetea ilicis*), in turn differentiated in the territory with the subassociations *typicum*, *phlomidetosum fruticosae*, *artemisietosum arborescentis* and *rhamnetosum alaterni*. In this contribution, the occurrence of several new forest nuclei dominated by the endemic subspecies are reported for the first time; after the finding of these nuclei, located on the southern slopes of Rocca Busambra, the local population range increases from 2 to about 90 hectares. The area falls within the homonymous Nature reserve located in the province of Palermo, and this site also corresponds to a Special Conservation Area (ITA020008) and a Special Protection Area (ITA020048) of the Sicilian Natura 2000 network. These are forest nuclei of average height varying between 2 and 6 m, extended in patches of 200-400 square meters, present along the steep slopes upstream of the rugged rocky cliffs, between the locaties Casale di Sopra and Contrada Marosa; they show a scattered distribution (from 800 m up to about 1340 a.s.l.), just below the main ridge of Rocca Busambra.From a bioclimatic point of view, the area is falls within the upper mesomediterranean and the lower supramediterranean thermotypes, with subhumid-humid ombrotype (Gianguzzi, 2004). The climacic potential of the territory is largely dominated by the holm oak series (*Aceri campestris-Querco ilicis* Σ), locally rather degraded and floristically impoverished, due to the massive deforestation carried out by man in the past. The only form of use compatible with the geomorphological characteristics of the site is represented by pastoralism; also the local pastures are periodically crossed by fires, which in turn triggers and speeds the processes of soil erosion, with consequent denudation of the limestone mother rock. Local micro-forests of *Celtis* tend to colonize the barest sites, in particular the tops prone to cryoclastic processes but protected from cold winds, or the small scree along the slopes. Considering its relictual meaning, this peculiar Mediterranean deciduous community deserves to be adequately protected, although it does not correspond to any of the forest habitats listed in the Annex I of 92/43 EEC Directive.

Fig. 1. Nuclei of vegetation dominated by *Celtis tournefortii* subsp. *aetnensis* on the slopes of Rocca Busambra.

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8 = The effects of land-use intensification on the spatial distribution of Small Woodlots Outside Forests and plant functional responses

Erika Bazzato[1], Erik Lallai[1], Maria Teresa Melis[2], Elisa Serra[1], Simona Maccherini[3], Michela Marignani[1]
[1]Department of Life and Environmental Sciences, University of Cagliari, Via S. Ignazio da Laconi 13, 09123 Cagliari, Italy; [2]Department of Chemical and Geological Sciences - Remote Sensing and GIS - TeleGIS Lab, University of Cagliari, Cittadella Universitaria - S.S. 554, 09042 Monserrato, Italy. [3]Department of Life Sciences, University of Siena, Via P.A. Mattioli 4, 53100 Siena, Italy

Trees outside forest (TOF; i.e., scattered trees, small woodlots - SWOFs, trees lines) represent important multifunctional resources, providing different ecosystem services at both global and local scales. Nevertheless, few studies provide an exhaustive census considering the effect of the surrounding land-use type on TOF functional responses, especially in Sardinia – a hotspot for biodiversity conservation located in the Mediterranean basin. To exemplify the effects of land-use intensification (from natural areas to urbanized zones) on different plant functional responses, we adopted a multiscale approach, describing the spatial distribution of SWOFs embedded in different land-use types at landscape scale, evaluating differences in terms of vegetation structure (i.e., the diameter at breast height, DBH) at local scale and focusing on seed mass variation of two common species (*Asparagus albus* L. and *Asparagus acutifolius* L.).
We adopted a multiphase sampling design, including two phases: pre-fieldwork phase and fieldwork phase.
The pre-fieldwork phase was carried out by photo-interpretation of digital aerial orthophotos to provide a Small Woodlots Outside Forests (SWOF) census in the study area. SWOFs were classified according to the regional land-use map into three main categories: SWOFs in natural and semi-natural areas (NAT), SWOFs in agricultural areas (AGR), and SWOFs in urban and artificial surfaces (URB). Hence, we randomly selected 30 SWOFs along the land-use intensification gradient (i.e., NAT, AGR, URB). The fieldwork phase was performed by applying a systematic sampling design for each SWOF using five plots of 1x1 m. Within and around each plot, we measured and collected (i) DBH of five individuals of tree and shrub for a total of 25 measures per SWOF, and (ii) mature seeds from healthy adult plants of the two *Asparagus* species. Seeds were cleaned and oven-dried at 80°C for at least 48 hours, or until equilibrium mass; hence, we weighted to 100 µg accuracy 10 seeds randomly chosen with 15 replicates from each SWOF.
We calculated summary statistics of spatial distribution of SWOFs embedded in different land-use types, DBH and seed mass variation along the land-use intensification gradient. We evaluated the differences among SWOFs embedded in different land-use types, using (i) a Kruskal-Wallis test and pairwise comparisons with the multiple Wilcoxon tests based on the coefficient of variation of DBH per SWOF, (ii) permutational univariate analysis of variance and PERMANOVA t statistic based on Euclidean distance of 15 replicates per SWOF for each *Asparagus* species.
We observed that SWOFs were widely spread along the land-use intensification gradient, also in human-modified land-use types: the SWOFs coverage increased following this gradient, reaching the highest value in URB areas (0.85%), and the lower in AGR and NAT areas (0.31% and 0.23%, respectively).
Analyses showed that land-use types significantly affected the SWOFs vegetation structure (i.e., coefficient of variation of DBH) and the seed mass variation at intra- and inter-specific level.
For the vegetation structure, significant differences were observed among NAT SWOFs vs URB ones, but also between URB SWOFs and AGR ones; whereas no significant contrasts among SWOFs of NAT and AGR areas were identified.
Considering the seed mass variation at intra-specific level, *A. acutifolius* seeds were significantly different at the extremes of the gradient (NAT vs URB areas), while *A. albus* showed significant differences both between NAT vs. URB areas, and among AGR vs. URB areas, revealing more sensitiveness to land-use change. At inter-specific level, we observed that the seed mass of the two species did not differ in SWOFs located in URB areas.
Overall, our findings highlighted the good levels of naturalness of small patches in agricultural land-use in terms of vegetation structure, but also the strong effect of urban areas on seed mass variability at inter-specific level, suggesting a homogenization among different species of this key plant trait.
Monitoring plant functional traits on TOFs can be useful to describe plants' responses to human disturbance and predict global changes. Understanding how and why these relations occur could improve our capacity to find adaptive strategies for SWOFs management and conservation, with potential benefits for natural conversion actions and nature-based solutions to contrast fragmentation effects.

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8 = Intra-annual and within-inflorescence variation in reproductive success of the annual *Silene canescens*: environmental maternal effect and resource competition

Angelino Carta, Daniela Ciccarelli, Alessio Mo
Department of Biology, University of Pisa, via Derna 1, 56126 Pisa, Italy; CIRSEC, Centre for Climatic Change Impact, University of Pisa, via del Borghetto 80, 56124 Pisa, Italy

Intra-specific variation in reproductive success is often found among populations but also at the intra-individual level. Differential environmental conditions during flower phenology and the arrangement of flowers within inflorescences may explain large amount of this variation. In annual plants environmental maternal effects are known to be particularly strong, especially concerning variation in seed traits.
Here, we studied flowering phenology and female reproductive success in one population of the interdunal annual plant *Silene canescens* Ten. (Caryophyllaceae). The goal of this study is to determine the effect of maternal environmental conditions and of intra-inflorescence flower position on reproductive success (fruit set, seed set and seed traits), while accounting for the con-specific individual densities and their vegetative functional traits (plant height, root depth and lateral extent, leaf area). This study is part of a two-year lasting project aimed at analyzing the impact of climate change on coastal and marine ecosystems, financed by the University of Pisa.
To this end, we selected 10 plots of equal area (80 m^2) visited three times over one reproductive season in the northern coasts of Tuscany. Each plot was randomly selected to ensure homogeneity and representativeness of the studied population. Within each plot we sampled 5 flowering individuals and annotated environmental characteristics (distance from the sea, altitude, disturbance), total vegetation cover and the total number of *S. canescens* flowering individuals. Reproductive success at the fruit level (fruit set) was calculated separately in three different parts of similar size within the inflorescence (i.e., bottom, middle, top) on the basis of the total number of flowers within each part. Reproductive success at the seed level was quantified by (a) calculating the seed set (on the basis of the mean number of ovules per fruit) in each inflorescence part, (b) measuring the average seed mass in each inflorescence part, and (c) estimating viability, primary dormancy and germination speed of seeds produced in each inflorescence part.
By using mixed regressive models and multivariate approach, we will be able to evaluate covariances and trade-offs between reproductive outputs, vegetative traits and environmental conditions, while considering genetic (individual) and spatial (plot) random effects. Overall, we expect to separate the effects of intra-inflorescence (resource allocation) from those of intra-population (maternal environmental conditions) on annual plant reproductive responses.

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8 = Response of arable plant communities and associated insects to different levels of agricultural intensity

Emanuele Fanfarillo[1], Claudia Angiolini[1], Giovanni Bacaro[2], Stefano Biagiotti[3], Daniele Calabrese[1], Paolo Castagnini[1], Stefano Loppi[1], Tommaso Martellini[1], Simona Maccherini[1]
[1]Department of Life Sciences, University of Siena, Via PA Mattioli 4, 53100 Siena, Italy; [2]Department of Life Sciences, University of Trieste, Via Licio Giorgieri 5, 34127 Trieste, Italy; [3]Telematic University Pegaso, Piazza Trieste e Trento 48, 80132 Napoli, Italy

A relevant proportion of European biodiversity is related to low-input agriculture. Arable vegetation is acknowledged to provide many services in agroecosystems, which are beneficial to both the environment and crop production. The intensification of agricultural practices considerably reduced plant diversity in arable land, with a detrimental effect also on associated groups of organisms. A notable objective of Green Deal of the EU is the increase of biodiversity in agricultural areas. In this study, we surveyed the communities of arable plants and insects in five fields of a traditional Elephant garlic crop (*Allium ampeloprasum* L., locally known as "Aglione della Valdichiana") of Tuscany (central Italy), along a gradient of management intensity.
To catch the different aspects of arable biodiversity along the season, the sampling was carried out twice: in spring, during crop growing, and in summer, after crop harvesting. The communities were surveyed by means of 44 plots of 2×2 m, randomly placed all over the surface of the fields (Fig. 1a). Percentage cover values and the number of individuals were used as abundance measures for plant and insect taxa, respectively. Based on the frequency of disturbance events (soil tillage, hoeing, fertilization, and weeding operations), we attributed a score of agricultural intensity to each surveyed field to be used as an explanatory variable of the observed biodiversity. We used univariate and multivariate analyses to assess the relationships between plant and insect assemblages and the effects of different agricultural management on the richness and composition of plant and insect communities.
Our results showed that increasing vascular plant richness caused an increase of insect richness (Fig. 1b). The decreasing disturbance level significantly affected species richness in arable plant communities (Fig. 1c,d). Furthermore, we highlighted a consistent co-occurrence of specific plant and insect community types. Differences in species composition of both plant and insect communities were significantly related to different management intensities.
Given the similar response of plants and insects to agricultural intensity, our results confirm the importance of low-input management for the conservation of plant diversity and connected organisms in arable ecosystems.

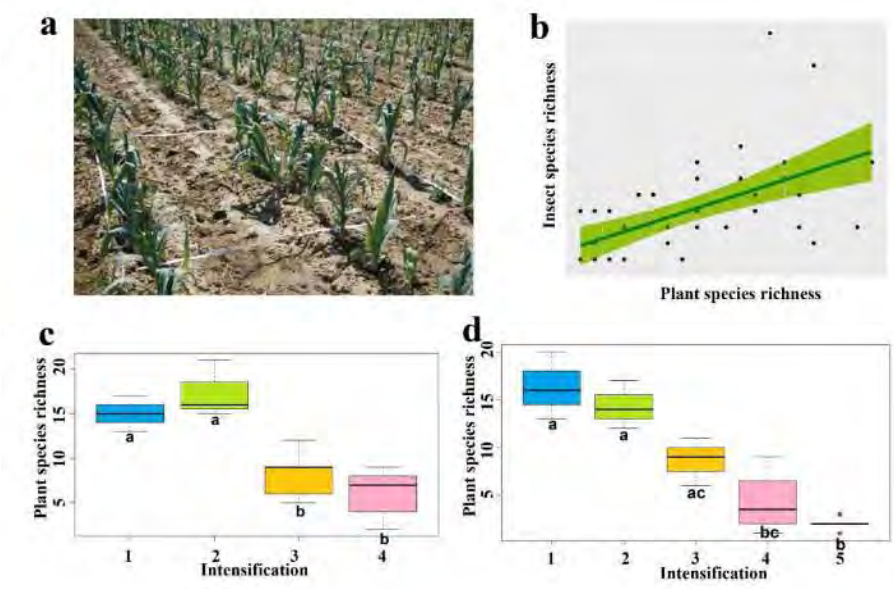

Fig. 1. a: sampling plot in an Elephant garlic field; b: relationship between plant and insect species richness ($p<0.001$; $R^2=0.31$); c and d: boxplots of plant species richness along the intensification gradient in spring (c) and summer (d) – different letters indicate statistically significant differences between the intensification levels.

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8 = The orchid flora of Cagliari (Sardinia, Italy)

Michele Lussu[1]
[1]Istituto Regionale per la Floricoltura, Via Carducci n. 12 – 18038 Sanremo (IM), Italy

In recent decades, human population has grown dramatically, moving more and more often to cities. In urban ecosystem, biodiversity is threatened by human activites such as soil consumption, habitat fragmentation and biotic homogenization. Orchids are recongnized as indicator of ecosystem health due to their life cycle which includes peculiar interactions with other organisms as fungi for seed germination and animals for pollination. Through a ten-year study (2010-2020), here, the orchid flora of the city of Cagliari (Sardinia, Italy) is described and anthropogenic threats are discussed. During this research, seventeen species, two subspecies belonging to six genera were found. The most rapresented genera are *Ophrys* (10) and *Anacamptis* (3) (Fig. 1) and most common species are *Himantoglossum robertianum*, *Ophrys lutea*, *Ophrys speculum*, *Serapias lingua* and *Serapias parviflora*. However, five specie were no longer found and three of which disappeared in the course of this investigation. The gathered data shows that seminatural habitats and garigues become refugee for many orchid species in the aftermath of the loss of their natural habitats. Although further studies are needed to clarify how terrestrial orchids respond to specific disturbances in urban ecosystems, based on these findings, orchid sites should be taken into consideration in programming integrated land management and develope conservation reserves.

Fig. 1. *Anacamptis fragrans* blooming in abandoned pneumatic.

Fig. 2. *Himantoglossum robertianum* in a public park.

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8 = Phenology and composition of annual interdunal plant communities in a climate change context

Alessio Mo, Daniela Ciccarelli, Angelino Carta
Department of Biology, University of Pisa, via Derna 1, 56126 Pisa, Italy; CIRSEC, Centre for Climatic Change Impact, University of Pisa, via del Borghetto 80, 56124 Pisa, Italy

Climate change can have an impact on ecosystems, overwhelming their capacity to mitigate extreme climatic and disturbance events. These may lead to an alteration of the structure, distribution and composition as well as changes in phenology at the species level. Annual plant populations may be particularly affected by these events, as they must regenerate each year primarily by seed.
Here, we studied the phenology and composition of annual plant communities while accounting for the species phylogenetic relatedness and their functional traits. We used as a study model the interdunal annual communities of fixed dunes present in the northern coasts of Tuscany. This study is part of a two-year lasting project aimed at analyzing the impact of climate change on coastal and marine ecosystems, financed by the University of Pisa.
To achieve our objectives, we identified 10 permanent transects visited once a month for an entire vegetative year with the aim of monitoring the presence and abundance of annual plant species. Each 20 m long transect was randomly selected to ensure homogeneity and representativeness of the study area. Within each transect we annotated environmental characteristics (distance from the sea, altitude, disturbance) and selected ten plots of 0.25 m^2 each to quantify plant community composition. For each species we gathered information concerning the following vegetative and reproductive traits that likely influence plant responses to climate conditions: plant height, SLA, LDMC, seed mass and seed dormancy class, as well as seed dispersal and pollination strategy.
In September 2020, no annual plants were detected in any plot. The first seedling emergences occurred in the first half of October soon after the early autumn rains. These seedlings belong to *Festuca fasciculata* Forssk., *Marcus-Kochia ramosissima* (Desf.) Al-Shehbaz, *Medicago littoralis* Rhode ex Loisel. and *Lagurus ovatus* L., which also dominated the communities throughout the year until the end of the vegetative and reproductive phases (July). During the autumn and winter months other frequently occurring species were *Cerastium ligusticum* Viv. and *Draba verna* L., which completely disappeared when spring temperatures raised, while *M. ramosissima* increased its coverage. An even different pattern characterizes *Odontites luteus* (L.) Clairv., detected since mid-autumn but its frequencies decreased in the winter months, increased in early spring and then decreased again. *Catapodium balearicum* (Willk.) H. Scholz was instead observed only in May.
By using a comprehensive multivariate approach, considering plant co-occurrences and their vegetative and reproductive traits, we will be able to evaluate covariances and trade-offs explaining plant responses to interannual variations in climatic conditions. Specifically, we expect to track phenology and composition of the communities and to understand annual plant species responses to extreme climatic events.

Acknowledgements
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8 = Consequences of extreme climatic phenomena in the Mediterranean area: the “Effects of climate environmental shifts on species, communities and ecosystems” Project

Maria Silvia Pinna, Donatella Cogoni, Pierantonio Addis, Gianluigi Bacchetta, Serenella Cabiddu, Alessandro Cau, Annalena Cogoni, Maria Cecilia Loi, Michela Marignani, Davide Moccia, Antonio Pusceddu, Andrea Sabatini, Erika Bazzato, Giuseppe Fenu
Department of Life and Environmental Sciences (DiSVA), University of Cagliari, Via Fiorelli 1, 09126 - Cagliari, Italy

Climate change (CC) has a significant impact on the increase in unpredictable and extreme weather phenomena concentrated in a short period of time [1,2]. Indeed, in the coming decades, a considerable intensification in the frequency and magnitude of weather extremes events has been foreseen over a broad range of ecosystems, along with an increased risk of drought and heatwaves and the likelihood of intense precipitation events and flooding [3,4]. Despite this, there is a lack of knowledge concerning the influence of extreme weather events on species, populations, communities and ecosystems, and their functions.
The Mediterranean Basin has been identified as one of the most susceptible to climate change areas of the world [1,4]. For this region, CC scenarios predict major droughts as well as changes in seasonality and temporal variability of precipitation [5]. In particular, Mediterranean islands, are expected to be adversely affected by climate change. Sardinia, as special hotspot of biodiversity within the Mediterranean, will be particularly exposed in the near future to a greater frequency and magnitude of extreme weather events. However, to date, their effects on biodiversity and ecosystem functioning have been almost entirely neglected.
In this frame, the project entitled "*Effects of climate environmental shifts on species, communities and ecosystems*", aims to respond to the urgent need for basic knowledge on the ecological consequences of extreme weather events. The project focused on different Sardinian terrestrial, freshwater, and transitional water habitats. Specifically, the effects caused by mechanical-physical variables such as, heavy rainfall events, sea storms, run-offs, variations in the water thermohaline structure on changes in composition, structure and abundance of populations, community and ecosystems are being analyzed, besides their repercussions on biogeochemical cycles.
A representative study area, including terrestrial, freshwater, and transitional habitats, was selected among those most exposed to extreme rainfall events localized in south-Sardinia and the available historical data was collected. In this natural laboratory, the effects of extreme rainfall events on populations (endemic riparian plant species, freshwater fish and invertebrates of transitional waters) and community (typical riparian plant communities and macrofauna communities in transitional aquatic ecosystems) levels are being investigated. Moreover, the effects at the ecosystem level are being analyzed by either field studies or manipulative experiments in mesocosm. By combining proprietary and historical data with the results of the experimentation in the field, carried out before and after extreme rainy events, we are investigating the resilience of the biological communities to anomalous climatic events in the medium/long term.
The project, which involves a multidisciplinary team of scientists from Cagliari University, will provide results that could be a basis for planning conservation measures also in other Mediterranean insular territories with similar environmental conditions.

The project “*Effects of climate environmental shifts on species, communities and ecosystems*” is funded by Fondazione di Sardegna (2018).

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8 = Risk Factors and Plant Management Activities for the Terraced Agricultural Systems on the Amalfi Coast (Italy)

Valentina Savo[1], Emanuela Cicinelli[1], Giulia Caneva[1]
[1]Department of Science, University of Roma Tre, Viale Marconi 446, 00146 Roma, Italy

Cultivated terraces are quite widespread across continents and many terraced systems are today considered cultural landscapes. These terraces are often managed with traditional techniques, which generally preserve natural biodiversity and ecological functionality. However, these features of terraced landscapes, along with their aesthetic values, are retained only in well-preserved terraces. The landscape of the Amalfi Coast (UNESCO Cultural Heritage site) is typified by rugged coasts, woodlands, and a mosaic of villages and terraced vineyards and orchards. Today, this unique landscape is currently threatened by the potential collapse of terraces once they start deteriorating after the abandonment of agricultural activities. The risk of these collapses is also amplified by the increasing incidence of heavy rainfall events. We combined semi-structured interviews with traditional farmers and vegetation surveys on in-use terraces to understand which are the most relevant perceived factors of risk and the management activities that are performed to maintain the cultural landscape of the Amalfi Coast (Fig. 1).
In total, we performed 30 interviews with local farmers and 34 vegetation surveys on their terraces (Fig. 2). Farmers were randomly selected within the study area; interviews were anonymous and performed following International Codes of Ethics. During our vegetation surveys, we collected data on the exposure, altitude, wall type, species diversity and species cover of each sampled plot. A species/survey matrix was prepared and subjected to Cluster analysis to evaluate the difference among the surveys. To better evaluate features, ecology, and potential dynamics of vegetation, we performed a syntaxonomical classification of the plant species. We identified four main ecological groups and, for each species belonging to the these groups, we calculated the medium value of each cover range (+ = 0,1%; 1 = 2,5%; 2 = 15,0%; 3 = 37,5%; 4 = 62,5%; 5 = 87,5%).
All the interviewed farmers still practice maintenance of the terraces even if they are no longer cultivating them, to avoid their collapse. According to most informants, the first key factor in the management of terraces is maintaining waterways clear from debris to ensure an optimal rainwater runoff. Indeed, most farmers highlighted the linkage between terrace collapses and heavy rainfall. The second most relevant factor pertains to the maintenance of the retaining walls, which entails managing the vegetation growing on the risers and repairing damaged structures. Farmers also mentioned fires and grazing animals as potential risk factors for the preservation of the integrity of terraces. We recorded a certain diversity of plant species, mostly herbaceous, and with a Mediterranean distribution (109 species, 42 families). The number of species varied greatly among the surveys (from a minimum of three species to a maximum of 22), with an evident influence on the total cover. The cluster analysis showed a separation of our surveys into two main clusters, which mostly differ for the contribution of Therophytes and Hemicryptophytes detected in the plots. The wider group of plants were referable to the *Stellarietea mediae* and *Artemisietea vulgaris* classes, which describe slightly-nitrophylous and nitrophylous ephemeral and perennial vegetation. However, when considering the cover values of the plants, we found a predominance of the nitrophilous vegetation of the *Parietarietea judaicae* class and the non-nitrophilous vegetation of the *Asplenietea trichomanis* class. The vegetation growing on the walls has a relevant role in the stability of terraces, according to our informants.
Abandonment of terraces is increasingly becoming a threat for the cultural landscape of the Amalfi Coast. According to our study, it seems that simple management actions can prevent, or at least reduce, the incidence of landslides and related risks. Maintaining vital the agriculture on the Amalfi Coast becomes a priority for preserving the cultural landscape that has contributed to making the area a UNESCO Cultural Heritage site.


Fig. 1. Cultural landscape of the Amalfi Coast.

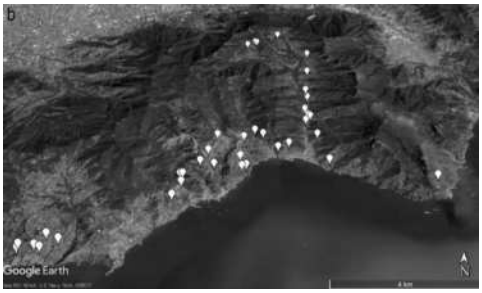

Fig. 2. Survey sites.

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8 = Diversity of plant communities in long-term disturbed areas - The case of Sacred Natural Sites in Italy

Piero Zannini[1], Fabrizio Frascaroli[1,2], Juri Nascimbene[1], Michele Di Musciano[1,3], Riccardo Guarino[4], Sara Landi[5], Giovanna Pezzi[1], Fabrizio Buldrini[1], Alessandro Chiarucci[1]

[1]Biodiversity & Macroecology Lab, Department of Biological, Geological and Environmental Sciences, Alma Mater Studiorum University of Bologna, Via Irnerio 42, 40126, Bologna, Italy; [2]Lòm Research, Via Re Manfredi 38, 03030, Rocca d'Arce (FR), Italy; [3]Department of Life Health and Environmental Science (MESVA), University of L'Aquila, Piazzale Salvatore Tommasi 1, 67100, Coppito (AQ), Italy; [4]Botanical Unit, Department STEBICEF, University of Palermo, Viale delle Scienze 16, 90128, Palermo, Italy, Italy; [5]GREENARCO, Spin-Off Company of the Alma Mater Studiorum University of Bologna, Viale G. Fanin 48, 40127, Bologna, Italy

It's largely known that Earth's biota has entered its sixth mass extinction. Indeed, biodiversity is currently being eroded at an unprecented rate, across taxa and biomes. Protection of large portions of Earth has been called for to halt the current biodiversity crisis. However, establishment of large protected areas (PA) is typically difficult due to costs, logistic and aversion of local communities. Small PA and other area-based conservation measures can complement large PA, while ensuring the connectivity of the network of PA. Sacred Natural Sites (SNS) represent instances of long-term disturbance across large spatial scales with varying degree of human pressure. On the other hand, SNS have demonstrated their potential for conserving biodiversity but large scale studies are lacking. We sampled vascular plant communities at 30 SNS across Italy and related control sites (Reference Sites, hereafter RS) and compared them in terms of species richness, rarity, locally-exclusive species, beta and gamma diversity. While species richness of SNS and RS were similar, we found that SNS had significantly rarer species assemblages, more locally-exclusive species and larger beta and gamma diversity. Moreover, woodlands and annual crops found at SNS showed greater conservation value in respect with RS, whereas we found no evidence for conservation value of grasslands and perennial crops of SNS. The moderate and continuous disturbance following traditional management practices, as well as social taboos and natural features of SNS are likely responsible for the observed patterns. A legal recognition of these sites is likely urgently needed to help local communities conserve these precious instances of informal PA, while enabling the persistence of moderate disturbance regimes. Finally, the different trends we found across macrohabitats points at the need to apply habitat-based conservation measures.

Fig. 1. Map of 30 Sacred Natural Sites sampled.

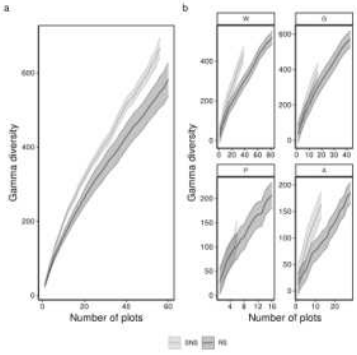

Fig. 2. Gamma diversity rarefaction curves.

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10 = Archaeobotany of *Olea europaea* in the Near East: first data (Early Bronze age) from Jebel al-Mutawwaq (Jordan)

Alessia D'Auria[1], Alessandra Caselli[1], Andrea Polcaro[1], Gaetano Di Pasquale [2]
[1]Department of humanities, ancient and modern languages, literature and cultures, University of Perugia, P.zza Università – 06123 – Perugia, Italy; [2]Department of Agricultural Sciences, University of Naples Federico II, Via Università 100, 80055, Portici, Italy

This species belongs to the first group of domesticated trees and it has dominated the Mediterranean landscape, culture and history. Its ancient history is at times questioned. In the Levant, the Early Bronze Age is characterized by an increase of olive cultivation especially in Southern Levant testified also by archaeobotanical evidence from Tell Fadous in northern Lebanon and from Tell Mastuma in northern Syria. These data indicate a significant olive exploitation in the Early Bronze II-III.
In this context, archaeobotanical data from the site of Jebel al-Mutawwaq (Jordan) are presented. Jebel al-Mutawwaq is located in the Wadi az-Zarqa Valley, on the top of a hill at around 550m a.s.l. The vegetation cover consists today in scattered evergreen shrubs and ancient olive-groves. The archaeological site consists of a 18 ha village surrounded by a demarcation wall and of a huge necropolis covering the entire hill surface. Jebel al-Mutawwaq was settled during Early Bronze Age I, a crucial period for Southern Levant civilizations, when the seminomadic communities started to establish permanent villages and consequently to increase the complexity of the society. For this reason, Early Bronze Age I is considered a proto-urban phase and the study of the socio-economic dynamics developed in this period is essential to understand the evolution of the urbanization process. Interestingly, first archaeobotanical analysis shows the presence of uncharred stones of olive dated to the 3500-2900 BCE (Early Bronze Age).

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10 = Pollen and molecular biomarkers from sedimentary archives: complementary tools to improve knowledge on the introduction of broomcorn millet in the central Po Plain (N Italy)

Assunta Florenzano[1], Eleonora Clò[1], Anna Maria Mercuri[1], Jérémy Jacob[2]
[1]Laboratorio di Palinologia e Paleobotanica, Dipartimento di Scienze della Vita, Università degli Studi di Modena e Reggio Emilia, Via G. Campi 287, 41125 Modena, Italy; [2]LSCE (UMR CEA-CNRS-UVSQ 8212), Orme des Merisiers, CEA/Saclay Bât. 714, 91191 Gif-sur-Yvette Cedex, France

Broomcorn millet (*Panicum miliaceum* L., Poaceae family) is a C4, warm-season annual grass growing in poor soils and hot dry weather. This cereal requires very little water and, as many of the other millets, is progressively being researched to provide farmers - facing climatic crisis - solutions to increase the diversity of crops.
P. miliaceum was domesticated in northeast China by 6000 BC; it was thought to have been adopted in Europe by Early Neolithic communities, but recent interdisciplinary research has cast doubt on this statement. Archaeobotanical evidence reveals that millet was widely spread in Europe from the 2nd millennium BC, but there are several uncertainties about the timing and the diffusion path of its adoption by European societies (Filipović et al. 2020, Scientific Reports 10:13698).
In Italy, the introduction of broomcorn millet cultivation probably occurred at the end of the 3rd millennium BC as testified by grains from early Bronze Age in north-eastern Italy, with further spread in the Middle Bronze Age within the Terramare culture (Po Plain area) and became a common culture during the Iron Age. However, archaeocarpological records often lack grains of broomcorn millet due to preservation issues, and hence further approaches to investigate its introduction and adoption are necessary.
Recent research is focused on the detection of miliacin, a molecular biomarker specific of *Panicum*, in sedimentary archives as a trace of past presence of millets in paleosols and sediments. Specifically, miliacin is a pentacyclic triterpene methyl ether that is enriched in grains of common/broomcorn millet and other few Poaceae. Combined with the evidence from pollen analysis, the presence of miliacin in sediments may be considered the molecular evidence of millet cultivation.
This paper relies on a project (2020 FAR-DSV UNIMORE, in collaboration with CNRS-LSCE) dealing with the integration of biomolecular techniques and pollen analysis to improve information on broomcorn millet adoption by the Terramare culture.
A specific objective of the project is to verify the correspondence between miliacin content and *Panicum* pollen concentration in 3 near-site stratigraphic sequences of the Terramara S. Rosa di Poviglio (central Po Plain).
The sediment records from the 3 long cores continuously span the last ~15,000 years, providing detailed and high-resolution information on vegetation history and land-use changes in the area. The combined evidence of *Panicum* pollen and sedimentary miliacin could testify the local presence of the plant even in absence of millet grains, and provides empirical evidence of the introduction of millet crops in the central Po Plain.

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11 = Comparing probabilistic and preferential datasets of Italian forests: A case-study through the LifeWatch infrastructure

Nicola Alessi[1], Gianmaria Bonari[2], Piero Zannini[1], Emiliano Agrillo[3], Fabio Attorre[4], Roberto Canullo[5], Laura Casella[3], Marco Cervellini[1], Stefano Chelli[5], Michele Di Musciano[1], Riccardo Guarino[6], Stefano Martellos[7], Marco Massimi[4], Stefan Zerbe[2], Alessandro Chiarucci[1]

[1]Dipartimento di Scienze Biologiche, Geologiche e Ambientali, Università di Bologna Via Irnerio 42, Bologna; [2]Facoltà di Scienze e Tecnologie, Libera Università di Bolzano, Piazza Università 5, Bolzano; [3]Istituto Superiore per la Protezione e la Ricerca Ambientale, Via Vitaliano Brancati 48, Roma; [4]Dipartimento di Biologia Ambientale, Sapienza Università di Roma, Piazzale Aldo Moro 5, Roma; [5]Scuola di Bioscienze e Medicina Veterinaria, Università di Camerino, Via Pontoni 5, Camerino; [6]Dipartimento di Scienze e Tecnologie Biologiche Chimiche e Farmaceutiche, Università di Palermo, Via Archirafi, 38 Palermo; [7]Dipartimento di Scienze della Vita, Università di Trieste, via Giorgieri 10, Trieste

LifeWatch infrastructure mainly aims to aggregate and analyze big data in the field of ecology, thus, offering to the public virtual services and tools to boost scientific research and dissemination. In this context, the inter-university centre PlantData began to collect and organize published and original datasets on plant diversity at the national level to obtain a standardized database from different sources. Different data collection methods were applied, due to different purposes. Most published field surveys employed a preferential sampling, i.e the sampling sites were subjectively chosen by the researcher in order to describe vegetation units, while the remaining data derive from a probabilistic sampling approach. Although such aggregated datasets are commonly used in large-scale ecological studies due to the large amount of species co-occurrence data they can provide, the statistical inferences on diversity patterns could be biased by the violated assumption of equal chance to select a site to survey. The evaluation of such bias is useful to improve the process of data aggregation, and thus to inform users of big data infrastructure about methodological pitfalls and limitations. To this end, a target dataset derived from a heterogeneous collection of vegetation plots in the Italian forests is compared with a probabilistic reference dataset to evaluate its potential gaps and limitations.

We assembled a dataset composed of three regional databases (AMS-VegBank, VPD-Sapienza University of Rome, Vegetation database of Habitats in the Italian Alps – HabItAlp, database of the Museum of Nature South Tyrol Bolzano), with about 18,000 vegetation plots from the whole Italy mainly following the preferential sampling – i.e. phytosociological approach. Our dataset was compared with the ICP-Forest dataset on plant diversity, which followed a probabilistic sampling design on the Italian forests, composed by 201 vegetation plots. We compared the two datasets in terms of broad forest types, which resulted from a multivariate regression tree (MRT) that classified plots by using species composition as well as bioclimatic variables. We obtained three forest types comprising (i) the warm temperate forest dominated by evergreen and deciduous *Quercus* spp.; (ii) the cool temperate forest dominated by *Fagus sylvatica* and *Pinus nigra*; (iii) the montane forest dominated by *Picea abies* and *Larix decidua*. An estimation of the occupied area by the three forest types was obtained using bioclimatic thresholds resulting from the MRT and remotely-sensed forest delimitation (Copernicus products).

The preferential dataset resulted spatially representative for the forest types in the study area, due to the great number of plots included. This last characteristic is also reflected in the taxonomic and ecological representativeness of the dataset through the occurrence of rare species. However, the preferential dataset showed an oversampling in the warm temperate area, suggesting a preferential accumulation of data for the (sub-)Mediterranean forests, while the coniferous forest of the Alps were undersampled.

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11 = High-throughput phenotyping experiments to understand lettuce morpho-physiological acclimation under changing VPDs and watering regimes

Chiara Amitrano[1], Astrid Junker[2], Veronica De Micco[1]
[1]Department of Agricultural Sciences University of Naples "Federico II", Via Università 100, Portici (Napoli) Italy; [2]Department of Molecular Genetics, Leibniz Institute of Plant Genetics and Crop Plant Research (IPK) Gatersleben, Stadt Seeland, Germany

Nowadays, about 50 % of the global yield loss are due to climate changes. Increasing Vapor Pressure Deficit (VPD) and drought are among the principal environmental stressors, affecting stomatal regulation and thus reducing plant photosynthesis and biomass accumulation. VPD and drought effects on plant growth are already well-studied, however how the interaction between these environmental factors affect plant morpho-physiological development and their capacity of acclimation under changing conditions, is still unclear. To fill this gap, in this study we used a high-throughput phenotyping facility (APPP-A small plants of the IPK-Gatersleben, Germany) to grow two lettuce cultivars (*Lactuca sativa* L. var. *capitata*) with green and red leaves under different VPDs (low and high) and watering regimes (well-watered, WW, and water deficit, WD, regimes). During cultivation, the plants were subjected to sudden changes in the VPD to evaluate their acclimation capability. More specifically, two trials were performed: the first trial was conducted at a VPD of 0.7 kPa (low VPD) and the second at 1.4 kPa (high VPD), both with WW and WD conditions. After 12 days of cultivation in the phenotyping chamber, the environmental conditions were switched, and plants were kept for 5 days at the opposite VPD to test the short-term acclimation. VPD conditions were obtained by keeping T fixed and changing RH %. RGB imaging was applied to track changes in morphological parameters, near-infrared camera (NIR) was used to estimate plant-water relationships, and FLUO camera to assess changes in photosystem II reflecting optimal/stressful conditions. At the end of the experimental trials, leaf samples collected in FAA fixative were characterized in terms of stomatal and mesophyll traits through light microscopy analyses. A specific focus was devoted to exploring how stomata regulation and water use efficiency affects carbon gain and biomass allocation in lettuces pre-acclimated to different environmental conditions (VPDs), and then subjected to sudden changes in the VPD. Results showed that lettuces grown at the two VPDs and water regimes developed a different leaf anatomical structure, which differently influenced their capacity in acclimation under the switch in environmental conditions. These results are fundamental to study crop adaptation under sudden fluctuation in environmental condition due to climatic changes and can improve knowledge in how stomatal regulation and carbon allocation influence productivity in warmer and drier climates, with wide impact also for the design of cultivation protocols for sustainable indoor farming.

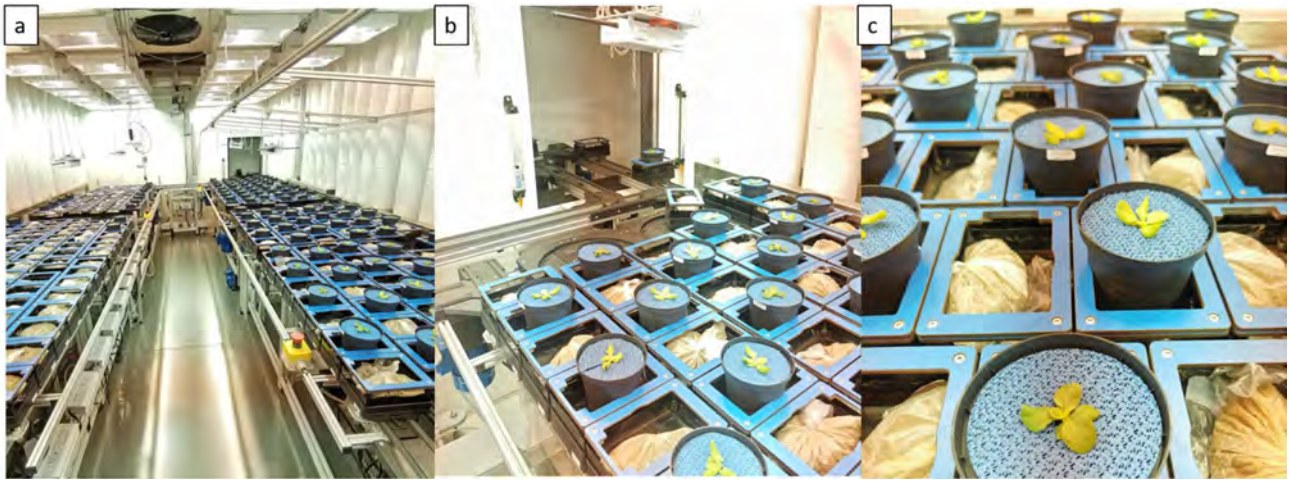

Fig. 1a,b,c. Lettuce plants in the phenotyping growth chamber.

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11 = The Herbarium Agrariae Pisa: a memory of agricultural research in the period 1920-1935

Iduna Arduini[1], Serena Trimarchi[1]
[1]Department of Agricultural, Food and Environmental Sciences, University of Pisa, Via del Borghetto 80, I-56124 PISA, Italy

A collection of vascular plants, consisting of 1250 exsiccata for a total of 705 species belonging to 99 families, is preserved at the Department of Agricultural, Food and Environmental Sciences of the University of Pisa (Fig. 1). The description of the specimen, without images up to now, is available on the international herbarium management system JACQ (http://www.jacq.org/#home) with the acronym PIAGR-Herbarium Agrariae Pisa. The value of this small herbarium does not rely on the rarity or origin of the preserved species, or on the reputation of the collector, but on being a 'living' testimony of the research activities carried out in the first half of the XX century at the Royal High Institute of Agriculture of Pisa. This institute inherited the knowledge of the first university School for Agriculture in the world, which was founded in Pisa at the behest of Cosimo Ridolfi, in 1842.
Most specimens were collected between 1923 and 1934, but it is not possible to trace with certainty the author of the collection or the client, because the labels of the core collection are anonymous. However, as Count Napoleone Pio Passerini, a botanist and agronomist of Florence, directed the R.H. Institute of Agriculture and taught agronomy in the same period, we can assume that he gave the assignment to arrange the herbarium, probably for didactic purposes, primarily. The interest of Passerini in collecting plants is demonstrated by his own collections of exsiccata, now preserved at the Botanical Museum of Pisa and at the University of Florence. Furthermore, several exsiccata were collected in Scandicci (FI) and Antignano (LI), where Passerini lived and had extensive possessions (Fig. 2). Plant specimens were identified and numbered following the New Analytical Flora of Italy that was published by Adriano Fiori in 1923-25. Personal contacts with Fiori could be hypothesized, based on the presence of some tables labeled A. Fiori in the herbarium. The collection also includes 32 plates legit Pontecorvo, dated 1920-21 and stamped 'Chair and Laboratory of Plant Pathology and Bacteriology', which suggest that plant collecting had been started in Pisa before the arrival of Passerini.
The scientific and historical interest of the Herbarium Agrariae Pisa, as well as its originality, rely on having preserved the species that were cultivated at the experimental farm and the garden of the Institute in the period 1920-30, thus giving us a picture of crop research at that time. The presence of over 100 species belonging to the *Fabaceae* suggests wide interest in forage research. However, a special value is deserved by the 23 plates of the genus *Triticum*, among which we find the ancient wheat varieties Ardito and Gentil Rosso, and several hybrids, which are a unique testimony of the wheat improvement research carried out in Pisa in those years.
The small Herbarium Agrariae Pisa demonstrates that a multidisciplinary approach that merges botanical and agronomical skills, can give an added value and enrich knowledge both for the past and future generations.


Fig. 1. PIAGR - Herbarium Agrariae Pisa.

Fig. 2. *Punica granatum* collected in Scandicci (FI).

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11 = Papua New Guinea: how to save a botanic paradise?

Fabio Attorre[1], Michele De Sanctis[1], Silvio Cianciullo[1], Luca Malatesta[1], Riccardo Testolin[1], Kipiro Qizac Damas[2], Miller Kawanamo[2], Penniel Lamei[2], Peter Homot[2], Gibson Lainza Sosanika[2], Daniele Cicuzza[3]
[1]Department of., University of Environmental Biology, Sapienza University of Rome, P.le A. Moro 5, 00185, Roma, Italy; [2]Papua New Guinea Forest Research Institute, P. O. Box 314, LAE, Morobe Province; [3]Faculty of Science, Environmental and Life Sciences, Brunei-Muara District, Brunei

Papua New Guinea (PNG) covers the eastern part of New Guinea and includes several smaller islands and archipelagos. It hosts the third largest tropical forest in the world after Amazon and Congo basins, and is a globally recognized centre of biological and cultural diversity. A recent floristic assessment accounted for 13634 species (68% endemic), 1742 genera and 264 families, suggesting that it is the most floristically diverse island in the world, with many more species still to discover. However, this botanic paradise is under threat due to increasing deforestation and forest degradation caused by the rapid population growth and fast-growing economy. A possible mechanism to prevent biodiversity loss in PNG, is the implementation of the REDD+ (Reducing Emission from Deforestation and Forest degradation) initiative established within the framework of the UNFCCC. REDD+ creates a financial value for the carbon stored in forests by offering incentives for developing countries to reduce emissions from forested lands and invest in low-carbon paths through the National Forest Monitoring System (NFMS), which is responsible for the measurement, reporting, and verification (MRV). In PNG, the National Forest Inventory (NFI) is an integral part of the NFMS and was conducted to establish a baseline of the carbon stock and sequestration as well as several biodiversity indicators including tree species, ferns, lianas, and ground herbaceous vegetation. To this aim 26 plots of 25 m radius were sampled along an altitudinal gradient: 8 in lowland primary forest, 12 in lowland disturbed forest, and 6 in the lower montane primary forest. Specimens were collected, stored and identified at the PNG National Herbarium of Lae. Differences among forest types and level of disturbances were assessed in terms of species richness, diversity and composition for all the analysed taxonomic groups. Results highlighted similar patterns among the groups with more marked differences between lowland and montane types then between disturbed and undisturbed forests. This was the first extensive assessment of the plant species richness and diversity in PNG, and data collected will be used to support the elaboration of effective REDD+ management activities and to monitor their impact on the conservation of a unique biodiversity hotspot.

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11 = Comparative studies on seed germination in montane related *Aquilegia* species (Ranunculaceae)

Silvia Bacci[1], Lorenzo Pinzani[1], Francesca Olivieri[1], Gianni Bedini[1], Angelino Carta[1]
[1]Department of Biology, University of Pisa, Via Derna 1, 56126 Pisa, Italy

Seed germination is a critical event in the life cycle of spermatophytes and its synchronization with the environment is crucial to avoid unfavorable conditions for seedling establishment. Seed dormancy is a complex adaptation that prevents germination until specific environmental requirements are met. Identifying the processes underlying seed germination and dormancy enables to understand plant adaptation and effectively contribute to plant conservation.
Seeds in the Ranunculaceae family are characterized by underdeveloped embryos at dispersal that require a time lapse to complete embryo growth before starting germination. This internal seed feature is considered as a morphological constraint on seed germination (usually defined as morphological dormancy; MD), which is sometimes coupled with physiological dormancy (MPD).
The genus *Aquilegia* L. is characterized by small and rudimentary embryos at dispersal, thus likely possessing either MD or MPD. So far, only a few studies on seed dormancy and germination behavior in *Aquilegia* have been conducted. Here, we investigate seed dormancy in 5 species and 11 populations of *Aquilegia* distributed on different mountain ranges of Northern Italy. The selected species are *Aquilegia alpina* L., *A. lucensis* E.Nardi, *A. reuteri* Boiss., *A. ophiolithica* Barberis & E.Nardi, and *A. bertolonii* Schott. To determine which conditions are required to break dormancy, different temperature treatments were included in the experiment: seeds were exposed to six different temperatures (5, 10, 15, 20, 25, 30°C) shortly after harvesting, while others were pre-treated either with warm stratification (25°C for 3 months), cold stratification (5°C for 3 months), or with a sequence of warm + cold stratification (3 months at 25°C + 3 months at 5°C). Moreover, a “move-along” experiment where the seeds were exposed to different seasonal temperature regimes was simultaneously conducted, complemented by periodic seed sections to monitor embryo growth over time. The seeds of all species show a deep level of MPD, as almost no germination at any condition was detected after 8 months currently. Nevertheless, seed sections highlighted a slow but progressive embryo growth in all populations during the “move-along” experiment.
This study will help to better understand the adaptive mechanisms underpinning seed germination in *Aquilegia*. Specifically, whilst these species are genetically related, different classification schemes have been proposed over the years: this study will help to understand the degree of functional differentiation among these taxa. Moreover, the acquired knowledge would serve to implement tailored strategies for the conservation of these species and to make predictions about their responses to climate change.

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11 = Traditional uses in Valmalenco (SO, Italy): from ethnobotany to the nutritional value of typical plant species

Martina Bottoni[1,2], Fabrizia Milani[1,2], Paola Sira Colombo[1,2], Lorenzo Colombo[1,2], Stefania Teocchi[3], Claudia Giuliani[1,2], Piero Bruschi[4], Daniela Erba[3], Gelsomina Fico[1,2]
[1]Department of Pharmaceutical Sciences, University of Milan, Via Mangiagalli 25, 20133 Milan, Italy; [2] Ghirardi Botanic Garden, Department of Pharmaceutical Sciences, University of Milan, Via Religione 25, 25088 Toscolano Maderno, Brescia, Italy; [3]Department of Food, Environmental and Nutritional Sciences, University of Milan, Via Celoria 2, 20133 Milan, Italy; [4]Department of AgriFood Production and Environmental Sciences, University of Florence, Piazzale delle Cascine 18, 50144 Florence, Italy

This work is part of the Italy-Switzerland Interreg research project *B-ICE* (ID. 63143) *Study and enhancement of a precious natural and cultural heritage in an open Alpin region, with innovative approaches to the future. Valmalenco (SO): study and enhancement of the plant and cultural biodiversity through an ethnobotanical survey*, which is aimed to promote a sustainable management model for the ongoing climate change in Valmalenco (Sondrio, Italy) and to reach new sources for the local enhancement. To this goal, traditional uses of autochthonous plant species represent an important part of the rich biocultural heritage locally available.
The ethnobotanical survey was performed in the municipalities of Chiesa in Valmalenco, Caspoggio, Lanzada, Spriana and Torre di Santa Maria, with the purpose of investigating, through the proposal of interviews to the local community, the traditional uses in the therapeutic, cosmetic, food, artisan, agropastoral, recreational and religious fields. In 2019 and 2020, 340 interviews were conducted. For each species, attention was paid to the: common and dialectal names of the plant, past or current use, plant risk category, ways of administration, recommendations of use and methods and forms of preparation. Concerning the food sector, the use of 123 species, belonging to 48 families, emerged: Rosaceae, Ericaceae and Asteraceae were the most represented. *Vaccinium myrtillus* L., *Rubus idaeus* L., *Fragaria vesca* L., and *Taraxacum officinale* Weber (s. l.) were the most cited species, mostly employed as fresh fruit or in jam. This work aims to investigate the nutritional properties of the most mentioned plant species of the Malenca culinary tradition, using the number of citations and the type of preparation as selection criteria; alcoholic preparations, as well as those with a high content of sugar or oil, were excluded *a priori*. On these bases, 6 target-species were selected: *Chenopodium bonus-henricus* L., whose boiled leaves are consumed in gnocchi, risottos, and frittatas; *Silene vulgaris* (Moench) Garcke, whose young leaves are employed raw in salads or boiled in rice soups; *Taraxacum officinale* Weber (s. l.), whose young leaves, sometimes with the apical portion of the root and the inflorescence, are collected to prepare salads, while the boiled fully-expanded leaves represent the basis for frittatas; *Rumex acetosa* L., whose herbaceous stems and young leaves are eaten as country snack; *Aruncus dioicus* (Walter) Fernald, whose boiled sprouts are used in frittatas and salads; *Urtica dioica* L., whose boiled young leaves are the basis for the preparation of gnocchi, risottos and filling. Therefore, for each species a working protocol including subsequent phases was developed: 1) selection of the traditional dish to be analysed; 2) identification of key-collectors; 3) creation of a timetable of the harvesting periods related to the "balsamic time" defined by the traditional knowledge; 4) identification of the collection sites; 5) definition of the procedure for the treatment and storage of samples until the time of analysis; 6) evaluation and beginning of the tests on the nutritional value. The nutritional quality will be analysed through the detection of the centesimal composition (percentages of moisture, proteins, lipids, total carbohydrates, and ashes), as well as of the amounts of some bioactive compounds known for their potential positive effects on human health (polyphenols, carotenoids, and minerals), in both raw and boiled samples according to traditional procedures. This work will also enhance the Valley's tradition from a nutritional point of view through the realization of a dedicated recipe book, including the following information: botanical description of the species together with its botanical sketch; its traditional use in the food sector documented in the study area; description of the typical dish according to the Malenca culinary tradition together with the detailed recipe; photo's archive of the work's steps, from the harvesting to the final dish; return of the scientific data to the general public, with the aim of reawakening the inhabitants' awareness and of enhancing the value of the Valmalenco biocultural heritage.

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11 = Orchid mycorrhizal fungi: time to re-think their ecology?

Jacopo Calevo[1-2], Samuele Voyron[1-3], Martino Adamo[1], Karl J. Duffy[2], Silvia Perotto[1-3], Mariangela Girlanda[1-3]
[1]Department of Life Sciences and Systems Biology, University of Turin, Viale Mattioli 25, 10125, Torino, Italy; [2]Department of Biology, University of Naples Federico II, Campus Monte Sant'Angelo - Via Cinthia, 80126, Napoli, Italy; [3]CNR-Istituto per la Protezione Sostenibile delle Piante, UOS Torino (CNR-IPSP), Viale Mattioli 25, 10125, Torino, Italy

There is growing evidence that many fungi have more complex niches than previously thought. The environmental factors explaining the distributions of orchid mycorrhizal (OrM) fungi remains elusive, due to the fact that some of these fungi are undetectable in soil outside the host rhizosphere.
A manipulation experiment, performed to assess the importance of neighbouring non-orchid plants and soil as possible reservoirs of OrM fungi for *Spiranthes spiralis*, showed that the removal of the surrounding vegetation and soil significantly affected the fungal colonization of newly-formed orchid roots but most OrM fungi were consistently associated with the host roots. Frequency patterns in differently aged roots suggested that these fungi colonized new orchid roots from either older roots or other parts of the same plant, which may thus represent an environmental source for the subsequent establishment of the OrM symbiosis.
Given that OrM fungi may be "ecologically obligate" orchid endophytes, these results have obvious implications for orchid conservation, since orchids depend on OrM fungi to complete their life-cycle. If OrM fungi are also present as orchid non-root endophytes, over-collection of plants in the wild would mean not only depauperating orchid populations, but also depriving the environment of OrM communities on which orchids depend.
While we need to further investigate the role of different fungal taxa in orchid germination and development we should be prepared to re-think and better understand OrM fungal ecology in order to have effective conservation strategies of both orchids and their symbiotic fungi.

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11 = Urban green: taxonomic and distributive update of phytoindividuals found in the "Giardino Inglese" of Palermo (Sicily)

Patrizia Campisi[1], Emilio Di Gristina[2], Maria Letizia Gargano[3], Filippo Bartoli[4], Francesco M. Raimondo[4]
[1]Department STEBICEF/Sect. of Botany, Anthropology and Zoology, University of Palermo, Via Archirafi 38, 90123 - Palermo, Italy; [2]Department of Agricultural, Food and Forest Sciences, University of Palermo, Viale delle Scienze, bldg. 4, 90128 - Palermo, Italy; [3]Department of Agricultural and Environmental Science, University of Bari Aldo Moro, Via Amendola 165/A, I-70126 Bari, Italy; [4]*PLANTA*/Center for Research, Documentation and Training, Via Serraglio Vecchio 28, 90123 - Palermo, Italy

After the first investigations started in 1980, when in addition to the city trees, the most representative gardens of the city of Palermo were examined such as Villa Bonanno, Villa Trabia, Villa Niscemi, Villa Belmonte, Villa Florio-Pignatelli, the garden of Palazzo of Normanni, the Garibaldi Garden, the English Garden and, among private gardens, the garden of Villa Whitaker in Malfitano, the systematic study of urban green of the Sicilian capital underwent an arrest at the beginning of 2000. The research in this regard will resume 15 years later, involving Villa Natoli in Boccadifalco and the "Giardinetto dello Spasimo", the latter in the historical center. Punctual studies of floristic character - aimed primarily at the elaboration of a catalog of trees, shrubs, and succulents cultivated in public and private accessible spaces - have been recently published. This contribution focuses on one of the historic gardens that had already been the subject of a previous study published in 2000, namely the Giardino Inglese, on Via Libertà.
It is the first romantic garden planted in Palermo between 1851 and 1856, designed by the young architect Giovan Battista Filippo Basile - who became famous for designing the Teatro Massimo - under the wise guidance of Vincenzo Tineo, director of the Botanical Garden at the time. The Giardino Inglese - being designed and built "in the English style" - in the last 20 years of management not very consonant with its historical and monumental character, has suffered further losses and alterations of architectural nature. For this reason, we review the floristic survey carried out at the time and consequently updating the map dating back to 2000. The list of species found is poor if compared to that of the species introduced in the garden, among which there were many herbaceous and suffruticose plants, some of which were rare, then obviously disappeared. Today, the garden hosts only a small part of those present in the lists of purchases, made at the time in the most relevant Italian nursery centers (Turin, Naples, Palermo) and abroad (Marseille). Tree and shrubby species, a few bulbous and some succulent were predominantly preserved. The reduction of the botanical species - taking into account also those introduced between the end of the 19th century and the first decades of the 20th century - dates back to the last post-war period and has been progressive. A further impoverishment has occurred in the last 20 years if we consider the data resulting from the mentioned study of 2000 in which only 119 specific and infraspecific taxa are listed, while those resulting from our unpublished census carried out in recent months, rebalance a bit the gap.
Based on the updating carried out, the florula of the garden is today constituted by 186 specific and infraspecific taxa divided into 127 genera of 60 botanical families. The most represented family is that of *Arecaceae* with 16 species referred to 9 genera; *Moraceae* follow with 12 species distributed in 4 genera, among which *Ficus* represented by 9 species; then *Cupressaceae* with 8 species afferent to 4 genera. Due to the showy seasonal flowering, it deserves a special mention *Brachychiton acerifolius* (A. Cunn. ex G. Don) F.Muell., *Sterculiaceae*, represented with other two species of the same genus. Considering the historical importance of the garden, it is useful to draw attention to the most significant plants in the garden, as well as the cases that were missed or misinterpreted in the first census. In some cases, these plants are new or very rare in European and Italian gardens, such as *Pisoniella arborescens* (Lag. & Rodr.) Standl. and *Deutzia scabra* Thunb. Important are also some trees such as: one plant of *Ficus benghalensis* L., two of *F. macrophylla* fo. c*olumnaris* (C. Moore) D.J.Dixon, and three of *Araucaria columnaris* (G. Forst.) Hook. (*A. cookii* R. Br. ex Endl.). One plant reported as *A. cookii* in the study published in 2000, was then considered its variety (*Araucaria cookii* var. *luxurians* Brongn. & Gris), and now recognized as a valid species [*A. luxurians* (Brongn. & Gris) de Laub.]. This last finding has a unique character in gardens all over Europe and the Mediterranean countries. Relevant cases, also under the aspect of "monumentality" or "singularity" are appropriately highlighted in the new map of phytoindividuals that has been elaborated and that is therefore presented. The legend summarizes all the elements that express the botanical value of the garden under study.

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11 = Biomonitoring of atmospheric Hg indoor pollution: comparison of the accumulation efficiency of three different bioindicators

Francesco Ciani[1], Silvia Fornasaro[1], Renato Benesperi[2], Luca Di Nuzzo[2], Lisa Grifoni[2], Elisabetta Bianchi[3], Pilario Costagliola[1,4], Valentina Rimondi[1,4]
[1]Department of Earth Science, University of Florence, Via G. La Pira 4, Firenze 50121, Italy; [2]Department of Biology, University of Florence, Via G. La Pira 4, Firenze 50121, Italy; [3]Department of Life Science, University of Siena, Via Mattioli 4, Siena 53100, Italy; [4]CNR-IGG, Via G. La Pira 4, Firenze 50121, Italy

Biomonitoring technique, i.e. the use of biological specimens as a tool to monitor air quality, represents a simple, efficient, and economic method to assess the presence of airborne persistent pollutants, in particular heavy metals. Both living and transplanted organisms, such as mosses and lichens, or plant portions, like tree barks or leaves, may be used as bioindicators. As a matter of fact, tree tissues are not commonly used as bioindicators as compared to other more classical bio-substrates. Tree barks are potentially very efficient for the accumulation and retention of aerosol particles because of structural porosity.
In this study, we tested the efficiency of Hg bioaccumulation of three different types of bioindicators: tree barks (*Pinus nigra* J.F.Arnold), lichens (*Pseudevernia furfuracea* L.), and mosses (*Hypnum cupressiforme* Hedw.). In particular, the efficiency of barks in retaining pollutants was compared with lichens and mosses ones, two classic and well-known organisms widely used as biomonitors. As pollutant we selected mercury (Hg) since it is possibly the ideal element to be monitored through barks because a) it is generally scarcely bioavailable i soils; b) its presence in the bark is mainly ascribable to the atmospheric transport c) it could be retained in tree barks both physically and chemically. Here, barks, lichens, and mosses have been exposed in the *Central Italian Herbarium* (Natural History Museum of the University of Florence, Italy) where recent studies proved high level of airborne Hg, both as gaseous elemental Hg and as particulate bound Hg.
The three bioindicators were collected from an unpolluted site in the Appennino Pistoiese (Cutigliano, Italy) to avoid Hg environmental contamination: lichens and mosses were taken from the bark of fir trees at approximately 1.5 m above ground level, while pine barks were sampled at the same height directly from the trees. Once in lab, samples were cleaned and stored at -20°C until the exposition and then placed for six weeks in four *Herbarium*'s rooms. The total Hg of each bioindicators was measured by a direct Hg analyzer (Milestone DMA-80 evo, Department of Earth Sciences, University of Florence) before exposure (zero-measurement), and after three and six weeks.
After only three weeks of exposure, all bioindicators showed a high Hg-uptake, with concentrations (µg/kg) almost double compared to the starting conditions and accumulation percentages mostly comparable: the differences in Hg-uptake are probably ascribable to the morpho-physiological properties of the bioindicators and to their capacity to intercept airborne particles. The highest Hg accumulation (µg/kg) of all the bioindicators was reached in the room mostly used by *Herbarium* staff for normal work activities, i.e. the handling of packages containing plant samples for consultation and cataloguing. These actions probably produce a resuspension of atmospheric particulate matter, suggesting that Hg accumulation in the bioindicators could be related mainly to Hg-rich particles rather than to the gaseous Hg species. At the end of the six weeks of exposure, mosses and lichens showed about the same amount of Hg (µg/kg), while the concentrations accumulated by the barks, although lower, are correlated with the Hg concentrations of the other bioindicators.
The results of the study clearly indicate a high Hg-uptake capacity of all bioindicators, with different accumulation rates based on the organism used and the atmospheric indoor conditions of the different *Herbarium*'s rooms: despite this, the performances in the Hg-uptake are comparable for barks, mosses, and lichens. Based on our knowledge, this is the first time that three different bioindicators are simultaneously used to assess their Hg-uptake efficiency in an indoor environment: from this point of view, the peculiar indoor conditions of the *Herbarium* give an excellent opportunity to check their performance.

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11 = Association between leaf and root functional spectra in Mediterranean coastal dune communities

Daniela Ciccarelli[1], Cleusa Bona[2], Giovanna Wisniewski Peletti[2], Angelino Carta[1]
[1]Dipartimento di Biologia, Università di Pisa, Via Luca Ghini 13, Pisa; [2]Department of Botany, Federal University of Paraná, Rua Coronel Francisco H. dos Santos, 100 – Jardim das Américas, Caixa Postal 19031 CEP 81531-980, Brazil

Plant traits can be used as proxies for functioning and performance, including survival, growth and resource capture. In general, there are limited data on root traits because analysis of below-ground structures is complex and time-consuming. We focused our research on coastal dune vegetation in northern Tuscany. Mediterranean coastal dunes are extreme environments featuring a strong environmental gradient from the shoreline to inland regions. The most important factors which have a critical effect on adult plant survival are salinity, water stress, substrate instability, sand burial, wind abrasion, high temperature and nutrient limitation.
The main aim of this research was to explore the association between leaf and root functional spectra, while considering phylogenetic relationships between species. This allowed us to identify key plant vegetation strategies in herbaceous coastal dune species. We considered four continuous leaf traits (SLA, LDMC, limb thickness and adaxial cuticle thickness), as well as four continuous root traits (RDMC, vessel diameter and frequency and vessel wall thickness) which are linked to relative growth rate (RGR), and acquisitive versus conservative syndrome.
To achieve our goals, we positioned three random transects perpendicular to the shoreline ranging from upper beach to fixed dunes. In each transect, we identified three habitats as follows: frontal dunes, back dunes and slack areas where water temporarily accumulates.
Multivariate analysis showed one trade-off aligned along the first axis between species with high SLA values and high root vessel frequencies, unlike plants which display conservative traits (thick leaves with high values of adaxial cuticle thickness, and large root vessels). Indeed, along the second axis, we found another leaf-root trade-off between high LDMC values and high values of root vessel wall thickness.
Specifically, the front and the slack habitats showed no link between leaf and root spectra, while in the back habitat the two spectra were closely correlated ($r=0.42$, $P<0.01$), highlighting a covariance in root and leaf traits and potentially greater stability in edaphic conditions in this habitat. However, in the slack habitat, where water is temporarily available, we observed a phylogenetic clustering of both root and leaf traits driven by the presence of Poales species which have adapted to humid sites. Overall, in the ecosystem studied, we observed an evolutionary convergence of traits within phylogenetically distant species sharing similar functional traits at leaf and root level.

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11 = New insights in food floral mimicry revealed by an optical physics approach. The study case of the Mediterranean orchid *Anacamptis papilionacea* (L.) R.M. Bateman, Pridgeon & M.W. Chase

Pierluigi Cortis[1], Daniela Scaccabarozzi[2], Antonio De Agostini[1], Elisabetta Costa[1], Marta Cardini[1], Annalena Cogoni[1], Pier Carlo Ricci[3]
[1]Department of Life and Environmental Sciences, University of Cagliari, Via Sant'Ignazio 13, 09123, Cagliari (CA); [2]School of Molecular and Life Sciences, Curtin University, Perth, Western Australia 6102, Australia; [3]Department of Physics, University of Cagliari, SS 554, bivio per Sestu, Monserrato, 09042 Cagliari, Italy

The majority of non-rewarding orchids that are pollinated via deceptive means, employ food deception falsely advertising floral signals of rewarding co-occurring species. However, the boundary between Batesian mimicry and generalised food deception is not always sharp and model species aren't obvious to identify. In generalised food deception, the orchid displays a general flower image that incorporates multiple floral traits of an assemblage of co-flowering rewarding species.
Anacamptis papilionacea (L.) R.M. Bateman, Pridgeon & M.W. Chase is a Mediterranean orchid species that possibly attracts generalist pollinators of Hymenoptera via food deception. As surrounded by diverse *Fabaceae* co-flowering species that superficially share similar floral traits, is an ideal species for testing for Batesian mimicry of model plants or generalised food deception.
Spectral reflectance measurements of floral components through Avantes Sensiline Avaspec-ULS-TEC Spectrometer in the bee visual range (300-700 nm) were undertaken to test if *Anacamptis* and the *Fabaceae* were unlikely discriminated by bees, and so perceived as identical. In 2021 ten *Anacamptis* plants were randomly selected in ten sites to perform optical physical measurements on their flowers (two flowers per plant). Fruit-set was then recorded in the same sites where plants were collected to test if fitness of *Anacamptis* varied in function of measured floral traits.
Based on preliminary observation and first data exploration, we expect that: i) *Anacamptis* employs Batesian mimicry towards rewarding *Fabaceae* species for being pollinated; ii) more than one model species are used by *Anacamptis* to improve pollination; iii) the orchid reproductive success is higher in sites where the mimic-model floral colour match (spectral reflectance) is more pronounced.
Anacamptis genus is pollinated by mimicry of co-flowering *Fabaceae* species, suggesting that the pollination system may represent a guild mimicry system. This will be one of the few tested cases of floral mimicry involving *Fabaceae*. To date, as the mimicry towards *Fabaceae* has been confirmed in South Western Australia in *Diuris* orchid genus, our study suggests an evolutionary convergence of mimicry in phylogenetically distant orchid genera characterised by a Mediterranean climate.

11 = *U-plant*, an online database of the living collections in the Botanic Garden of Pisa

Marco D'Antraccoli[1], Leonardo Cocchi[1], Gemma Giannetti[1], Pietro Picconi[2], Davide Borgioli[2], Luca Francesconi[2], Dario Besseghini[2], Lorenzo Peruzzi[1]
[1]Pisa Botanic Garden and Museum, University of Pisa, Via Luca Ghini 13, 56126 Pisa, Italy; [2]Sistema Informatico di Ateneo, University of Pisa, Via Lungarno Pacinotti 43-44, 56126, Pisa, Italy

An efficient system to record plant accessions is strategic for the activities of a botanic garden, which has among its institutional scopes the management of documented living collections. The garden needs to easily locate, track and store information about every cultivated individual: in a certain way, the documentation tasks can be considered as an integral part of the cultivation process.
The new online database of the Botanic Garden of Pisa is called *U-plant* and has been adopted in 2020 in substitution of a former plant record system implemented in Microsoft Access, whose records migrated in the new system. *U-plant* is coded in C-Sharp and .Net framework and was planned to satisfy international standards for a botanic garden documentation system and to offer future chances of implementation.
The staff of the Botanic Garden can access to the database through three different types of credentials: 'Administrator', 'Operator', or 'Reader'. The former populates (and possibly modifies) the database primary data and acts as a supervisor, validating the accession records entered by the staff having 'Operator' credentials. Eventually, users with 'Reader' credentials can only visualise the database.
The homepage is composed by a left panel with tools to populate the database primary data (Administration tables), entry form for new accessions, search modules for basic and advanced queries, and download tools. Other than a shortcut menu, in the bottom part of the home page lies a table providing the summary statistics of the collections cultivated in the Botanic Garden. Despite it should be an obvious task for a botanic garden, only thanks to the new database we have been able to have a continuously updated overview on how many species we cultivate and where they are located in the garden. To date, we have 2,567 labelled plants in our garden, corresponding to 1,692 species or subspecies, 791 genera and 169 families. In our nursery, we are currently cultivating 221 taxa, while 69 taxa are available for our *Index Seminum*.
U-plant holds a wide-range of information about accessions that are - or were - cultivated in the garden or stored as propagules in the Germplasm bank. An accession number is composed by 8 number digits, where the first four correspond to the accession's year and those following represent a progressively increasing number (e.g., 2021-2847). If the IPEN (International Plant Exchange Network) code of the plant material was not provided during the accession creation, the database automatically assigns the appropriate IPEN according to data entered. Among the main contents of the accession record there are: scientific name and identification data, acquisition modality, origin of the material (e.g., cultivated, wild), gathering data (if applicable), and the list of the individuals linked to the accession. Individuals are defined as plants which have a definite physical location in the Botanic Garden. They can range from plants displayed to the public, seedlings in cultivation in the nursery, or stored germplasm. The record of a given individual includes several information such as its exact location in the garden, the register of horticultural treatments directly updated by 'Operator' users and a virtual folder where to store photographic documentation.
The implementation of a freely accessible version of the database, namely *U-plant DISCOVER*, is currently ongoing. The platform is primarily planned to offer a visiting tool in double language (Italian/English) through mobile devices. It allows a simple query by scientific name or advanced queries according to multiple criteria. The search also allows to check the exact location of the individual in a georeferenced map of the Botanic Garden and to visualize a selected pool of information extracted from *U-plant*. In addition, the visitor can access a gallery of photographic documentation linked to each individual.

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11 = Microbiological characterization of the root nodules of *Genista tinctoria* of the Mefite of the Ansanto Valley (southern Italy)

Emanuela Di Iorio[1], Olga De Castro[1], Eleonora Manzo[1], Monika Marek-Kozaczuk[2], Wojciech Sokołowski[2], Sylwia Wdowiak-Wróbel[2], Michał Kalita[2]
[1]Department of Biology, Botanical Garden, University of Naples Federico II, Via Foria 223, 80139 Naples, Italy; [2]Department of Genetics and Microbiology, M. Curie- Sklodowska University, Akademicka 19, 20-033 Lublin, Poland

In southern Italy near Rocca San Felice municipality (Avellino's province), there is one of the most important extreme environments of the world, whose name is the Mefite in the Ansanto Valley (Fig. 1). This locality is a desolate and spectral small landscape of lunar-like appearance of clayey-calcareous nature of tectonic origin derived from the uplift phases of the Apennine chain in the Pleistocene, and characterized by an imposing non volcanic gas emission area where gases are constantly released at ambient temperatures. The gas in its maximum emission forms a pool of bubbling mud from which a river of gas is generated that flows down the valley that can kill humans and animals. In fact, the gas emission is composed of carbon dioxide (ca 98%) and other gases in minimal concentrations (< 2% nitrogen, methane, and hydrogen sulfide). In this area, an aberrant population of *Genista tinctoria* L. (Fabaceae) was described in 1821 by Michele Tenore as *Genista anxantica* Ten. for its distinctive morphological characteristics, as well as for its presence in this extreme environment of the Mefite. Currently, *G. anxantica* is a synonym of *G. tinctoria* and to avoid confusion in the poster, the *G. tinctoria* specimens present in the Mefite will be referred to as "Anxantica-Type", while individuals from the populations of the other localities, corresponding to the typical *G. tinctoria*, will be referred to as "Tinctoria-Type".
The purpose of the work is to highlight possible differences caused by this extreme environment in the symbiotic interactions present in the roots of Anxantica-Type. In fact, it is well documented that the plants belonging to the Fabaceae present symbiotic relationships roots-bacteria (rhizobia). Rhizobia are soil bacteria capable of forming nitrogen-fixing nodules on roots and, in some cases, on stems of leguminous plants.
For this purpose, nodule from Anxantica-Type and neighboring Tinctoria-Type (Fig. 2) were sampled for a phylogenetic analysis. For each sampling site, the soil was also collected for chemical analyses (e.g., pH, TOC, TN and CSC).
Five to twenty nodules from 33 individuals were sampled, for a total of 234 nodules. Bacterial strains were isolated from nodules and the genomic DNA was extracted and amplified the 16S gene as preliminary step of DNA barcoding characterization. The sequences obtained were used as queries in the BLASTn program to search the GenBank database for identity sequences.
According to the preliminary data of DNA barcoding characterization, the genus *Micromonospora* (Actinobacteria) and *Bradyrhizobium* (Proteobacteria) were identified in the 10% and 5 % of nodules analysed, respectively.
As future perspectives, our goals are: (1) to conclude the preliminary phylogenetic characterization with 16S gene and the chemical analyses; and (2) to implement additional molecular markers as *nod*C and *nif*H genes (N-acetylglucosaminyltransferase and nitrogenase, respectively). In fact, these genes are the biomarkers most widely used to study the ecology and evolution of nitrogen-fixing bacteria.

Fig. 1. The Mefite of the Ansanto Valley.

Fig. 2. Geographical distribution of the sampling sites of Tinctoria Type (green) and Anxantica-Type populations (red).

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11 = Anatomical and biochemical characterization of *Spartium junceum* vessels infected by *Xylella fastidiosa* subsp. *multiplex*

Sara Falsini[1], Corrado Tani[1], Gina Sambuco[1], Alessio Papini[1], Paola Faraoni[2], Sara Campigli[3], Luisa Ghelardini[3], Gianluca Bleve[4], Domenico Rizzo[5], Massimo Ricciolini[5], Ilaria Scarpelli[5], Lorenzo Drosera[5], Alessio Gnerucci[6,2], Francesco Ranaldi[2], Francesca Peduto Hand[7], Guido Marchi[3], Silvia Schiff[1]
[1]Dipartimento di Biologia, Università degli studi di Firenze, via P.A. Micheli 3, 50121, Firenze, Italy; [2]Dipartimento di Scienze Biomediche, Sperimentali e Cliniche, Università degli Studi di Firenze, viale G. Pieraccini 6, 50139, Firenze, Italy; [3]Dipartimento di Scienze delle Produzioni Agroalimentari e dell'Ambiente, Università degli Studi di Firenze, Piazzale delle Cascine 28, 50100 Firenze, Italy; [4]Istituto di Scienze delle Produzioni Alimentari, Consiglio Nazionale delle Ricerche, Lecce, Italy; [5]Regione Toscana, Servizio Fitosanitario Regionale e di Vigilanza e Controllo Agroforestale, Via A. Manzoni 16, 50121 Firenze, Italy. [6]Dipartimento di Fisica e Astronomia, Università di Firenze, Via Sansone 1, 50019, Sesto Fiorentino (FI); [7]Department of Plant Pathology, The Ohio State University, Columbus, OH 43220, USA

Xylella fastidiosa (Xf) is a xylem-limited bacterium that causes destructive diseases on many host species including grapevine (*Pierce's disease*), olive (*Olive Quick Decline Syndrome*), and citrus (*Citrus variegated chlorosis*). Its recent and progressive spread in Europe, in which it was most likely introduced by plants global trade, is caused by hemipteran insect vectors that feed on xylem sap, and is facilitated by the presence of a wide range of suitable hosts, some of which are indigenous species of the Mediterranean landscape like *Spartium junceum* (Spanish Broom).
In this work, we describe the anatomy of naturally infected *S. junceum* plants that were collected from the recent outbreak area of Monte Argentario (Tuscany, Italy). According to our reconstruction, the infection process by *X. fastidiosa* subsp. multiplex phylotype ST87, as confirmed by means of molecular characterization (Multi locus sequence typing) and immunohistochemistry, initiates with the colonization of a single xylem vessel by proceeding through its perforation plates and continues by digesting the pit membranes to invade adjacent xylem cells (Fig 1). Moreover, we observed that in the colonized vessels the bacterium is often associated to a pink/red stained gel matrix, that seems to be secreted by the adjacent parenchyma cells (Fig. 2). We speculate that the matrix is involved in the attempts of the host to curb bacterial spread inside the vessel lumen, which stained blue when bacteria were mostly dead. In the secondary xylem, *X. fastidiosa* subsp. *multiplex* ST87 was only found in the last growth ring boundary, meanwhile the older vessels were filled by the gel-matrix and free from bacteria.

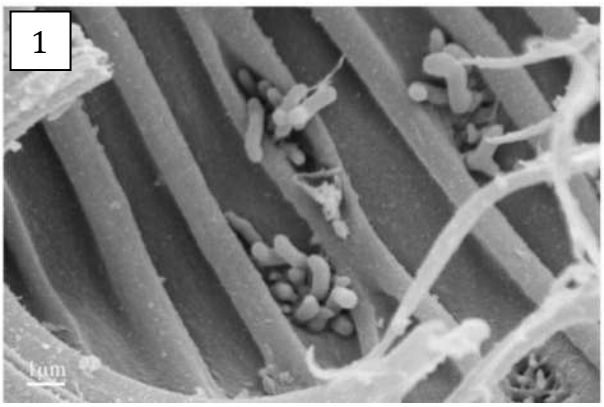

Fig. 1. SEM image of longitudinal sections of young shoots of *S. junceum*. Bacteria crossing the pits of the adjacent vessel.

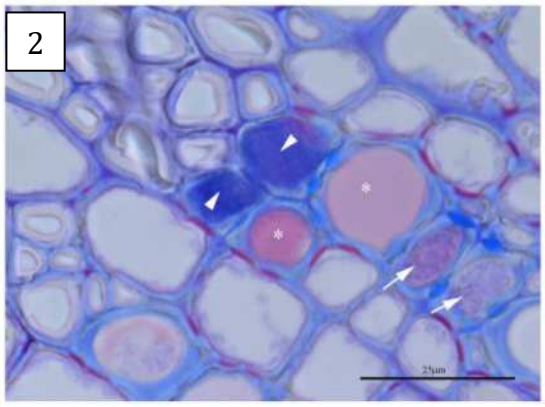

Fig. 2. LM image of *S. junceum*. Cross-section of a green shoot with polysaccharide (pink/violet indicated by asterisk) and phenolic (blue indicated by triangle) matrices stained with Toluidine blue.

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11 = Preliminary investigations of ecophysiological traits in *Abies nebrodensis* (Lojac.) Mattei (Madonie, Sicilia)

Anna Geraci[1], Rosario Schicchi[2], Luisa Friulla[1], Elisabetta Oddo[1]
[1]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, via Archirafi 38, I-90123 Palermo, Italy; [2]Department of Agricultural, Food and Forest Sciences, Viale delle Scienze Ed. 4, I-90128 Palermo, Italy

Abies nebrodensis (Lojac.) Mattei (Pinaceae) is an endemic relict species occurring in a small area of the territory of Polizzi Generosa, within the Madonie Natural Park (Sicily). It has been categorized as a "Critically Endangered" ("CR") species according to IUCN Red List Criterion D. The natural population consists of only 30 individuals, 25 of which produce fertile cones. In the last 20 years several actions within both National and European funded projects have been carried out, in order to avoid extinction and to increase the natural population (reintroductions, gradual elimination of non-indigenous fir species in the surrounding area, propagation by grafting, production of genetically pure seeds by means of controlled pollinations, etc.).
Within the activities of the LIFE4FIR project (LIFE 18 NAT/IT/000164) started in 2019, we performed some ecophysiological measurements to monitor the response of potted plants to dry conditions.
Key physiological traits such as leaf stomatal conductance to water vapour (g_s), leaf water potential (Ψ_L), turgor loss point (Ψ_{TLP}) threshold and hydraulic traits of branchlet xylem were assessed. Measurements were carried out on two sets of *A. nebrodensis*, one of 4-year-old and one of 10 to 12-year-old individuals, growing in 1.5 L pots. Plants came from the Piano Noce Nursery (Polizzi Generosa) and were grown for one year at the Botanical Garden of Palermo.
Pressure-Volume (PV) curves were constructed with the bench dehydration method using a PMS 1505D pressure chamber, and stomatal conductance was measured with a Decagon SC-1 leaf porometer. Hydraulic traits were based on anatomical observations of branchlet cross-sections stained with phloroglucinol and analysed with ImageJ software. Air temperature and RH, PAR and soil water potential were measured and stored with a Decagon EM50 datalogger. No significant differences in leaf water potential at turgor loss point or in leaf osmotic potential at full turgor were found between age groups or comparing summer and winter PV curves. After a 6-day drought stress experiment, the 10/12-year-old individuals reached Ψ_{TLP} (-2.1 MPa) at midday, while the 4-year-old individuals showed higher midday Ψ_L values (-1.7 MPa). The younger plants showed g_s levels twice those of older plants during water deficit imposition, and a slightly greater recovery of g_s upon re-watering.
These analyses provide useful information for planning targeted irrigation and transplantation interventions for young individuals grown in nursery. Our data suggest planning irrigation at 5 d intervals in periods with air temperatures up to 25 °C. Non-destructive monitoring of stomatal conductance allows the detection of stress onset when a 40 – 60% reduction in gs is observed. Older individuals showed greater signs of stress, possibly due to root constriction. Younger and well consolidated saplings seem therefore more suitable for reintroduction interventions in areas of the Madonie Park.

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11 = Life Drylands: a project for the conservation of lowland continental dry habitats

Gabriele Gheza[1], Juri Nascimbene[1], Matteo Barcella[2], Patrizia Berera[3], Francesco Bracco[2], Maura Brusoni[2], Davide Cavalletti[1], Alessandro Chiarucci[1], Michael Maerker[2], Emanuela Martino[2], Paola Nola[2], Luisa Pellegrini[2], Giovanna Pezzi[1], Chiara Vallese[1], Silvia Assini[1]
[1]BIOME Lab, Department of Biological, Geological and Environmental Sciences, Alma Mater Studiorum – University of Bologna, Via Irnerio 42, 40126 Bologna, Italy; [2]Department of Earth and Environmental Sciences, University of Pavia, Via S. Epifanio 14, 27100 Pavia, Italy; [3]Network of Botanical Gardens of Lombardia, Piazza Matteotti 27, 24100 Bergamo, Italy

Open dry habitats (acidic and calcareous dry grasslands, dry *Calluna vulgaris* heathlands), which are protected by the European Union according to the 1992/43/EEC Directive ("Habitats Directive"), are increasingly rare and threatened in all of Europe. In the Po Plain they occur in a particularly critical situation due to the high human impact, that, together with the lack of proper management, caused their degradation, fragmentation and, in many cases, disappearance. An active management is fundamental for their conservation, which is needed because such habitats (1) host a rich biodiversity, (2) provide several relevant ecosystem services that benefit also humans, (3) have a great phytogeographic value, being located at the southernmost edge of their range and therefore hosting a peculiar floristic composition in which central-European, atlantic and mediterranean elements occur together, (4) host biological soil crusts rich in terricolous lichens, which at present can be found exclusively within such habitats in the lowlands.
With the aim of preserving the Natura 2000 Habitats 2330 ("Inland dunes with open *Corynephorus* and *Agrostis* grasslands"), 4030 ("European dry heaths") and 6210 ("Semi-natural dry grasslands and scrubland facies on calcareous substrates (*Festuco-Brometalia*)" – acidophilous subtype) in 8 Natura 2000 Sites of the western Po Plain (Lombardia and Piemonte), the LIFE NAT/IT/000803 "LIFE DRYLANDS" project started in Autumn 2019 and will conclude in 2024.
The general objectives of the project are the restoration of the target habitats to a favourable conservation status and the creation of core areas and ecological corridors to reduce their fragmentation and increase their connectivity. These objectives will be achieved by means of: (1) restoration of the vertical and horizontal structures of the target habitats by means of mowing, raking, cutting of native trees, sod cutting and top soil inversion, according to a dynamic approach that preserves pioneer (bare soil and soil biological crusts), typical (perennial herbs/forbs and/or dwarf shrubs) and mature aspects (dense shrubby patches at the contacts with the forest communities), since this approach is consistent with the dynamic nature of the Po Plain, takes into account the processes driving the formation of the target habitats, and results in higher biodiversity if compared to a static approach that preserves only single aspects of the target habitats; (2) control/reduction of the invasive woody species most responsible for biodiversity loss in the target habitats (*Ailanthus altissima*, *Prunus serotina*, *Quercus rubra*, *Robinia pseudoacacia*) by means of repeated cutting; (3) improvement of the floristic composition, by means of introduction/reinforcement of selected typical plant species; (4) *ex-novo* creation of new patches of the target habitats. Further actions will concern: (5) production, transfer and replication of guidelines for the management and the monitoring of the target habitats on the basis of the project results, with the aim of providing widely applicable management models for an evidence-based conservation; (6) raising awareness in the general public and the stakeholders about the importance of Natura 2000 Habitats by promoting the project and its results by means of the official website (https://www.lifedrylands.eu/), the social networks (https://www.facebook.com/lifedrylands and https://www.instagram.com/lifedrylands) and events in attendante, including educational activities with the schools.
The final hope is that this project can involve, in addition to improving the quality of the target habitats, which is its main purpose, also an increase in awareness among the general public in relation to (1) the huge value of habitats that are generally wrongly considered to be of scarce or no value because considered unproductive or little appreciated from an aesthetic point of view, (2) the importance of organisms that are poorly or not considered because "non-charismatic", such as terricolous lichens, (3) the urgent need and greater effectiveness of protecting entire habitats, to preserve biodiversity, instead of protecting single or few "charismatic" species.

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11 = Impact of yeast metabolic activity on the neuro-active properties of nectar

Emanuele Giordano[1], Massimo Guarnieri[1], Andrey Yurkov[2], Moritz Mittelbach[3], Pietro Buzzini[4], Benedetta Turchetti[4], Massimo Nepi[1]

[1]Department of Life Science, University of Siena, Via P.A. Mattioli 4, 53100 Siena, Italy; [2]Leibniz Institute DSMZ - German Collection of Microorganisms and Cell Cultures, Inhoffenstraße 7B, 38124 Braunschweig (Germany), [3]Senate Department for the Environment, Transport and Climate Protection, Am Köllnischen Park 3, 10179 Berlin (Germany), [4]Department of Agricultural, Food and Environmental Sciences, University of Perugia, Borgo XX Giugno 74, 06121 Perugia (Italy)

Floral nectar is commonly colonized by nectarivorous microorganisms, most often yeasts and bacteria, that exploit it as sugar resource. The attention of the researchers has recently focused on the impact that such microorganisms may have on nectar chemistry, lowering the total sugar and total amino acid content as well as altering the sugar and amino acid profiles. Since nectar chemistry is related to plant-pollinator interaction, it appears that nectar-exploiting microorganisms may be responsible of indirect effects on pollinating insects. Although a large number of studies show what microorganisms take from nectar, very little is known about what microorganisms add to nectar. A suggestive hypothesis is that the metabolic activity of microorganisms such as yeasts may be responsible for the production of neuroactive substances affecting directly the behaviour of pollinators. We tested this hypothesis, inoculating an artificial nectar with three species of specialized nectar yeast, namely *Metschnikowia reukaufii*, *Metschnikowia chrysoperlae* and *Metschnikowia gruessii*. The presence of biogenic amines have been determined by HPLC-DAD in the culture medium before inoculation and after one, two, three and seven days. Our results show that the metabolic activity of tested yeasts changes the aminic profile of the artificial nectar, generating tryptamine and serotonin *ex novo* and significantly modifying the amount of tyramine. It is evident that in all cases, the aminic profile changes according to time and it is different compared to controls (time 0) (Fig.1). Since biogenic amines are known to be neuroactive molecules for insects, the potential impact of yeast activity on plant-insect interaction is clear. Specifically, for all the amines detected, the involvement in neural circuits that influence aggressiveness, memory and olfactory abilities of various insects has been demonstrated. The results highlight for the first time that common nectar-dwelling yeasts can be responsible for new neuro-active properties of nectar and open innovative perspectives about multi-order interactions based on the trophic exploitation of nectar.

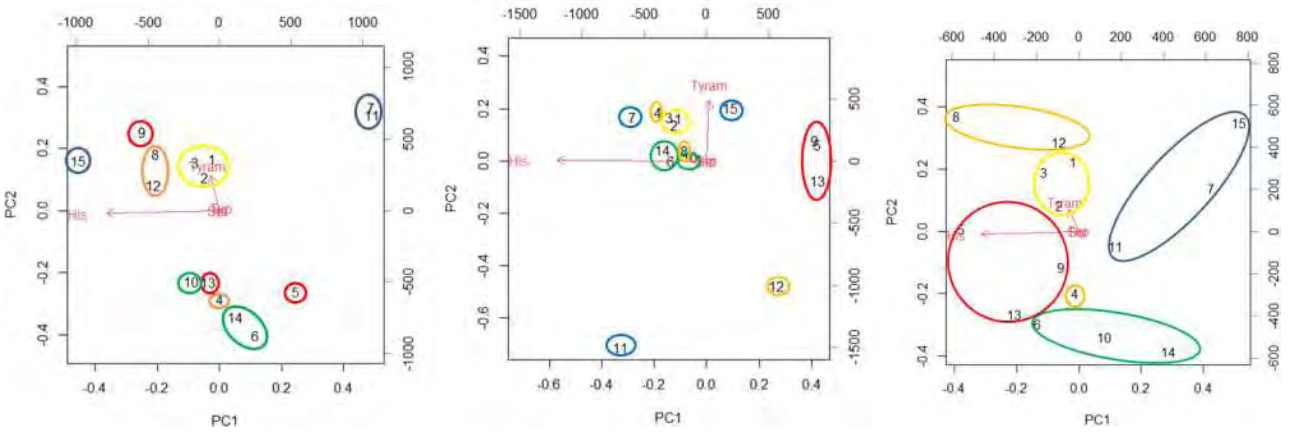

Fig. 1 Principal component analysis (PCA) for the aminic profile of the artificial nectar inoculated with *Metschnikowia reukaufii* (left), with *Metschnikowia gruessii* (middle) and with *Metschnikowia chrysoperlae* (right). The arrows in red show the vectors of the original variables tyramine (Tyr), histamine (His), serotonin (Ser) and tryptamine (Trip). In all three cases the percentage of variance explained by the first two principal components exceeds 90%.

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11 = Hilly and sub-mountain areas are rich in plant agrobiodiversity: the case of Italy

Luca Giupponi[1,2], Davide Pedrali[1], Valeria Leoni[1], Alessia Rodari[1], Annamaria Giorgi[1,2]
[1]Centre of Applied Studies for the Sustainable Management and Protection of Mountain Areas–CRC Ge.S.Di.Mont., University of Milan, Via Morino 8, 25048 Edolo (BS), Italy; [2]Department of Agricultural and Environmental Sciences-Production, Landscape and Agroenergy-DiSAA, University of Milan, Via Celoria 2, 20133 Milan (MI), Italy

Landraces are agri-food and historical-cultural heritage but they are undergoing losses worldwide. During the last century, the progressive substitution of these traditional cultivars with modern, high yielding varieties, determined a reduction of about 75% of global agrobiodiversity. Italy is taking action to counteract this problem by following European guidelines. One of the most important measures is the Agrobiodiversity National Register (ANR; Law n. 194/2015), but many Italian regions currently appear without any landraces and around 80% of landraces listed are trees, with less detailed data on herbaceous species. The aim of this study was to investigate the state of Italian herbaceous landraces preserved on farms (*in situ*) by merging and analysing data contained in the main databases on plant agrobiodiversity in Italy. Data were georeferenced, organized by botanic families and Italian regions, and analysed by GIS and R software.
A total of 1615 herbaceous landraces were found (versus the 416 recorded in the ANR) subdivided into cereals (321), legumes (535), potatoes (67), tomatoes (135), onions (72), other (485). Poaceae, Fabaceae and Solanaceae together comprise 70% of all herbaceous landraces and mostly preserved/grown in areas between 150 and 800 m a.s.l. Their distribution throughout Italy was analysed, highlighting how some hilly and sub-mountain areas of the Apennines and Alps are hotspots of herbaceous landraces (Fig.1) due to anthropic and environmental factors. It is hoped that the database will be useful to enrich the ANR; in fact, the total number of herbaceous landrace varieties could increase by about 290% due to this study.
The results of this research contributed to the knowledge on herbaceous landraces cultivated in Italy, that require scientific attention, and they could trigger actions of characterization, conservation and promotion of these plant resources. The data of this inventory are available open source at the following link: https://www.unimontagna.it/servizi/mappatura-agrobiodiversita-vegetale/ .
The database will be supplemented with maintainer details, cultivation, usage, and specific threats and that information and periodically updated to monitor changes in on-farm maintained landrace and agrobiodiversity.

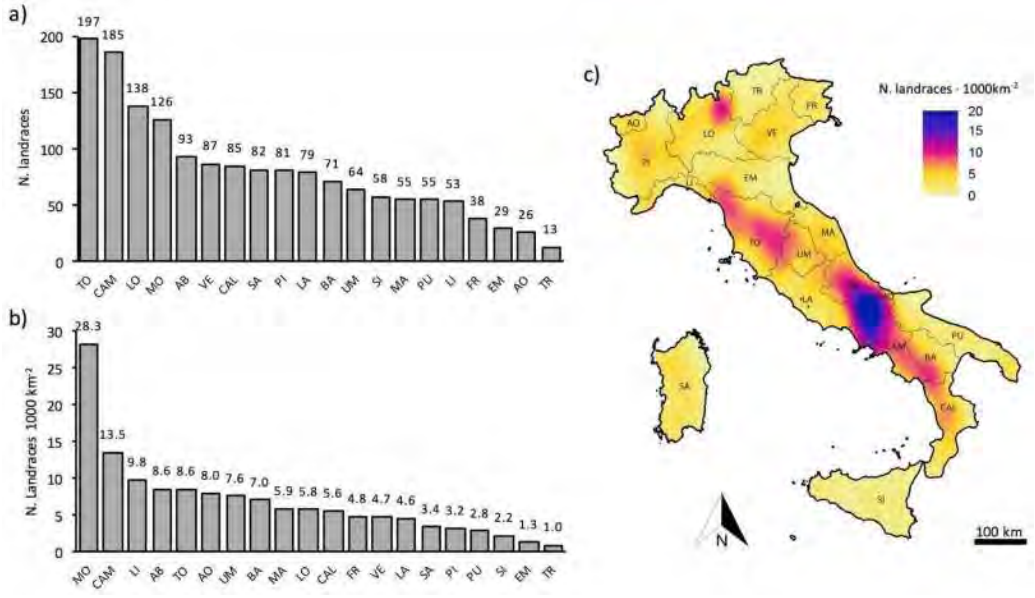

Fig.1. Number of herbaceous landraces (a) and mean number of landraces per 1000 km^2 (b) of each Italian region, and maps of landrace hotspots (c). Key: AO, Aosta Valley; PI, Piedmont; LO, Lombardy; TR, Trentino-Alto Adige; VE, Veneto; FR, Friuli-Venezia Giulia; LI, Liguria; EM, Emilia-Romagna; TO, Tuscany; UM, Umbria; MA, Marche; AB, Abruzzo; LA, Lazio; MO, Molise; CAM, Campania; PU, Apulia; BA, Basilicata; CAL, Calabria; SI, Sicily SA, Sardinia.

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11 = Pollen from 13 cultivars of *Prunus armeniaca* L. differently reacts to temperatures in a climate change scenario

Maurizio Iovane, Luigi Gennaro Izzo, Aurora Cirillo, Leone Ermes Romano, Antonio Bruno, Claudio Di Vaio, Giovanna Aronne
Department of Agricultural Sciences, University of Naples Federico II, via Università 100, 80055 Portici

Pollen functionality is essential for most crop species in which fertilization is needed to produce seeds and /or fruits. Environmental factors, especially temperature and humidity are well known to strongly affect pollen longevity. In the framework of the global temperature increase and the increasing frequency of unexpected rises and drops in temperature (heat/cold waves), studies on the interaction between pollen and environmental factors rise economic interest. In *Prunus armeniaca* L. (apricot), as well as in most of crop species of the Rosaceae family, pollen needs to stay viable and germinable along its journey from the anther to the stigma (pollination), to finally succeed in the double fertilization and trigger fruit development. *P. armeniaca* is widely cultivated in the Southern regions of Italy, including the area of Vesuvius volcano in Campania. In this area apricot has been cultivated since ancient times, therefore leading to the selection of numerous cultivars particularly adapted to the Vesuvian environmental and edaphic factors.
The aim of our study was to assess pollen functionality of apricot cultivars traditional from the Campania region in a climate change scenario. More specifically, the screening was performed to identify cultivars whose pollen functionality might best tolerate the effect of abrupt increseases or sudden drops of temperature during flowering season of *Prunus armeniaca*.
Pollen from both early and late flowering cultivars was sampled from flowers at "balloon" stage (Fig. 1, BBCH 57) in which anthers were still undehisced. The effect of temperature on pollen functionality was assessed through viability and germinability tests. According to the minimum and maximum mean temperatures recorded in the field during flowering, pollen was incubated at 5°C and 15°C, respectively. In addition, a third temperature treatment (25°C) was chosen to test pollen response in a heat wave scenario.
Diaminobenzidine (DAB) reaction was used to compare initial pollen viability among genotypes (Fig. 2). Successively, pollen germination was assessed after 48-hours incubation at 5°C, 15°C, and 25°C (70% RH) (Fig. 3.). We further investigated on capability of pollen in preserving its functionality over time by storing pollen for 14 days 5°C, 15°C and 25°C before germination.
Results showed high initial viability of pollen from flowers at "balloon" stage with no significative differences among cultivars, highlighting that pollen from both early and late flowering cultivars did not experienced any environmental constraints during microsporogenesis. Pollen germinability at 5°C, 15°C, and 25°C resulted significantly different both among cultivars and temperatures. Overall, most cultivars showed highest pollen germination at 15°C, whereas 5°C and 25°C treatments resulted in a drastic loss of germinability compared to pollen initial viability. Despite the great variability in germination responses, early flowering cultivars clearly distinguished from others for best pollen tolerance to higher temperatures. Results from pollen germination tests after 14-days storage revealed that 5°C treatment was the most effective for the conservation of pollen functionality throughout flowering. Particularly, pollen from early flowering cultivars showed revealed the highest germination/viability ratio after 14 days. These findings highlighted the possibility of crossing early flowering genotypes with late flowering genotypes.
Results of this study showed that the occurrence of high temperature during flowering can severely affect pollen viability and germinability in *P. armeniaca* and that pollen response is different in different cultivars. Moreover, in the scenario of the ongoing climate changes, our results highlighted that in self-incompatible species as *P. armeniaca*, the assessment of pollen functionality is critical and should be adopted as a criterium to identify cultivars to be used as pollen donor plants in the orchards.

Fig. 1. Flowers at "balloon" stage.

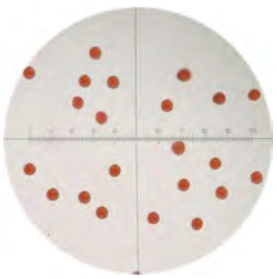

Fig. 2. Pollen positive to DAB test.

Fig. 3. In vitro germinated pollen.

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11 = Ways to guide root growth orientation on Earth and in space

Luigi Gennaro Izzo, Giovanna Aronne
Department of Agricultural Sciences, University of Naples Federico II, Via Università 100, 80055, Portici, Italy

Tropisms are directional growth responses to directional stimuli allowing plants to maximise resource harvesting and stress avoidance. On Earth, plant roots sense and respond to many environmental stimuli, consequently adjusting their growth orientation. In this context, gravity is a primary stimulus ever since plants colonised terrestrial environments. Besides gravity, several other stimuli mediate directional responses of plant roots, including light, water, touch, nutrients, electromagnetic fields, and even sounds. In most cases, root tropisms have been well characterized mainly using model species as *Arabidopsis thaliana*, but some mechanisms, pathways, and sensors, remain unknown especially for minor tropisms and other species. Furthermore, most studies focused on single tropisms, although it is arguable that the integration of multiple stimuli determines the final growth of plants in natural circumstances with possible differences among species.

On Earth, gravitropism is considered dominant on other tropisms and the alteration of gravity stimulus can severely affect root growth orientation. In this regard, studies on multiple interactions between tropisms could advance the frontiers of tropism research with possible application of cultivation strategies to maximise resource-use-efficiency. Studies aimed at increasing the knowledge on root directional growth are claimed for the design of plant-based life support systems in space where tropism interaction is altered by microgravity, partial-gravity, or hypergravity conditions. In addition, an in-depth understanding of the interaction among tropisms is valuable for applications in natural and controlled environments on Earth to advance plant production systems.

In our experiments, we evaluated the interaction of multiple stimuli in a wide range of gravity conditions to assess the relative importance of tropisms and their effectiveness in guiding root growth orientation. Specific experimental tools and environments were used to alter the gravity stimulus, i.e., clinostats, random positioning machines, large diameter centrifuge, and laboratories of the International Space Station. Experiments are part of a broader research on plant tropisms, which involves also studies founded by the Italian Space Agency (e.g., MULTITROP: interaction of gravity, nutrient, and water stimuli for root orientation in microgravity) (Figure 1) and the European Space Agency (e.g., ROOTROPS: tackling the roots of bending) (Figure 2). Our experiments conducted in real or simulated microgravity indicate that tropistic stimuli other than gravity can play a significant role in orienting root growth, as in the case of root chemotropism. Conversely, on Earth, the dominant effect of gravitropism masks other tropisms, whereas increasing gravity can affect directional growth to a certain extent. In this framework, phototropism resulted effective in guiding root growth under a wide range of gravity levels, from simulated microgravity up to 20*g*, although with differences due to light spectra.

We are currently targeting these studies to exploit the potential applicability of environmental stimuli in controlled environments not only for plant cultivation in space but also in the framework of sustainable agriculture on Earth.

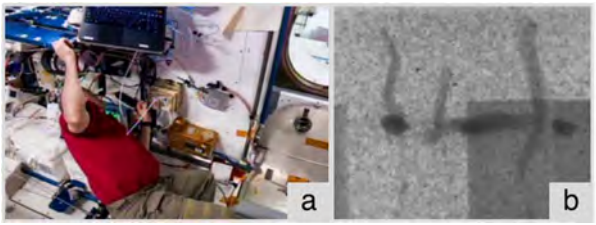

Fig. 1. MULTITROP experiment performed in microgravity; a) experiment activation onboard the International Space Station; b) X-ray microtomography of seedlings grown in microgravity under chemotropic and hydrotropic stimuli.

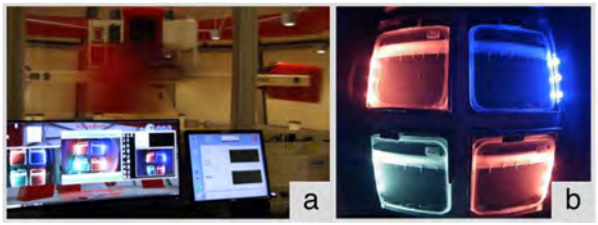

Fig. 2. ROOTROPS experiment performed at the European Space Research and Technology Centre; a) experiment running within gondolas of the Large Diameter Centrifuge; b) seedlings growing at 20*g* under different light treatments.

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11 = Identification of genes involved in fruit development and senescence

Simona Masiero, Sara Forlani, Annamaria Massafra, Chiara Mizzotti,
Dipartimento di Bioscienze, Università degli Studi di Milano, via Celoria 26, 20133 Milano

Fruits represent the key of the successful evolution and spread of angiosperms. They play a fundamental role in the worldwide economy, directly affecting the global market, and provide essential nutritional elements for the human consumption. Fruit yield and quality are therefore key features to be controlled in the agricultural production, and improvements of fruit characteristics depend on the comprehension of the mechanisms controlling their development and maturation. In order to identify genes involved in such processes, an RNA sequencing on the fruit of the model plant *Arabidopsis thaliana*, called silique, was performed. In order to cover the entire fruit formation and maturation, different developmental stages were considered, from the initial development to the final senescence stage. Bioinformatics and statistical analyses of the data led to the identification of about thousand genes differentially expressed between early and late stages of development. In order to find genes involved in the regulation of such a process, we focused our attention on a family of transcription factors known to be involved in fruit growth and maturation: the NAC (NAM, ATAF, CUC) family of transcription factors. We selected some NAC genes from the transcription factors resulted to be differentially expressed, and we characterized the corresponding mutant lines in Arabidopsis. Our data indicate that an intricate network of NAC proteins participates to proper silique maturation.

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11 = Monitoring activities of plant species protected by Habitat Directive (92/43/CEE) in Tuscany (Central Italy)

Francesca Olivieri[1], Lorenzo Pinzani[1], Lorenzo Peruzzi[1], Giovanni Astuti[1], Gianni Bedini[1]
[1]Department of Biology University of Pisa, Via Derna 1, 56126 Pisa, Italy

The Habitat Directive (92/43/CEE) aims at achieving the satisfactory conservation of species and habitats of community interest, thanks to the establishment of Special Areas of Conservation (SACs). Within these areas, it is crucial to set up a periodical monitoring assessment that evaluates species and habitats trends over time, throughout the European territory. In Tuscany, this task has been assigned to the Universities of Pisa, Firenze and Siena through the agreement of two research projects, namely Monito-Rare and NaTNet, in 2018 and in 2019, respectively. Both provide for field activities to monitor species and habitats and acquire ecological knowledge to improve conservation measures and objectives within the Tuscan SACs. As part of these projects, the University of Pisa is in charge of monitoring activities concerning plant species present in the Annexes II, IV and V of Directive. From January to October 2018, field surveys verified species occurrence in locations selected from bibliography, reporting population threats, numerical consistency and extension of populations. In May-August 2020 and for the entire period scheduled for May-September 2021, field data were collected through a smartphone app (Epicollect5) directly in the field. For each population, data relating to position, altitude, date, environment, occupied area, conservation status, recovery possibilities, pressures, threats, number and status of individuals and quality of the population coupled with photographic documentation were collected and uploaded to a database. Here, we present a summary of the results related to protected vascular plants occurring in Tuscany (Fig. 1). In total, 98 populations of 16 species occurring in 36 SACs were surveyed. Local extinction of *Aldrovanda vesiculosa* L., *Hibiscus pentacarpos* L., *Liparis loeselii* (L.) Rich., and *Caldesia parnassifolia* (Bassi) Parl. is confirmed. The information collected so far represents an accurate and updated picture about plant conservation related to Habitat Directive in Tuscany. This workframe is of strategic importance for determining the effectiveness of the implementation of the Community Directives on biodiversity, but also a reference to identify priorities and criticalities and to measure the achievement of the envisaged targets with the ultimate aim of extending the monitoring of plant species to the entire Tuscany.

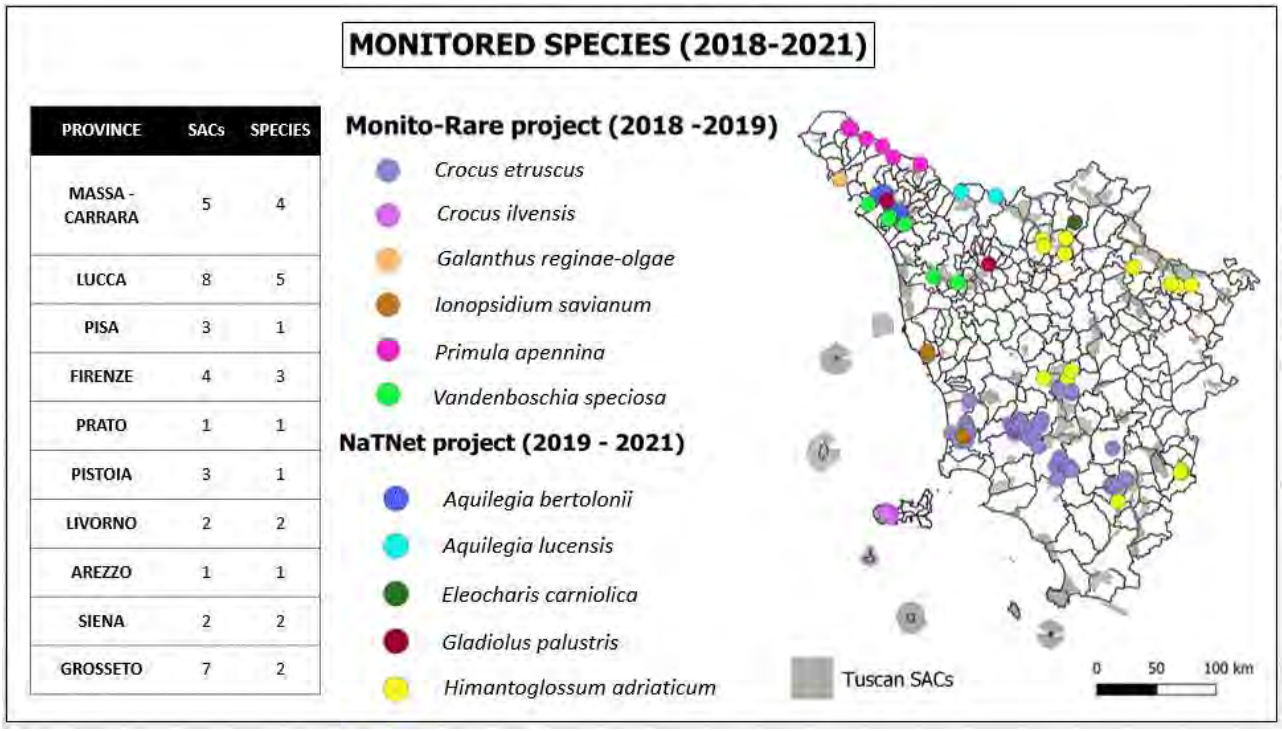

Fig. 1. Monitored species (2018 - 2021)

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11 = Biocontrol of *F. oxysporum* in *Crocus sativus*: a case study of "Zafferano dell'Aquila DOP"

Loretta Pace[1,2], Beatrice Farda[1,2], Marika Pellegrini[1], Rihab Djebaili[1], Maddalena Del Gallo[1]
[1]Department of Life, Health & Environmental Sciences, University of L'Aquila Via Vetoio, 1, Coppito, 67100 L'Aquila (Italy); [2]Botanical Garden of Campo Imperatore, Gran Sasso d'Italia, University of L'Aquila

Saffron (*Crocus sativus* L.) is an autumnal flowering geophyte well known for its dried stigmas, with flavouring, colouring and bioactive properties. Italy is one of the most important producing countries, with several protected designations of origin - DOP. Among the latter, the "Zafferano dell'Aquila DOP" is cultivated in L'Aquila territory (Abruzzi, Central Italy). Saffron cultivation is obtained by corms planting during their dormant period in summer (August) and flowers are produced after 8–10 weeks in autumn (October-November). After harvest, saffron plants are left in the soil undisturbed and allowed to grow throughout the winter. In the spring, 4–10 daughter corms are formed above the old ones, which shrivel and eventually rot away. Since the corm, as a sub-terranean organ, is susceptible to diseases caused by fungi, bacteria, nematodes, and viruses, the new corms are harvested and subjected to chemical disinfestation treatment before replanting. However, many fungal pathogens are resistant to this treatment. *Fusarium oxysporum* ff. spp. is one of the most destructive fungal diseases, which led to severe yield losses. The main symptoms of the disease occur during the flowering period in which infected plants show a dropping, yellowing, and wilting of shoots, basal and corm rot. The pathogen survives in infected corms and soil as mycelium, chlamydospores, macroconidia and microconidia, spreading increasingly all over the crops. Plant-microbe interactions can increase crop resistance toward phytopathogens and the use of bacteria inoculants is a valid strategy for the biocontrol of several plant diseases.
In this perspective, the present work aimed at: i) isolate strains of *F. oxysporum* from soil and rotting corm; ii) evaluate the inhibition ability of selected strains of bacteria against *F. oxysporum* ff. spp.; iii) obtain an *in vitro* culture of saffron corms free of pathogens to conserve the "Zafferano dell'Aquila DOP" crop. Pathogenic *Fusarium* strains have been isolated from soil and corms of "Zafferano dell'Aquila DOP" plants with *Fusarium* wilt. After isolation, the fungal strain identification was carried out based on morphological and molecular characterizations and pathogenicity assays. Selected plant growth-promoting bacteria (PGPB) with interesting biocontrol properties were tested *in vitro* against *F. oxysporum* ff. spp. by dual culture and microscopic observations. The "Zafferano dell'Aquila DOP" corms were also subjected to surface sterilization, explant cultivation, and multiplication on Murashige and Skoog medium (MS), enriched with 6-benzylaminopurine (BAP – 400 µg) and 1-naphthaleneacetic acid (NAA – 100 µg).
Three different strains of *F. oxysporum* (FIS1, FIS2, FIS3) were isolated and identified as *formae speciales* responsible for saffron wilt. Five PGPB strains (*i.e. Bacillus pumilus*, WB1, WB2, WG6, BC6 AII) presented a good *in vitro* biocontrol activity against all *F. oxysporum* isolates, showing the capability to induce hyphae disgregation and vacuolization. The *in vitro* tissue multiplication also allowed the establishment of a "Zafferano dell'Aquila DOP" free of pathogens culture.
The selection of plant growth-promoting bacteria (PGPB) as promising biocontrol agents could help to counteract the *Fusarium* threat. While the establishment of an *in vitro* free of pathogens culture could help the conservation of this important DOP. Future studies should be directed towards the *in planta* biocontrol ability of the selected PGPB strains to counteract *Fusarium* wilt and the *ex vitro* cultivation of saffron corms. In any case, these preliminary results represent a valid basis for future investigation on the valorization and protection of an important and valuable plant.

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11 = Urban green: restoration of unusual historical trees, the case study of coral trees (*Erithryna caffra*) in the city of Trapani (Sicily)

Franco Palla[1], Francesco M. Raimondo[2], Filippo Salerno[2], Giuseppe Venturella[3], Gianvito Zizzo[2]
[1]Department STEBICEF/Section of Botany, Anthropology and Zoology, University of Palermo, Via Archirafi 38, 90123– Palermo, Italy; [2]*PLANTA*/Centre for Research, Documentation and Training, Via Serraglio Vecchio 28, I-90123, Palermo, Italy; [3]Department of Agricultural, Food and Forest Sciences, University of Palermo, Viale delle Scienze, bldg. 4, 90128 – Palermo, Italy

Street trees play several important roles in requalifying and revitalizing city areas. In the second half of the nineteenth century, in the historic center of the city of Trapani several individuals of the South African Erythrina caffra (Fabaceae) were used as road trees. This action was performed not only to support the fashion of exoticism, pervading throughout Europe, but also to introduce the coral tree as "symbol of coral craftsmanship". Years later erythrina trees began to give concern to the city administrators, due to the easy detachment of branches or trunk collapse, not only in Trapani but also in other Sicilian cities such as in Palermo. Considerable resources were spent on the management of these unusual trees performing partial or total felling, as happened in Trapani. Due to the old age and deterioration condition of those historic and symbolic erythrina trees, 16 technical felling were recommended out of the more than 75 monitored. A citizen committee, strongly opposed to this action, promoted a conservative solutions through an experimental safety plan, later adopted by the city administration. The project includes three phases: first, pruning to lighten and reconstitute the crown; second, rehabilitation on old cuts, with the elimination of widespread wood tissue deterioration, including dendro-surgery ("slupatura", mainly on trunk and large branches cavities) and treatment of healthy wood surfaces by green conservative products, as Origanum vulgare L. (Lamiaceae) essential oil and hydro-alcoholic solutions. These extracts mainly contain phenolic monoterpenes, carvacrol and thymol, and their biosynthetic precursors γ-terpinene and p-cymene. Specifically, GC-MS analysis reveals 4,04% carvacrol and 27.18% thymol in EO, instead carvacrol= 0,04%; and thymol= 0.05% in hydro alcoholic extract. If necessary, treatment by HA and EO solutions can be repeated several times, since it is a green-base procedure, i.e. safe for plant, human and environment. The final phase is based on covering the mouths of several open and healed cavities by specifically shaped corks, to avoid rainwater infiltration and pest colonization. These structures will be removed during the dry season, implementing the air flow circulation inside the cavities, making the microbial re-colonization monitoring easier over the time. Cork structures will be made by students of Master Degree in Conservation and Restoration of Cultural Heritage, University of Palermo.

a  b  c 

Erythrina trees in Trapani city center. Trunks: peculiar morphology (a); large cavities (b); sampling of deteriorated wood structure.

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11 = When isolation matters: Relationships among pedoclimatic niches in phylogenetically related species of the genus *Aquilegia* (Ranunculaceae)

Lorenzo Pinzani[1], Angelino Carta[1], Francesca Olivieri[1], Gabriele Casazza[2], Gianni Bedini[1]
[1]Dipartimento of Biologia, Università di Pisa, Via Derna 1 56126 Pisa, Italia; [2]Dipartimento di Scienze della terra, dell'ambiente e della vita (DISTAV), Università di Genova, Viale Benedetto XV 5, 16132 Genova, Italia

The delimitation of taxa boundaries is a crucial element for research in systematics and evolution, and a fundamental requirement for the implementation of any conservation measure. Traditionally, limits are set using morphological characters and molecular data, whereas the use of ecology, as a tool to support taxonomy, is still poorly considered. However, according to several authors, high ecological differentiation among species contributes significantly to their genetic isolation.
The aim of the present study is to verify the contribution of ecological niche differentiation in the circumscription of five closely related species of the genus *Aquilegia* (Ranunculaceae). This genus has approximately 70 species distributed throughout the temperate zones of the northern hemisphere, which are the result of a rapid process of recent diversification. Despite the attention received in evolutionary biology, knowledge of phylogenetic relationships within this genus is still incomplete. In this study we examined the relationships among the pedo-climatic niches of five species from central-northern Italy typical of mountain and subalpine environments: *A. alpina* L. (Central-Western Alps), *A. reuteri* Boiss. (Western Alps), *A. ophiolithica* Barberis & E.Nardi (Ligurian Apennines), *A. lucensis* E.Nardi (Tuscan-Emilian Apennines) and *A. bertolonii* Schott (Apuan Alps).
We characterized the edaphic and climatic niche of each species using occurrence points, bioclimatic variables and soil features. Then we calculated the niche overlap in the environmental space. Occurrence points (ca. 5200) were obtained from herbaria, bibliographic sources, and highly reliable databases; bioclimatic layers were downloaded from the Chelsa dataset and edaphic layer were downloaded from the SoilGrids dataset. The method of niche analysis and comparison consisted of three steps: [1] calculation of the density of occurrences and environmental factors along the axes of a multivariate analysis; [2] measurement of niche overlap (Schoener's D metric) in multivariate space; [3] execution of statistical tests of niche equivalence and similarity by randomization.
The results show high pedoclimatic differentiation among the studied species, which tend to occupy different climatic and edaphic niches with low degree of overlap. Overall, similarity tests indicate that the observed differentiation does not derive from a specific ecological preference of the species, but rather to the unavailability of climatic conditions typical of the comparison species. Indeed, although niche differentiation among species is high, these differences are not due to a "choice" of the species.
Nevertheless, *A. bertolonii* shows some degree of niche conservatism as it exhibits significant similarity indices in several comparisons. This evidence indicates that *A. bertolonii* (phylogenetically far from the others) is less dependent on ecological barriers to maintain its systematic independence, whereas other closely related species might require other barriers like geographic isolation to remain distinct.
To further investigate this aspect, the next phase of the work will involve the comparison of the niches at group level, comparing all species belonging the *A. alpina* group sensu Nardi (*A. alpina* L., *A. reuteri* Boiss., *A. ophiolithica* Barberis & E.Nardi and *A. lucensis* E.Nardi) from Northern Apennines and Western Alps and all species representing the *A. bertolonii* group sensu Nardi (*A. bertolonii* Schott, *A. apuana* (Marchetti) E.Nardi, *A. magellensis* F.Conti & Soldano, *A. champagnatii* Moraldo, E.Nardi & La Valva and *A. marcelliana* E. Nardi) from Central- Northern Apennines.
The comparison of niches within and between two important morphological groups along the Apennine ridge and the western Alps, will broaden our perspective, to better understand the importance of ecology in speciation processes of *Aquilegia* in mountain systems of South Europe.

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11 = GLORIA ITALIA NETWORK - 20 years of monitoring activities in the Italian mountains

Francesco Porro[1], Graziano Rossi[1]
[1]Department of Earth and Environmental Sciences, University of Pavia, Pavia, Italy

Global warming is affecting natural habitats worldwide, especially cold regions such as the Arctic and Alpine biomes, and it is projected to have a large impact on these ecosystems' biodiversity. Within this context, the world-wide long-term observation network GLORIA has collected vegetation and climatic data in alpine habitats since 2001. In Italy, since the beginning of the project, crucial data have been gathered to assess plant diversity and vegetation changes at a regional scale, on seven GLORIA study sites (Target Regions - TR) located on the Alps and Apennines (Fig.1). So far, the application of the GLORIA standardized method in the Italian TRs has produced key results for understanding how the high summit flora is responding to climate change at a regional scale. Overall, after the first 20 years of monitoring activities, we observed changes in species richness, plant cover and signals of thermophilization of the flora, as well as local plant diversity losses across the Italian mountains. Nevertheless, a comprehensive overview of the climate change impacts on mountain vegetation at a national scale is missing. Therefore, on the occasion of the next GLORIA-EUROPE monitoring scheduled for 2022, all the institutions that are managing the Italian GLORIA sites agreed to implement a national network that brings themselves together. Here we announce the formation of the "GLORIA ITALIA NETWORK" and present an up-to-date overview of the principal changes that have occurred in the Italian high mountain flora and plant diversity in the last 20 years. This newly formed network will provide better coordination among the GLORIA sites managers, leading to research and monitoring activities being carried out at a national level, greatly enhancing the visibility of the project for stakeholders and the public.

Fig. 1. The Italian GLORIA TR.

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11 = The phyllosphere of *Quercus ilex* provides an ecosystem service in the abatement of atmospheric pollution in the urban area of Naples: epiphytic microorganisms dislodgment protocol for metagenomic analysis

Alessia Postiglione[1], Antonello Prigioniero[1], Maria Tartaglia[1], Daniela Zuzolo[1], Pierpaolo Scarano[1], Maria Antonietta Ranauda[1], Maria Maisto[1], Rosaria Sciarrillo[1], Carmine Guarino[1]
[1]Department of Science and Technology, University of Sannio, Via Francesco De Sanctis SNC, 82100, Benevento, Italy

Quercus ilex is an evergreen tree widely used in urban forests and in the urban context of green infrastructures. It provides a series of ecosystem services including the abatement of atmospheric pollution. In recent years, the microorganisms that inhabit the leaves of plants have acquired a growing interest as they play a role in the degradation of pollutants, especially organic ones such as Polycyclic Aromatic Hydrocarbons (PAHs). Epiphytic microorganisms (bacteria and fungi) colonize the leaf surfaces of the holm oak leaf. In particular, the functional traits of the leaf (such us the star shaped trichomes present on the leaf lower face) can retain a great amount of PM that carries the PAHs, and can represent a substrate for microorganism communities. A protocol to dislodge epiphytic microorganism, washing the leaf with 0.9 M NaCl solution and then filtered through 0.45 µm bacteriological filter, has been conducted and validated, allowing to collect a good amount of microorganism from *Quercus ilex*. Filter were used to conduct a DNA extraction for a metagenomic study to evaluate if atmospheric contaminations in 5 different places of Naples urban area can induce spatio-temporal variations in bacteria and fungi community composition and differences in hydrocarbon degradation pathway abundance.

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11 = Biomonitoring through lichens and tree rings: time exposure and pollutant thresholds

Sonia Ravera[1], Claudia Cocozza[2]
[1]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo, Via Archirafi 38, 90123 Palermo, Italy; [2]Department of Agriculture, Food, Environment and Forestry (DAGRI), University of Florence, Via San Bonaventura 13, 50145 Florence, Italy

Environmental monitoring benefits of contaminants data representative of pollution as long as possible, especially where the monitoring of emissions has been short term or not existent. The use of biomonitoring for assessing atmospheric levels and deposition patterns of trace elements includes approach based on repeated surveys of trace elements both in native accumulative species collected each time ad hoc in the study area and in transplants of biological samples collected in the background area. But in highly polluted urban sites, both the difficulty of finding a sufficient quantity of native samples and of keeping transplants viable during the exposure can invalidate a research. Considering the ability of trees to take up and incorporate trace elements in tree-rings, their profiles could be a powerful tool reflecting the variation of pollutant concentrations in the environment. An additional advantage to collect tree cores is the easy get sample in urban and peri-urban areas and in remote places, frequently unreachable by traditional air monitoring stations. In recent years, studies concern few main issues leaving open questions by stimulating research in: (i) differences in accumulation capacity between species, (ii) differences between the content of trace elements in earlywood and latewood to define temporal patterns of atmospheric pollution, (iii) the definition of the specificity of chemical species in the definition of pollutants in biomonitors, (iv) the assessment of threshold of pollutant concentration in the environment able to induce uptake in biomonitors. The present study was based on biomonitors, plant material, tree rings and lichens, for the reconstruction of environmental pollution in the city of Terni, one the most polluted urban area in Italy especially for the PM pollution, characterized by the presence of both urban and industrial emission of pollutants. The hypothesis of the study is that the accumulation of pollutants in biomonitors is mainly related to exposure and level of pollution, that especially in urban areas define complex conditions, where sources are difficult to discriminate and consequently the effects in plant materials, namely uptake and storage. In 2015, trace elements were detected in tree rings of *Quercus pubescens* and in lichen transplants of *Evernia prunastri* in the major sectors of the city: industrial, urban and residential (natural and semi-natural environments). The approach allowed us to provide a contribution on (1) the effect of pollutant thresholds in biomonitors uptake and (2) the biomonitors sensitivity to time exposure to pollutants.

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11 = Allergenic risk assessment of urban parks: towards a standard index

Chiara Suanno[1], Iris Aloisi[1], Luigi Parrotta[1], Delia Fernández-González[2,3], Stefano Del Duca[1]
[1]Department of Biological, Geological and Environmental Sciences, University of Bologna, Via Irnerio 43, 40126, Bologna, Italy; Department of Biological, Geological and Environmental Sciences, University of Bologna, Via Irnerio 43, 40126 Bologna, Italy; [2]Institute of Atmospheric Sciences and Climate, ISAC-CNR, Via Piero Gobetti 101, 40129 Bologna, Italy; [3]Department Biodiversity and Environmental Management, University of León, 24071, Callejón Campus Vegazana, s/n, 24007 León, Spain

Allergenicity indices are a powerful tool to assess the health hazard posed by urban parks to pollen allergic subjects. Nonetheless, only three indices have been developed and applied to urban vegetation in the last decade, among which the Urban Green Zones Allergenicity Index (I_{UGZA}) and the Specific Allergenicity Index (SAI) are the best known. While SAI has been employed only a few times and mainly on anthropogenic, spontaneous vegetation, I_{UGZA} had a considerable success after its publication, and it has been applied to many urban parks in several European cities. However, the rapid spread of this index was not preceded by a method standardisation, especially for the sampling design, making it difficult to compare results obtained from incomparable datasets. In this work, the two indices SAI and I_{UGZA} were calculated for the same urban park (Botanical Garden of Bologna, Italy) using different sampling methods and inclusion criteria. Vegetational data were collected by systematic sampling in 2019 and by complete census of the woody species in 2020. Species richness, Shannon diversity index, SAI, and I_{UGZA} were then calculated on both datasets, for all the spermatophytes or for the woody species only. The collection method and the exclusion of herbaceous species did not significantly affect the allergenicity indices results, indicating systematic sampling as a reliable approximation of the total census, and supporting the hypothesis that the allergenicity of urban green areas is driven by woody species. On the other hand, the allergenic risk posed by the Botanical Garden resulted moderate to high according to SAI, and very low according to I_{UGZA}. Based on the parameters they consider and on their relationship with the allergenic species diversity, I_{UGZA} was deemed more reliable in estimating the allergenicity of an enclosed green space.

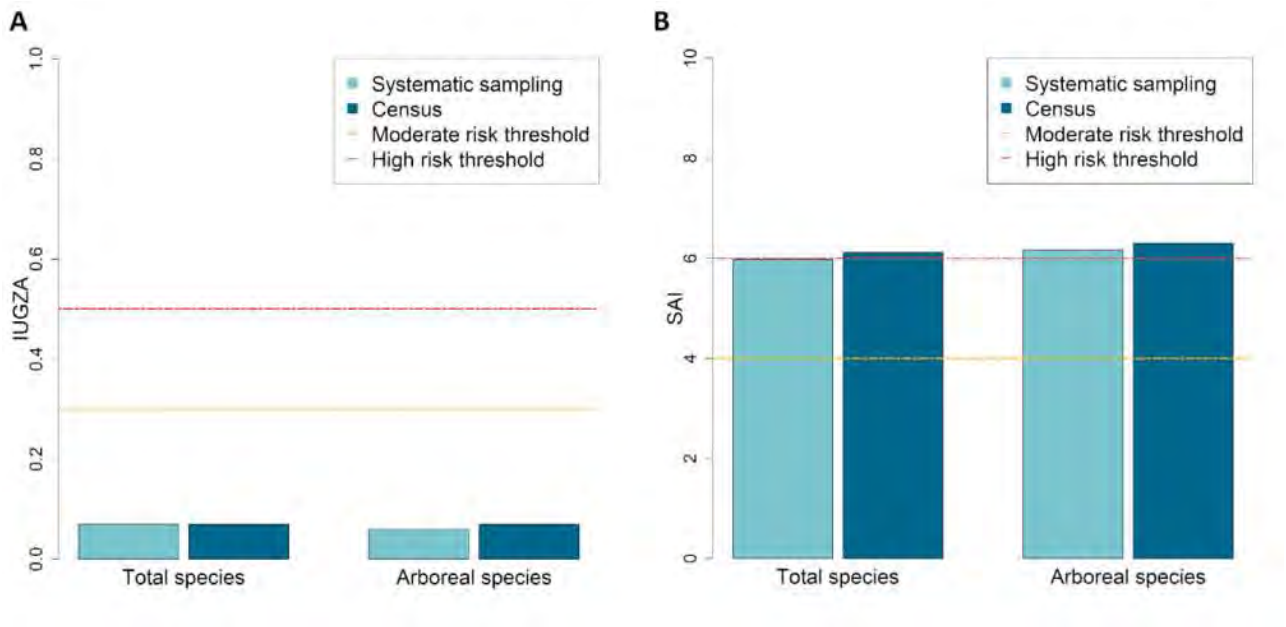

Fig. 1 Comparison of I_{UGZA} (A) and SAI (B) values for the Botanical Garden of Bologna, calculated on different datasets and vegetation groups. Light blue bars: data from systematic sampling of 2019; dark blue bars: data from arboreal census of 2020. Dotted lines indicate the risk thresholds: yellow for moderate and red for high risk.

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11 = Patterns of diversity and phylogenetic structure of terricolous lichen communities in a Mediterranean Mountain: from the field to the paper

Chiara Vallese[1], Luca Di Nuzzo[2], Gabriele Gheza[1], Michele Di Musciano[1,3], Renato Benesperi[2], Valter Di Cecco[4], Luciano Di Martino[4], Paolo Giordani[5], Chiara Lelli[1], Lucia Muggia[6], Juri Nascimbene[1]
[1]BIOME Lab, Department of Biological, Geological and Environmental Sciences, Alma Mater Studiorum University of Bologna, via Irnerio 42, 40126 Bologna, Italy; [2]Università di Firenze, Dipartimento di Biologia, Via la Pira 4, 50121 Firenze, Italia; [3]University of L'Aquila, Department of Life, Health and Environmental Sciences, Piazzale Salvatore Tommasi 1, 67100 L'Aquila; [4]Parco Nazionale della Majella, Via Badia, 28, 67039 Sulmona, Italia; [5]Università di Genova, Dipartimento di Farmacia, viale Cembrano, 4, 16148, Genova, Italia; [6]Department of Life Sciences, University of Trieste, 34121 Trieste, Italy

Mediterranean Mountains are a unique reserve of biodiversity. These peculiar environments are expected to be much more affected by the rapid variation in climatic components due to global change than the other mountain systems of Europe. In particular, an increase in drought conditions has been predicted as a result of a reduction in annual precipitation and an increase in the mean temperature. This dramatic situation causes latitudinal and altitudinal range shifts in the communities of high elevation organisms. The poikilohydric nature of lichens makes them extremely sensitive to climatic factors, this applies to high elevation ranges where communities are mainly composed of terricolous cold-adapted species that may suffer increasing warming and drought. Thanks to a scientific collaboration started in 2017 between the administration of the Majella National Park and the University of Bologna, a sampling design has been elaborated to study the biodiversity of lichen and the climate change response dynamic of these highly sensitive organisms along the Massif. We focused our attention on lichens not only for their physiology and sensitivity to climate, but also to provide more information on this taxon. In fact, despite the long tradition of botanical explorations which has seen the recent publication of a checklist of vascular plants including 2286 infrageneric taxa, the lichen biota of this area was still poorly investigated. Thus, we conducted two works in parallel. The first aimed at summarizing all available information on the occurrence of lichens in the Majella National Park, retrieved from previous literature, herbarium material and original data produced by new sampling design and expedition. The latter explored the relationships between climatic variables and both taxonomic and phylogenetic diversity of terricolous lichen communities along an elevational gradient in the Majella Massif. We analyzed (1) species richness patterns in terms of heat-adapted, intermediate, and cold-adapted species; (2) phylogenetic diversity and evolutionary distinctiveness indices; (3) phylogenetic structure indices that should reflect specific assembly mechanisms. In total, we reported the presence in the National Park of 335 accepted taxa, among them 26 have an arctic-alpine distribution and form disjunct populations in the summit area of the Massif. Most of these specialized and cryophiles species were reported into the list of species that we sampled along the Massif from 1800 to 2800 mt of elevation. Our results indicate that lichen richness decreases in cold-adapted species with increasing mean temperature while enhances in all temperature-affinities groups with higher annual precipitation. The communities living in the wettest sites show the highest values in terms of phylogenetic diversity resulting in more evolutionarily isolated and less closely related than expected by chance (overdispersion) as a result of competitive exclusion. These dynamics indicate that species loss across the gradient is the main expected response of terricolous lichens in a climate change scenario. This is mainly due to the loss of the cryophilous component that is also the most phylogenetically fostered. We conclude that increasing warm and drought conditions will dramatically impact the taxonomic and phylogenetic diversity of terricolous lichen communities in the Mediterranean Mountains.

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11 = Musealisation of the remains of historic trees: the case study of an old pine (*Pinus pinea)* in Partinico (Sicily)

Giuseppe Venturella[1], Franco Palla[2], Donato S. La Mela Veca[1], Filippo Bartoli[3], Francesco M. Raimondo[3]
[1]Department of Agricultural, Food and Forest Sciences, University of Palermo, Viale delle Scienze, bldg. 4, 90128 – Palermo, Italy; [2]Department STEBICEF/Sect. of Botany, Anthropology and Zoology, University of Palermo, Via Archirafi 38, 90123– Palermo, Italy; [3]*PLANTA*/Center for Research, Documentation and Training, Via Serraglio Vecchio 28, 90123, Palermo, Italy

On May 1st of 2021, shortly before midnight, a centuries-old pine tree (*Pinus pinea* L.) in the town of Partinico (province of Palermo), a point of reference for several generations of inhabitants, crashed to the ground, fortunately without causing any damage other than knocking down a grating and obstructing the roadway of a busy city street. Collapsed as a result of the failure of the root system, weakened in several places by a widespread attack of *Heterobasidion annosum* (Fr.) Bret. (Bondarzewiaceae), the crash of the large pine tree has caused so much apprehension among the residents and administrators of the town. In function of the presumed age of more than 150 years and of the dimensions (6 m circumference at 1,30 m from the ground and a height of about 18 m), the tree - for its notorious age and monumentality - has represented for many generations of inhabitants of the village, an irreplaceable symbol, a certain point of reference. All the citizens felt the sudden collapse as a loss of identity. For several days the local media, associations, and individual citizens have not been spared in spreading the news by expressing their sorrow for the loss of the historic pine tree and presenting to the municipal administration every kind of request to save the memory of the historic tree. Consulted by the Administration, after having carried out the necessary surveys to establish the cause of the sudden collapse - in the absence of wind and adverse weather conditions - it was recommended, rather than the removal of the fallen tree, the musealization on site of the most intact part of the trunk and the main branches, thus maintaining the physical memory of the great tree. Thus, the salient dendrometric data were collected and the determination of the plant's age was started following the dendrochronological methods. In the end, a project was developed that in principle foresees: a) the partial debarking of the trunk and the total debarking of the branches of the first, second, and partly third-order; b) the recomposition of the colossal find in the public space where the plant lived; c) the conservative and protective treatment of the entire wooden surface; d) the realization of a transparent cover for the whole length of the find (about 15 m); the realization of some benches to be placed around the find to allow the public to visit the site. 15 m); the realization of some benches to be placed around the find with the wood obtained from the big branches broken after the impact of the foliage on the asphalt, to allow the public to stop; e) the setting up of special panels to be placed on the sides and under the cover, in which the main news about the tree and its history will be reported.
An example, therefore, of a virtuous path that has brought together citizens and administrators, to bring to life, albeit in a symbolic way, particular trees that will thus continue to "live" as cultural assets, because they tell their story and the one at the same time lived, for several generations, by the city community.

Fig. 1. Images following the crash of the century-old *Pinun pinea* L. tree in the town of Partinico (Palermo).

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11 = *Juncus* sect. *Tenageia* in the Herbarium Centrale Italicum: confirmations and novelties for Italy

Daniele Viciani[1], Valeria Gambirasio[1], Lorenzo Lazzaro[1], Lorenzo Lastrucci[2]
[1]Museum System, Museum of Natural History of the University of Florence, Botany, via La Pira 4, 50121 Florence; [2]Department of Biology, Via La Pira 4, 50121 Florence

We carried out a revision of the Italian specimens belonging to *Juncus* L. subgenus *Agathryon* Raf. sect. *Tenageia* Dumort stored in the Herbarium Centrale Italicum of the Museum of Natural History of the University of Florence. By re-examining the 650 specimens present, we find that all the taxa belonging to this Section are present in the herbarium. This work also allowed us to highlight several novelties concerning species distribution at the regional level, thus confirming the importance of studying herbarium material. On the other hand, the work calls attention to a lack of information at the regional level for some taxa (e.g., *Juncus sphaerocarpus*), showing it would be desirable for botanists to continue to send samples to the herbarium to fill these gaps.
Table 1. Occurrence of *Juncus* sect. *Tenageia* species in Italy, updated following this study. Abbreviations: "P" occurring as native species, "PC" present as cryptogenic, "NP" recorded by mistake, "NC" no longer recorded, "D" doubtfully occurring, empty cells = absence.

	Valle d'Aosta	Piemonte	Lombardia	Trentino-Alto Adige	Veneto	Friuli-Venezia Giulia	Liguria	Emilia-Romagna	Toscana	Marche	Umbria	Lazio	Abruzzo	Molise	Campania	Puglia	Basilicata	Calabria	Sicilia	Sardegna
Juncus bufonius	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P	P
Juncus foliosus							NC					D			P		P	P	NC	P
Juncus hybridus	NP		P		P	NC	NC	P	P	P	P	P	P	P	P	P	P	P	P	P
Juncus minutulus	NC	P	NC	P	NC	P		NC	P	NC									P	P
Juncus ranarius	PC	NC	P	P		P		P	P	NC					NC			D	NC	P
Juncus sorrentinoi									P										NC	P
Juncus sphaerocarpus	P	P	P	P	P															
Juncus tenageia	P	P	P		P	P	P	P	P		P	P			P	NC		P	P	P

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