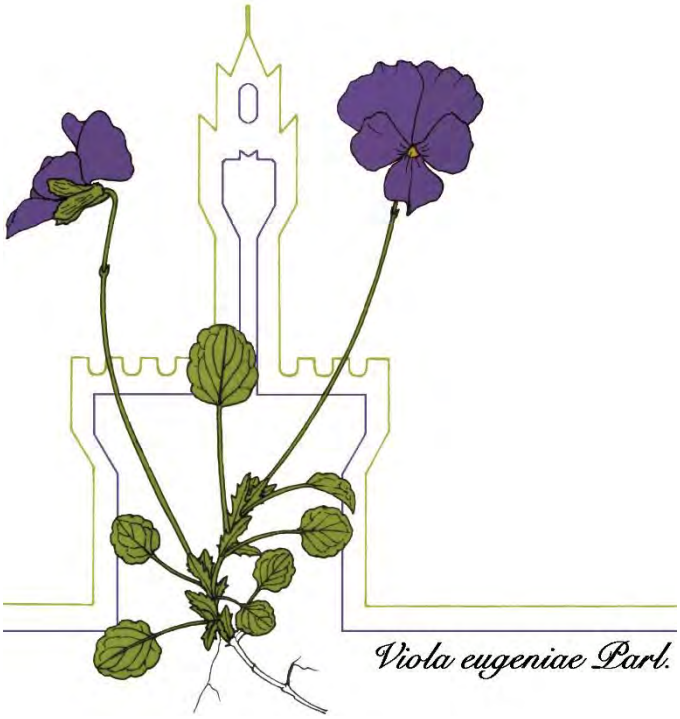
117° Congresso della Società Botanica Italiana

VIII INTERNATIONAL PLANT SCIENCE CONFERENCE (IPSC)

BOLOGNA, 7 - 10 SEPTEMBER 2022



ABSTRACTS

KEYNOTE LECTURES, COMMUNICATIONS, POSTERS

ISBN 978-88-85915-27-5

Scientific Committee

Alessandro Chiarucci (Università di Bologna)
Presidente
Stefania Biondi (Università di Bologna)
Antonella Canini (Università di Roma Tor Vergata)
Giovanni Cristofolini (Università di Bologna)
Stefano Del Duca (Università di Bologna)
Vincenzo De Feo (Università di Fisciano)
Gianniantonio Domina (Università di Palermo)
Goffredo Filibeck (Università della Tuscia)
Marta Galloni (Università di Bologna)
Maria Paola Germanò (Università di Messina)
Lorenzo Gianguzzi (Università di Palermo)
Marcello Salvatore Lenucci (Università del Salento)
Stefano Loppi (Università di Siena)
Filippo Maggi (Università di Camerino)
Michela Marignani (Università di Cagliari)
Lorella Navazio (Università di Padova)
Fabio Orlandi (Università di Perugia)
Rossella Pistocchi (Università di Bologna)
Ferruccio Poli (Università di Bologna)
Gianni Sacchetti (Università di Ferrara)
Laura Sadori (Università La Sapienza, Roma)
Luigi Sanità di Toppi (Università di Pisa)
Solveig Tosi (Università di Pavia)
Giuseppe Venturella (Università di Palermo)

Local Committee

Iris Aloisi (iris.aloisi2@unibo.it)
Fabiana Antognoni (fabiana.antognoni@unibo.it)
Stefania Biondi (stefania.biondi@unibo.it)
Fabrizio Buldrini (fabrizio.buldrini@unibo.it)
Marco Cantonati (marco.cantonati@unibo.it)
Davide Cavalletti (davide.cavalletti@unibo.it)
Alessandro Chiarucci
(alessandro.chiarucci@unibo.it)
Stefano Del Duca (stefano.delduca@unibo.it)
Maura Ferri (maura.ferri@unibo.it)
Manuela Mandrone
(manuela.mandrone2@unibo.it)
Umberto Mossetti (umberto.mossetti@unibo.it)
Juri Nascimbene (juri.nascimbene@unibo.it)
Luigi Parrotta (luigi.parrotta@unibo.it)
Laura Pezzolesi (laura.pezzolesi@unibo.it)
Rossella Pistocchi (rossella.pistocchi@unibo.it)
Ferruccio Poli (ferruccio.poli@unibo.it)
Mirko Salinitro (mirko.salinitro2@unibo.it)
Annalisa Tassoni (annalisa.tassoni2@unibo.it)
Alessandra Zambonelli
(alessandr.zambonelli@unibo.it)

With the patronage of

ALMA MATER STUDIORUM
UNIVERSITÀ DI BOLOGNA

Sponsor

consorzio della bonifica Renana

Koeltz Botanical Books

WALZ 50 SINCE 1972
ITALY - LOMBARD & MAROZZINI
High Quality Systems for PLANT SCIENCES

EDIZIONI IDELSON GNOCCHI dal 1908

MICOPERI BLUE GROWTH

PICCIN

TEsseLab www.tiesselab.com

ZANICHELLI

117° Congresso della Società Botanica Italiana onlus

VIII INTERNATIONAL PLANT SCIENCE CONFERENCE (IPSC)

Complesso Belmeloro, Alma Mater Studiorum Università di Bologna, 7 - 10 September 2022

Programme

Wednesday 7 September 2022

9:00-12:00 Registration and poster installation

12:00-13:00 Welcome lunch

13:30-14:00 **Opening ceremony**

Macro Symposium

THE ROLE OF PLANT ECOLOGY AND VEGETATION SCIENCE IN ECOSYSTEM RESTORATION: THEORY AND PRACTICE

Symposium 1

VEGETATION SCIENCE AND PLANT LANDSCAPE RESTORATION: RESEARCHES AND PERSPECTIVES

(Chairpersons G. Bazan and S. Casavecchia)

Key words: vegetation dynamics, habitat restoration, habitat assessments, plant landscape conservation, landscape planning

14:00-14:30 • **Carlo Blasi**, Sapienza University of Rome, Italy (25 + 5 min)
"New tools of plant ecology and plant sociology to assess, recover and restore the natural capital"

14:30-15:45 **Communications**

- **Miris Castello**, Alfredo Altobelli, Francesco Liccari et al. (12 + 3 min)
"Vegetation survey and mapping of Lake Doberdò: an analysis for the assessment of habitat conservation status of a disappearing lake of the Classical Karst (NE Italy)"
- **Marco Canella**, Marco Baldin, Simona Bardi et al. (12 + 3 min)
"Project FORESTALL: connecting habitat restoration to social inclusion in the Ramsar site of Valle Averto, southern lagoon of Venice (N-Adriatic Sea)"
- **Giulio Tesei**, Paride D'Ottavio, Mattia Di Silvestri et al. (12 + 3 min)
"The landscape of Central Apennine wooded grasslands: space-time dynamisms and future perspectives"
- **Carmelo Maria Musarella**, Silvio Bagnato, Giuseppe Bombino et al. (12 + 3 min)
"Urban green infrastructures design for the ecological and landscape restoration: the case of the Metropolitan City of Reggio Calabria (southern Italy)"
- **Pietro Minissale**, Salvatore Cambria, Fabio Cilea et al. (12 + 3 min)
"Plant conservation and restoration in the coastal wetlands of Sicily: the case studies of the Natura 2000 sites "Saline di Priolo" and "Pantano Ponterio""

15:45-16:15 Coffee Break

Symposium 2

RESTORATION OF PLANT COMMUNITIES: ECOLOGICAL BACKGROUND AND PRACTICAL EXPERIENCES
(Chairpersons S. Maccherini and G. Filibeck)

Key words: plant community restoration, historical ecosystem references, restoration monitoring, restoration and climate change, rewilding

16:15-17:00 • **Rasmus Ejrnæs**, Aarhus University, Denmark (35 + 10 min)
"The restoration of natural plant communities – disturbing baselines and even more disturbing animals"

17:00-18:00 **Communications**

- Silvia Assini, **Gabriele Gheza**, Juri Nascimbene et al. (12 + 3 min)
"Restoration of dry-acidic grasslands and heathlands in the western Po Plain (northern Italy – Life Drylands project)"
- **Pierluigi Cortis**, Antonio De Agostini, Donatella Cogoni et al. (12 + 3 min)
"Seed conservation and development in metallicolous and non-metallicolous orchid populations"
- **Benedetta Gori**, Carla Lambertini, Alessandra Zambonelli (12 + 3 min)
"Riparian vegetation management along artificial channels: first effects on plant community ecology of "Life Green4Blue""
- **Alessia Masi**, Cristiano Vignola, Lucrezia Masci et al. (12 + 3 min)
"Rewilding vs. cultivation, what plants can tell us about demography after pandemics"

18:00-19:30 **Poster session**

Thursday 8 September 2022

Symposium 3

BIODIVERSITY: A CHALLENGE FOR NATURE CONSERVATION AND ENVIRONMENTAL MONITORING
(Chairpersons S. Loppi and J. Nascimbene)

Key words: biodiversity; nature conservation; environmental monitoring

9:00-9:30 • **Bettina Weber,** University of Graz, Austria (25 + 5 min)
"Cryptogamic communities: their biodiversity, functionality, and roles in global biogeochemical cycling in times of global change"

9:30-10:45 **Communications**

- **Francesco Maria Sabatini**, Borja Jiménez-Alfaro, Ute Jandt et al. (12 + 3 min)
"Global patterns of vascular plant alpha diversity"
- **Stefano Martellos**, Pier Luigi Nimis, Matteo Conti et al. (12 + 3 min)
"Digitization and geolocation of specimens from Italian lichen herbaria"
- **David Dolci**, Alessandro Chiarucci, Lorenzo Peruzzi (12 + 3 min)
"Identification of multi-species suitable areas for assisted colonization under climate change scenarios"
- **Simonetta Bagella**, Claudia Angiolini, Marco Caccianiga et al. (12 + 3 min)
"Robotic Monitoring of Habitats: good news from the dune front"
- **Sergio Enrico Favero-Longo**, Enrica Matteucci, Maria Giuseppina Ruggiero (12 + 3 min)
"Rock drawings in Valle Camonica: Monitoring lichen diversity and the efficacy of biodeterioration control towards a joint conservation of the cultural and natural heritage"

10:45-11:15 Coffee Break

Symposium 4

PLANT SPECIES CONCEPTS IN THE 21TH CENTURY

(Chairpersons G. Cristofolini and G. Domina)

Key words: taxonomy, speciation, evolution, natural selection, genome

11:15-11:45 • **Dirk Carl Albach**, University of Oldenburg, Germany (25 + 5 min)
"The species in the age of phylogenomics - Types, hybrids and convergence"

11:45-13:00 **Communications**

- **Jacopo Franzoni**, Giovanni Astuti, Gianluigi Bacchetta et al. (12 + 3 min)
"Preliminary genetic analyses of central Mediterranean taxa in the *Dianthus virgineus* complex (Caryophyllaceae) suggest taxonomic inflation"
- **Antonio Giacò**, Lucia Varaldo, Gabriele Casazza et al. (12 + 3 min)
"An integrative taxonomic study of *Santolina* (Asteraceae) from southern France and north-eastern Spain"
- **Eleonora Manzo**, Salvatore Tomasello (12 + 3 min)
"Integrative taxonomy in the 21st century. An example from the intricate plant genus *Xanthium* (Ambrosinae, Asteraceae)"
- **Martina D'Agostino**, Giulia Albani Rocchetti, Marta Carboni et al. (12 + 3 min)
"Study of the Italian and French populations of *Vicia cusnae* (Fabaceae)"
- **Giulio Barone**, Emilio Di Gristina, Gianniantonio Domina (12 + 3 min)
"On the concept of species in cultivated plants and its consequences in alien species and CWR"

13:00-14:00 Lunch, **Poster session**

Symposium 5

PRIMARY AND SECONDARY METABOLITES PRODUCED BY AQUATIC PHOTOTROPHS WITH IMPLICATIONS FOR THE ENVIRONMENT AND BIOECONOMY

(Chairpersons R. Pistocchi and M. Cantonati)

Key words: biodiversity, biotechnology, environmental issues, infochemicals

14:00-14:30 • **Ana Bartual Magro,** Universida de Càdiz, Spain (25 + 5 min)
"Allelopathic interactions in the diatom phycospheres: ascertaining the ecological role of polyunsaturated aldehydes in the ocean"

14:30-15:45 **Communications**

- **Saverio Savio**, Riccardo Turchi, Debora Paris et al. (12 + 3 min)
"Biorefining the diatom *Staurosirella pinnata* for drug discovery"
- **Mara Simonazzi**, Laura Pezzolesi, Tânia K Shishido et al. (12 + 3 min)
"Unveiling new potential toxic metabolites from cyanobacteria through genome mining: a case study from an Italian drinking water reservoir"
- **Tomas Morosinotto**, Francesca Bucci, Alessandra Bellan (12 + 3 min)
"Algae biomass as sustainable source of protein and lipids"
- **Damiano Spagnuolo**, Viviana Bressi, Antonio Manghisi et al. (12 + 3 min)
"Influence of aqueous phase from hydrothermal carbonization (AHL) of *Sargassum muticum* (Phaeophyceae) on germination and growth of *Phaseolus vulgaris* (Fabaceae)"
- **Simona Armeli Minicante**, Lucia Bongiorni, Amelia De Lazzari (12 + 3 min)
"Bio-based products from Mediterranean seaweeds and their opportunities in the bioeconomy field"

15:45-16:15 Coffee Break

Symposium 6

URBAN GREEN-SPACE PLANNING AND MANAGEMENT: FROM FUNCTIONAL ASPECTS TO ECOSYSTEM BENEFITS

(Chairpersons M. Galloni and F. Orlandi)

Key words: urban ecosystems, urban green areas, ecosystem benefits, citizen science, environmental education, urban ecological corridors, urban flora

16:15-16:45 • **Mario Balzan**, Malta College of Arts, Science and Technology, Malta (25 + 5 min)
"Mainstreaming nature in urban green space planning and management"

16:45-18:00 **Communications**

- **Marta Pianta**, Mariasole Calbi, Alexandre Changenet et al. (12 + 3 min)
"Plant functional groups and vegetation dynamics within urban ecosystems: the ECOLOPES' project approach"
- **Giulia Capotorti**, Laura Zavattero, Sandro Bonacquisti et al. (12 + 3 min)
"On biodiversity friendly Green Infrastructure planning: contribution of plant ecology to the monitoring and valorization of peri-urban environments in the Metropolitan City of Rome (Mediterranenan Italy)"
- **Giulia Santunione**, Alice Barbieri, Elisabetta Sgarbi (12 + 3 min)
"Analysis of particulate matter trapped by four different shrub species in an urban forest: quantification and characterization"
- Marco Fornaciari, Federico Rossi, **Desirée Muscas** et al. (12 + 3 min)
"Multiple ecosystem benefits of urban green-space: two case studies"
- **Chiara Acquaroli**, Nicola Lothar Herrmann, Simone Flaminio et al. (12 + 3 min)
"Students 4 pollinators: a citizen science project in urban green areas to teach plant diversity and pollinator conservation to young generations"

18:00-19:00 **General Meeting of the Italian Botanical Society (members only)**

20:30-23:30 **Congress dinner**

Friday 9 September 2022

Symposium 7

TRANSLATING PLANT RESEARCH FROM LAB TO LIFE: CELLULAR, MOLECULAR AND BIOTECHNOLOGICAL APPROACHES FOR A SUSTAINABLE DEVELOPMENT

(Chairpersons L. Navazio and M. Lenucci)

Key words: plant cell and molecular biology, plant biotechnology, plant growth and differentiation, plant environment interactions, productivity and sustainability challenges

9:00-9:30 • **Zoe Popper**, National University of Ireland Galway, Ireland (25 + 5 min)
"A wall of opportunities: from adaptation to exploitation"

9:30-10:45 **Communications**

- **Sofia Montanari**, Mirko Salinitro, Annalisa Tassoni (12 + 3 min)
"Root growth response to localized selenium enrichment in soil: a comparison between hyperaccumulator and non-accumulator species"
- **Camilla Badiali**, Valerio Petruccelli, Fabio Sciubba et al. (12 + 3 min)
"Innovative approaches for controlling *Botrytis cinerea* infections in tomato by using poly (lactic-co-glycolic acid) nanoparticles for the vehiculation of antifungals"

- **Paola Malaspina**, Cristina Danna, Laura Cornara et al. (12 + 3 min)
  "Characterization of *Eucalyptus cinerea* and *E. nicholii* by-products for agricultural applications"
- **Maria De Benedictis**, Antonia Gallo, Pietro Roversi et al. (12 + 3 min)
  "*Arabidopsis thaliana* tolerance to cadmium stress is mediated by endoplasmic reticulum stress sensing"
- **Gianmaria Oliva**, Giovanni Vigliotta, Mattia Terzaghi et al. (12 + 3 min)
  "Effect of Plant Growth-Promoting Rhizobacteria on maize seedlings cultivated on saline soil: changes in root morphologies and amelioration of saline stress response"

10:45-11:15 Coffee Break

11:15-12:30 **Communications**

- Chiara Suanno, Elisa Tonoli, **Stefano Del Duca** et al. (12 + 3 min)
  "Pollensomes cargo and markers in *Actinidia chinensis* Planch."
- **Marco Giovannetti**, Arianna Capparotto, Daniele Castelli et al. (12 + 3 min)
  "Leveraging plant natural variation to investigate root phosphate homeostasis"
- **Patrizia Cesaro**, Nadia Massa, Daniela Campana et al. (12 + 3 min)
  "Effects of mycorrhizal inoculation on responses of *Solanum lycopersicum* in the early stages of the symbiosis"
- **Martina Cerri**, Sara Cannavò, Alma Costarelli et al. (12 + 3 min)
  "Disentangling the interaction between rice and *Azolla filiculoides*"
- **Ylenia De Luca**, Niklaus Zemp, Antonia Cristaudo et al. (12 + 3 min)
  "Development of molecular markers for rapid sex screening in *Cycas revoluta* and in *Ginkgo biloba*"

12:30-14:00 Lunch

Symposium 8

FUNGI: α-BIODIVERSITY, ECOSYSTEM SERVICES, OPEN SCIENCE

(Chairpersons G. Venturella and L. Nicola)

Key words: biodiversity, conservation, species distribution, social network data sharing web sites, ecosystem services, citizen science

14:00-14:30 • **Gregory M. Mueller**, Chicago Botanic Garden, Illinois, USA (25 + 5 min)
"Building on interactions between the professional and non-professional mycological communities to advance fungal conservation"

14:30-15:45 **Communications**

- **Claudia Perini**, Irene Mazza, Elena Salerni (12 + 3 min)
  "The Fungal communities of Sienese "biancana" badlands"
- **Marta Elisabetta Eleonora Temporiti**, Lidia Nicola, Solveig Tosi (12 + 3 min)
  "The influence of plastic pollution on soil fungal biodiversity"
- **Federica Spina**, Viktoria Ilieva, Davide Ferrero et al. (12 + 3 min)
  "Exploiting the fungal community against plastic polymers"
- **Simone Di Piazza**, Grazia Cecchi, Paolo Vassallo et al. (12 + 3 min)
  "Truffle and ecosystem services: the hidden potential of the Ligurian countryside"
- **Nicola Baldoni**, Mara Rondolini, Francesco Prosperi et al. (12 + 3 min)
  "Preliminary approach to mycorrhization of micropropagated linden sprouts with *Tuber borchii*"

15:45-16:15 Coffee Break

Symposium 9

FROM MEDICINAL PLANTS TO HEALTH PRODUCTS THROUGH SPECIALIZED METABOLISM
(Chairpersons M. Germanò and F. Maggi)

Key words: medicinal plants, crude drugs, specialized metabolites, biological activities, natural products

16:15-16:45 • **Juha-Pekka Salminen**, University of Turku, Finland (25 + 5 min)
"Plant tannin analytics and its importance to understanding tannin bioactivity"

16:45-18:00 **Communications**

- **Marta Ferrati**, Giovanni Benelli, Roman Pavela et al. (12 + 3 min)
"Development of a highly stable *Carlina acaulis* essential oil nanoemulsion for managing agricultural pests"
- **Lorenzo Marincich**, Margherita Bellucci, Mariacaterina Lianza et al. (12 + 3 min)
"Red-fleshed apple: polyphenol profile characterization, antioxidant activity evaluation and development of a green extraction method for anthocyanins recovery"
- **Marinella De Leo**, Valentina Parisi, Valentina Santoro et al. (12 + 3 min)
"Exploring *Punica granatum* L. peels from different southern Italian varieties for their potential healthy value"
- **Milena Masullo**, Zahra Sadeghi, Antonietta Cerulli et al. (12 + 3 min)
"*Perovskia artemisioides* Boiss roots as source of diterpene constituents with *in vitro* anti-inflammatory activity"
- **Antonella Smeriglio**, Cristina Danna, Miriam Bazzicalupo et al. (12 + 3 min)
"The phytochemical profile of leaf and rizhome of *Peucedanum ostruthium* (L.) W. D. J. Koch influences the antioxidant, anti-inflammatory, and wound healing properties of this medicinal plant"

18:00-18:30 **Congress Closure**

Saturday 10 September 2022

Evento Post-Congress

(Aula A del Complesso Belmeloro, Alma Mater Studiorum Università di Bologna)

"PIÙ NATURA NEGLI ECOSISTEMI URBANI PER IL FUTURO SOSTENIBILE E RESILIENTE DELLE CITTÀ"

9:00-10:30 **Saluti delle Autorità e introduzione al Simposio**
(Coordina C. Blasi)

- **Giovanni Molari**, Magnifico Rettore Alma Mater Studiorum Università di Bologna
- **Maria Cristina Messa**, Ministra dell'Università e della Ricerca
- **Roberto Cingolani**, Ministro della Transizione Ecologica
- **Enrico Giovannini**, Ministro delle Infrastrutture e della Mobilità Sostenibile
- **Antonio Pietro Marzo**, Generale C.A. Comandante delle Unità Forestali, Ambientali e Agroalimentari dell'Arma dei Carabinieri
- **Marco Marchetti**, Presidente Fondazione Alberltalia
- **Alessandro Chiarucci**, Presidente Società Botanica Italiana
- **Giovanni Maria Flick**, Presidente Emerito della Corte Costituzionale
"Il significato culturale, storico e ambientale dell'inserimento di biodiversità ed ecosistemi nella Costituzione Italiana"

10:30-11:30 **Prima sessione**

- **Maria Carmela Giarratano**, Capo Dipartimento amministrazione generale pianificazione e patrimonio naturale, Ministero della Transizione Ecologica
- **Alessandra Stefani**, Direttrice Generale Ministero delle Politiche Agricole, Alimentari e Forestali
  "Vivaismo forestale al servizio della Biodiversità e del Capitale Naturale"
- **Massimo Labra**, Coordinatore scientifico spoke 'Urban Biodiversity' del Centro Nazionale per la Biodiversità
  "Biodiversità funzionale della città: la sfida del Centro Nazionale del MUR"
- **Gemma Calamandrei**, Direttrice del Centro di riferimento per le Scienze comportamentali e la Salute mentale, Istituto Superiore di Sanità
  "Aree verdi, resilienza e salute mentale: evidenze sperimentali ed epidemiologiche"

11:30-13:30 **Seconda sessione**
(Coordina A. Chiarucci)

- **M.C. Siniscalco**
  "Torino città verde: l'evoluzione degli ultimi anni"
- **R. Gentili**, G. Galasso, C. Montagnani, S. Citterio
  "Flora e vegetazione di aree marginali o degradate da recuperare nel Comune di Milano"
- **S.P. Assini**, P. Nola
  "Il verde urbano e periurbano nelle ricerche dell'Università di Pavia: passato, presente e futuro"
- **S. Landi**, G. Angelini
  "Sviluppo di protocolli per quantificare i servizi ecosistemici delle foreste urbane"
- **L. Zavattero**, G. Capotorti, S. Bonacquisti, E. Del Vico, F. Manes, V. De Lazzari, G. Scarascia Mugnozza, R. Salvati, G. Eusepi, M. De Horatis, B. Bertani, M. Marchetti
  "Scienza della vegetazione applicata alla forestazione urbana e periurbana della Città Metropolitana di Roma capitale"

Dibattito

- **A. Canini**, R. Braglia, E.L. Redi, F. Scuderi
  "Piante autoctone per la rigenerazione sostenibile degli ecosistemi urbani"
- A. Lallai, **L. Podda**, M. Sarigu, G. Bacchetta
  "Programmare la sostenibilità delle città del futuro: l'esempio del progetto sul verde pubblico del comune di Assemini (Cagliari)"
- **C.M. Musarella**
  "Naturali, sostenibili e... botaniche: le buone pratiche nella pianificazione di interventi naturalistici nella Città Metropolitana di Reggio Calabria"
- **G. Venturella**
  "Più natura in città: casi studio in Puglia e Sicilia"

Dibattito

- **Carlo Blasi -** Conclusioni e linee programmatiche per il prossimo futuro

The timetable may be modified

INDEX

Communications

SYMPOSIUM 1.
"VEGETATION SCIENCE AND PLANT LANDSCAPE RESTORATION: RESEARCHES AND PERSPECTIVES"

SYMPOSIUM 2.
"RESTORATION OF PLANT COMMUNITIES: ECOLOGICAL BACKGROUND AND PRACTICAL EXPERIENCES"

SYMPOSIUM 3.
"BIODIVERSITY: A CHALLENGE FOR NATURE CONSERVATION AND ENVIRONMENTAL MONITORING"

SYMPOSIUM 4.
"PLANT SPECIES CONCEPTS IN THE 21TH CENTURY"

SYMPOSIUM 5.
"PRIMARY AND SECONDARY METABOLITES PRODUCED BY AQUATIC PHOTOTROPHS WITH IMPLICATIONS FOR THE ENVIRONMENT AND BIOECONOMY"

SYMPOSIUM 6.
"URBAN GREEN-SPACE PLANNING AND MANAGEMENT: FROM FUNCTIONAL ASPECTS TO ECOSYSTEM BENEFITS"

SYMPOSIUM 7.
"TRANSLATING PLANT RESEARCH FROM LAB TO LIFE: CELLULAR, MOLECULAR AND BIOTECHNOLOGICAL APPROACHES FOR A SUSTAINABLE DEVELOPMENT"

SYMPOSIUM 8.
"FUNGI: α-BIODIVERSITY, ECOSYSTEM SERVICES, OPEN SCIENCE"

SYMPOSIUM 9.
"FROM MEDICINAL PLANTS TO HEALTH PRODUCTS THROUGH SPECIALIZED METABOLISM"

Posters

3. "BIODIVERSITY: A CHALLENGE FOR NATURE CONSERVATION AND ENVIRONMENTAL MONITORING"

7. "TRANSLATING PLANT RESEARCH FROM LAB TO LIFE: CELLULAR, MOLECULAR AND BIOTECHNOLOGICAL APPROACHES FOR A SUSTAINABLE DEVELOPMENT"

8. “FUNGI: α-BIODIVERSITY, ECOSYSTEM SERVICES, OPEN SCIENCE”

9. "FROM MEDICINAL PLANTS TO HEALTH PRODUCTS THROUGH SPECIALIZED METABOLISM"

10. FREE TOPIC

KEYNOTE LECTURES

New tools of plant ecology and plant sociology to assess, recover and restore the natural capital

Carlo Blasi
Department of Environmental Biology, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy

The European strategies on farm to fork, biodiversity and Green Infrastructures, highlight all the centrality of protecting the biodiversity of natural ecosystems, their services and their resilience. In the decade 2010-2020, the biodiversity strategy had given more space to the recognition and protection of the full functionality of ecosystem services. All this promoting not only knowledge, but also the cartography of the ecosystem services. Currently for the decade 20-30 the importance of biodiversity is again emphasized and a whole series of actions consistent with the ecology of restoration are proposed.
After a careful analysis of the innovations introduced by the European strategies, the Next Generation EU and the RRNP, the useful skills of botanists, plant ecologists, phytosociologists for the achievement of European and national objectives are highlighted.
As a conclusion, the relationships between restoration ecology, ecosystems restoration and ecological restoration are analyzed and the concept of ecosystem restoration is discussed, clearing the role of human actions and the role of natural systems. The ecosystem restoration involves the recovery of resilience, not the total restoration of the functions and structures that are instead the responsibility of natural systems themselves.

The restoration of natural plant communities – disturbing baselines and even more disturbing animals

Rasmus Ejrnæs[1], Camilla Fløjgaard[1], Christopher Sandom[2], Pil Birkefelt Møller Pedersen[3], Jens-Christian Svenning[3]
[1]Department of Ecoscience, Aarhus University, CF Møllers Allé, Bygning 1110, 8000 Aarhus C, Denmark; [2]School of Life Sciences and Sussex Sustainability Research Programme, University of Sussex, Brighton, BN1 9RH, UK; [3]Department of Biology, Aarhus University, Ny Munkegade 114-116, 8000 Aarhus C, Denmark

Plant communities represent assemblies of plants growing together in response to the abiotic conditions – but also to the intensity, frequency and duration of physical disturbances such as coastal erosion, sand drift, flooding, wildfire and grazing animals. Here, we will focus on the importance of large herbivores and the obstacles associated with the restoration of natural communities of large herbivores.
The Danish professor Thyge W Böcher, who studied grassland communities on steep slopes around the Danish coasts in first half of the 20th century hypothesised that these communities were naturally open due to drought and coastal erosion keeping trees away. Today Böcher's famous slopes are 95% covered in dense scrubs of Prunus spinosa, Crataegus spp, Rosa spp, and in some places also Fraxinus excelsior, Quercus robur, Corylus avellana and Ulmus glabra. In hindsight, it is obvious that the grasslands were kept open by grazing cattle, horses and sheep.
Most biologists have met the view that these species-rich grassland communities were really a creation of the grazing of domestic animals kept by a farmer. This interpretation overlooks the obvious, namely that the large species-pool of species-rich temperate grassland has evolved over a long evolutionary timespan predating the arrival of modern humans and surely also the onset of agriculture and domestic animals. It also appears obvious that grazing as a process occurred everywhere in the landscape, with important ecological effect not only in grassland, but also in mires, meadows and fens and even in forests.
But where should we look for a natural reference, a baseline, a restoration target, if not in the traditional extensive farming practices? My colleagues and I have identified three different approaches to the challenge of finding a useful baseline for restoration. 1) Paleo-ecological inference from the study of pollen and macrofossils predating the onset of agriculture or the arrival of modern humans. 2) Studying present day near-intact faunas inside well-protected nature reserves. 3) Experiments where large herbivores are reintroduced to nature reserves with a minimum of human intervention – so-called rewilding.
Paleoecology clearly documents that our current European biota of large herbivores is exceptionally poor in species – particularly the megaherbivores have gone missing. And all evidence point to human extirpation. Looking at nature reserves support the notion that only in Africa we can find evidence for natural herbivore communities approaching carrying capacity. Rewilding experiments indicate that our current landscapes are either undergrazed or overgrazed – with detrimental consequences for biodiversity.
Each of our three approaches has its pros and cons, but together they provide a convincing and disturbing account of a modern world with pervasive lack of wild large herbivores at natural densities – all due to human regulation and replacement of natural grazing with domestic animals herded under regulations set in the common agricultural policy of the EU.

Fig. 1 Grazed ecosystem of Okavango, Botswana

Fig. 2. Rewilding in Mols Bjerge, Denmark

Cryptogamic communities: their biodiversity, functionality, and roles in global biogeochemical cycling in times of global change

Bettina Weber
Department of Biology, University of Graz, Holteigasse 6, 8010 Graz, Austria

Cryptogamic communities (CC) are composed of lichens, bryophytes, cyanobacteria, and algae in varying proportions. They occur on and in soil, rocks and epiphytically on the bark and even the leaves of shrubs and trees. The overall cryptogam diversity is high, and with a large percentage of taxa being not described, yet, they are considered to contribute about 40% to the overall diversity of plants.
It is well known that CC are important primary colonizers, contribute to weathering and soil formation, and influence the local water balance, but also react quite sensitive upon air pollution. In our research, we want to learn, if, besides these functional roles, they also influence global biogeochemical cycling processes.
For this, we studied the role of CC in global carbon (C) and nitrogen (N) cycling. By means of data analysis and modelling approaches, we analyzed their global C and N fixation and investigated the N cycling processes and the related emission of gaseous atmospherically relevant N compounds. Here, we observed that CC are highly relevant in global N fixation and release both reactive and climate relevant compounds in the atmosphere.
In a recent study conducted in the Amazon rain forest, we analyzed if epiphytic CC release also biogenic volatile organic compounds (BVOCs), a process up to now mainly ascribed to vascular plants. These BVOCs are highly reactive, influence the local ozone concentrations and the lifetime of greenhouse gases, and can form aerosol particles, which influence cloud formation and climate. Our measurements showed that bryophytes release major amounts of sesquiterpenoids, whereas lichens take up atmospheric oxidation products. A first calculation suggests that the amount of sesquiterpenes released by bryophytes is in a similar range as the quantities described for vascular vegetation.
One key community of cryptogams, described as biological soil crusts (in short: biocrusts), occurs on and within the uppermost millimeters of soils in drylands or wherever dry microclimatic conditions occur. In a modeling approach, we could show that they cover about 12% of the global land surface. As these biocrusts excrete gelatinous sheaths and tightly entangle soil particles, they effectively stabilize dryland soils and prevent erosion by wind and water. In a recent modeling approach, we showed that biocrusts reduce the global atmospheric dust emissions by about 60%, preventing the release of about 700 tons of dust per year. However, until 2070 biocrust coverage is expected to be reduced by 25-40%, caused by both climate and land use change, with potential detrimental effects on future dust scenarios.
Overall, our studies show that CC are relevant in various global processes, which up to now have not or only barely been considered. These mechanisms need to be included in global modeling approaches to obtain a more thorough understanding of the processes and drivers and to evaluate the role of CC in current and future environmental scenarios.

The species in the age of phylogenomics - Types, hybrids and convergence

Dirk C. Albach[1]
Botanical Garden, Carl von Ossietzky-University, 26111 Oldenburg, Germany

The species is the central unit in biology. However, its definition continues to cause controversies, in general as well as in specific cases. Among the problems encountered by plant taxonomists are evolutionary processes such as gene flow among species as well as selection causing convergence in characters. Detecting these processes and its extent among species has been problematic in the past and many names and types have been generated describing the variation between species. High-throughput DNA sequencing have not just allowed to detect such problems. Using examples from *Veronica* subg. *Pseudolysimachium* (*V. spicata*, *V. longifolia* and relatives), a group of approximately 30 species, I demonstrate that lineage-specific patterns of gene flow and convergence can be integral parts of species definitions. For example, *Veronica longifolia* and *V. spicata* seem to readily hybridize in both directions in sympatry and form morphologically plastic hybrid plants.In other cases hybridization seems to be unidirectional towards high-alpine species in the Altai Mountains. Prominent examples of convergence involve the indumentum, previously used to characterize species such as *V. incana* and *V. paczoskiana*. Assigning names to lineages, however, requires in all these cases thorough typification and inclusion of types in phylogenomic analyses. The large number of 400 names available for the 30 species in the groups demonstrates that there is a large complexity in choosing the right name for a taxon and determining whether a type represents a hybrid or the species. An example will be presented involving the southern Romanian group of *V. orchidea*, *V. barrelieri*, *V. spicata* and their hybrids.

Fig. 1: Linnean specimen of *V. spicata* (BM) of doubtful affinity

Fig. 2: *Veronica spicata* from Eastern Austria

Allelopathic interactions in the diatom phycospheres: ascertaining the ecological role of polyunsaturated aldehydes in the ocean

Ana Bartual Magro
Universida de Càdiz, Spain

In the early 19 th century, Augustin Pyramus de Candolle was the first to suggest that substances or exudates released by plant roots interacted with the species itself. This region surrounding the root and enriched in these substances was later named as rhizosphere. At 1937, Molisch coined the term "allelopathy" from greek words *allelon* ("to each other") and *pathos* ("to suffer") as negative effect on one on the other. Allelopathy was then defined as the process in which plants releases into the environment one or more chemical compounds that inhibit the growth of another plants living in the same or nearby habitat. In an analogous way, the region surrounding a phytoplanktonic cell is called phycosphere, with the peculiarity that, in this case, the surrounding medium is water. Throughout this region, phytoplankton cell interacts with viruses, bacteria and other contemporary phytoplankton species. Diatoms are one of the most important and widely distributed phytoplankton groups and it was the first phytoplankton group where polyunsaturated aldehydes (PUA) were isolated (Miralto et al., 1999) and to which have been attributed allelopathic and infochemical effects. Here, I would like to summarize our advances in the last decade on the ecological role of PUA, especially on the natural distribution of these molecules in ocean waters, by ranges and types at different temporal and spatial scales. Currently, our work focuses on the interactions of PUA-producing diatoms with other non-PUA producing diatoms, bacteria and cyanobacteria.

Mainstreaming nature in urban green space planning and management

Mario V. Balzan
Institute of Applied Sciences, Malta College of Arts, Science and Technology (MCAST), Paola, PLA9032, Malta
mario.balzan@mcast.edu.mt

Policy and institutions are increasingly looking at integrating nature in decision-making, investment, and action because of its benefits to communities and as it can contribute to biodiversity conservation goals. Within this context, nature-based solutions, or solutions that harness nature and natural processes to alleviate well-defined societal challenges by delivering multifunctional solutions as part of governance and business, can be prioritised to expand existing green infrastructure while addressing climate resilience, natural hazards, urbanisation and environmental quality, inequalities in availability and access to green space, public health concerns, and biodiversity loss. However, the mainstreaming of nature, and nature-based solutions as socio-ecological-technological innovations, in urban planning and decision-making in cities remains underexplored. Through case-study analyses, this presentation will analyse barriers and enablers to mainstreaming nature in decision-making and promoting nature-based solutions uptake and evaluates their current deployment to address societal challenges and sustainable development goals. Current bottlenecks include knowledge gaps regarding the scope, cost-effectiveness and benefits arising from nature-based solutions, and limited practical experience with implementation. The effectiveness of current urban green space management in providing benefits to communities, and opportunities for mainstreaming nature in policy and planning to address inequalities in availability and access to urban nature, are also discussed. Finally, the presentation will draw on the case-study analyses and literature to explore strategies for mainstreaming nature-based solutions in policy-making and urban green space management in response to biodiversity and sustainability objectives.

A wall of opportunities: from adaptation to exploitation

Zoë A. Popper
Botany and Plant Science, School of Natural Sciences, and The Ryan Institute for Environmental, Marine and Energy Research, College of Science and Engineering, National University of Ireland Galway, Galway, Ireland
Zoe.popper@nuigalway.ie

Possession of a polysaccharide-rich cell wall is the primary unifying feature of Eukaryotic photosynthetic organisms. However, brown algae, glaucophytes, red and green algae, and land plants, last shared a common ancestor more than 1,600 Million Years Ago, and have diversified considerably in that time. Cell walls are highly dynamic and play important roles the biomechanical properties of the cell and plant, and are central to physiology and development. The fact that cell walls are principally composed of polysaccharides is key to their properties, adaptability, and exploitation. Over ten monosaccharide residues commonly occur in plant and algal cell walls, and each can be present in different forms e.g. isomers and enantiomers, modified by e.g. acetylation and methylation, and linked to different sugar residues, at different positions. A frequently cited example of how carbon chemistry enables diversity is that 1892 distinct tetrasaccharides can be made from a single sugar residue in ring form. Changes in cell wall composition occur during development and in response to stress. Cell wall composition may also differ between different plant organs, tissues, cell types; differences are even observed at the subcellular level. However, these differences are underlain by the plant's biosynthetic machinery, meaning that diversity is fundamentally controlled at taxonomic level. Evolution has resulted in a wide range of polysaccharides, sustainable resources, with varying properties. Many cell wall-derived polysaccharides are commercially valuable e.g. in food, feed, and textile industries, with current research is unveiling further potential applications.

Building on interactions between the professional and non-professional mycological communities to advance fungal conservation

Gregory M. Mueller
Negaunee Institute for Plant Conservation Science and Action, Chicago Botanic Garden, Glencoe USA, 60022 and IUCN SSC Fungal Conservation Committee

Fungi occur throughout the world in virtually every habitat, natural and human dominated. They play essential roles as decomposers; mutualists of plants and animals; plant and animal pathogens; and important food resources for humans and other animals. Even so, they are rarely considered by the conservation community or in conservation actions even though they are not immune to the stressors that threaten animals and plants including habitat loss, over harvesting, and climate change. Preserved specimens housed in natural history collections, along with their associated metadata, are critical resources for documenting species diversity and distributions. However, the data in these collections are too incomplete to provide an accurate estimate of the distribution, population size, or change in population size of most fungal species. Unfortunately, the relatively small number of professionally employed mycologists cannot generate and compile the information needed by themselves. Because fungal diversity and distributions are still poorly documented, there is great opportunity for discovery. Thus, amateur, and other citizen scientists can make significant contributions. There is a long history of amateur mycologists and other field naturalists contributing important data, but for the most part, their efforts have not been coordinated and the results of their findings were often not captured. Websites like iNaturalist now provide a platform to capture observation data. On 1 June 2022, iNaturalist had over 6 million fungal observations posted by 500,000 observers! However, the quality of these observations and the metadata provided varies greatly. This variance in quality limits the utility of the data on these sites for documenting diversity, distributions, and plant and habitat associations of fungi. Additionally, the data being compiled on these sites are for the most part random observations, and efforts are not coordinated to target gaps in knowledge on species of interest. Several recent initiatives are addressing these issues, e.g., Australia's *fungi*map, UK's *Lost and Found Fungi Project*, and the US's Fungal Diversity Survey (FunDiS). In the US, FunDiS integrates three core citizen science projects – focused fieldwork targeting species of interest, curating a database of high-quality observations, and facilitating DNA sequencing of targeted species. Such citizen science initiatives provide data of high utility for documenting fungal distributions and fungal species of conservation concern. The high-quality data from these initiatives have been used in red list assessments, to refine lists of species of conservation concern used by land managers, to formulate conservation action plans, and to highlight the diversity and conservation need of fungi to the public. It is important to have continued conversations among these inititives and the conservation/research community to agree on data and data standards, and manage expectations, processes, and timelines to build on the potential of these citizen science initiatives.

Plant tannin analytics and its importance to understanding tannin bioactivity

Juha-Pekka Salminen
Department of Chemistry, University of Turku, Finland

Tannins are one of the most common groups of specialized metabolites in the plant kingdom. They show perhaps also the most complex structures and for that reason they may be a bit underestimated or non-understood from their biological activity and chemical diversity points of view. Especially their analytics can be demanding, since one plant species may produce hundreds of different tannin structures. In my talk, I will give an overview of the different types of tannin families found in the plant kingdom, and the analytical challenges they may require us to overcome. In addition, I will give insights into how tannin analytics as such could be more efficiently used to estimate both the tannin diversity but also tannin bioactivity in the species that are or could be scientifically important for our research.

COMMUNICATIONS

Vegetation survey and mapping of Lake Doberdò: an analysis for the assessment of habitat conservation status of a disappearing lake of the Classical Karst (NE Italy)

Miris Castello, Alfredo Altobelli, Francesco Liccari, Giovanni Bacaro, Livio Poldini
Department of Life Sciences, University of Trieste, Via L. Giorgieri 10, 34127 Trieste, Italy

Lake Doberdò is a well-known example of karst lakes, temporary lakes that seasonally fill and empty through springs and swallow holes connected to the underground waters. It is one of the few karst lakes in Italy and one of the most important examples of karst hydrology at the international level.
It is an area of exceptional interest for geological-geomorphological and biodiversity conservation, and it is part the Regional Nature Reserve of Doberdò and Pietrarossa Lakes and of the Natura 2000 network. Its peculiar hydro-ecological nature allows an impressive variety of species and habitats also of high conservation value, thanks to its strong ecotonal character. However, plant biodiversity and habitats are currently threatened by several factors: a reduction of the water level, the abandonment of management of the water body, the spread of invasive alien plant species.
A research project focused on the vegetation of the karst lake was recently started to provide updated basic knowledge essential for the assessment of the conservation status of its habitats, their monitoring and the planning of conservation strategies to be undertaken also in response to the rising problem of climate change that is threatening wetlands at the global level. The research was based on the following main objectives: a) vegetation analysis based on the phytosociological approach to identify plant communities and Annex I habitat types; b) vegetation mapping of plant communities and habitats, using high resolution drone orthoimagery as a baseline and fieldwork; c) a temporal comparison of the vegetation of the area after 50 years, based on a vegetation survey carried out in the 1960s; d) the identification of the most frequent alien species and the most affected habitats.
In the area liable to seasonal high water (ca. 47 ha) 43 phytocoenoses were found, and 21 communities are attributed to 8 Annex I Habitats of the 92/43/EEC Directive. The particular hydrological features, the resulting ecotonal nature and the high value for biodiversity of the disappearing lake mirror the fundamental essence of habitat 3180* modelled on Irish turloughs, allowing to refer Lake Doberdò to this priority habitat type. Compared to the past, various communities show a clear change in their distribution while some valuable coenoses were not found.
The vegetation survey confirms the high value of Lake Doberdò for biodiversity conservation but highlights an ongoing process of environmental change due to both natural and human-related causes including modifications of the hydrological regime and abandonment of traditional farm practices and claims the need for active conservation interventions for the preservation and the recovery of such a precious natural area.

Project FORESTALL: connecting habitat restoration to social inclusion in the Ramsar site of Valle Averto, southern lagoon of Venice (N-Adriatic Sea)

Marco Canella[1], Marco Baldin[2], Simona Bardi[3], Stefano Borella[3], Pierpaolo Campostrini[4], Isabel Cavalli[2], Vanessa Cazzin[1], Licurgo Chiorboli[5], Chiara Dall'Angelo[4], Francesco Gervasutti[1], Francesco Marcone[3], Alessandro Meggiato[4], Enrico Rinaldi[4], Francesco Scarton[2], Valerio Volpe[5]
[1]Cooperativa Sociale Primavera, Via Guglielmo Marconi 33, 30035 Mirano (VE); [2]SELC Società Cooperativa, Via dell'Elettricità 3/d, 300175, Venezia; [3]WWF Italia, Via Po 25/c, 00198 Roma; [4]CORILA, Consorzio per il coordinamento delle ricerche inerenti al sistema lagunare di Venezia, San Polo 19, 30125 Venezia; [5]Provveditorato Interregionale per le Opere Pubbliche per il Veneto, Trentino Alto Adige e Friuli Venezia Giulia, Rialto 19, 30125 Venezia

Project FORESTALL is funded by European Union's Programme for the Environment and Climate Change (LIFE). Conservation actions took place in the WWF oasis of Valle Averto, in the Southern Lagoon of Venice. The area has been ascribed to the "Wetlands of International Importance" of the Ramsar Convention since 1989. Moreover, Valle Averto is part of a Special Area of Conservation (IT3250030) and a Special Protection Area (IT3250046). Habitat restoration actions are currently ongoing and include: hydraulic works to improve water circulation and quality; protecting and increasing 7210* and 91E0* habitat, reduction of plant invasive species occurrence. An additional purpose of FORESTALL is to connect nature conservation to social inclusion and local policies. All the mentioned field operations involve disadvantaged people, as workers of the project partner Cooperativa Sociale Primavera ONLUS. Efforts spent on work-related activities by disadvantaged people are expected to reach 1000 working hours up to the end of the project.
Restoration in wet habitats is strongly linked to water circulation and water table depth. Hydraulic works are a key action to allow the management of water regime in the net of channels and water ponds of Valle Averto. To do this, nine sluice gates have been placed and 220 meters of channels have been dredged. In addition, 500 meters of channel banks will be protected from erosion by the setting of fascines. Before planting, topsoil removal has been performed in most project areas from a depth of 10 cm up to 60 cm. These earth movements have the double purpose of 1) guaranteeing the periodic flooding of the restored areas and 2) removing organic matter in excess.
Considering 7210* habitat, 14300 seedlings have been planted for a total area of 6.19 ha. *Cladium mariscus* (L.) Pohl is the main species (10000 seedlings), while additional species are *Carex elata* All., *Lycopus europaeus* L., *Iris pseudacorus* L., *Euphorbia palustre* L., *Jacobaea paludosa* (L.) G. Gaertn., B. Mey. & Scherb., *Stachys palustris* L. (4400 seedlings overall).
About 5800 tree seedlings have been planted for the restoration of alluvial forest habitat 91E0* for a total area of 11.6 ha. Tree species are *Fraxinus angustifolia* Vahl subsp. *oxycarpa* (M. Bieb. ex Willd.) Franco & Rocha Afonso, *Alnus glutinosa* (L.) Gaertn., *Ulmus minor* Mill.. Additional shrubs seedlings have been planted as e.g.: *Salix cinerea* L., *Viburnum opulus* L., *Crataegus monogyna* Jacq., *Rhamnus cathartica* L., *Euonymus europaeus* L. (1500 seedlings overall). Moreover, herbaceous seedlings complete the habitat restoration: *Carex elata* All.*C acutiformis* Ehrh., *Iris pseudacorus* L. (8200 seedlings overall).
In parallel with habitat restoration, eradication of invasive plant species has been performed. The target species are: *Baccharis halimifolia* L. and *Robinia pseudoacacia* L.. Both the species present competitive traits as: reshooting, vegetative propagation, high production of seeds, capability to grow in oligotrophic soils. A seasonal control of re-shooting is critical for eradication and is currently ongoing. Concerning *R. pseudoacacia*, 885 mature plants have been cut down and substituted by 4070 seedlings of endemic species. *B. halimifolia* is a recently introduced alien species for North East Italy and studies for its control in the Venice Lagoon, as well as in the whole Italy, are still lacking. Five eradication methods are currently in testing: log cut, winter and summer manual eradication, phytosanitary treatment pure or diluted.
Maintenance activities will carry on up to the end of the project in November 2023, both for habitat restoration and alien species eradication. Maintenance foresees: cut the re-shooting of alien plants and eradication of eventual new individuals found, conservational mowing in the planting areas, replacing of eventual death seedlings due to physiological mortality (expected 10-20%). Preliminary observations suggest the restoration success of project areas depends to: depth of topsoil removal, pre-existing vegetation, growth stage of seedlings, frequency and method of conservation mowing.

The landscape of Central Apennine wooded grasslands: space-time dynamisms and future perspectives

Giulio Tesei[1], Paride D'Ottavio[1], Mattia Di Silvestri[1], Demetra Giovagnoli[1], Diego Giulietti[1], Debora Solforati[1], Laura Trozzo[1], Matteo Francioni[1], Marco Bianchini[1], Giuliano Bonanomi[2], Marina Allegrezza[1]
[1]Department of Agricultural, Food and Environmental Sciences, Marche Polytechnic University, Via Brecce Bianche, 10, 60131 Ancona, Italy; [2]Department of Agricultural Sciences, University of Naples "Federico II", Via Università, 100, 80055 Portici (Napoli), Italy

Wooded grasslands are agroforestry systems maintained through grazing, haymaking and silviculture and represent distinctive socio-cultural local traditions and economic histories. The under-use and abandonment of the traditional management practices in the Mediterranean basin, especially in mountain marginal areas, have favored afforestation of the grasslands. It is therefore important to identify actions for their restoration and conservation as also underlined by the inclusion of the "Temperate wooded pasture and meadow" habitat within the European Red List of Habitats and qualified as vulnerable (Red List Criteria A1). The objectives of the study, conducted in a protected area of the Central Apennines, were: i) to describe the floristic and ecological characterization of the wooded grasslands in relation to different management practices; ii) to assess the role of the wooded grasslands in the dynamic processes of afforestation following the abandonment of the traditional management practices; iii) to define management options for the restoration and conservation of this peculiar Apennine grassland landscape. Three wooded grassland sites under three different use intensities were selected in the Monte San Vicino and Monte Canfaito Special Protection Area (IT5330025 SPA): medium (mowed and grazed), low (grazed), abandoned (not used). For each site, four isolated beech (*Fagus sylvatica*) trees on the grassland were selected. For each tree, whose structural parameters were measured, floristic and vegetation along transect in the four cardinal points, with contiguous 1 m^2 plot starting from the trunk up to 2 meters outside the projection of the canopy, were assessed. In each plot, vascular species with their relative percentage coverage were listed. The filtered light available on the ground, the height of the shrubs and the distance of the crown branches from the ground were also measured. The plots were then grouped in four positions along the transect: T for plots close to the trunk, IN for plots under the canopy, E for plots falling within the crown projection and P for plots outside the canopy projection. The results highlighted that the amount of the filtered light available on the ground represents one of the main driving factors of the floristic gradient from the T to P position. This condition determines the presence, under the canopy, of three vegetation belts with specific ranges of ecological variables and floristic bioindicators. The P position recorded the highest floristic and structure variations in relation to the use intensity: herbaceous species dominate in the sites under low and medium intensity, while shrubs dominate in the abandoned site. A significant floristic variation of herbaceous species was also found in the E position of managed wooded grasslands: grasslands species for the medium intensity and heliophilous edge species for low intensity.
The presence of three vegetation belts under the canopy, that are in spatial and dynamic connection with each other, suggests that if grazing (i.e., low use) were stopped, conditions would be created for rapid afforestation of the grasslands starting from the ecotone E position. Thus, the floristic-vegetation monitoring has to be concentrated in the ecotone position, also providing indications for the modalities (e.g., mowing) useful for the restoration and conservation of the wooded grasslands.

Urban green infrastructures design for the ecological and landscape restoration: the case of the Metropolitan City of Reggio Calabria (southern Italy)

Carmelo Maria Musarella, Silvio Bagnato, Giuseppe Bombino, Pasquale Antonio Marziliano, Giuseppe Modica, Andrea Rosario Proto, Giovanni Spampinato
Department of AGRARIA, Mediterranea University of Reggio Calabria, Località Feo di Vito, 89122 Reggio Calabria, Italy

The Metropolitan City of Reggio Calabria and the Department of Agraria of the Mediterranea University of Reggio Calabria have started from the year 2020 a technical and scientific collaboration for the planning, financing and designing of urban forestry activities. In particular, the aforementioned working group is now dealing with a forestation plan of the hill of the Department of AGRARIA ("AGRARIA's hill").
This project is based on the principles of restoration and natural enhancement as a strategic factor for recovering the functionality of the urban ecosystem. The creation of a forest with native species is envisaged, constituting a biotope with the typical physiognomy of the Mediterranean environment. The choice of this area for urban forestation interventions regulated by the "Experimental Program for Urban Reforestation", falls within a perspective of the redevelopment of peri-urban spaces near the inhabited centre of Reggio Calabria, which will allow to carry out the recovery of urban/rural fringe areas which at present are continuously subject to anthropic disturbance (grazing, fires, illegal landfills, etc.).
In this context, a forest will represent the project's final result that creates a morphological and ecological continuity between the lowland system and the lines of the hilly landscape that "close" the intervention area to the east and north. Indeed, there is a short distance between this site and the "Collina di Pentimele" SAC (IT9350139), with which the "AGRARIA's hill" can create a valuable ecological functional connection and aid to the landscape defragmentation (in agreement with the National Biodiversity Strategy, the European Green Infrastructure Strategy and the Habitats Directive).
The presence of residual forests of both natural and artificial origin causes the fall of the reforestation project within the pre-existing plant communities in order to enhance its connective and ecological role with the local natural context. In particular, there are residual oak forests with Quercus pubescens Willd. subsp. pubescens [= Q. virgiliana (Ten.) Ten.] mixed with Ampelodesmos mauritanicus (Poir.) T. Durand & Schinz (Fig. 1).
The forestry activity is based on the vegetational approach and on pedological, bioclimatic and morphological analyses of the area. In particular, the structural and ecological functionality of the plant communities already present are analysed, as well as their dynamic series with respect to the climax that will be obtained.
Therefore, the creation of a Mediterranean biotope is envisaged, with the aim of: a) reviving the area currently characterized by grasslands with A. mauritanicus (Fig. 2) favoured by continuous fires; b) increasing the CO2 absorbing surface; c) creating forest communities and Mediterranean fringe scrubs, useful for regenerating natural forest environments. Specifically, in order to create a habitat of environmental, landscape and naturalistic value, autochthonous plant species of the Mediterranean basin will be selected whose ecological, physiological and mechanical characteristics are compatible with the environmental conditions of the site and with the need to maximize the effectiveness of the intervention and minimize the commitment and costs of plant care. The phytocoenoses that will characterize the interventions, in addition to guaranteeing reliability and high operating performance during the life of the work, will be consistent with the environmental context: just enough not to create deformations, decompositions, loss of identity and character of the landscape.

Fig. 1. Residual oak forest with *Quercus pubescens* subsp. *pubescens*

Fig. 2. *Ampelodesmos mauritanicus* grassland

Plant conservation and restoration in the coastal wetlands of Sicily: the case studies of the Natura 2000 sites "Saline di Priolo" and "Pantano Ponterio"

Pietro Minissale[1], Salvatore Cambria[1], Fabio Cilea[2], Gianpietro Giusso del Galdo[1], Gianmarco Tavilla[1], Giovanni Spampinato[3], Saverio Sciandrello[1]
[1]Department of Biological Geological and Environmental Sciences, University of Catania, Via A. Longo 19, 95125 Catania, Italy; [2] Lipu, Riserva Naturale Orientata "Saline Di Priolo" C.da Pantano Pozzillo 96010 Priolo Gargallo (SR)
[3]Department of Agricoltura, Università Mediterranea di Reggio Calabria, Località Feo di Vito, 89122 Reggio Calabria

Plant community restoration is a hot topic right now, and it provides a challenge for vegetation scientists. Scholars are constantly seeking for new approaches to carry out restoration activities based on potential vegetation and ecological features of a territory. In the case of wetlands, several factors are at play, including not only the climate and the soil but also the level and quality of water, potential management, etc. The cases presented here concern two coastal wetlands in Sicily, the Pantano Ponterio in eastern Sicily, which is part of the "Pantani della Sicilia sud-orientale" SPA and the Saline di Priolo nature reserve, also a Natura 2000 site. The first site was restored a few years ago as part of a European project SIMBIOTIC (P.O. Italia Malta 2007-2013), and the second is currently being restored as part of a project on wetlands (MedIsWet) funded by the MAVA foundation.
In the first study area, the problems to be overcome essentially concerned the removal of waste, the restoring the hydraulic functionality and the subsequent planting of species suitable for the site obtained from locally collected seeds, taking into account the micro-zonation of the vegetation belts. For the second area the main challenge is the spread of alien species and we are attempting to control their occurrence, particularly that of *Acacia saligna* (Labill.) H.L. Wendl. and *Lantana camara* L. Each restoration action was preceded in both cases by an accurate flora and vegetation survey in order to focus on the existing plant communities, or to be rebuilt, according to the identified vegetation potential, followed by a restoration plan based on the processed data.
In the case of Pantano Pontiero, eight years after the interventions, monitoring highlighted that the vegetation had recovered well despite the absence of management authority. In the example of Saline di Priolo, which was carefully managed by the LIPU, it was possible to see how the control of alien species requires massive resources and, above all, a continuous control effort following the primary intervention to neutralize the regrowth and the emergence of new seedlings. Especially in the latter case, which is part of a territory heavily degraded by extensive industrial activities with a high environmental impact, these restoration activities serve as means of engaging the community, local administrators, and economic actors in environmental recovery issues. Finally, the constant commitment of the site managers has led to a strong consensus and support by the local stakeholders for the renaturation and rewilding of this area.

Restoration of dry-acidic grasslands and heathlands in the western Po Plain (northern Italy – Life Drylands project)

Silvia Assini1[1], Gabriele Gheza[2], Juri Nascimbene[2], Alessandro Chiarucci[2], Giovanna Pezzi[2], Matteo Barcella[1]
[1]Department of Earth and Environmental Sciences, University of Pavia, Via S. Epifanio 14, 27100 Pavia, Italy; [2]BIOME Lab, Department of Biological, Geological and Environmental Sciences, Alma Mater Studiorum – University of Bologna, Via Irnerio 42, 40126 Bologna, Italy

Open dry-acidic habitats, protected in Europe through the Habitats Directive (92/43/EEC), are increasingly rare and threatened throughout Europe. In the Po Plain, they are in a particularly critical situation: the high anthropic impact, the atmospheric nitrogen depositions and the lack of management led to their fragmentation, degradation and, in many cases, their disappearance. Here, these habitats (1) have a high phytogeographical value, being at the southern limit of their European distribution range, where central European, Atlantic, Mediterranean and steppe plants mix, and (2) host communities rich in terricolous lichens, which at low altitudes are now exclusive to these habitats.
The "LIFE18 NAT/IT/000803 DRYLANDS" project was proposed with the aim of restoring 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*) (*important orchid sites)" acidophylous subtype) in eight Natura 2000 sites in the western Po Plain.
The ecosystem references were defined on the bases of previous phytosociological studies and ecological knowledge. For the H2330, the association *Spergulo vernalis-Corynephoretum canescentis* (R. Tx. 1928) Libbert 1933 (Assini et al. 2013) was identified as reference ecosystem, also considering the terricolous lichen associations reported from this habitat (Gheza et al., 2016). For H4030, the associations *Jasiono montanae–Callunetum vulgaris* Cerabolini, Brusa, Ceriani, Armiraglio, De Molli & Pierce 2017 (Lombardy heaths) and *Salici rosmarinifoliae–Callunetum vulgaris* Cerabolini, Brusa, Ceriani, Armiraglio, De Molli & Pierce 2017 (Piedmont heaths) were identified as reference ecosystems (Cerabolini et al., 2017). For H6210 (acidophilous subtype), the *Koeleria pyramidata* and *Artemisia campestris* community and the *Chrysopogon gryllus* and *Armeria plantaginea* community (of the *Koelerio-Phleion phleoidis* Korneck 1974) (Ticino River) were mainly identified as reference ecosystems (Assini and Sartori, 2004), together with the association *Poo bulbosae-Festucetum trachyphyllae* Lonati & Lonati 2007 (Sesia River) (Lonati and Lonati, 2007). The *Thero-Airion* Tüxen ex Oberdorfer 1957 associations, which often form a mosaic with the *Koelerio-Phleion* communities (Assini and Sartori, 2004), and the terricolous lichen communities reported from this habitat (Gheza et al., 2019), were also considered.
A dynamic approach was applied to restore the vertical and horizontal structures of the target habitats. It allows to preserve: pioneer aspects (bare soil and soil biological crusts); typical aspects (perennial herbs/forbs and/or dwarf shrubs); and mature aspects (dense shrubby patches at the contacts with the forest communities). This approach is consistent with the dynamic nature of the Po Plain, that hosts the target habitats, which is the result of different past and present causes, both natural (river dynamics and natural vegetation dynamics) and human (unpredictable land use). It thus takes into account the processes driving the formation of the target habitats, and results in higher biodiversity; in comparison with a static approach that preserves only one aspect of the target habitats, and results in lower biodiversity. The main used techniques included: mowing and removal of herbs (because the target habitats are oligotrophic); cutting back of native woody species, maintaining the larger specimens, under which ecotone species can refuge; sod cutting and top soil inversion (to create pioneer conditions and reduce the nutrients in the soil).
Other acitivities aimed to restore the floristic composition of the target habitats included: (1) removal/reduction of invasive woody species, by means of: cutting, removal of stumps, and stem injection with plant protection products, and (2) improvement of the floristic composition, planting typical habitat species produced by specialized nurseries.
Furthermore, new patches of the target habitats were created ex-novo using suitable propagation material (surface sands rich in the seeds of typical species for H2330; harvested seeds for H6210; cuttings of *Calluna* for H4030).
As part of the project, a sample design was defined aimed at the *ex-ante* and *ex-post* monitoring of the vegetation in the intervention areas, included in 26 patches, where 99 circular plots of 3 m radius were analyzed, collecting data about vegetation structure and % cover of vascular plants, bryophytes and lichens. The comparison between *ex-ante* and *ex-post* data will allow to preliminarly assess the success of the restoration actions.

Seed conservation and development in metallicolous and non-metallicolous orchid populations

Pierluigi Cortis, Antonio De Agostini, Donatella Cogoni, Annalena Cogoni, Giuseppe Fenu
Department of Life and Environmental Sciences, University of Cagliari, Via Sant'Ignazio da Laconi 13, 09123 Cagliari, Italy

Mining has been considered one of the principal sources of environmental pollution worldwide. Mines have a substantial impact on local and regional environments during the production phase and even after their closure and abandonment they may continue to degrade surrounding land, water bodies and air. The management of abandoned mines is challenging yet urgent and represents an often-unresolved environmental problem. The reclamation of abandoned mines could however endanger the conservation of peculiar and rare metallicolous flora settling there. This could be the case of the heavy metal polluted tailing dump of Barraxiutta (Domusnovas, South-Western Sardinia, Italy), which hosts a metallicolous population of *Epipactis tremolsii* (Orchidaceae). In this pilot study, we evaluated the suitability of seed packet-based field experiments to explore different conservation options for this population. Four different sowing treatments (full crossed experimental design) were set up in the tailing dump and in a near unpolluted site (control site). The field phase of the experiment lasted 10 months in which the experimental seed bank preservation and incipient seed development were observed and tested by parametric or non-parametric tests corrected for multiple comparisons and by analysis of variance. For the first time the seed packet technique was proved to be suitable to explore orchid seed bank conservation and incipient seed development in extreme, post-mining environments. The 10-month field phase duration was appropriate to test seed bank persistence and incipient, non-mycorrhizal, seed development in polluted tailing dump substratum, nevertheless a longer field phase is recommended to explore seed-soil fungi interactions. The site-specificity in seed development dynamics that emerged in the study case needs to be considered in future restoration approaches to the tailing dump orchid population: seed-based conservation approaches should employ metallicolous seeds. The validation of this field technique will be a valuable tool in the present study case and in similar ecological issues to evaluate the better conservation options via translocation and quasi in-situ approaches.

Riparian vegetation management along artificial channels: first effects on plant community ecology of "Life Green4Blue"

Benedetta Gori[1], Carla Lambertini[2], Alessandra Zambonelli[1]
[1]Department of Agriculture and Food Science, University of Bologna, Viale G. Fanin 40-50, 40127 Bologna, Italy; [2]Department of Bioscience, University of Milan, Via Celoria 26, 20133 Milan, Italy

Lowland alluvial areas are nowadays among the most disturbed and human-modified landscapes, because of intensive agriculture, industrial and urban activities. Anthropogenic pressures seriously threaten the few still existing natural and semi-natural ecosystems, which are affected by fragmentation, biodiversity loss, water pollution and eutrophication. The Po Plain within the provinces of Bologna and Ferrara is an area that suffers such an anthropogenic pressure: the main land use is agriculture, and urban and industrial settlements are scattered in the agricultural landscape, together with hunting wetlands and some isolated Natura 2000 protected sites.
The "Life Green4Blue" project aims at increasing the ecosystems services provided by the artificial network of channels that was traditionally built to ensure irrigation water to agriculture and hydraulic safety. Thanks to this Life project, the channels are supposed to turn into "green-blue infrastructures" (*sensu* EU Biodiversity Strategy 2020) improving connection among seminatural habitats and alleviating the effects of habitat fragmentation and biodiversity loss.
For this purpose, a new vegetation management along the artificial channels has been experimented in the project area for the past two years (since 2020) with the aim of replacing the traditional clear-cut of the banks with a "target mowing". This new technique consists in leaving a strip of vegetation close to the water, corresponding to the ecotonal belt, to allow aquatic and riparian vegetation to grow, but it also controls the establishment of woody vegetation that can decrease hydraulic safety. The upper part of the banks, usually close to fields and tracks, is kept mown, so that water managers are guaranteed a safe and easy access to channels banks for network maintenance.
The first effects of the new mowing management on plant communities were investigated with floristic surveys aimed at detecting ecological changes in species composition and cover compared with traditionally mown sites. Ecological changes were recorded as changes in biological forms, species' most common habitat types and Grime's adaptive strategies of plant communities. Overall, differences between target-mown and traditionally mown banks were still modest, as the existing plant community is adapted to decades of clear-cut mowing, disturbance and high nutrient loads in soil and water. The dominant biological forms were, in fact, hemicryptophytes, geophytes and therophytes even in target mowing, typical of plants adapted to above-ground biomass removal. Nevertheless, in target-mown sites, the number of species of each biological form was higher than in traditionally mown sites, indicating that the interruption of mowing has allowed the existing community to fully develop. Additionally, in target-mown sites, the number of aquatic and hygrophilous plants increased, confirming a lower pressure on ecotonal vegetation as promoted by the project. Changes were also detected in the adaptive strategies of the plant community. At target-mown sites, the number of competitive plants in nutrient-rich environments ("C-plants") decreased, while stress-tolerant plants adapted to nutrient limitation and/or disturbance ("S-plants") increased: such a shift from adaptation to eutrophication and disturbance to stress-tolerance can perhaps be considered the very initial phase of the secondary succession. On the other hand, the control of woody vegetation prevents the evolution of the green-blue infrastructure towards the climax community of this area, i.e., the floodplain forest. This makes the infrastructure unstable and dependent on human management.
Differences in cover and species composition were statistically significant. Some species, like *Phragmites australis*, occurred only in target-mown sites all year around. At these sites, occasional bare soil spots were present due to bank landslides, whereas traditionally mown sites had a higher percentage of bare soil, especially in winter when all vegetation is clear-cut and erosion risk is high. *Phragmites australis* was dominant at all target-mown sites and determined, through competition, which species could co-exist in the infrastructure during the vegetative and the winter seasons. This made all target-mown sites plant communities more similar in species composition to each other than were traditionally mown site communities.
The results of these very first analysis of vegetation communities is only the first step of a longer monitoring programme, which will be carried out for the whole duration of "Life Green4Blue" project. The target-mowing technique is currently applied to some of the artificial canals on an experimental basis, but is intended to become the ordinary vegetation management of most of the channels in that area.

Rewilding vs. cultivation, what plants can tell us about demography after pandemics

Alessia Masi[1,2], Cristiano Vignola[1,2], Lucrezia Masci[3], Jordan Palli[4,5], Gianluca Piovesan[5], Adam Izdebski[2], Laura Sadori[1]

[1]Department of Environmental Biology, Sapienza University of Roma, Piazzale A. Moro 5, 00185 Roma, Italy; [2]Paleo-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 Roma, Piazzale A. Moro 5, 00185 Roma, Italy; [4]Department of Agriculture and Forest Sciences (Dafne), SS675, 01100 University of Tuscia, Viterbo, Italy; [5]Department of Ecological and Biological Sciences (Deb), University of Tuscia, Largo dell'Università, snc, 01100, Viterbo, Italy

Pandemics strongly impact populations in the past, and today as well. Above all the Black Death (1347–1352 AD) affected not only the demography but also the social economic history of Europe. The current knowledge of the effects of this pandemic is limited to the fragmented written sources, available for some areas of Europe, and some pioneer studies on ancient DNA. Since in historical times the connection between human impact and vegetation is extremely strict, the study of vegetation changes can be used as a tool for better appreciate such events and their consequence in terms of land use. We applied a pioneering approach named 'big data palaeoecology', based on fossil pollen data, to evaluate the landscape impact of the Black Death on a regional scale across Europe. Palynological data from 261 coring sites (lakes and wetlands), located in 19 present-day European countries, have been selected after checking quality and resolution of the chronologies. We evaluated whether changes in the landscape reconstructed at the time of the Black Death agree with the hypothesis that a large portion of the population, upwards of half, died within a few years in the 21 historical regions we studied.

In particular, we grouped pollen in four indices supported by Ellenberg light indicator and Niinemets and Valladares shade tolerance scale. These indices describe (1) landscape with high human pressure: *Cereals*, (2) evidence of pastureland: *Herding*, (3) abandonment of the land: *Fast succession,* and (4) rewilding: *Slow succession*. The assumption is that cultivation testifies the highest level of human presence in a territory. A strong reduction in population brings to the reorganization of the land as pasturelands, that require less workforce, or to the abandonment at different scales with a consequence increase of tree pollen. The indices have been compared before and after the pandemic arrival with different time extent. In fact, we checked for possible changes in land management occurred 50, 100 and 150 years before and after the pandemic. We found that in some regions the Black Death had a devastating impact with a clear decrease in cereal cultivation. On the other hand, some regions had negligible or no impact with an increase both in *Cereals* and *Herding* indices. It is interesting to note that the increase in cultivation is often linked to the reduction of arboreal pollen but the land abandonment brings to the rewilding only in sparse regions (Fig. 1). This aspect could be useful for the conservation experts in the research of the historical baseline of ecosystems for the purposes of restoration ecology. Such an inter-regional difference in the Black Death's mortality as well as in the land transformation demonstrates the significance of cultural, ecological, economic, societal and climatic factors that mediated the dissemination and impact of the disease. This successful result has been the starting point for a new investigation on the Justinian plague (541–549 AD), for which the database has been improved in terms of both chronological resolution and number of sites.

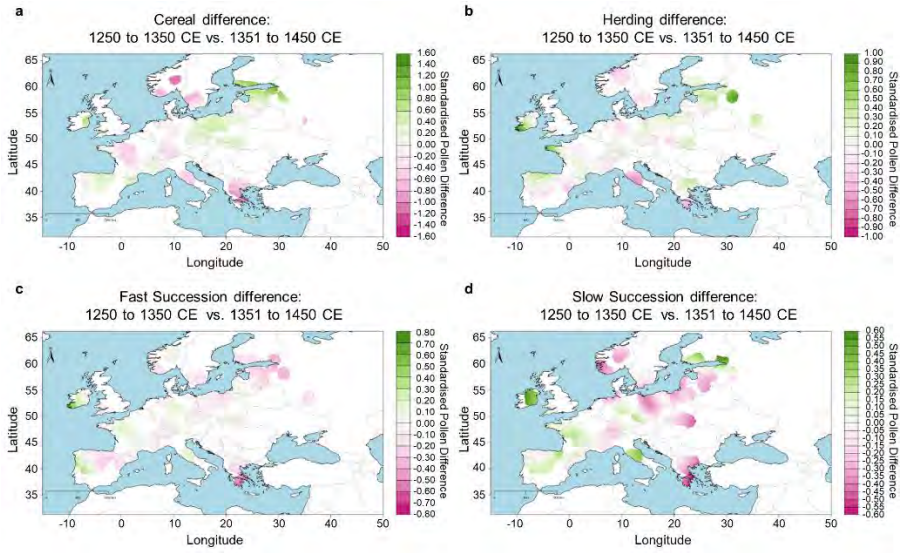


Fig. 1. Regional extrapolation of Black Death impact showing the increase (green) or decrease (magenta) of the pollen indicators in the comparison of the 100 years before (1250–1350 AD) and after (1351–1450 AD) the pandemic (Izdebski et al. 2022. Nature E&E, 6(3), 297-306).

Global patterns of vascular plant alpha diversity

Francesco Maria Sabatini[1,2,3], Borja Jiménez-Alfaro[4,2], Ute Jandt[3,2], Milan Chytrý[5], Richard Field[6], Michael Kessler[7], Jonathan Lenoir[8], Franziska Schrodt[6], Susan K. Wiser[9], Helge Bruelheide[3,2], the sPlot Consortium
[1]BIOME Lab, Department of Biological, Geological and Environmental Sciences (BiGeA), Alma Mater Studiorum University of Bologna, Via Irnerio 42, 40126, Bologna, Italy; [2]German Centre for Integrative Biodiversity Research (iDiv), Halle-Jena-Leipzig, Puschstr. 4, 04103 Leipzig, Germany; [3]Martin Luther University Halle-Wittenberg, Institute of Biology/Geobotany and Botanical Garden, Am Kirchtor 1, 06108 Halle (Saale), Germany; [4]Biodiversity Research Institute (CSIC/UO/PA), University of Oviedo, Campus de Mieres, Gonzalo Gutierrez Quiros 33600 Mieres, Spain; [5]Masaryk University, Faculty of Science, Department of Botany and Zoology, Kotlářská 2, 611 37 Brno, Czech Republic; [6]University of Nottingham, School of Geography, University Park, NG7 2RD, Nottingham, UK; [7]University of Zurich, Systematic and Evolutionary Botany, Zollikerstrasse 107, 8008, Zurich, Switzerland; [8]UMR CNRS 7058 "Ecologie et Dynamique des Systèmes Anthropisés" (EDYSAN), Université de Picardie Jules Verne, 1 Rue des Louvels, 80037, Amiens Cedex 1, France; [9]Manaaki Whenua - Landcare Research, Ecosystems and Conservation, 54 Gerald Street, 7608, Lincoln, New Zealand

Global patterns of regional (‘gamma’) plant diversity are relatively well known, but whether these patterns hold for local communities, and the dependence on spatial grain, remain controversial. Based on 170,272 georeferenced vegetation plots, we applied machine learning (boosted regression trees) to model the relationships between vascular plant species richness at different grains and 20 global datasets on current and past climate, soil and topography. We created global maps of alpha diversity (i.e., local species richness) for vascular plants at three different spatial grains, for forests and non-forests.
We show that alpha diversity is consistently high across grains in some regions (e.g., Andean-Amazonian foothills), but regional ‘scaling anomalies’ (i.e., deviations from the positive correlation) exist elsewhere, particularly in Eurasian temperate forests (disproportionally higher fine-grained richness) and many African tropical forests (disproportionally higher coarse-grained richness). The influence of different climatic, topographic and biogeographical variables on alpha diversity also varies across grains. Our multi-grain maps return a nuanced understanding of vascular plant biodiversity patterns that complements classic maps of biodiversity hotspots and will improve predictions of global change effects on biodiversity.

Digitization and geolocation of specimens from Italian lichen herbaria

Stefano Martellos[1], Pier Luigi Nimis[1], Matteo Conti[1], Juri Nascimbene[2]
[1]Department of Life Sciences, University of Trieste, Via Giorgieri 10, 34127 Trieste, Italy; [2]Biome-Lab, Department of Biological, Geological and Environmental Sciences, Alma Mater Studiorum, University of Bologna, Via Irnerio 42, 40126 Bologna, Italy

An exhaustive taxonomic and biogeographic knowledge is important for developing effective conservation strategies targeted to both species and ecosystems. Poor datasets undermine any modeling approach on biodiversity, reducing the reliability of results and, ultimately, preventing the building of awareness on the threts to which biodiversity is exposed. Natural history specimens are an invaluable source of falsifiable data on the presence of taxa in space and time, so that their digitization is fundamental for building a reliable knowledge-base on the changes in biodiversity during the last 2-3 centuries.
While retrieving species occurrence data from digitized natural history collections, some intrinsic challenges need to be addressed, mainly the difficulty of properly geo-referencing *a posteriori* each record, and the need of revising the identification of ancient specimens on the light of recent taxonomic knowledge.
A relevant effort for geo-referencing occurrence records from the digitization of natural history collections of lichens in Italy has recently started. The work was limited to modern herbaria, thus reducing the need of taxonomic investigations for many specimens. Digitized occurrence records of specimens from 11 Italian lichen herbaria were geo-referenced in Decimal Degrees (WGS84). For each record, coordinates and uncertainty of the point were estimated, on the basis of information from the original label. All occurrence records which were already geo-referenced using different reference systems were converted to Decimal Degrees (WGS84). A simplified data-structure was adopted for all herbaria, which will permit an easy updating of the general database by the curators of the individual herbaria.
On May 30, 2022, 58.942 records were geo-referenced. They cover all Italian administrative regions and 76% of the taxa hitherto known to occur in Italy. These data, which will be published online in the form of dot-maps, will be freely downloadable in csv format (following the Darwin Core standard) from a new version of ITALIC, the information system on Italian lichens, released in June 2022.

Identification of multi-species suitable areas for assisted colonization under climate change scenarios

David Dolci[1], Alessandro Chiarucci[1], Lorenzo Peruzzi[2,3]
[1]Centro Interuniversitario per la Biodiversità Vegetale Big Data - PLANT DATA, Department of Biological, Geological and Environmental Sciences, Alma Mater Studiorum University of Bologna, Via Irnerio 42, 40126 Bologna, Italy; [2]Dipartimento di Biologia, Università di Pisa, Via Derna 1, 56126 Pisa, Italy; [2]CIRSEC, Centre for Climatic Change Impact, University of Pisa, via del Borghetto 80, 56124 Pisa, Italy

Climate change represents perhaps the biggest threat for plant conservation in the 21st century. The past century experienced the strongest warming trend of the last millennium due to high levels of greenhouse gas emissions. Average temperature rose by about 0.6 °C, and future temperature rises are likely to be faster than in those observed in the 20th century. Under the pressure of a changing climate, plant species tend to move to higher elevations and latitudes. Numerous studies confirmed that such changes in plant distribution are occurring. Nevertheless, not all species are capable to adapt their distribution to new climatic conditions. Many of the species at greatest risk of extinction are narrow endemics that face insuperable dispersal barriers. A pragmatic conservation strategy, capable to deal with fast change of climatic conditions, is assisted colonization.
In the framework of the studies carried out since more than 15 years by the Working Group for Nature Conservation of the Italian Botanical Society, and taking advantage of the dataset recently made available for 188 Italian endemic species listed in the national IUCN Red List (Casazza et al. 2021), we carried out a study aimed at identifying areas potentially suitable to concentrate the highest possible number of these species. To achieve this, maximum entropy algorithm implemented in Maxent software (v. 3.3.4) was used to calculate the potential distributions of these taxa. All 188 species were modeled using three non-collinear and biologically meaningful climatic variables (annual mean temperature, annual precipitation, and annual potential evapotranspiration), calculated by using mean monthly climatic data for three temporal ranges (1981–2010 'current times', 2041–2070, 2071–2100) downloaded from CHELSA. All species were modeled in current times, then projected to future climatic scenarios. All future environmental data were derived from IPSL-CM6A-LR climatic models and related to three Shared Socioeconomic Pathways (SSP): SSP1-2.6 (CO_2 emissions cut to net zero around 2075), SSP3-7.0 (CO_2 emissions around current levels until 2050, then falling but not reaching net zero by 2100) and SSP5-8.5 (CO_2 emissions triple by 2075). Despite the actual distribution of species could be affected by several factors, the use of few biologically meaningful variables allows to identify areas capable to satisfy at least their fundamental physiological needs concerning temperature and water balance.
The analysis of potential distributions allowed to identify suitable areas for multi-species assisted colonization. Only areas potentially suitable for accommodating at least 60 species were considered. The calculation performed on current climate highlights the availability of 9,917 2×2 km cells. Most of this surface is located in the northern part of Italy (Subalpine region, Maritime Alps, Apuan Alps, Northern Apennines, and Elba Island). Only few areas are identified also in central-southern Italy (Gargano, Sorrentine Peninsula, Aspromonte, and a small portion of Nebrodi and Madonie). Under SSP1-2.6 climatic scenario, potentially suitable areas are reduced to 3,757–3,329 2×2 km cells between 2070–2100, but the general pattern did not change compared with the estimation based on current climatic conditions. Under SSP3-7.0 climatic scenario, potentially suitable areas are drastically reduced to 2,829–1,285 2×2 km cells between 2070–2100, located in a few mountain regions in northern Italy. Finally, under SSP5-8.5, potentially suitable areas almost disappear (2,037–456 2×2 km cells between 2070–2100) and only two areas are highlighted: the mountain region around lake Garda and a small portion of the Graian Alps. Despite the high potentialities of environmental niche modeling to plan assisted colonization, only a limited number of areas are suitable to potentially host multi-species assisted colonization. These areas could be regarded as 'hotspots' for the creation of new protected areas to mitigate the effect of climate change on Italian endemic plant species, whose conservation is under full responsibility of our country.

Robotic Monitoring of Habitats: good news from the dune front

Simonetta Bagella[1]; Claudia Angiolini[2]; Marco Caccianiga[3], Daniela Gigante[4]; Giovanni Rivieccio[1]; Franco Angelini[5]; Pierangela Angelini[6], Laura Casella[6]; Mathew J. Pollayil[5], Manolo Garabini[5]
[1]Department of Chemical, Physical, Mathematical and Natural Sciences, University of Sassari, Via Piandanna 4, 07100 Sassari, Italy; [2]Department of Life Sciences, University of Siena, Via Aldo Moro 2, 53100 Siena, Italy; [3]Department of Biosciences, University of Milano, Via Celoria 26, 20133 Milano, Italy; [4]Department of Agricultural, Food and Environmental Sciences, University of Perugia, Borgo XX giugno, 06021 Perugia, Italy; [5] Department of Information Engineering, Research Center "E. Piaggio", University of Pisa, Largo Lucio Lazzarino 1, 56122 Pisa, Italy; [6] Italian National Institute for Environmental Protection and Research, Via Vitaliano Brancati 60, 00144 Roma, Italy

According to the Directive 92/43/EEC of the European Council (Habitats Directive), EU countries are required to periodically assess habitat monitoring campaigns to evaluate the effectiveness of conservation measures and the achievement of conservation targets, which is crucial for the preservation of biodiversity. Nowadays, these tasks are carried out only by highly trained human operators. The H2020 Project "Natural Intelligence for Robotic Monitoring of Habitats - NI " (call H2020-ICT-2020-2, ICT-47-2020 "Research and Innovation boosting promising robotics applications") aims to develop quadruped robots, ANYmal, able to move autonomously in the unstructured environment of several habitat types (dunes, grasslands, forests, screes). Our study case focused on dune habitats (2110-2120 Annex I Habitats). We brought the robot to selected stands in the Special Area of Conservation "Stagno e ginepreto di Platamona" and the National Park Parco dell'Asinara (Sassari, Italy). Here, we tested the robot's locomotion abilities on sand substrata in the field. The robot faced and successfully overcame multiple challenges, including autonomously moving on highly uneven, slipping, and irregular terrains and managing unexpected contacts and impacts with vegetation. The second round of tests aimed to prove the NI robot's ability to gather floristic and structural data according to the Manual for Italian Habitat Monitoring (ISPRA, 2016). The robot was equipped with a RGB-D Intel Real Sense D435camera on each side. The four cameras had a field of view of 77° x 69.4° x 42.5° and recorded full-HD photos and videos. Also, the robot carried a Velodyne VLP-16 Puck LITE LiDAR. For this first mission, ANYmal focused on four target species: *Thinopyrum junceum* (L.) Á. Löve and *Achillea maritima* (L.) Ehrend. & Y.P. Guo (typical of habitat 2110) and *Calamagrostis arenaria* (L.) Roth subsp. *arundinacea* (Husn.) Banfi, Galasso & Bartolucci (typical of the habitat 2120) and the invasive alien species *Carpobrotus acinaciformis* (L.) L. Bolus. It first created a 3D map of its surroundings thanks to the LiDAR-generated point cloud. Based on this map, it located itself and operated autonomous missions at specified waypoints. For each waypoint, the robot recorded four images and four videos. The total vegetation cover was assessed and the target species were recognized based on the two-dimensional images. The point cloud was used to segment the tuft size (circumference and height) of the *Poaceae* as an indicator of "structure and functions" of the habitat. To evaluate the quality of the data collected by the robot, two expert botanists made the same assessments in parallel. The results of this first mission are very encouraging and open new challenges for future developments that will allow botanists to have support for habitat monitoring, particularly for repetitive and time-consuming activities.

Fig. 1. ANYmal

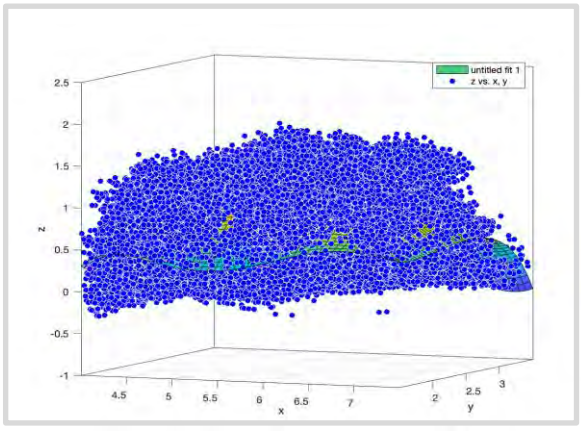


Fig. 2. Point cloud representing a *C. arenaria* tuft

Rock drawings in Valle Camonica: Monitoring lichen diversity and the efficacy of biodeterioration control towards a joint conservation of the cultural and natural heritage

Sergio E. Favero-Longo[1], Enrica Matteucci[1], Maria Giuseppina Ruggiero[2]
[1]Department of Life Sciences and Systems Biology, University of Torino, Viale Mattioli 25, 10125 Torino, Italy; [2]Direzione Regionale Musei della Lombardia, Palazzo Litta, Corso Magenta, 24, 20123, Milano, Italy

Archaeological and monumental areas have been widely recognized as biodiversity hotspots and potential refuges for the local vascular flora, often threatened in the surrounding territory by a higher urbanization. Accordingly, dedicated strategies of botanical planning have been traced to protect species and vegetation types of conservation interest, and to disseminate their naturalistic value, taking care of avoiding any negative impact on the stone heritage structures. Similarly, a high lichen diversity is a common feature of several heritage sites, which, in the case of the saxicolous component, is directly linked with the stone surfaces. Early proposals to recognize a positive value in lichen richness on the stone cultural heritage were thus obviously in contrast with the standpoint of art conservators. Lichen colonization can indeed cause aesthetic damage and obscure surface details; moreover, lichen growth on and within the mineral substrates is responsible for biogeo-physical and -chemical processes affecting their stability and durability. Although these processes variously imply negative (biodeterioration) and/or positive (bioprotection) effects depending on the species and lithologies involved, and the (micro-)environmental conditions, the removal of lichens is usually considered a necessary step of conservation interventions. However, the practicability of controlling lichens, and other lithobionts, on outdoor stone substrates strongly depends on the type of heritage surfaces and their degree of involvement in the natural environment. In particular, this latter point has implications on the suitability of traditional methods for the devitalization of lichens and microbial biofilms, which should necessarily precede their removal to make a cleaning intervention effective.
All these issues are of particular interest for rock-art sites, in which lichens and other lithobionts threaten the conservation and legibility of engravings, but are also the biodiversity components which naturally occur on the rock outcrops. With this regard, approaches to combine the conservation of both the cultural and natural heritage seem desirable, but practicable management strategies are still generally lacking.
In this study, we investigated lichen biodiversity in the Rock Engravings National Park of Naquane, in middle Valle Camonica (UNESCO world heritage site n. 94; Brescia, Italy). Surveys on 23 engraved rocks (n=54 plots 50×50 cm), differing in conservation history and characterized with respect to (micro-)environmental features, showed that tree sheltering and water flow from adjacent vegetated terrains are primary conditional factors of rapid (re-)colonization following restoration interventions. Microscopical analyses of cross-sectioned samples mostly showed a negligible to poorly pervasive lichen penetration within the local sandstone, with some specific exceptions. The effectiveness of biocidal treatments, often combined with the mechanical removal of thalli in past restoration interventions, was experimentally assayed, and different application methods compared, showing the potential risk of spreading chemicals without reaching the devitalization goal. Moreover, following the removal of lichens and other lithobionts and the application of different preservative treatments, 33 rock parcels (30x30cm) were seasonally monitored for four years with respect to the (re-)colonization dynamics by fluorimetric, colorimetric and image analyses. Crossed influences of microenvironmental conditions and the different products in determining the (re-)colonization rate and the composition of the secondary communities were recognized. On the basis of these findings, the combination of preventive strategies to limit factors favouring lithobiontic colonization and the application of effective devitalization treatments was tested on engraved rocks needing restoration, and monitored for its efficacy. Positive results after three years indicate the opportunity of focusing accurate preventive and devitalization interventions on selected rock-art surfaces -potentially followed by a periodic maintenance with the preservative products which showed the best results-, thus guaranteeing a longer duration of cleaning effects. Such practices, however, may be hardly sustainable on the overall outcrops in the Park. Accordingly, dedicated itineraries are under construction to guide visitors to acknowledge lichens as natural colonizers of rock outcrops and appreciate their biodiversity value, rather than denouncing their presence as decaying factor. The suitability of such an approach for the joint conservation of the cultural and natural heritage in rock art sites and others will be finally discussed.

Preliminary genetic analyses of central Mediterranean taxa in the *Dianthus virgineus* complex (Caryophyllaceae) suggest taxonomic inflation

Jacopo Franzoni[1], Giovanni Astuti[2], Gianluigi Bacchetta[3], Liliana Bernardo[4], Cristian Brullo[5], Fabio Conti[6], Gianniantonio Domina[7], Duilio Iamonico[1], Lucia Varaldo[8], Hirzi Luqman[9], Ivana Rešetnik[10], Alex Widmer[9], Simone Fior[9], 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]Centre for Conservation of Biodiversity (CCB), Department of Life and Environmental Sciences, University of Cagliari, V.le S. Ignazio da Laconi 13, 09123 Cagliari, Italy; [4]Department of Biology, Ecology and Earth Sciences, University of Calabria, 87036 Arcavacata di Rende (Cosenza), Italy; [5]Department of Biological Geological and Environmental Sciences, University of Catania, Via A. Longo 19, I-95125 Catania, Italy; [6]School of Biosciences and Veterinary Medicine, University of Camerino, Camerino, Italy; [7]Department of Agricultural, Food and Forest Sciences, University of Palermo, Viale delle Scienze ed. 4, 90128 Palermo, Italy; [8]Department for the Earth, Environment and Life Sciences (DISTAV), University of Genoa, Genova, Italy; [9]Institute of Integrative Biology, ETH Zürich, Universitätstrasse 2, 8092 Zürich, Switzerland; [10]Faculty of Science, University of Zagreb, Marulićev trg 20/II, HR-10000 Zagreb, Croatia

Delimiting species only on morphology may lead to taxonomic inflation, biasing conservation studies and actions, particularly in organisms recently diversified. For instance, the central Mediterranean *Dianthus virgineus* L. complex belongs to a lineage that evolved rapidly in Eurasia, and is characterized by high phenotypic variation. In the central Mediterranean, 21 taxa are currently accepted, albeit solely described according to qualitative morphological and phenological variation. Our objective is to apply an integrative taxonomic approach, including morphometry, karyology, and genetics, to test these taxonomic hypotheses. We genotyped thousands of genome-wide biallelic, unlinked SNPs from 123 populations from southern France, peninsular Italy, Corsica, Sardinia, and Sicily, through ddRAD sequencing and conducted an explorative PCA and STRUCTURE analysis to detect genetic differentiation.
All across the study area there is an overall low genetic variability, albeit structured according to geographical distribution of
populations. In particular, we detected a NW-SE cline from southern France to Calabria, and a second, orthogonal cline from central Italy to Sardinia through the Tuscan Archipelago and Corsica. Conversely, plants from Sicily form a genetic cluster that does not appear to follow clinal patterns recovered in the Italian Peninsula. We deduce that the number of genetically recognizable groups of the *D. virgineus* complex in central Mediterranean is much lower than the current taxonomic hypotheses, highlighting the importance of an integrative taxonomic approach to better circumscribe systematic units. However, taxonomic conclusions await thorough morphometric and karyological analyses, which are currently underway.

Acknowledgements
This work is supported by the "Progetto di Ricerca di Rilevante Interesse Nazionale" (PRIN) "PLAN.T.S. 2.0 - towards a renaissance of PLANt Taxonomy and Systematics" led by the University of Pisa, under the grant number 2017JW4HZK (Principal Investigator: Lorenzo Peruzzi) and by the Croatian Science Foundation under the project UIP-2017-05-2882 (AmphiAdriPlant).

An integrative taxonomic study of *Santolina* (Asteraceae) from southern France and north-eastern Spain

Antonio Giacò[1], Lucia Varaldo[2], Gabriele Casazza[2], Daniele De Luca[3], Paolo Caputo[3], Marco Sarigu[4], Gianluigi Bacchetta[4], Llorenç Sáez[5], Lorenzo Peruzzi[1]
[1]Department of Biology, University of Pisa, Via Derna 1, 56126 Pisa, Italy; [2]Department of DISTAV, University of Genoa, Corso Europa 26, 16132, Genoa, Italy; [3]Department of Biology, University of Naples "Federico II", via Cupa Nuova Cintia, 21, 80126, Naples, Italy; [4]Department of Life and Environmental Sciences, University of Cagliari, V.le S. Ignazio da Laconi 11-13, Cagliari, Italy; [5]Department BABVE (Systematics and Evolution of Vascular Plants, associate unit to CSIC), Faculty of Biosciences, Autonomous University of Barcelona, 08193, Bellaterra Barcelona, Spain

Santolina L. (Asteraceae, Anthemideae) is a taxonomically critical genus composed of approximately 26 subshrub species endemic to the western Mediterranean Basin. Recent studies resolved taxonomic issues for the Iberian taxa and for populations from Corsica and Sardinia. However, continental diploid populations of the *S. chamaecyparissus* L. group, occurring across Spain and southern France, still lack quantitative taxonomic investigation. The three native species endemic to that area are *S. benthamiana* Jord. & Fourr. (Spanish and French eastern Pyrenees), *S. decumbens* Mill. (Provence), and *S. ericoides* Poir. (widespread from eastern Spain to south France). The morphological distinction of populations from type localities is quite easy; however, several morphological intermediates are known among these three taxa. In order to properly address the taxonomic treatment of this group of species, we sampled the three type localities (all occurring in France) and five additional populations (two from Spain and three from France). We used an integrated taxonomic approach including morphometry, molecular systematics (*trnH-psbA*, *trnL-trnF*, *trnQ-rps16*, *rps15-ycf1*, *psbM-trnD*, and *trnS-trnG* plastid markers), and ecological niche analysis. For morphometry of flowering individuals, we sampled 20 individuals for each population. A subset of three individuals was used for molecular analyses. For diaspore morphometry, cypselae features were obtained for each population. Finally, for niche analysis, occurrence points were obtained from online databases (INPN, SILENE, and GBIF), personal communications, and field research. Based on our results, we infer that the current taxonomic circumscription does not adequately reflect the new systematic knowledge. We can conclude that populations from the lower altitudes of eastern Pyrenees, formerly attributed either to *S. benthamiana* or *S. ericoides*, are actually different from these two species both on molecular and morphological grounds. Based on our evidences, these populations can be considered as a distinct species, namely *S. intricata* Jord. & Fourr., a name that was previously considered as a tentative heterotypic synonym of *S. benthamiana*. On the other hand, *S. ericoides* and *S. benthamiana* share the same group of haplotypes. Despite this, they can retain the species level, thanks to significant differences on both morphology and environmental conditions. Finally, *Santolina decumbens* shows high morphological infraspecific variability, and it is possible to circumscribe three allopatric, closely related, systematic units.

Acknowledgements
This work is supported by the "Progetto di Ricerca di Rilevante Interesse Nazionale" (PRIN) "PLAN.T.S. 2.0 - towards a renaissance of PLANt Taxonomy and Systematics" led by the University of Pisa, under the grant number 2017JW4HZK (Principal Investigator: Lorenzo Peruzzi).

Integrative taxonomy in the 21st century. An example from the intricate plant genus *Xanthium* (Ambrosinae, Asteraceae)

Eleonora Manzo, Salvatore Tomasello
Department of Systematics, Biodiversity and Evolution of Plants (with Herbarium), George-August University of Göttingen, Untere Karspüle 2, 37077, Göttingen

Natural history collections are extremely important for studies aiming at resolving taxonomies in intricate groups. The use of herbarium material helps both while sampling, especially for widespread taxa, and foremost because the inclusion of name-bearing types facilitates tremendously the nomenclatural work. Herewith, we aim at delimiting evolutionary lineages in the taxonomically intricate genus *Xanthium* L., employing types and using phylogenomics, morphometrics, and coalescent-based species delimitation approaches.
Xanthium is a peculiar genus of the Asteraceae, characterized by spiny, wind-pollinated, female capitula (burs). Most of the morphological characters used to delimit taxa in the genus are features of burs. Due to their extreme morphological variability, taxonomic treatments have been inconsistent in the past and several names have been given by different authors. A recent study has proven that most of the taxa can be grouped into five species complexes (Tomasello 2018: https://doi.org/10.1016/j.ympev.2018.05.024). However, some of these extremely variable complexes remained widely unresolved.
To address this issue, we examined several herbaria and collected over 270 samples, 39 of which being types (more than 70% of recognisable types), covering a wide range of the morphological variation and geographic distribution of the genus. We retrieved high-resolution specimen images for morphometric analyses of leaf shapes and burs traits, and small leaf fragments for DNA extraction and subsequent phylogenomic analyses. For the latter, we combine standard extraction kits with methods used for ancient DNA and archaeobotanical remains. Thus, we were able to extract DNA and produce sequences from 200 years old samples. We applied target enrichment techniques (Hyb-Seq), and the commercially available "Asteraceae COS baits kit" to retrieve sequence data from hundreds of nuclear loci. Using integrative approaches, we delimit evolutionary lineages and link them to names.

Fig. 1. Dr. Salvatore Tomasello samples old leave material.

Fig. 2. *Xanthium orientale* at the French coastline.

Study of the Italian and French populations of *Vicia cusnae* (Fabaceae)

Martina D'Agostino[1], Giulia Albani Rocchetti[1], Marta Carboni[1], Rodolfo Gentili[2], Sandra Citterio[2], Thomas Abeli[1]
[1]Department of Science, Roma Tre University, Viale Guglielmo Marconi 446, Roma, Italy; [2]Department of Earth and Environmental Sciences, University of Milano-Bicocca, Milano, Italy

Vicia cusnae Foggi et Ricceri is a rare vascular plant occurring only in three geographically isolated mountain locations, two in France (Western Alps) and one in Italy (Northern Apennines).
Based on global and regional assessments, the conservation status of this species is Near Threatened (NT) according to the IUCN Categories and Criteria, due to extreme summer heat waves, lack of consistent monitoring data on the existing populations' trends, restricted and fragmented area of occupancy.
Although some studies suggest that *V. cusnae* is taxomically close to *V. canescens*, *V. variegata* and *V. sirinca*, comparative study between the French and the Italian populations is missing.
To assess whether the Italian and French populations belong to the same species or to two different taxa, genetic investigations, comparison of germination behaviors and initial growth rates were carried out between the Italian population from the southern slope of Mount Cusna (2120 m a.s.l., Tuscan-Emilian Apennines) and the French population from Montagne d'Aurouze, Devoluy area (Western Alps).
Investigations through DNA barcoding and flow cytometry (Fig. 1) on the Italian and French populations did not exhibit differences in the nucleodite sequences of rbcL and ITS regions and in the DNA contents, indicating belonging to the same species. This were the first results of this kind on *V. cusnae*, as there were no sequences of *V. cusnae* recorded in the NCBI database (nor elsewhere) and data on DNA content in literature. Moreover, our study confirms that *V. cusnae* likely belong to the "*V. cracca* section" and shows genetic similarities with *V. rechingeri* Chrtkova and *V. aucheri* Boiss, thus being able to hypothesize a possible ancient Middle Eastern origin of *V. cusnae*.
The mean final germination was marginally higher in the French population (97.92%) than in the Italian (96.22%), but this difference was statistically significant (Wald $\chi 2 = 19.192$; df = 1; p-value < 0.001). the growth rate until the fifth week was higher in the Italian than in the French population (test: t= -2.31; df =33; p-value =0.027).
From this first comparative analysis of the *V. cusnae* populations, it was possible to take a step forward towards the knowledge of this species and opening a new direction its conservation.

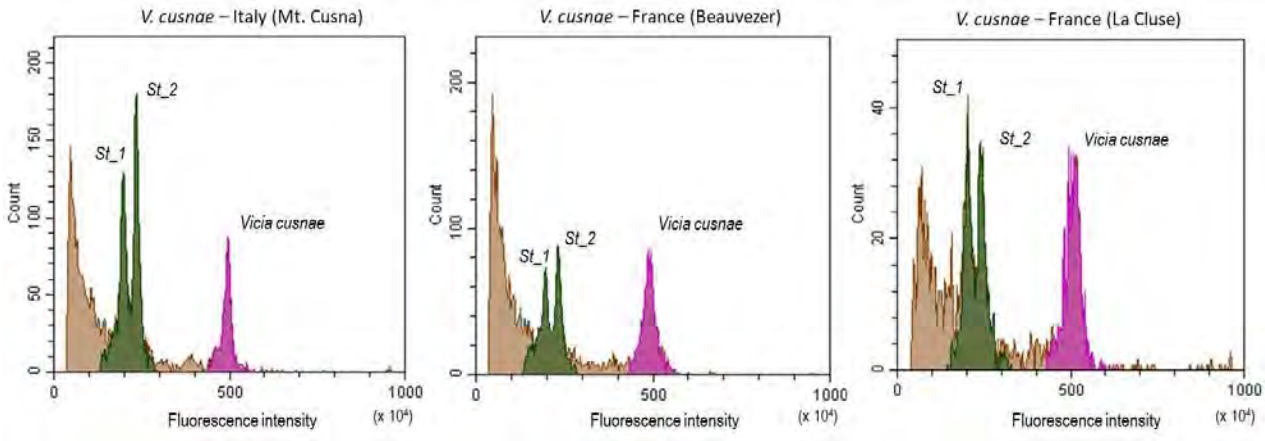


Fig. 1. Flow cytometry data of the DNA content analysis of *Vicia cusnae* samples.

On the concept of species in cultivated plants and its consequences in alien species and CWR

Giulio Barone[1], Emilio Di Gristina[2], Gianniantonio Domina[2]
[1]Department of Architecture, University of Palermo, Viale Delle Scienze Bldg. 14, 90128 Palermo, Italy; [2]Department of Agricultural, Food and Forest Sciences, University of Palermo, Viale delle Scienze Bldg.4, 90128, Palermo, Italy

In 1942 Ernst Mayr proposed the Biological Species Concept based on reproductive compatibility within populations of organisms. Mayr's approach is one of the bases of modern biology and is taught in schools, although biologists agree that the species problem is too multidimensional to be "solved" by a single concept. Cultivated taxa add a further level of difficulty to defining the concept of species in plants. In fact, plants in cultivation, as a rule, do not have a natural population structure, do not occupy natural areas, nor natural habitats and are not only products of evolution through natural selection in its widest sense, but also, to a large extent, of unconscious and conscious selection by man; therefore, they are often extremely polymorphic. These difficulties have led many botanists not only to abandon studies on cultivated plants but also to avoid them; the classification of most cultivated taxa, thus, has been influenced by persons not trained in taxonomy. This phenomenon was called by Edgar Anderson in 1952 "the greater paradox". In 1953 has been published the International Code of Nomenclature for Cultivated Plants (ICNCP), to accommodate the needs of plant scientists working on agriculture, forestry and horticulture. The morphological variation of cultivated plants was considered too wide to be assessed by the same standards as those governing the nomenclature of wild organisms of algae, fungi, fossils and plants. As long as botanists and plant scientists work on different taxonomic groups or on the same groups at different levels, the resulting annoyances are tolerable. The main problems emerge when cultivated plants have to be treated by botanists. This is the case of cultivated plants that naturalize, of wild ancestors of cultivated plants, or when cultivated and wild plants are assigned to the same species. To a lesser extent, the problem arises with weeds and parasites of cultivated plants. The most complex cases are found in genera in which the wild forms exhibit complex variation, often paralleling variation in the cultivated crop, where obviously domestication has happened repeatedly, concurrently with escapes into the wild as well as hybridization and introgression between wild and cultivated forms have for long been happening.

Such diversified taxonomic concepts have led to different approaches. Linnaeus, who standardized the nomenclature of biological taxa, chose to classify cultivated plants subordinate to their wild relatives but otherwise use the same nomenclatural conventions for both. Some authors in mid XX Century have proposed different infraspecific categories for spontaneous and cultivated plants. Others at the end of the XX Century proposed super-specific units grouping wild and cultivated species between which gene transfer is possible. The problem remains, taxon definition is arbitrary and left to the judgement of individual researchers.

There are different approaches to the variation in spontaneous and cultivated plants but also in spontaneous plants evolution the direct or indirect human interventions should be taken into consideration. In the Mediterranean, for example, numerous wild species have been described in *Brassica* and *Pyrus* but only a single cultivated one is recognized. For both genera there are no reproductive barriers between cultivated and wild plants. For *Olea* and *Ceratonia*, cultivated and wild plants tend to be considered a single species. On the other hand, there are species known only in cultivation for which natural populations are not known with certainty. This is the case of *Vicia faba* L. Similar problems are found with aliens for which new species (sometimes hybrids or hybrid derivatives) have been described outside their natural generic range, as in *Amaranthus* and *Oenothera*.

The solution, if at all feasible, must pass through integrated taxonomic studies that consider classical morphology, genetics, reproductive strategies, pollen and archaeological finds, this to infer the phylogenetic relationships between cultivated and wild plants. These approaches should take into consideration also the practical aspects of the use of names. Meetings, seminars and congresses between botanists s. s. and plant scientists s. l. would help seek shared interpretations for the taxonomic and nomenclatural treatment of these taxa.

Biorefining the diatom *Staurosirella pinnata* for drug discovery

Saverio Savio[1], Riccardo Turchi[1], Debora Paris[2], Marianna Carbone[2], Letizia Ciavatta[2], Katia Aquilano[1], Carlo Rodolfo[1], Roberta Congestri[1]
[1]Department of Biology, University of Rome 'Tor Vergata', Via della Ricerca Scientifica snc, 00133 Rome, Italy; [2]Institute of Biomolecular Chemistry (ICB) of National Research Council (CNR), Comprensorio Olivetti, Via Campi Flegrei 34, 80078, Pozzuoli, Italy

Diatoms are currently recognized as an important platform for drug discovery and conspicuous information exists on their bioactivity. However, despite the promising reports on extract screenings, little focus has been paid so far on the identification and purification of the bioactive metabolites and their target cellular pathways. This work started studying hydrophilic extracts obtained from three diatom strains selected to incorporate a range of ecological and phylogenetic diversity: one planktonic model organism, *Phaeodactylum tricornutum* Bohlin, and two benthic, field-isolated strains belonging to *Cylindrotheca closterium* (Ehrenberg) Reimann & J.C. Lewin and *Staurosirella pinnata* (Ehrenberg) D.M. Williams & Round. Preliminary bioactivity screenings of the extracts were carried out on melanoma cells, using cytofluorimetric analyses that showed the highest level of cell death induction for *S. pinnata.*

Based on this, we focused on *S. pinnata* to implement a drug-discovery project to move beyond the preliminary phase of microalgae prospecting. Thus, we followed a multidisciplinary approach including: i) diatom biomass cultivation in large scale system and sequential extractions; ii) fractionation and characterization of extracts to obtain purified compounds; and iii) cellular and molecular biology sound setups, to elucidate the effects on human cells and to unravel modulation of target cell pathways.

Mass cultivation of *S. pinnata* was carried out in photobioreactor (indoor, 10 L), after identifying optimal culture conditions (80 μmol m^{-2} s^{-1}, 21 °C, 12:12h light/dark cycle) to maintain stable biomass yields (5 g L^{-1}, after 21 days) and biochemistry. At the end of the stationary phase the biomass was sequentially extracted and methanolic and lipidic extracts were obtained.

Characterization of extracts was then started using Nuclear Magnetic Resonance (NMR) and Gas Chromatography-Mass Spectrometry (GC-MS, FID), this included fractionation of the extracts, through a hydrophilic gradient for the methanolic one, leading to identify two anti-grazing compounds with cytotoxic activity against copepods, the rare oxylipins (7E)-9-ketohexadec-7-enoic acid and (7E)-9-hydroxyhexadec-7-enoic acid.

Fraction bioactivity was then assessed on human cancer cell lines (A375 and CHL-1) and normal keratinocytes (HaCaT), evidencing cell death induction on cancer cells (53%) and cell cycle alteration, as resulted by the drastic reduction of cells in the G2/M phase. Meanwhile, the lipidic extract bioactivity was tested on white adipose tissue cells (3T3-L1) by analyzing the expression of proteins involved in target cellular pathways. Results indicated effects on mitochondrial dynamics, e.g., modulation of p-DRP1 and OPA-1 proteins, and enhancement of lipolysis and thermogenesis.

Finally, in the context of an ecology-driven approach to drug discovery, we began to analyze the effect of exposing *Staurosirella pinnata* cells to high irradiances (200 and 600 μmol photons m^{-2} s^{-1}). The low-molecular weight fractions of the extracts obtained from cultures after 54 hours exposure were tested on melanoma cancer cells revealing a selective cytotoxic effect of the 600 μmol photons m^{-2} s^{-1} culture. Metabolome evaluation by an enrichment analysis, evidenced a marked metabolic shift, with the up regulation of metabolites involved in lipid synthesis and stress response, for *S. pinnata* exposed to the highest light conditions.

Unveiling new potential toxic metabolites from cyanobacteria through genome mining: a case study from an Italian drinking water reservoir

Mara Simonazzi[1], Laura Pezzolesi[1], Tânia K. Shishido[2], David P. Fewer[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 Microbiology, University of Helsinki, Viikinkaari 5, 00790 Helsinki, Finland

Freshwater ecosystems are characterized by a complex trophic web that comprises different microorganisms, including cyanobacteria. In such environments, cyanobacteria may proliferate into dense blooms, outcompeting other organisms and affecting bio-physical-chemical characteristics of the water column. The frequency and intensity of cyanobacterial blooms in surface water has recently increased worldwide, likely as a consequence of climate change and eutrophication, posing serious threats to water bodies used for recreational or drinking purpose. Indeed, cyanobacteria produce a wide array of specialized metabolites with diverse bioactivity, including harmful toxins, i.e. cyanotoxins. To date, more than 2000 cyanobacterial metabolites have been described, of which known cyanotoxins represent about 18% of the total. So far, the discovery of cyanotoxins has been investigated after poisoning events, in bloom samples or in cultured isolates, via chemical analyses and bioassays. In recent years, the structure of known metabolites has been used to guide the discovery of biosynthetic gene clusters (BGCs) in cyanobacterial genomes, i.e. clustered groups of genes in prokaryotes responsible for the synthesis of specialized metabolites and cyanotoxins, e.g. microcystins and nodularins. Advances in genomics have further expanded the potential to discover unknown metabolites from cyanobacteria, even in the absence of a known chemical structure to guide the process.
In this study, a cyanobacterial strain, *Limnothrix* sp., was isolated from a shallow lake located in Rimini province (Emilia-Romagna, Italy), providing water for the local drinking plant. Potential toxicity of aqueous extracts was tested by *Artemia* sp. bioassay, resulting in 98-100% mortality of the crustacean within 24h. Nonetheless, preliminary chemical analysis of methanolic extracts did not highlight the presence of known cyanotoxins. The genomic DNA of *Limnothrix* sp. was extracted and sequenced by Illumina Miseq, obtaining a genome completeness of 99.64%. The prediction of BGCs was performed with antiSMASH, that confirmed the absence of known cyanotoxins gene clusters but revealed the presence of a trans-AT PKS region. This region seems highly conserved in the genus *Limnothrix*, and is responsible for the synthesis of polyketides, a family of bioactive compounds potentially involved in toxicity. The product of the trans-AT PKS region in our strain has not been detected yet, thus further investigations are needed. Similarly to our bioassay results, toxicity of hydrophilic extracts of another *Limnothrix* strain was observed by other authors, who suggested the synthesis of an unknown toxin, i.e. "limnothrixin". Therefore, possible implication for drinking water should not be excluded.

Algae biomass as sustainable source of protein and lipids

Tomas Morosinotto[1,2], Francesca Bucci[1], Alessandra Bellan[1]
[1]Department of Biology, University of Padova, 35121 Padova, Italy; [2]Botanical Garden, University of Padova, 35123 Padova, Italy

Global population growth is driving a continuous increase of food demand that in the next decades will need to be satisfied by simultaneously decreasing the negative impacts of present agricultural practices. There is thus a strong need to develop new, sustainable, strategies for food production. A promising perspective is to complement plant crops cultivation with microalgae, photosynthetic organisms that use solar energy to fix CO_2 and produce biomass with high nutritional value.
We here describe two research projects in collaboration with private companies to develop algae strains for food applications. In the first example, the objective was to develop a protein source, especially suitable for vegetarian diets. After selecting algae strains with high protein content and rich in essential aminoacids, random mutagenesis approaches were applied to optimize the biomass pigment composition in order to improve colour and taste of the final product.
In the second example, an oil rich strain was genetically modified to change its lipid profile by targeting the genes involved in lipids' biosynthesis. The final result is that the oil produced by this strain has a strong similarity with the profile of palm oil, with the remarkable difference of also containing 10% of polyunsaturated fatty acids. This strain thus represents a highly valuable sustainable and healthier alternative to palm oil.

Influence of aqueous phase from hydrothermal carbonization (AHL) of *Sargassum muticum* (Phaeophyceae) on germination and growth of *Phaseolus vulgaris* (Fabaceae)

Damiano Spagnuolo[1], Viviana Bressi[2], Antonio Manghisi[1], Maria Azzarà[1], Maria Teresa[1] Chiofalo[1], Claudia Espro[2], Giuseppa Genovese[1], Marina Morabito[1], Patrizia Trifilò[1]
[1]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Salita Sperone, 31, 98166 Messina, Italy; [2]Department of Engineering, University of Messina, Salita Sperone, 31, 98166 Messina, Italy

In the last twenty years, climate change has severely affected agricultural productivity. Socio-economic effects of loss in crops yield are expected to be exacerbated in the next future, also as a result of the forecasted increase in population growth and, consequently, in food requirement. Thus, finding and/or fine-tuning practices limiting climate-change-driven loss of crop yield, aiming at circular economy and sustainable agriculture, is very urgent. Macroalgae are often used as plant fertilizer and/or biostimulant and provide also numerous molecules used in industry, including hydrochar by thermo-chemical process called hydrothermal carbonization (HTC). In the last few years, HTC has been reconsidered as an actual alternative to process wet biomass into a value-added products. Unlike conventional dry thermochemical processes (e.g., combustion, pyrolysis, and gasification), HTC does not require an expensive or energy intensive preliminary drying step, as it directly exploits the water retained in digestate as a solvent during the process. In particular, the HTC process takes place between 180 °C and 300 °C and 10 bars and 80 bars, with residence time ranging from few minutes to several hours. The HTC process converts wet feedstock into a carbonaceous solid fraction (hydrochar) and a liquid phase termed aqueous HTC liquid (AHL). AHL phase is often discarded in carbonization process and revaluation will be an incentive to use HTC in the practical problems of resource management. Wet biomass may be derived from macroalgal species and some studies have been carried out about chemical characterization and applications on algal hydrochar but not on AHL. Macroalgae may be selected from anthropized environment, where they produce abundant biomass that negatively affects human activities and, in part, also other organisms, so they must be dredged and disposed of in some way. The aim of the present work is to use AHL deriving from a process of HTC in *Sargassum muticum* to probe their potential effects on plant productivity of *Phaseolus vulgaris*. Different HTC protocols were tested, differentiating temperatures, to obtain slightly dissimilar hydrochar. AHL obtained from all different HTC protocols were supplied to seeds of *Phaseolus vulgaris* and germination rate and plant growth were recorded. To exclude a mere osmotic influence, isotonic solutions were also tested in parallel. Growth as well as water relations were monitored until fruits were developed.
Among results, seed germination of about 80% was recorded in all studied treatments and it could be noted that 180 °C AHL seed priming treatment induced higher productivity in *P. vulgaris*, in terms of higher photosynthesis rate, as supported, likely, by higher stomatal density values, together with higher shoot/root ratio, as driven by higher whole leaf area and stem biomass. Nevertheless, it can be noted that plants treated with 240 °C AHL showed the higher root dry mass value and, as a consequence, the lower root/shoot ratio values, but higher photosynthesis rate than control samples. Plant treated with 300 °C AHL showed higher leaf mass area values respect to all treated samples. Further studies need to be performed to investigate the effect of these discarded solutions in plants growing in mild water stress, to explore their possible use for triggering plant water stress resistance and obtain high productivity at low water demand. Overall, our findings suggest a promising use of AHL in agriculture in the framework of resource management and circular green economy.

Bio-based products from Mediterranean seaweeds and their opportunities in the bioeconomy field

Simona Armeli Minicante, Lucia Bongiorni, Amelia De Lazzari
Institute of Marine Sciences, National Research Council, Arsenale 101-104, Castello 2737F, 30122 Venezia, Italy

The ocean-based natural capital provides to humans a wide range of ecosystem services (ESs), including natural goods like food, raw materials and genetic resources but also functions like purification of air and water, climate regulation, pollination, protection against extreme events and many others ecosystem functions which benefit human well-being. The ESs framework was indeed designed with the aim of including those benefits into decision-making processes in order to facilitate the transition of the global economy towards a sustainable state. Among biotic resources, seaweeds are attracting increasing attention as alternative healthy food sources to feed the growing human population and renewable source of food supplements and drugs, and for providing essential ecosystem services such as the reduction of greenhouse gasses. Although the analysis of seaweed production and their industrial use exists on a global scale, there are no surveys focusing on the entire Mediterranean area.
In this work, we analyze the state of seaweeds bio-based products and research in the Mediterranean Sea in the last 20 years. Among the various phyla, Rhodophyta resulted to be the most investigated. A large amount of published data referred to antimicrobial, followed in minor percentage by antioxidant and anti-inflammatory activities of seaweed extracts, whereas only few studies reported seaweed bio-products, such as biofuels and bioplastics. Among Mediterranean countries, Tunisia, Italy and Egypt were the ones with the largest number of published papers focusing on the potential applications of Mediterranean seaweeds, including Non-Indigenous Species (NISs). Lastly, we focus on the Italian situation - including researches, companies and legislations on seaweed production - and we discuss gaps, perspectives, and challenges for the potential development of a sustainable seaweed industry according to the Sustainable Blue Economy.

Plant functional groups and vegetation dynamics within urban ecosystems: the ECOLOPES' project approach

Marta Pianta[1], Mariasole Calbi[1], Alexandre Changenet[2], Jens Joschinski[3], Anne Mimet[4], Wolfgang Weisser[4], Enrica Roccotiello[1]
[1]Department of Earth, Environment and Life Sciences, University of Genoa, Corso Europa 26, 16132 Genoa, Italy; [2]Department of Forest Resource Management, Swedish University of Agricultural Sciences, Skogsmarksgränd, SE-901 83 Umeå, Sweden; [3]Studio Animal-Aided Design, Dresdner Straße 26 D – 10999 Berlin, Germany; [4]Technical University Munich, Terrestrial Ecology Research Group, School of Life Sciences, D-85354 Freising, Germany

The United Nations predict that about 70% of world's population will live in cities by 2050.
Urban contexts are complex, heterogeneous, fragmented ecosystems, subjected to different anthropic influence. To make cities more sustainable and liveable, governments and organizations worldwide have started turning from 'grey' to 'green' building infrastructures. Nevertheless, current approaches to urban greening limit the natural evolution of vegetation and do not consider the dynamic network of interactions between biotic components. This resulted in 'static' green systems, exclusively aimed at providing ecosystem services to humans.
The project H2020 FET-Open ECOLOPES (ECOlogical building enveLOPES: a game-changing design approach for regenerative urban ecosystems) aims at overcoming this human-centred vision through a new integrated ecosystem approach that focus equally on humans, plants, animals, and microbiota. The overall goal is to develop technological tools that, by integrating ecological and architectural knowledge and data, will support the design of innovative building envelopes all over the world.
One of the key elements is the spatially explicit ecological model able to simulate the temporal and spatial dynamics of different natural components (soil-microbiota, plant, and animal) as a function of abiotic conditions and biotic interactions, including human management. In this context, the plant sub-model plays a key role in predicting natural dynamics at the building/neighbourhood level. The aim of the current study is to reduce the complexity provided by species through a methodology that can be applied worldwide.
To this aim, plant functional groups (PFG) have been obtained by using relevant species traits, extracted from the global database of plant functional traits TRY according to seven ecological dimensions (considering different aspects of plant life), and an aesthetic filter chosen by the user. The intersection of all the dimensions potentially defines the ecological niche space of every plant species of the world. In addition, the validation of the PFG building methodology is being done through field data in the form of a green roof and urban plots experiment. Urban plots are subjected to different level of anthropic pressures, classified using the codes of the official EU threat/pressure list, and the green roof is subjected to different level of human management practices (e.g., mowing, fertilization, irrigation). At both sites, plants are allowed to naturally colonise the studied areas. From the urban plots, the data collection has been carried out through phytosociological relevées of spontaneous plant communities. The lists of species collected are being analysed with TRY database to define traits that are associated with typical urban anthropic pressures (e.g., the presence of roads). Likewise, shall be done for the green roof to define traits associated to maintenance. The list of plant functional traits collected are being processed to obtain PFG. These will later be checked against the theoretical PFG to check the effectiveness of the current PFG method.
The final output of the plant sub-model will be a map showing the distribution of PFG in different time step on the base of the architect design, i.e., geometry of the buildings, local abiotic conditions, soil characteristics, human management, interactions with the other biological components, and the natural evolution of the ecological systems.
The originality of this functional approach lies in the definition of groups that are generic and hence valid for the entire world.

On biodiversity friendly Green Infrastructure planning: contribution of plant ecology to the monitoring and valorization of peri-urban environments in the Metropolitan City of Rome (Mediterranenan Italy)

Giulia Capotorti[1], Laura Zavattero[2], Sandro Bonacquisti[2], Eva Del Vico[2], Laura Facioni[3], Duilio Iamonico[1], Alessandro Montaldi[1], Simone Valeri[1]
[1]Dipartimento di Biologia Ambientale, Università di Roma 'La Sapienza', P.le Aldo Moro 5, 00185 Roma; [2]Centro di Ricerca Interuniversitario "Biodiversità, Servizi ecosistemici e Sostenibilità" (CIRBISES), c/o Università di Roma 'La Sapienza', P.le Aldo Moro 5, 00185 Roma; [3]Ministero della Transizione Ecologica, Direzione Generale per il Patrimonio Naturalistico e Mare, Via Cristoforo Colombo 44, 00147 Roma

In Europe, Green Infrastructure (GI) represents a strategic tool for supporting the sustainable development of land, conceived for bringing natural and semi-natural areas together to form a physically and/or functionally connected network across rural and urban areas. In particular, the greening of urban and peri-urban areas, that is the integration of GI into urban planning, is expected to provide a crucial contribution to the "bringing back nature into our lives" goal of the new Biodiversity Strategy for 2030.
In Italy, this goal is being further reinforced by the NRRP investment on "Urban and extra-urban forestation", which is stimulating the recovery of tree cover in metropolitan cities in order to counteract air pollution, climate change and biodiversity loss.
Within this framework, the Metropolitan City of Rome is actively involved in deploying GI networks that are capable of facing main environmental, social and economic challenges in the Capital City and surrounding municipalities. An interdisciplinary approach is therefore informing different initiatives promoted by the local administration, with plant ecologists especially engaged in the definition of suitable conservation and restoration actions.
The general objective of the present contribution is to highlight how plant and ecological sciences are allowing GI planning processes to become truly biodiversity-friendly in the area. In particular, the project concerning the realization of a cluster of GIs in Valle Galeria, aimed at the environmental restoration and requalification of the western sector of the Metropolitan City, is considered. Such a cluster should aid the multiple objectives of biodiversity conservation, ecological connectivity improvement, air and water quality amelioration, soil consumption containment, and traditional agricultural landscape valorisation, to be addressed.
The methodological approach adopted for a specific GI primarily devoted to the containment of biological invasions, together with the recovery of ecological corridors and mitigation of artificialization pressures, will be therefore presented. The approach enhances the usefulness of scientific knowledge in terms of: i) ecological basis for large area planning; ii) mitigation of conflicts between forestation measures and conservation of vulnerable plant species and habitats; iii) enhancement of synergies between forestation measures and regulating/provisioning ecosystem services capacity. To avoid giving ill-founded recommendations, the deep need for fine-scale definition of ecosystem typology and conditions, and for the completion of remotely acquired data with field observation, will be also stressed by means of concrete examples from the case study.

Analysis of particulate matter trapped by four different shrub species in an urban forest: quantification and characterization

Giulia Santunione, Alice Barbieri, Elisabetta Sgarbi
Department of Life Science, University of Modena and Reggio Emilia, Via Amendola, 2, 42124 Reggio Emilia, Italy

In last decades the strong urbanization phenomenon has followed by a series of severe implications: among them, air pollution is one of the current problems in most of countries all over the world and according to World Health Organization (WHO), it is estimated that it is involved in million deaths every year. In fact, it is associated with several negative health outcomes for urban residents, such as cardiovascular and respiratory illness, neurological disorders and cancer. One of most important pollutants is particulate matter (PM), a complex mixture of solid and liquid particles, hovering in the air. It is classified by aerodynamical diameter, and it comprises inorganic compounds such as heavy and transition metals and organic ones as polycyclic aromatic hydrocarbons. PM comes from different sources, including anthropogenic ones, such as exhaust and non-exhaust emission, heating system and industries 'emissions, but also natural ones as volcanic eruptions and soil erosion. Like other air pollution components, it has severe health implications, so it's important to find solutions to reduce PM concentrations in the atmosphere.
As several studies have highlighted, rows of trees and urban forest provide important ecosystem services in the environment of the cities: among them, besides reducing the high temperature during summer periods, vegetation has the key role to improve air quality. Many authors have underlined as vegetation is able to reduce the atmospheric particulate in terrestrial ecosystems, thanks to adsorption of particles by leaves surfaces. However, the capability of PM adsorption by vegetation depends on a lot of factors, including leaf shape, waxy cuticles, extent of leaf pubescence, adhesiveness, roughness and leaf surface wettability (water retention). Moreover, the deposition process and particle interception by trees are affected by many factors such as the canopy characteristics, wind speed, temperature, particle size.
This work presents the results of an experimental urban forest case-study, settled in collaboration with the Municipality of Reggio Emilia. The urban forest was planted in 2018- 2019 inside the "Acque Chiare" Park, in an area of 1200 m^2. It sees a boundary of buildings on the S-O side and open green areas on the N-E side. Four plant species have been chosen: *Tilia cordata, Morus alba, Celtis australis* and *Fraxinus ornus,* settled in rows of 9 plants each, through 3 rows for each species. All the species have been selected in according with Guidelines (Project REBUS – Emilia-Romagna Region): plant habitus, growth rate, resistance, adaptability, origin, leaf morphology, etc. The aim of the study was to analyze PM deposition on leaves, comparing the capability of PM adsorption by different plants species across three following years of monitoring. Leaves samplings have been performed twice a year: first samples have been collected at the end of April, the other one at the end of September. Leaf samples were conventionally fixed in 3% glutaraldehyde in phosphate buffer (pH 6.9), acetone dehydrated and finally processed in a Critical-Point Dryer. Samples were observed under a Nova NanoSEM 450 (Fei) for leaf micromorphology and to detect PM trapped by the leaves; all images have been analysed through ImageJ software, in order to calculate the number of particles and their size. Furthermore, PM was analysed through Energy Dispersive X-ray Spectroscopy (X-EDS, Bruker QUANTAX-200) with the aim to get a semi-quantitative analysis of their chemical composition. The amounts of PM captured during the two different seasons have been compared for all the three years of monitoring, in order to analyse if the species involved could provide a significative particulate's trapping into urban context.
The analysis gave a significant difference in terms of number of PM between April and September mainly on leaves of *F. ornus*. This species seems to be able to trap particles during all warm period, with a similar trend throughout three years of monitoring. High variability in terms of amount of trapped PM occurred in the other three species.
F. ornus and *C. australis* showed the greater ability to retain particles of pollutants compared with other species. The chemical analysis of PM recorded the presence of several elements: some of them (as Silicium) are normally contained into the soil then naturally present on leaves surfaces, others are likely products of anthropogenic activities (Iron, Copper, Titanium, etc.). These first results provide some important findings to correlate plant species and leaf micromorphology with the efficiency in scavenging PM pollutants from urban environment. Nowadays, this capacity can have important ripples on human health in preventing acute and chronic illnesses.

Multiple ecosystem benefits of urban green-space: two case studies

Marco Fornaciari[1], Federico Rossi[2], Chiara Proietti[1], Mirko Filipponi[2], Desirée Muscas[1], Beatrice Castellani[2], Luigia Ruga[1], Fabio Orlandi[1]
[1]Department of Civil and Environmental Engineering, University of Perugia, borgo xx giugno 74, 06121 Perugia, Italy; [2]Department of Engineering, University of Perugia, Via G. Duranti 93, 06125 Perugia, Italy

During this climate crisis, people and administration need to become more conscious of urban green space's mitigation functionality to better understand its environmental potentiality and economic value and correctly manage it. Among a collaboration between four different medium-sized Mediterranean cities, the Life Clivut Project works in this sense by developing a web app (Fig. 1) that integrates a growth model and software that permits common people and municipalities to account for the ecosystem services provided by the urban forest (CLImate Value of Urban Trees - LIFE18 GIC/IT/001217). Within this case study were assessed five mains e.s. provided by trees in two Italian parks ("Chico Mendez" in Perugia and "11 September 2001" in Bologna): CO_2 absorption and storage, PM10 absorption, cooling effect by shadow, cooling effect by evapotranspiration, and cooling effect by the increased albedo. These ecosystem services are species-specific, and some of them are closely related to tree dimensions, so they were estimated through a growth model based on plant parameters, i.e., dbh, height, and crown width, manually measured during data collection.
The CO_2 emissions' sequestration was estimated through volumetric equations based on the tree's fresh biomass, converted to dry weight by wood's densities as reported by McPherson (2016) to calculate C content and then expressed in stored CO_2 equivalent. To quantify the amount of particulate matter (PM10) absorbed, the I-Tree formula defined by Nowak (1988) was modified considering also Leaf Area Index, the deposition rate, the local PM10 monthly concentration (µg/m3) in atmosphere, the number of monthly days when the leaves are present, and a 0,5-resuspension rate coefficient. The shadow effect has been estimated based on the tree's volume calculated, considering the crown's shape, height, width, and density. The total shaded area includes the sum of all trees effect except crown interference. The effect of evapotranspiration was expressed both in terms of "cooling effect" estimating the energy (Watt) extracted by the atmosphere, that considering the CO_2 emission "compensation effect" setting the reduction of radiation force (ΔRF) due to water energy absorption during its phase transition equal to the variation in radiation force (ΔRF) generated by CO_2 emissions. Exploiting the same principle, increasing the albedo of 1 m^2 area by 1% permits a CO_2 compensation of 1,60 kg of CO_{2eq} emissions at Perugia's latitude and 1,57 kg of CO_{2eq} at Bologna's latitude, so the albedo effect was calculated by multiplying it by the albedo variance compared to the used asphalt and by the crown's projection area.
According to the methodology described above, it has been observed that the main species identified within the Chico Mendez Park (812 trees spread over a 25000 m^2 area), in addition to having stored 309 tons of CO_2 at the census (2020), are able to reduce the temperature below their crown by 1,7 degrees on average if considering the May-September period of 2020, to adsorb a total of 153 kg of PM10, and to ensure during their life a cooling effect enough to compensate 296.75 t of $CO_{2\ eq.}$ emissions into the atmosphere (4.89 t compensated by increasing the albedo and 291.86 thanks to evapotranspiration). In the same way, the main species selected within the "11 September 2001" Park in Bologna (44 trees on about 3000 m^2 surface) showed a total cooling effect of 0.794 t of CO_2 compensated for the albedo variance and 38.30 of CO_2 compensated by evapotranspiration in addition to 47.92 t of CO_2 already stored at the census (2020), and 18 kg of PM10 adsorbed. Moreover, thanks to the shadow cooling effect, it has been possible to estimate a temperature of 1.13 °C lower on average from May to September 2020 under the tree crown. At "11 September 2001" Park, the most significant contribution to the evapotranspiration effect was given by *Platanus acerifolia*, *Populus spp*., and *Ulmus spp*., which also have a high value of stored CO_2, ΔT°, and PM10 absorbed. While *Quercus ilex*, *Aesculus hippocastanum*, P*opulus nigra*, *Cupressus sempervirens*, and *Acer campestre* at the Chico Mendez Park are the species that provide the most significant contribution to stored and compensated CO_2, with interesting values also for cooling for shading contribution.

Fig. 1. LIFE-CLIVUT Webapp

Students 4 pollinators: a citizen science project in urban green areas to teach plant diversity and pollinator conservation to young generations

Chiara Acquaroli[1], Nicola Lothar Herrmann[2], Simone Flaminio[3,5], Fortunato Fulvio Bitonto[1], Elise Maria Keller[1], Giovanna Dante[1], Fabrizio Tenna[4], Marta Galloni[1]
[1]Department of Biological, Geological and Environmental Sciences, University of Bologna, Via Irnerio 42, 40126 Bologna, Italy; [2]SMA Botanic Garden and Herbarium, University of Bologna, Via Irnerio 42, 40126 Bologna, Italy; [3]CREA Research Centre for Agriculture and Environment, Via di Corticella 133, 40128 Bologna, Italy; [4]Centrale Valutativa, Monitoring and Evaluation Centre, 00162 Roma, Italy; [5]Laboratoire de Zoologie, Université de Mons, Mons, 7000, Belgium

Pollination is fundamental for the sexual reproduction of flowering plants. Many plants require a vector that transfers pollen from one flower to another: for about 90% of known plant species, the vectors are animal pollinators. Apart from being indispensable for life, pollination is also an enormously important ecosystem service for humans, but pollinator populations are declining worldwide. Conservation measures are necessary to counterbalance this loss, however, efforts cannot be made unless people are properly informed and aware. To reach this objective, several citizen science activities are foreseen within the LIFE 4 Pollinators EU project (LIFE18/GIE/IT000755). Among them, the school project "Students 4 Pollinators" has been implemented in Italy (Bologna) in 2021 and 2022, involving so far more than 500 pupils from 24 classes of secondary schools of I and II level. The educational activity foresees three phases: a preliminary lesson; a practical activity, based on standard protocols and field-sheets to record plant-pollinator interactions; a "restitution" session where the results of practical monitoring are presented and discussed. Here we present the methodology, materials and common protocols adopted, and we show the results obtained until now, both in terms of data collected as well as of project success. Overall, considering only the last school year, monitoring time of plant-pollinator interactions reached more than 20 hours of observations. Records were analyzed together to obtain qualitative pollination networks, that have been discussed with the students during final sessions. In addition to plant and pollinator diversity data, several information can be extracted from these analyses, such as the most visited flowering plant taxa or the most abundant pollinator groups, the most generalized/ specialized interactions. All this information may be useful for urban green management purposes. To check the accuracy and reliability of the data collected by students, random parallel surveys were performed by an expert. Project success was assessed through questionnaires administered after the end of the activity, including students' self-perception as well as specific questions to assess knowledge level and behavioral change. Almost three hundred questionnaires have been analyzed so far, showing a general increase in knowledge and the intention to adopt pollinator-friendly everyday comportments.

Fig. 1 Students self-perception on knowledge and behaviors

Fig. 2 Practical activity

Root growth response to localized selenium enrichment in soil: a comparison between hyperaccumulator and non-accumulator species

Sofia Montanari[1*], Mirko Salinitro[1*], Annalisa Tassoni[1]
[1]Department of Biological, Geological and Environmental Sciences, University of Bologna, Via Irnerio 42, 40126 Bologna, Italy
* first co-authors

Selenium has been recognised since the 1970s as an essential nutrient for humans and other animal species, as its deficiency has been proved to cause negative impacts on their health. Nonetheless, the difference between selenium deficiency and selenium toxicity is very narrow, resulting in both conditions being critical problems worldwide. In this context, the use of selenium hyperaccumulators, i.e. plants able to accumulate more than 1000 µg Se g-1 in their tissues in natural conditions, has the potential both to remove excess selenium in Se-enriched soils and to provide biofortified biomass to alleviate Se deficiencies in humans and animals.
The aim of this study was to determine whether the hyperaccumulator species *Stanleya pinnata* preferentially forages selenate (SeO_2^{4-}) or selenite (SeO_2^{3-}) in selenium-enriched soils and to compare its behaviour with a non-hyperaccumulator species of the same family, *Brassica juncea*.
Plants were grown in rhizotrons (23x23 cm petri dishes, Fig. 2) each containing two individuals separated by a polystyrene septum. The central part of the petri was filled with selenium-enriched soil, whereas the outer parts contained control soil (Fig. 2). Two different forms of selenium were tested (selenate and selenite) and rhizotron completely filled with control soil were also included. The seedlings were transferred at the interface between selenium-enriched and control soils and plants were cultivated for 3 weeks. At the end of this period, roots were photographed to assess their distribution. Shoots and roots were separated from the soil, weighted and oven-dried at 40°C. Plant and soil samples were digested and trace elements were quantified through ICP-OES analysis. Overall, *S. pinnata* proved to actively forage for selenium in both selenate and selenite-enriched soils, although the response was significantly stronger in the presence of selenate (Fig. 2a). In fact, the root density resulted particularly high (+30%) in the part spiked with selenate (40 mg/kg, centre of the petri). *B. juncea*, on the other hand, resulted unaffected by the presence of selenite-enriched soil and it showed clear signs of avoidance in the presence of selenate. As shown in Fig. 2b, in the petri with selenate-enriched soil, *B. juncea*'s root growth resulted more abundant (+35%) in the outer part of the petri, where control soil was present. The same trend was supported also by the plants' biomass: while in *S. pinnata* the average fresh weight was highest when grown in selenate-enriched soils (0.33 g $plant^{-1}$), *B. juncea*'s biomass resulted greatest in control conditions (0.40 g $plant^{-1}$).

Fig. 1. Petri dish set-up

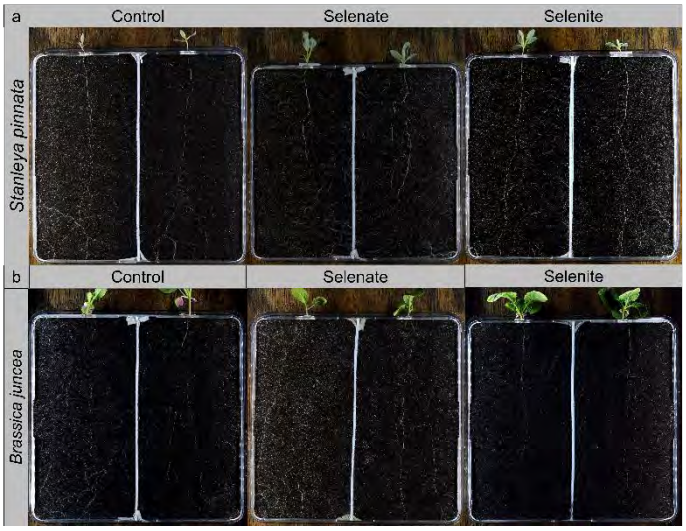


Fig. 2. Root growth in *S. pinnata (a)* and *B. juncea (b)* in the three different treatments.

Innovative approaches for controlling *Botrytis cinerea* infections in tomato by using poly (lactic-co-glycolic acid) nanoparticles for the vehiculation of antifungals

Camilla Badiali[1], Valerio Petruccelli[1], Fabio Sciubba[1,3], Marzia Beccaccioli[1], Cleofe Palocci[2], Laura Chronopoulou[2], Irene Muratore[1], Massimo Reverberi[1], Elisa Brasili[1,3], Gabriella Pasqua[1,3]
[1]Department of Environmental Biology, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy; [2]Department Department of Chemistry, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy; [3]NMR-Based Metabolomics Laboratory (NMLab), Sapienza University of Rome, Italy

Effective pathogen management is a major challenge in modern agriculture, with a need to consider efficacy, cost affordability, environmental safety, toxicity towards non-target organisms, and sustainability of the production system. A solution could be provided by lowering the amount of the agrochemicals delaying resistant strains' appearance and/or researching novel natural products with antimicrobial activity. Nanotechnologies combined with agrochemicals and/or natural compounds could offer a smart solution for controlling crop diseases.
In this research, poly(lactic-co-glycolic acid) (PLGA) nanoparticles (NPs) were used for controlling fungal diseases. PLGA has been extensively studied and its biocompatibility has been proved, and it has shown great potential for use in the development of nano-based delivery systems for plants. PLGA has been approved by the FDA, WHO, and other regulating agencies. Recently our laboratory demonstrated the ability of PLGA NPs to enter Vitis vinifera plants and phytopathogenic fungi. In this study PLGA NPs were loaded with fluopyram, an antifungal widely used in agriculture, or pterostilbene, a bioactive natural compound. The research was focused on a highly remunerative fresh-market variety of tomato (*Solanum lycopersicum* L.), variety ciliegino F1 and one of its fungal pathogens, i.e. *Botrytis cinerea*, an airborne plant pathogen with a necrotrophic lifestyle which is the causal agent of the grey mold, one of the most problematic diseases for tomato cultivation. PLGA NPs uptake in the fungal cell of *B. cinerea* was recently confirmed by fluorescence microscopy observing fungal mycelium after the administration of PLGA NPs loaded with the fluorophore coumarin-6. The effect of empty or drug loaded PLGA NPs on *B. cinerea* has been evaluated by performing a minimum inhibitory concentration (MIC) assay. In vitro antifungal activity revealed the PLGA NPs loaded with fluopyram or pterostilbene were more active against *B. cinerea* than free administered compounds.
The influence of empty or drug loaded PLGA NPs on *B. cinerea* infected plants has been evaluated by 1H-NMR-based metabolomic analysis, quantitative profiling of fatty acids and oxylipins by LC-MS, chlorophyll and carotenoids quantification by spectrophotometric analyses. Chlorophyll and carotenoids analysis showed PLGA NPs, empty or drug loaded, do not cause variations in the parameters analyzed. Differently, the untargeted metabolomic analysis allowed us to highlight that PLGA NPs act as elicitors of plant defense response by stimulating the flavonoid pathway. Fatty acids and oxylipins quantitative profiling were also affected by PLGA-NPs administration.

Characterization of *Eucalyptus cinerea* and *E. nicholii* by-products for agricultural applications

Paola Malaspina[1], Cristina Danna[1], Laura Cornara[1], Pierluca Aicardi[2], Sheridan Lois Woo[3], Marina Papaianni[4], Flavio Polito[5], Vincenzo De Feo[5].
[1]Department of Earth, Environment and Life Sciences, University of Genova, 16132 Genova, Italy; [2]Coldiretti Savona, 17100 Savona, Italy; [3]Department of Pharmacy, University of Naples Federico II, 80055 Portici, Italy; [4]Department of Agricultural Sciences, University of Naples Federico II, 80055 Portici, Italy; [5]Department of Pharmacy, University of Salerno, 84084 Fisciano, Italy

The cultivation of *Eucalyptus*, globally widespread, is increasing and extending also in Italy. Particularly in Liguria, several *Eucalyptus* species are cultivated for their foliage that is cut and sold in Northern Europe for floral compositions. *Eucalyptus* plantations are constantly pruned to maintain production of juvenile branches, more suitable for the market. Due to the selection of fronds, floriculture produces annually large volumes of waste biomass that is mainly burned or buried for elimination. However, this by-product is still rich in bioactive compounds that could be recovered and used in agriculture.
Our study focuses on two species, *E. cinerea* F. Muell. ex Benth. (EC) and *E. nicholii* Maiden & Blakely (EN), analyzing macro- and micromorphological features of the leaves, the chemical composition of two essential oils (EOs) and their biological properties, including phytotoxicity and antimicrobial activity.
The leaves of EC and EN show different shapes, and variable colours depending on the abundance and arrangement of waxes. EC leaves are opposite, sessile, oval/rounded, blue grey, and densely reticulated. EN leaves are alternate, pedunculated, elongated, and dark green. In both species the leaves are glaucous, with a smooth and thick cuticle, and more-or-less prominent papillae. The leaves of both species are amphistomatic, with actinocytic and anomocytic stomata types. Crust waxes were found in both species, crystalloid waxes (rosettes) in EN, and tubular waxes in EC, especially within epistomatal cavities.
Many secretory cavities containing EOs emerge on the leaf surface. Particularly, EN shows significantly higher oil gland density than EC. This data is in agreement with higher yield of EOs in EN (3.67%) compared with that of EC (2.56%), based on leaf dry weight. The EOs, extracted by hydro-distillation, were analysed by GC and GC/MS. In both species, the major component was 1.8-cineole (67.7% in EC; 79.5% in EN), followed by α-pinene (7.3% in EC, 3.7% in EN), α-terpinyl acetate (5.2% in EC), α-terpineol (3.9% in both species), and α-terpinene 3.3% (in both species). The potential herbicidal activity of EOs was evaluated by phytotoxicity tests on weeds (*Lolium multiflorum* Lam., *Portulaca oleracea* L., *Chenopodium album* L.), crops (*Raphanus sativus* L., *Pisum sativum* L., *Cucumis sativus* L.), and on the aromatic *Ruta graveolens* L. EO from *E. cinerea* showed phytotoxic activity against the radicle elongation of all the plant species tested, and it also inhibited the germination of radish and rue. Instead EO of *E. nicholii* inhibited both germination and radicle growth of ryegrass.
The antimicrobial activity of EO was also tested on bacteria plant pathogens *Xanthomonas campestris* pv *camprestis (Xcc), Enterobacter cloacae* and *Citrobacter freundii* of agricultural interest.
The antibacterial activity depended on the concentration of the essential oil used, whereby total inhibition (100%) was observed with undiluted essential oils concentration for all the bacterial strains tested.
Moreover, differences were noted on the plant species origins of the extracts, the Minimum inhibitory concentration (MIC) used, and the target bacteria tested: EO from *E. cinerea* had a MIC of 10^{-4} (essential oil dilution) on all three bacteria, whereas EO from *E. nicholii* had a MIC of 10^{-3} (essential oil dilution) for *Xcc* and *Enterobacter cloacae*, and a MIC of 10^{-2} (essential oil dilution) for *Citrobacter freundii*.
This study suggests that there can be added-values of *Eucalyptus* EC and EN by-products for possible diverse applications in agriculture. The allelopathy and antimicrobial activities exerted by these EOs represent a safe strategy for crop management programs, replacing synthetic herbicides and pesticides, provide options for recycling of vegetative wastes meeting principles of Circular Economy, all concepts important for a more sustainable economic development.

Arabidopsis thaliana tolerance to cadmium stress is mediated by endoplasmic reticulum stress sensing

Maria De Benedictis[1], Antonia Gallo[1], Pietro Roversi[2], Angelo Santino[1]
[1]Institute of Sciences of Food Production, C.N.R., Unit of Lecce, Via Monteroni, 73100 Lecce, Italy; [2]Institute of Agricultural Biology and Biotechnology, C.N.R., Unit of Milan, Via Edoardo Bassini, 20133 Milan, Italy

About one third of all Eukaryotic proteins are folded and glycosylated in the Endoplasmic Reticulum (ER) under the control of a sophisticated machinery called Endoplasmic Reticulum Quality Control (ERQC), which ensures only properly folded glycoproteins can reach their final secretory destination. By contrast, glycoproteins that are not able to attain their intended fold are degraded by a second ER-resident machinery, called ER-Associated Degradation (ERAD). The ER homeostatic balance between glycoprotein folding capacity and glycoprotein folding demand is strictly controlled by the cell, because accumulation of misfolded glycoproteins in the ER is an important cause of cell stress. In this context, all eukaryotic genomes also encode the components of the Unfolded Protein Response (UPR) signalling pathway with the aim to restore glycoprotein homeostasis by reducing (mis)folded glycoprotein accumulation in the ER and guarantee cell survival. When ER stress is severe, UPR can also trigger programmed cell death (PCD).
UPR activation has been studied in plants in response to several biotic and abiotic stresses, but knowledge about its role in response to heavy metal stress is fairly limited, since the regulation of UPR key genes expression depends specifically on the stress applied. For example, the *Arabidopsis thaliana* (*A. thaliana*) double mutant *Atbzip28/bzip60*, lacking the two main transcription factors (TFs) involved in UPR activation, shows higher tolerance to heavy metals, but a higher sensitivity to tunicamycin and biotic stresses. These results highlight the variegated and complex regulation of UPR.
In this work, we studied the effects of chronic and temporary cadmium (Cd) treatment on ER stress using *A. thaliana* as a model system. In detail, we used: (i) Wt plants to investigate the consequences of plant exposure to Cd on the ERQC, ERAD, UPR and PCD pathways; (ii) *Atbzip28/bzip60* double mutant plants to monitor the consequences of down-regulation of UPR genes on the ERQC, ERAD and PCD pathways.
Our data clearly suggest that ER stress is part of the *A. thaliana* response to Cd and that this response varies with stress time and heavy metal concentration. Cd treatment leads to an upregulation of key ERQC and UPR genes, also it modulates the induction of PCD pathway when the ER stress is severe, with the upregulation of the membrane-associated transcription factor NAC089.
Conversely, Cd treatment did not greatly impact on ERAD and UPR marker genes in the *Atbzip28/bzip60* double mutant, suggesting a reduced sensitivity of this mutant to Cd induced ER stress.
Taken together these results suggest that the modulation of ER stress sensing could be a useful tool to increase crop plants tolerance to Cd.

Effect of Plant Growth-Promoting Rhizobacteria on maize seedlings cultivated on saline soil: changes in root morphologies and amelioration of saline stress response

Gianmaria Oliva, Giovanni Vigliotta, Mattia Terzaghi, Angela Cicatelli, Francesco Guarino, Stefano Castiglione
Department of Chemistry and Biology "A. Zambelli" University of Salerno, Via Giovanni Paolo II 132, 84084 Fisciano, Italy

Salinization is a process causing a progressive accumulation of salts, as: sulphates, sodium or chlorides into the soils. It is typical of arid and semi-arid areas, in which soluble salts precipitate and accumulate on the surface layers of the soil, causing desertification phenomena. Salt accumulation has different origins: through natural processes (primary salinization) or through anthropic activity (secondary salinization). In this regard, a saline soil has a lower availability of water, a certain toxicity caused by some ions such as sodium and/or chlorine, causing significant imbalances on the biochemistry, physiology, and morphology of plants, influencing the germination of seeds and plant growth. The increased level of salt in soil has significant effects on glycophyte plants as rice, maize, and wheat, which are staple foods for the world's population. Consequently, it is important to develop biotechnologies that improve crops and clean up the soil. Among the saline soil's remediation methods, there is an environmentally friendly approach to ameliorate the cultivation of glycophyte plants in saline soil: the use of microorganism tolerant to salt with growth promoting features. Plant Growth Promoting Rhizobacteria (PGPR) can improve plant growth by colonizing their roots and playing a vital role in helping plants to establish and grow in nutrient-deficient conditions. The aim of our research was to characterize the PGPR strains isolated in a previous work (Castiglione et al., 2021) by our research team, and inoculate them for improving maize seedlings growth cultivated in semi-hydroponic conditions in the presence of sodium chloride. The results showed an increase in biomass and sodium tolerance in seedlings inoculated with a PGPR bacterial consortium (Staphylococcus succinus Bacillus stratosphericus) over the control. Finally, the ability of S. succinus to alter root morphology of maize seedlings exposed to saline stress was noted, mainly in the production of fine roots. As a result, maize seedlings inoculated with the PGPR consortium show not only an increased biomass, but a higher salt tolerance stocking large amounts of sodium salt in the seedling organs. Adding PGPR to plants or seedlings can be an effective strategy to enhance sustainable agriculture in saline soils because they protect plants with inhibitory effect.

Pollensomes cargo and markers in *Actinidia chinensis* Planch.

Chiara Suanno[1], Elisa Tonoli[2], Enzo Fornari[3], Maria Pia Savoca[2], Iris Aloisi[1], Luigi Parrotta[1,4], Claudia Faleri[5], Giampiero Cai[5], Elisabetta Verderio-Edwards[2], Stefano Del Duca[1,4]

[1]Department of Biological, Geological, and Environmental Sciences, University of Bologna, Via Irnerio 42, 40126 Bologna (BO), Italy; [2] School of Science & Technology, Nottingham Trent University, Clifton Campus NG11 8NS3, UK; [3] Healthy stuff, 1A Bonington Complex, Trent Ln, Castle Donington DE74 2PY, UK; [4] Interdepartmental Centre for Agri-Food Industrial Research, University of Bologna, Via Quinto Bucci 336, 47521 Cesena, Italy; [5] Department of Life Sciences, University of Siena, Via Mattioli 4, 53100 Siena (SI), Italy

In the last decade, there has been a growing interest in the characterisation of the total proteins secreted by pollen during germination (secretome). In fact, pollen tube elongation inside the pistil is believed to be guided and mediated by a complex dialogue between pollen and the female tissues, although the exact pathways of this process and the molecules involved are still largely unknown. Thus, proteins released by pollen at this stage might be pivotal for a successful fertilisation, and understanding their role in this process might help in the improvement of fertilisation rates and crop yields in agriculture.

An important discovery in this field has been made in 2014 by Prado and colleagues, who have demonstrated the release of extracellular nanovesicles, termed "pollensomes", during pollen germination. These vesicles are significantly smaller than those originated from the plasma membrane by outward budding, having a diameter ranging from 28 to 60 nm, but their biosynthetic route has not been defined yet. However, it is postulated that they could be exosomes, a type of extracellular nanovesicle of endocytic origin involved in long-distance signalling. Unfortunately, scientific literature on plant exosomes is scarce, and little is known about their possible molecular markers and their role in plant cell physiology.

In this work, pollensomes were isolated from both germinated and hydrated kiwi (*Actinidia chinensis* Planch.) pollen, by sequential filtrations and differential centrifugations. Total proteins from pollen, pollensomes, and vesicle-free medium were then isolated, quantified, and analysed by immunoblotting, mass spectrometry (RP-HPLC-ESI-MS/MS), and immunofluorescence. Immunoblotting was employed to screen the pollensomes for possible molecular markers of different membrane-bound subcellular compartments: Clathrin heavy chain for vesicular compartments, H+ATPase for the plasma membrane, COXII (Plant Cytochrome oxidase subunit II) for mitochondria, and ARF1 (ADP-ribosylation factor 1) for the Golgi membrane. Proteins from pollensomes were also probed for the presence of UGPase (UDP-glucose phosphorylase), considered a molecular marker of the cytoplasm, to confirm the absence of cell debris and cytoplasmatic contamination. Mass spectrometry provided the nanovesicles proteomics, that was then compared with similar studies on pollensomes and pollen secretome in general. Finally, immunofluorescence was performed on the pollen tube to visualise the distribution of possible markers of conventional and non-conventional secretion pathways (ARF1, BRO1-domain containing protein, Clathrin heavy chain) (Fig.1). Proteomics results are in line with published papers on pollen germination, confirming that pollensomes contain proteins involved in endocytosis, unconventionally secreted proteins, and protein families common in exosomes, including some putative plant exosomes markers.

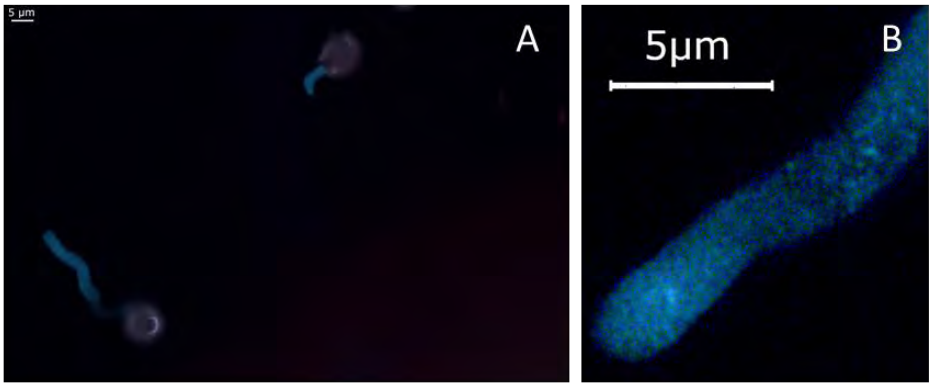


Fig. 1 Indirect immunofluorescence labelling of kiwi pollen with FITC-conjugated secondary antibodies, probed for ALIX. A: whole pollen grains, B: detail of the apical region of the pollen tube.

Leveraging plant natural variation to investigate root phosphate homeostasis

Marco Giovannetti[1]*, Arianna Capparotto[1], Daniele Castelli[1], Stanislav Kopriva[2], Gabriel Schaaf[3], Wolfgang Busch[4]
[1]Department of Biology, University of Padova, Padova, Italy; [2]University of Cologne, Botanical Institute and Cluster of Excellence on Plant Sciences (CEPLAS), Cologne, Germany; [3]Institute of Crop Science and Resource Conservation, University of Bonn, Bonn, Germany; [4]Salk Institute for Biological Studies, Plant Molecular and Cellular Biology Laboratory, and Integrative Biology Laboratory, La Jolla, CA, USA

Phosphate represents a major limiting factor for plant productivity. Plants have evolved different solutions to adapt to phosphate limitation ranging from a profound tuning of their root system and metabolic profile to the evolution of mutualistic interactions, such as arbuscular mycorrhizal symbiosis. We elucidated plant responses and their genetic basis to different phosphate levels in *Lotus japonicus* and compared it with *Medicago truncatula* by means of Genome Wide Association Studies applied to root traits that were acquired with a high-throughput root phenotyping approach. Our data advances our understanding of the continuum of genotype by phosphate level interaction existing throughout dicot plants. Moreover, we identified signs of convergent evolution at the genomic locus of a phosphoinositide phospholipase C (PLC2). This is the key enzymatic step that cleaves plasma membrane phospholipids and releases diacylglycerol and inositol 1,4,5-phosphate (InsP3) in the cytoplasm. A comprehensive analysis of the impact of PLC2 and its allelic diversity on developmental, metabolic and cellular root responses to phosphate in *L. japonicus* will be presented.

Effects of mycorrhizal inoculation on responses of *Solanum lycopersicum* in the early stages of the symbiosis

Patrizia Cesaro[1], Nadia Massa[1], Daniela Campana[1], Valeria Todeschini[1], Elisa Bona[2], Flavio Anastasia[1], Roberto Barbato[2], Guido Lingua[1]
[1]Department of Science and Technological Innovation, University of Piemonte Orientale, Viale Teresa Michel 11, 15121 Alessandria, Italy; [2]Department of Sustainable Development and Technological Transition, University of Piemonte Orientale, Piazza Sant'Eusebio 5, 13100 Vercelli, Italy

Arbuscular mycorrhizae represent the most common type of plant symbiosis. It is commonly considered mutualistic; however, considering that the interactions between organisms can change during their life cycle, a more correct interpretation describes it as a *continuum* between mutualism and parasitism. The arbuscular mycorrhizal (AM) fungus, belonging to the phylum Glomeromycota, supplies nutrients to the host plant and in return it receives photosynthates to complete its life cycle. Numerous evidences suggest that this symbiosis can modify different plant physiological aspects.
AM fungi are generally inoculated as mixtures of spores and root colonized fragments. In these conditions, hyphae or germinating spore tubes present in the inoculum colonize the roots of the germinated seedling and the fungus does not yet have a dense hyphal network to absorb water and minerals. Until the fungus reaches full development, the extra-radical mycelium is built only at the expense of the carbon provided by the plant. Conversely in natural conditions the colonization of the fungus is often carried out starting not only from the spores, but also from the extraradical hyphae of the fungi linked to other mycorrhizal plants, that form a dense network called Common Mycorrhizal Network (CMN). Under these conditions the fungus already has a large hyphae network capable of receiving the carbon that it needs from the plants previously colonized.
Few works describe what happens to the plants during the early stages of AM fungi colonization. For this reason, the aim of this work was to assess the effects of AM fungus *Funelliformis mosseae* BEG12 on *Solanum lycopersicum* cv. Rio Grande applying two different methods of inoculation: i) a system in which the fungus has a widely developed external hyphal network and ii) a system in which it requires nutrients from the plant itself in order to grow and develop its own mycelium. At each harvest, mycorrhizal colonization, shoot and root weights, morphometric parameters and photosynthetic efficiency were evaluated. In both the tested experimental systems few fungal effects were observed and no stress symptoms were detected suggesting that the fungus did not behave as a parasite.

Disentangling the interaction between rice and *Azolla filiculoides*

Martina Cerri[1], Sara Cannavò[2], Alma Costarelli[2], Agnese Bertoldi[2], Francesco Paolocci[3], Stefania Pasqualini[2], Lara Reale[1]
[1]Department of Agricultural, Food and Environmental Sciences, University of Perugia, Perugia, Italy; [2]Department of Chemistry, Biology and Biotechnology, University of Perugia, Perugia, Italy; [3] Institute of Bioscience and Bioresources (IBBR), National Research Council (CNR), Perugia, Italy

Rice is one of the major staple foods worldwide, thus an increment in its production is desirable and necessary, especially in view of ongoing climate change and the expected world population increase. *Azolla filiculoides* is an aquatic tiny fern belonging to the family of Salviniaceae. It lives in symbiosis with *Trichormus azollae,* a bacterium which fixes atmospheric nitrogen for crops. By virtue of this characteristic, *Azolla* has been extensively grown in rice paddies for thousands of years in the Far East, to increase rice production by more than 50%.
In this research, we co-cultivated rice (*Oriza sativa* ssp. *japonica* cv. Kitaake) with *Azolla filiculoides*, with the aim to phenotype rice plants and monitor the qualitative and quantitative effects of this interaction on this crop.
We investigated the morphology of the aerial part of rice plants (leaves area and length, number of tillers, fresh and dry weight) and the structure of the root apparatus (weight, number of adventitious roots and lateral roots) at three different developmental stages. The root apparatus of rice demonstrated to be significantly different when plants were cultivated together with *A. filiculoides*, as a different number of lateral and adventitious roots were observed. To verify the signals involved we checked the presence of NO in adventitious roots; differences were detected in the first stages of plant development suggesting a possible role of NO as trigger signal.
We also investigated the reproductive cycle of rice plants, to understand if there were any difference between rice plants cultivated with or without *Azolla*, in the same conditions. In particular, we measured the length of the reproductive cycle and the productivity of plants, in terms of number of primary and secondary branches of the panicles, number and weight of grains. The first data obtained suggested a higher productivity in co-cultivated rice plants.
Results obtained demonstrated the positive effect of *Azolla* in rice cultivation so the next step will be to understand the molecular and hormonal signals involved in this interaction.

Development of molecular markers for rapid sex screening in *Cycas revoluta* and in *Ginkgo biloba*

Ylenia De Luca[1], Niklaus Zemp[2], Antonia Cristaudo[3], Simone Fior[2], Alex Widmer[2], Salvatore Cozzolino[1], Donata Cafasso[1]

[1]Department of Biology, University of Naples Federico II, Via Cinthia, 80126, Napoli, Italy; [2]Department of Environmental Systems Science ETH Zurich; Institut für Integrative Biologie CHN G 21.1, Universitätstrasse 16, 8092 Zürich, Switzerland; [3]Department of Biological, Geological and Environmental Sciences (BIOMLG), University of Catania, Via Empedocle, 58, 95128 Catania

Dioecy is the predominant feature of gymnosperms being present in approx. 65% of the total recognized species. Among these, Cycads and *Ginkgo biloba* are the most primitive extant seed-producing species. These 'living fossil' are known to have existed as a recognizable species at least since the Jurassic and, with their fascinating forms, both Cycads and *G. biloba* are popular in landscape gardening. Management for their utilization in public and private gardens, therefore, requires information on the sex of introduced individuals. In fact, their introduction into public greenery is almost exclusively related to male individuals since female individuals are not suitable for ornamental uses. For example, seeds of *G. biloba* disintegrate when they fall to the ground thus causing a foul odor due to the presence of carboxylic acids, prompting probable aesthetic and public health problems. Whereas Cycad seeds contain several toxic glycosides that pose a serious risk of poisoning through accidental ingestion by kids and pets. Traditionally, sex identification of these plants was done morphologically using the reproductive structures of mature adult plants. However, *Ginkgo* and Cycad plants only reach sexual maturity after ca. 30 to 40 years and endure significant costs for the cultivation of female plants that are then subsequently discarded. Therefore, the use of rapid diagnostic tools that can determine the sex at the seedling stage would represent a strong advantage for plant breeders/florists. In our study, we aimed to develop molecular markers capable of discriminating sex at a very early stage of plant development by taking advantage of genome-scale sequencing technology using two different molecular approaches. For Cycads, we used a representative-reductive genomic analysis (ddRAD) approach that was applied to individuals of already known sex to identify molecular markers associated with sex in terms of probability. We identified a 284 bp sex-linked marker that was present in all males but absent in female individuals. For *G. biloba*, we tested a sex-linked genomic region already identified from a comparison of male and female sequenced genomes. We designed PCR primers specific for amplification of a male sex-determining region and we test them on a collection of *G. biloba* plants of known sex and on a small collection of plants with undetermined sex present at the University of Naples Federico II. We detected an amplification product in all males and no amplification in female plants. These markers, which have successfully passed the validation stage, may eventually be submitted for patenting, and a low-cost rapid screening kit may be developed for greenhouses, allowing significant rationalization in terms of both production and labor costs for plant breeding that requires a selection of plant material based on sex. In the next future, it would also be interesting to use these markers to evaluate the sex ratio in experimental crosses (e.g., 50% male and 50% female offspring, in the case of a pattern of inheritance of sex chromosomes as in humans) and/or the possible influence of epigenetic and/or environmental factors on sex determination.

The Fungal communities of Sienese “biancana” badlands

Claudia Perini[1], Irene Mazza[2], Elena Salerni[1]
[1]Department of Life Science, University of Siena, P. Mattioli 4, I 53100 Siena, Italy; [2]Department of Biology, University of Florence, La Pira 4/ Micheli 1-3, I 50121 Florence, Italy

Badlands, somehow meaning “eroded lands difficult to cross”, are multivariate, self-enhancing systems that can be contemplated globally due to its distinctive even if varying topography. Generally inappropriate for agricultural activities, they are considered a peculiar and fascinating cultural landscape. The “biancana” badlands are located in the “Crete Senesi”, one of the most interesting landscapes in Tuscany. They are characterized by a series of white small domes that arise gently from micropediments, with the slopes more or less steep covered by eroding pipes and rills.
Studies deals principally with their genesis, pedologic-hydrologic aspects, and erosion processes. As far as other research is concerned, from a botanical point of view, a protected species (*Artemisia caerulescens* subsp. *cretacea*) and various orchid species grow here and the sparse and dynamic vegetation revealed the presence of plant communities of high conservation interest at European level (like the semi-natural dry grasslands and scrubland facies on calcareous substrates, *Festuco-Brometalia,* hosting various orchids, and the pseudo-steppe with grasses and annuals of the *Thero-Brachypodietea*). Finally, the area of Crete Senesi, recognized as an area of geological or geomorphological interest, is also codified as IT5190004 “Crete di Camposodo e Crete di Leonina” by the Nature 2000 Network.
In this peculiar site, for the first time the presence and diversity of epigeous macromycetes was investigated. The purpose was to quantify its specific wealth, describe the macromycetes’s diversity in different environments, evaluate the distribution among trophic groups and increase the knowledge on species ecology. The investigated site was mapped through the choice of plots with dimensions of $2m^2$, moreover some fungal collections took place along the way from one plot to another.
The habitats were: pediment with sparsely herbaceus cover, grassland, shrubby areas, mossy islands, bare soil. In the period from April to December 2019 epigeous macromycetes, visible by naked eye greater than 1 mm, were identified and their fruiting bodies counted. The Funga of the Sienese “biancana” badlands was surprising rich, with 136 species. The most represented family was the *Bolbitiaceae* with 28 species belonging to *Conocybe* and including 4 interesting taxa for Italy: *Conocybe anthracophila*, *C. herbarum*, *C. monikae*, *C. roseipes*. The habitat with the greatest fungal diversity was the grassland with 39 species and 334 fruiting bodies. Among the species collected, some have been found in a different habitat respect to the one reported in bibliography, thus leading to an increase of their ecological knowledge. *Agrocybe paludosa* and *Arrhenia lobata* are the only species assessed according to the IUCN categories as "NT" and "CR", respectively. *A. lobata* was found only on the pediment, a characteristic environment of Crete Senesi sparsely colonized by the pioneer vegetation given by *A. caerulescens* subsp. *cretacea*.
This landscape, exceptionally dynamic and threatened by nearby agricultural activities, if not protected with appropriate management, is going to disappear at least within 30 years. Thanks to this study, to just investigated aspects like the interesting geomorphology and the precious diversity of habitats, or the Flora and Fauna sometimes very specific and threatened, the knowledge on Funga is added. Many scientists, from different disciplines, have made various proposals for the protection of this area and fungi can become another theme to push for action plans to conserve this ensemble of peculiar habitats and fascinating landscape with white domes, steep slopes, pipes and rills.

The influence of plastic pollution on soil fungal biodiversity

Marta Elisabetta Eleonora Temporiti, Lidia Nicola, Solveig Tosi
Department of Earth and Environmental Science, University of Pavia, Via Sant'Epifanio14, 27100 Pavia, Italy

Plastic pollution is a growing environmental problem. As a result of their versatility, plastic materials have been increasingly used, reaching global production of almost 370 million tonnes in 2020. After use, plastics that are not recycled or incinerated for energy recovery are discharged in landfills or abandoned in the environment. This results in the accumulation and persistence of plastic in land, freshwater, and oceans for many decades. A further problem associated with plastic pollution is the formation of small particles called microplastics, which originate from plastic fragmentation. Soil contamination by plastics and microplastics could affect the microbiota, changing the activity, composition, and diversity of communities. The aim of this work was to analyse the biodiversity of soil fungal communities of three areas with different degree of plastic pollution in the province of Pavia (Italy).
Eleven soil samples were collected in former landfill soil (D), an agro-environment (VD), and in a natural reserve forest (SN), all situated in the province of Pavia (Italy). Total soil DNA was extracted and the ITS1 segment of the 18S ribosomal cDNA was used as target for the metabarcoding of fungal communities. Moreover, the microbial functional diversity was studied using Biolog Ecoplate™, a technique able to analyse the metabolic footprint of soil microbial communities. Physico-chemical analyses and plastic fragments counts were also carried out on the soil samples.
The metabarcoding analysis revealed the presence of nine phyla: Mortierellomycota (from 46% VD to 52% in SN), Ascomycota (from 23% in SN to 39% in VD), Basidiomycota (from 11% in VD to 19% in SN), Mucoromycota (from 0,02% in VD to 5% in SN), Monoblepharomycota (0.05% in SN to 0.24% in VD), Rozellomycota (from 0.06% in D and SN to 0.1% in VD), Aphelidiomycota (from 0,04% in D to 1.2% in VD), Basidiobolomycota (0,08% only in VD), and Kickxellomycota (0,2% only in SN). The analyses of the beta-diversity of the communities highlighted a significant difference in the soil fungi between the natural reserve forest (SN) and the other two more polluted areas (D and VD). Moreover, the results of the functional analyses with the EcoPlate™ showed a general preference for phenolic compounds in all samples, while a lower capacity to metabolise amine was observed in landfill samples (D). Macroplastic and microplastic amounts, evaluated for each sample, were significantly higher in landfill soil (D) than in the natural reserve forest (SN). In conclusion, the present research contributed to the study of the effect of plastic pollution to the environment, specifically targeting soil fungal biodiversity and soil microbial metabolism.

Exploiting the fungal community against plastic polymers

Federica Spina[1], Viktoria Ilieva[2], Davide Ferrero[1], Giovanni Di Benedetto[2], Iolanda Perugini[1], Pierangiola Bracco[2], Marco Zanetti[2], Giovanna Cristina Varese[1]
[1]Department Of Life Science and System Biology, University of Turin, Viale P.A. Mattioli 25, 10125 Torino, Italy; [2]Department of Chemistry, University of Turin, via Pietro Giuria 7, 10125 Torino, Italy

When proper plastic disposal does not happen, both intentionally or unintentionally, natural ecosystems are the main recipients. Conventional plastic is persistent due to the high stability to chemical and physical stressors. In 2018, European plastics production reached 61.8 million tons, while only 29.1 million tons were collected. It has been estimated that worldwide, 2 billion people have no access to adequate waste collection system. When plastic reaches aquatic and terrestrial ecosystems, the effects can be seen along the entire trophic chain, also due to fragmentation in microfragments. On the other hand, bioplastics are considered a safe alternative, even though they cannot be underestimated. When not properly disposed, their degradation may be slow and partial, jeopardizing the ultimate sustainability of the process.
Likely, nature responds to bioplastic presence by evolving and adapting an active microbiota. The study of the plastisphere and its metabolome is therefore fundamental for the development of several biotechnological applications. This work aims to analyze the mycobiota associated with plastics in a terrestrial environment and investigate their degrading skills against plastic polymers. Extreme polluted environments were studied, including plastic-polluted landfill soil and compost plants, with different anthropic impacts. A rich fungal biodiversity was isolated and identified.
Fungi have been studied to evaluate their capability to mediate an active transformation of plastic polymers. Many strains confirmed this assumption. The actual capability of some fungi to degrade plastic was followed by means of growth studies and integrated analytical methods, as ATR-FTIR analyses and SEM investigation. As expected, at first, polymer biodegradation started as a superficial erosion process: the microorganisms colonize the surface and adhere, with the production of extracellular enzymes and other secretions to form a biofilm. Many fungi have been found capable to actively transform not only conventional plastic but also bioplastic, posing the basis for the development of bio-integrated technologies to strengthen their degradation leaving a harmless environment.
Trials were performed both in solid and liquid conditions, investigating few factors that may affect the process, as the temperature or the presence of other accessible C source to activate the primary metabolism. During the degradation in liquid media, HPLC-RI analyses highlighted the presence of several degradation metabolites, confirming then the depolymerization ability. The degradation products completely matched the monomer components of the tested polyesters, no unknown peaks were detected. However, their transformation rate was not always comparable, indicating that fungi may have a peculiar affinity towards few compounds. To investigate this aspect, the selected fungi was used to treat monomers at different concentrations, highlighting concentration effect and different assimilation rate.
Additional efforts are now aimed to better characterize the mycobiota of plastisphere, identify the best fungal degraders and the enzymes involved, and to enlarge the studied plastic polymers.

Truffle and ecosystem services: the hidden potential of the Ligurian countryside

Simone Di Piazza[1], Grazia Cecchi[1], Paolo Vassallo[2], Chiara Paoli[2], Maurizio Bazzano[3], Mirca Zotti[1]
[1]Laboratory of Mycology, DISTAV Department of Earth, Environmental and Life Science,University of Genoa, Corso Europa 26, 16132 Genoa, Italy; [2]DISTAV Department of Earth, Environmental and Life Science, University of Genoa, Corso Europa 26, 16132 Genoa, Italy; [3] A.T.T.L. ASS. TARTUFAI E TARTUFICOLTORI LIGURI Millesimo Piazza IV Novembre - 17017 - Millesimo (SV) Italy

In the collective imagination truffles (*Tuber* spp.) are high-quality edible products characterized by a high economic value. However, not all truffle consumers know that truffles are fungi and thanks to their symbiosis with different trees (*Quercus* spp, *Populus* spp, *Tilia* spp, etc) have a key role in natural ecosystems contributing to the forest development, health, and different ecosystem services for rural communities. In fact, in addition to supply services, truffle ecosystems contribute also to regulation and support services through soil formation and protection, water regulation, carbon cycle, etc. Moreover, thanks to their great historical and cultural value and great attractiveness, truffles significantly contribute to cultural services in rural areas.
In recent decades, the global truffle business has steadily expanded, generating a growing demand for most famous species with a consequent increase in harvesting pressure on natural sites. For cultivable species, such as *Tuber melanosporum* Vittad. and *T. aestivum* Vittad., this problem is partly solved through cultivation in vocated areas, thus reducing the pressure on natural sites. On the contrary, *T. magnatum*, instead, is not yet rationally cultivable and its high economic value (in 2022 around EUR 6,000 per kg) makes the preservation of natural sites difficult. These factors cause great pressure on truffle natural sites entailing a slow decrease of natural production. Shrinking productive natural areas forced many Italian regions to propose measures to counteract the loss of these valuable environments. A strategy to safeguard the truffle resource involves raising awareness among rural populations and local authorities. A way of emphasizing the importance of this resource to everyone is to quantify the ecosystem services that these ecosystems provide not only to individual truffle hunters but to all local communities.
This work describes the activities aimed at safeguarding the truffle heritage in Liguria (northwestern Italy). Such activities were carried out over the last 15 years in collaboration with the “Tartufai e Tartuficoltori Liguri” Association and several local administrative bodies: the main goals involved the mapping and safeguarding of harvesting areas, the mapping of areas potentially suitable for truffle-growing operations, and the enhancement of the truffle heritage.

Preliminary approach to mycorrhization of micropropagated linden sprouts with *Tuber borchii*

Nicola Baldoni, Mara Rondolini, Francesco Prosperi, Leonardo Baciarelli Falini, Maurizio Micheli, Domizia Donnini
Dipartimento di Scienze Agrarie, Alimentari e Ambientali, Università degli Studi di Perugia, Borgo XX giugno, 74 - 06121 Perugia (PG), Italy

Linden is a host plant particularly interesting given that in nature it forms ectomycorrhizas with all *Tuber* species of economic value. The propagation of the linden results to be quite problematic and according to Magherini and Nin especially the production of seed material because the seed germinates with great difficulty both for the incapacity to obtain viable seed and for the leathery integument. In the genus *Tilia* there is a strong dormancy, especially the physiological dormancy is dominant and, to overcome it, the intervention of hormones is necessary. Consequently, in the truffle nursery market it is difficult to have mycorrhized linden trees available, indeed, also because of the high incidence of costs for propagation.
In our opinion, a possible solution may be the possibility of propagating *Tilia* in-vitro and achieve mycorrhization still in partial asepsis conditions. In vitro plant propagation techniques allow the regeneration of different forms of propagules, such as somatic embryos (bipolar organs) or adventitious sprouts (unipolar organs). Therefore, it is very interesting the possibility of establishing a mutual symbiosis between fungus and plant already during the aseptic condition, which is realized after the rooting phase, in order to allow the seedlings to overcome the first phases of the ex-vitro acclimatation, always delicate and dangerous situation, thanks to the interaction with the symbiont fungus. The aim of the work is to obtain vitro-derived plantlets with root system on which, in the next phase, make a sporal inoculum of *Tuber borchii* Vittad. (bianchetto or marzuolo) thus obtaining mycorrhized linden plants.
For this reason, it has been identified an effective micropropagation protocol, which allowed to proliferate in vitro and to root in mixed vitro-vivo in conditions sprouts of *Tilia platyphillos* Scop. which will be mycorrhized later with inoculum of *T. borchii.*
Initial plant material, as uninodal portions, equipped with buds and without leaves, was excised from shoots of the year; then they have been subjected to decontamination, in order to obtain sterile explants and ready to be grown. The completely decontaminated explants were launched to the multiplication phase, subjected to the effect of a different nutritional component, which could stimulate the vegetative activity. The shoots that have reached a size considered suitable for the next steps of the experiment have been used for the subsequent rooting phase on soil. The rooting method on soil is now a widespread system and is defined as "mixed vitro-vivo system". The main advantage is to obtain roots that are structurally much more similar to the definitive and partially functional root systems. Therefore, the rhizogenesis in *Tilia* was induced by IBA and the results showed all its efficiency as an auxin suitable to stimulate rootin. The inductive treatment with IBA powdered has allowed to obtain 58% of the rooted shoots, while in the remaining 42% of the cases, the characteristic enlargement of the part of tissue treated with auxin has been recorded. The rooted shoots have developed an average of 2.1 roots/shoot with an average length of 16.6 mm. Finally, after only 4 months from the inoculum, up to 80% of mycorrhization with *T. borchii* was observed by morphological analysis and after 6 months the average of mycorrhization was 45%. These results, although still preliminar, are very interesting and promising in order to obtain micropropagated and mycorrhized lindens with *T. borchii*.

Development of a highly stable *Carlina acaulis* essential oil nanoemulsion for managing agricultural pests

Marta Ferrati[1], Giovanni Benelli[2], Roman Pavela[3,4], Eleonora Spinozzi[1], Giulia Bonacucina[1], Diego Romano Perinelli[1], Riccardo Petrelli[1], Filippo Maggi[1]
1Chemistry Interdisciplinary Project (ChIP), School of Pharmacy, University of Camerino, Madonna delle Carceri, 9/B, 62032 Camerino, Italy; 2Department of Agriculture, Food and Environment, University of Pisa, via del Borghetto 80, 56124, Pisa, 3Department of Plant Protection, Czech University of Life Sciences Prague, Kamycka 129, 16500, Praha 6, Suchdol, Czech Republic, 4Department of Crop Research Institute, Drnovska 507, 16106, Prague 6, Ruzyne, Czech Republic

In recent years, the need to safeguard the environment, natural resources and biodiversity has increased as well as the attention to food safety and improvement of the quality of life. Chemical pesticides are responsible for problems related to extensive pollution, the serious threats due to the presence of residues in food, the decrease in biodiversity and the development of resistance by harmful secondary organisms. In the agricultural field, the development of botanical pesticides is the concrete response to attention to sustainability. Despite a huge number of research products on the topic, moving plant-based insecticides from laboratory to field is still difficult, due to both formulation and regulatory issues. For these purposes, the in-depth study of essential oils (EOs), their safety, and their efficacy on different insect species are needed. Furthermore, efficient formulations for the application of these products are necessary to prevent their negative effects and to avoid waste. *Carlina acaulis* L. is a perennial herb belonging to the Asteraceae family, which originates from central Europe. The part typically used is its root which has a long history as a medical remedy. Its corresponding EO, composed of more than 95% of carlina oxide, has already been shown to be effective against *Musca domestica* and *Culex quinquefasciatus.*
In the present study, the EO was obtained from *C. acaulis* roots by hydrodistillation and its chemical composition was investigated by gas chromatography–mass spectrometry (GC–MS) confirming the polyacetylene carlina oxide as the main constituent. The insecticidal activity was evaluated on the Mediterranean fruit fly, *Ceratitis capitata* and on olive fly, *Bactrocera oleae*, adults using protein baits formulations. The LC_{50} values were 1094 ppm and 706 ppm, respectively. Moreover, *C. acaulis* EO was evaluated for its toxicity against 1st instar larvae of the European grapevine moth, *Lobesia botrana*, a major vineyard pest. The LC_{50} values was 7.299 µL/mL. Our work focused also on the development of formulations to cope with the poor solubility in water and the rapid degradation by UV light and oxygen of the *C. acaulis* EO. We developed nanocarrier-based formulations, namely micro- and nanoemulsions (ME and NE, respectively) containing *C. acaulis* EO. MEs showed limitation in the amount of encapsulated oil phase, contrary to NEs. This latter, was able to encapsulate 6% (w/w) of *C. acaulis* EO, showing a mean diameter of around 140 nm and a SOR (surfactant-to-oil ratio) of 0.6. Therefore, the NE was evaluated for its toxicity against *L. botrana* showing an LC_{50} value of 9.044 µL/mL.
The EO safety to mammals was finally investigated by studying its acute toxicity on the stomach, liver, and kidney of rats after oral administration. Only the highest dose (1000 mg/kg) of the EO caused modest neurological signs and moderate effects on the stomach, liver, and kidney. The other doses, which are closer to the practical use of the EO when formulated in protein baits, did not cause side effects. Overall, our findings strengthen the possibility for agrochemical industries to use *C. acaulis* EO and the respective NEs as botanical pesticides.

Red-fleshed apple: polyphenol profile characterization, antioxidant activity evaluation and development of a green extraction method for anthocyanins recovery

Lorenzo Marincich[1], Margherita Bellucci[1], Mariacaterina Lianza[1], Roberto Gregori[2], Stefano Tartarini[2], Roberto Mandrioli[1], Fabiana Antognoni[1]
[1]Department for Life Quality Studies, University of Bologna (QuVi), Rimini Campus, Corso d'Augusto 237, 47921, Rimini, Italy; [2]Department of Agricultural and Food Sciences, University of Bologna (DiSTAL), Viale Giuseppe Fanin 46, 40127, Bologna, Italy

Thanks to the high presence of antioxidant compounds, a diet rich in fruits and vegetables can significantly reduce cancer and cardiovascular risks. Among fruits, apples contain such a high amount of phenolic compounds to be considered one of the major sources of polyphenols in the western diet.
Apple (*Malus* x *domestica* Borkh) is one of the most cultivated and commercialized fruits all over the world and can be consumed either raw or in processed form. After orange juice, apple juice is the most popular one in the world and its processing generates a huge amount of agro-industrial waste. This waste, known as apple pomace, could be still rich in bioactive compounds such as phenolic acids and flavonoids. Among these, anthocyanins have been demonstrated to possess several applications, not only as supplements because of their health-promoting effects, but also as natural pigments in the food and cosmetic sectors. Among apples, red-fleshed apples and derived products, pomace included, could be particularly rich in anthocyanins just because of the nature of the starting matrix.
In the present study, starting from non-processed fruits of three varieties of red-fleshed apples (Red Moon®, Red Love® and Polpa Rossa UNIBO), the respective phenolic acid and flavonoid composition, and antioxidant activity, were investigated. In addition, a green extraction method for anthocyanin recovery was developed. First, for each apple variety, pulp and skin were separated, then the raw material was freeze-dried and ground to a fine powder. For phenolic acids and flavonoids, an acidic MeOH:H_2O extraction was performed from ground material. The extract was used to quantitatively determine the flavonoids and the free forms of phenolic acids content and, after basic hydrolysis, the soluble-conjugated forms of phenolic acids. Extract analysis was carried out by HPLC-PDA and the main flavonoids and phenolic acids were quantified. On the hydrolysed extract, the in vitro antioxidant activity was assessed with the Oxygen Radical Absorbance Capacity (ORAC) assay. The green anthocyanin extraction method was performed by combining the Ultrasound Assisted Extraction (UAE) technique with green solvents (water and ethanol), for both pulp and skin. The method was developed considering as independent variables the ethanol/water ratio, the solid/liquid ratio, and the sonication time, and as the dependent variable the total anthocyanin yield. For the best extraction conditions, the main anthocyanins were detected and quantified using HPLC-PDA.
After having assessed the polyphenol composition of these red-flashed apples, the next task will be to evaluate the total amount of polyphenols that can be recovered from their pomace. In addition, the selected green extraction method will be used on red-fleshed apple pomace to verify its efficacy in recovering anthocyanins.

Exploring *Punica granatum* L. peels from different southern Italian varieties for their potential healthy value

Marinella De Leo[1] Valentina Parisi[2], Valentina Santoro[2], Giuliana Donadio[2], Maria Laura Bellone[2], Alessandra Braca[1], Fabrizio Dal Piaz[3,4], Nunziatina De Tommasi[2]
[1]Department of Pharmacy, University of Pisa, Via Bonanno 33, 56126 Pisa, Italy; [2]Department of Pharmacy, University of Salerno, Via Giovanni Paolo II 132, 84084 Fisciano (SA), Italy; [3]Department of Clinic Pharmacology, University Hospital San Giovanni di Dio e Ruggi d'Aragona, Via San Leonardo, 84125 Salerno, Italy; [4]Department of Medicine, Surgery and Dentistry, University of Salerno, Via S. Allende, 84082 Baronissi, Italy

Punica granatum L. is a small deciduous shrub belonging to Lythraceae family, commonly called pomegranate. Already known since ancient times, pomegranate is nowadays widely cultivated throughout the Mediterranean region, as well as Africa, Asia, and South America. Its attractive red-purple fruit is considered one of the oldest edible fruit still consumed worldwide fresh or as a juice. Traditionally employed for the treatment of various ailments, pomegranate is included today among the richest fruits in health-promoting substances, including polyphenols as anthocyanins, phenolic acids, flavonoids, and both hydrolyzable and condensed tannins. As a by-product of juice production, pomegranate peels are a bio-waste of environmental impact. On the other hand, pomegranate peels are attracting great attention for their beneficial properties, still known in traditional medicine and recently discovered due to scientific studies reporting anti-inflammatory, antimicrobial, woundhealing, and antioxidant properties attributed due to their high content in polyphenols. The cultivation of pomegranate has a long tradition in the Southern Italian internal areas and recently is strongly encouraged to promote the rural and agricultural development of many depopulated internal villages. Taking into account all the benefits of promoting pomegranate products, the aim of this study was the exploration of fourteen pomegranate varieties from internal areas of South Italy, focusing the investigation on both the chemical composition and biological properties of the peels.
The whole fruits were separated into arils and peels, thus the latter were extracted using ethanol-water (70% v/v) by ultrasound-assisted extraction for 15 min. The dried extracts were subjected to an ultra-high performance liquid chromatography coupled to a high resolution electrospray ionization source mass spectrometer (UHPLC-HR-ESI-MS) for the chemical profiling. Furthermore, DPPH and $ABTS^{+}$-based radical scavenger assay, MTT assay, and *in vitro* anti-inflammatory assay monitoring of three typical markers of rheumatoid arthritis (IL-6, IL-1β, and TNF-α) were performed.
Through LC-MS metabolomic fingerprint 55 compounds were identified, including ellagitannins, flavonoids, and anthocyanins, evidencing superimposable profiles for most of the studied varieties. Quantitative estimation showed punicalagin as the major component in all extracts, followed by ellagic acid. Among anthocyanins cyanidin 3-*O*-glucoside was the most represented. All the extracts showed good antioxidant properties in both radical scavenger essays. No cytotoxicity was observed by MTT assay in all varieties. When the extracts were tested on THP-1 derived macrophage cells co-stimulated with lipopolysaccharide, modulation of IL-6 and IL-1β pro-inflammatory cytokines were observed; instead, TNF-α was slightly modulated by pomegranate treatment. Anyway, the different varieties showed a similar effect.
In conclusion, the results of the present work confirmed that pomegranate peels, considered a by-product of juice production, are a rich source of different classes of phenolic compounds exerting antioxidant and anti-inflammatory properties with a good chance in the management of rheumatoid arthritis.

Perovskia artemisioides Boiss roots as source of diterpene constituents with *in vitro* anti-inflammatory activity

Milena Masullo[1], Zahra Sadeghi[2], Antonietta Cerulli[1], Stefania Marzocco[1], Mahdi Moridi Farimani[2], Sonia Piacente[1]
[1]Department of Pharmacy, University of Salerno, Via Giovanni Paolo II n. 132, 84084 Fisciano (SA), Italy; [2]Department of Phytochemistry, Medicinal Plants and Drugs Research Institute, University Shahid Beheshti, 1983969411 Tehran, Iran

Perovskia artemisioides Boiss., belonging to the family Lamiaceae, is a perennial and aromatic plant that grows in Iran and Pakistan. In traditional medicines, extracts of *Perovskia* species are used as remedies for their antibacterial, anti-inflammatory, stomachic, expectorant, and carminative activities. Chemical investigations on *Perovskia* species have resulted in the occurrence of diterpenoid derivatives belonging to the abietane, icetexane, and isopimarane classes. In the context of the abietane class, tanshinones are phenolic diterpene compounds characterized by a phenanthrenequinone core, including ortho-quinones (i.e., tanshinone I) and para-quinones (i.e., isotanshinone I) derivatives. Tanshinones have attracted great attention for their anti-cancer properties and activity against cardiovascular and cerebrovascular diseases. So far, few articles have been reported on *P. artemisioides*, focused mainly on the chemical composition of its essential oil and the evaluation of its antimicrobial activity.
With the aim to identify the specialized metabolites occurring in *n*-hexane extract of *P. artemisioides* roots, an analytical approach based on LCESI/LTQOrbitrap/MS/MS was carried out (Fig. 1). To define the structures of unknown compounds and to confirm the other compounds, isolation and structure elucidation were performed. In this way, nineteen known diterpenoid compounds, mainly belonging to the class of tanshinone and abietane derivatives, along with six diterpenoids never reported before, were unambiguously characterized by 1D- and 2D-NMR experiments.
Diterpenes are reported for anti-inflammatory activity. In particular, tanshinone IIA has shown an anti-inflammatory effect through the inhibition of nuclear factor-kB (NF-kB) activity and inflammatory mediators such as nitric oxide (NO), TNF-α, interleukin- 1 (IL-1), and interleukin-6 (IL-6) in macrophage cells. Furthermore, tanshinone IIA has been reported to reduce LPS-induced lung injury, to attenuate lung inflammatory responses, and to reduce lung cell apoptosis. Considering the anti-inflammatory activity reported for tanshinone derivatives, the diterpenoids isolated from *Perovskia artemisioides* roots were evaluated in LPS-stimulated J774A.1 macrophage cell line. Some of the tested compounds, at the concentration of 50 μM, significantly inhibited NO release and iNOS expression. Furthermore, some of the tested compounds also significantly inhibited COX-2 expression in macrophages. Finally, compounds with the highest activity in reducing NO release were also tested for their antioxidant potential. The obtained results indicated for some of them a significant inhibition of ROS release and nitrotyrosine formation at all tested concentrations (12.5-50.0 μM) in macrophages, thus highlighting both an anti-inflammatory and antioxidant potential.

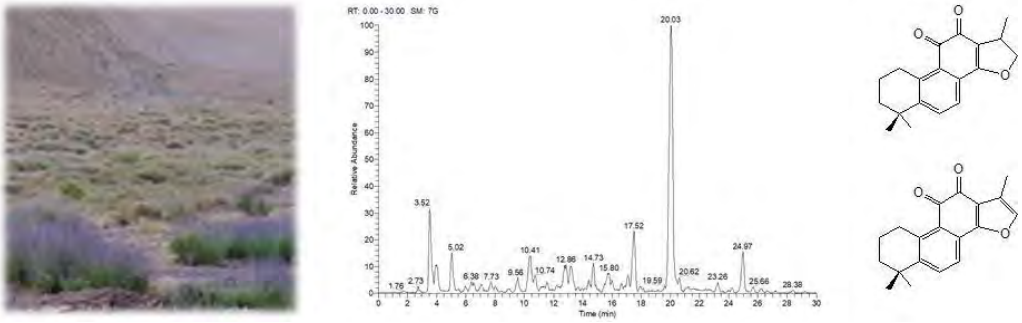


Fig. 1 LCESI/LTQOrbitrap/MS analysis of *n*-hexane extract of *P. artemisioides* roots

The phytochemical profile of leaf and rizhome of *Peucedanum ostruthium* (L.) W. D. J. Koch influences the antioxidant, anti-inflammatory, and wound healing properties of this medicinal plant

Antonella Smeriglio[1], Cristina Danna[2], Miriam Bazzicalupo[3], Mariarosaria Ingegneri[1], Bruno Burlando[4], Domenico Trombetta[1], Laura Cornara[2]
[1]Department of Chemical, Biological, Pharmaceutical and Enviromental Sciences (ChiBioFarAm), University of Messina, Viale Ferdinando Stagno d'Alcontres 31 - 98166 Messina, Italy; [2]Department of Earth, Environment and Life Sciences (DISTAV), University of Genova, Corso Europa 26, 16132 Genova, Italy; [3]CREA - Research Centre for Vegetable and Ornamental Crops, Council for Agricultural Research and Economics, Corso Inglesi 508, 18038 Sanremo, Italy; [4]Department of Pharmacy, DIFAR, University of Genova, Viale Benedetto XV 3, 16132, Genova, Italy

Peucedanum ostruthium (L.) W. D. J. Koch, commonly known as master wort, is a rhizomatous perennial species belonging to the Apiaceae family. Native to the mountains of Central-Southern Europe, it has been widely introduced outside its native range, and generally grows in rivers banks, wet grassy, and anthropic areas. Historical and modern ethnobotanical studies have reported several uses of this plant. The rhizome is officially recognized in traditional medicine, being included in the European Belfrit list of phytotherapeutic remedies for superficial injuries and wounds. However, similar uses of the leaves have also been locally reported. The aim of the present study was to compare the micromorphological features of leaves and rhizome and to develop and optimize a hydroalcoholic extraction procedure, respecting the traditional use of the plant, to investigate the plant phytochemical profile and to evaluate its antioxidant, anti-inflammatory, and wound-healing properties. Plant material was collected in the Gran Paradiso National Park (Aosta Valley, Italy). Rhizomes and leaves were morphologically characterized by light and scanning electron microscopy, while their extracts (RE and LE, respectively) were firstly screened by colorimetric assays (total phenols, flavonoids, flavan-3-ols, and proanthocyanidins) and then investigated by LC-DAD-ESI-MS analysis. A total of 24 compounds were identified and quantified, with a predominance of coumarins in RE and of flavonoids in LE (p<0.01). These different phytochemical profiles explain the differences found in terms of biological activities. *In vitro* tests with sub-cytotoxic extract concentrations revealed stronger antioxidant and anti-inflammatory activities of LE. Wound-healing assays showed stronger activity of LE on HaCaT keratinocytes, and similar activity of LE and RE on L929 fibroblasts. Finally, the phytochemical profile of LE is also favourable from the toxicological point of view due to its lower coumarin content, paving the way to a very promising use of this extract in the nutraceutical and cosmeceutical fields.

Fig. 1. *Peucedanum ostruthium* (L.) W. D. J. Koch plant (a) and detail of the rhizome (b).

POSTERS

3 = Natural microalgae consortia for the removal of nitrogen and phosphorus from urban wastewater streams: the case of the thickening effluent from the wastewater treatment plant of Ferrara

Costanza Baldisserotto[1], Sara Demaria[1,2], Roberta Marchesini[1], Lorenzo Ferroni[1], Linda Benetti[3], Marcello Zanella[3], Simonetta Pancaldi[1,2]
[1]Department of Environmental and Prevention Sciences, University of Ferrara, C.so Ercole I d'Este, 32, 44121 Ferrara, Italy; [2]Terra&Acqua Tech Laboratory, Technopole of Ferrara University, Via Saragat, 13, 44122 Ferrara, Italy; [3]HERA SpA – Direzione Acqua, Via C. Diana, 40, 44044 Cassana (Ferrara), Italy

Wastewaters (WW) produced as the result of normal human activities (urban, industrial, agricultural, etc.) cause a wide range of environmental pollution problems. Remediation of urban polluted waters usually occurs in wastewater treatment plants (WWTPs), which serve small up to large urban areas. WWTPs are essential to prevent environmental problems, like eutrophication due to excessive release of nutrients (nitrogen and phosphorus) into aquatic environments. During WW treatments in traditional WWTPs, sludge is produced; sludge itself needs further treatments (thickening, dewatering, anaerobic digestion), which are responsible for the production of nutrient-rich effluents recirculating inside the WWTP, as they do not meet the limits required by EU and national legislation for a safe discharge into natural water bodies. At the same time, the availability of nutrient-rich WWs can represent an opportunity: they can be used as culture media for microalgae production, as these microorganisms use the nutrients they contain for their growth, and leading, as the final result, to phytoremediation of the original effluent. Microalgae are, in fact, photosynthetic microorganisms, excellent candidates for a wide range of biotechnological applications, including the removal of nutrients from urban wastewaters. Thus, microalgae-based plants can be integrated into conventional WWTPs to improve the water depuration process. The choice of the best microalgae candidates for N and P removal is a strong issue to guarantee an effective phytoremediation. Besides collection microalgae, the isolation of strains from effluents represents a further opportunity. Within this latter approach, the employment of natural microalgae consortia for nutrient remediation deserves to be investigated. Indeed, isolated consortia tend to be more responsive to variations of the environmental conditions and easier to cultivate than algal mono-cultures. The present study is meant to investigate the potential of an isolated consortium formed by three autochthonous microalgae for the removal of N and P from WW effluents deriving from the thickening stage of sludges, produced at the WWTP of Ferrara managed by HERA SpA. Growth and nutrients removal ability of the microalgal consortium were compared using the thickening effluent and a synthetic medium, prepared with the same N and P concentrations as in the effluent. Relative proportion of each algal component of the consortium was monitored to better understand dynamics inside the consortium. The content of total proteins and photosynthetic pigments of microalgae was evaluated to highlight biotechnological potential of the harvested biomass; in parallel, the photosynthetic efficiency of PSII was evaluated to detect stress conditions. Cells cultivated in the effluent grew and removed nutrients better than those cultivated in the synthetic medium. Ammonium and phosphorus were removed by about 60-70% in 14 days of algae cultivation, while no nutrient removal occurred in the synthetic medium. These results indicate that the consortium was efficient in the phytoremediation process and that algal biomass can be proposed as feed supplement or agricultural biofertilizer, since it is rich in proteins and photosynthetic pigments.

This work was granted by a POR-FESR 2014-2020 Project – Emilia-Romagna, Italy (VALUE CE-IN "VALorizzazione di acque reflUE e fanghi in ottica di economia CircolarE e simbiosi INdustriale").

3 = Hybridization does not limit the distribution of the endangered *Orchis patens*

Miriam Bazzicalupo[1-2], Martino Adamo[3], Karl J. Duffy[4], Laura Cornara[1], Jacopo Calevo[4,5]
[1]Department of Earth, Environment and Life Sciences, University of Genova, Corso Europa 26, 16132 Genova, Italy; [2]CREA Research Centre for Vegetable and Ornamental Crops, Council for Agricultural Research and Economics, Corso degli Inglesi 508, 18038 Sanremo, Italy; [3]Department of Life Sciences and Systems Biology, University of Turin, Viale Mattioli 25, 10125 Torino, Italy; [4]Department of Biology, University of Naples Federico II, Via Cinthia, 80126 Naples, Italy; [5]Department of Ecosystem Stewardship, Jodrell laboratory, Royal Botanic Gardens, KEW, Richmond, Surrey, UK

Hybridization frequently happens because of generalist interactions with pollinators in Mediterranean orchids. When rare and common orchid species co-occur, share pollinators, and overlap in flowering time, hybridization is likely resulting in introgression and ultimately in the loss of the rare species. To verify if hybridization plays a role in limiting the distribution of the rare and endangered *Orchis patens* Desf., we investigated the possible interactions with the more common *O. provincialis* Balb. *ex* Lam. & DC*,* and their natural hybrid *O.* ×*fallax* (De Not.) WillK. & Lg. We tested whether hybridization among the parental taxa is bidirectional and leads to different vegetative traits (namely a green stem or a purple stem) of the hybrids; we also examined variation in genetic and morphometric traits for each taxon. As orchids depend on mycorrhizal fungi to germinate and often vary in the fungal taxa they associated with, we evaluated if mycorrhizal associations contribute to hybrid establishment.
Genetic analyses based on SCoT markers confirmed that hybridization between *O. patens* and *O. provincialis* can be bidirectional, with the green and purple stem forms of the hybrid having *O. provincialis* and *O. patens* as female parental species, respectively. Both hybrid forms showed a spur of intermediate length between *O. patens* and *O. provincialis*, while labellum and arm areas were larger in the hybrids and in *O. provincialis.* Orchid mycorrhizal (OrM) fungi were shared among all taxa, thus not limiting the establishment of new individuals of any taxon.
Differences in pollen competitive ability and pollen germination rates between taxa was assessed by *in vitro* tests, where we measured pollen tube growth rate, and ovary fertilization using epifluorescence microscopy. Hand-pollination experiments were carried out to evaluate selfing (autogamous and geitonogamous) and crossing (intra- and interspecific) ability. We also evaluated the possible competition among hybrid and conspecific pollen on the stigmatic surfaces of *O. patens* by applying these different pollen types simultaneously on the stigma. The highest percentage of *in vitro* pollen germination and speed were recorded for *O. patens* (69,58 ± 9,48% and 5,78 ± 1,58 µm/h) followed by *O. provincialis* (64,1 ± 5,35% and 3,07 ± 0,45 µm/h), while the two forms of the hybrid showed near-sterility performances (both ranging around 15% and 2 µm/h). Data on pollen tube growth on stigmas and within ovaries, together with information on fruit set, highlighted that both parental species are self-compatible, can cross and, if used as pollen donors, can backcross with the two forms of the hybrid. On the contrary, fruiting failure was recorded in almost all crosses involving the hybrids as pollen donor. However, as highlighted by the successful fruit formation in double-crossing experiments, the presence of hybrid sterile pollen on the stigma putatively did not hinder conspecific pollen germination in *O. patens*.
This confirms that in sympatric populations there is a high probability of hybridization and introgression, and that the frequent establishment of hybrid plants may be allowed also by the shared OrM fungi. Considering that the pollen of *O. patens* germinates quickly, and that seeds are highly viable and can germinate with a widespread fungus, the limited distribution of this rare species may be mainly due to strict habitat requirements, and to its low adaptability to adverse abiotic factors and environmental changes.

3 = Addressing data quality issues of Wikiplantbase #Toscana

Gianni Bedini[1], Gianmaria Bonari[2], Katia Caparelli[3], Marco D'Antraccoli[4], David Dolci[5], Giulio Ferretti[6], Giovanni Gestri[7], Giuliano Pacifico[8], Brunello Pierini[9], Lorenzo Pinzani[1], Francesco Roma-Marzio[4], Lorenzo Peruzzi[1]

[1]Department of Biology, University of Pisa, Via Derna 1, 56126 Pisa, Italy; [2]Faculty of Science and Technology, Free University of Bozen-Bolzano, Piazza Università 5, 39100 Bolzano, Italy; [3]Piazza G. Guerra 28, 50053 Empoli, Italy; [4]Botanic Garden and Museum, University of Pisa, via Ghini 13, 56126 Pisa, Italy; [5]Department of Biological, Geological, and Environmental Sciences, University of Bologna, via Irnerio 42, Bologna, Italy; [6]Botanic Garden, University of Florence, via Micheli 4, 50121 Florence, Italy; [7]via Bonfiglioli 30, 59100 Prato, Italy; [8]Via Ponte del Vescovo 32/D, Massa, Italy; [9]Via Zamenhof 2, 56127 Pisa, Italy

Floristic inventories, a primary tool for biodiversity research, are increasingly available as online, freely accessible digital archives, which spare researchers the need to cherry-pick data from myriads of publications or natural history specimens. Furthermore, digital archives promise to keep pace with the dynamic identity of floras and nomenclatural updates much quicker than traditional floras. However, multiple researchers are questioning the data quality, especially in large repositories relying on many providers such as GBIF (www.gbif.org). The debate on this issue is still lively, with many suggestions about data pre-processing before using them in research applications.

Wikiplantbase #Toscana (hereafter WPB) is an online floristic database storing about 400K georeferenced records for Tuscany, an Italian administrative region. Pro bono collaborators provide data entry via a user interface designed to minimize the error rate, taxonomic inconsistency, and spatio-temporal uncertainty relative to the whole source. In addition, appointed coordinators verify each record before it goes online. Nevertheless, some quality issues persist. In this work, we present some problems that affect the use of this resource in biodiversity-related research.

During an analysis to inform future field explorations, we queried the archive to extract unconfirmed floristic records for Tuscany. First, we selected only "presence" records (as opposed to "excluded" or "doubtful" ones). We then partitioned the dataset into old (i.e. documented before 1 Jan. 1950) and current (collected after or on 1 Jan. 1950) records. Next, we compared the two species lists (from the old and current partitions) and retained only the species represented exclusively in the old records. We then benchmarked the names and their occurrence status in Tuscany against Floritaly (dryades.units.it/floritaly) and recorded any discrepancy between our data and the benchmark.

Table 1. Floritaly occurrence status of 260 unconfirmed species from Wikiplantbase #Toscana

Floritaly status	Count
Occurring	97
Historical records	62
Doubtful	40
Alien	21
No data	12
Recorded by mistake	11
Not listed for Tuscany	11
Extinct	6

Our dataset included 260 species originating from 780 unconfirmed records. Tab. 1 summarises their occurrence status in Floritaly, and multiple issues arise from our results. Floritaly lists 110 unconfirmed species for Tuscany, approximately half of our dataset. Strikingly, our dataset does not include the whole Floritaly unconfirmed set (stored as "historical records"), but only 62 names out of 110. In some instances, our dataset comprises recent bibliographic or observational records overlooked in Floritaly, leading to species regarded as unconfirmed in Floritaly but not in Wikiplantbase: for example, the alien *Tulipa gesneriana* L. subsp. *gesneriana*, *Ricinus communis* L., *Salvinia molesta* D.S.Mitch, and *Oenothera sinuosa* W.L.Wagner & Hoch.

On the other hand, 97 species recorded with an "occurring" status in Floritaly are unconfirmed in WPB. In some cases, WPB is simply falling behind in data entry, as in the case of *Oxybasis chenopodioides* (L.) S.Fuentes, Uotila & Borsch.

Eleven species from the WPB dataset are marked as "recorded by mistake" in Floritaly. These are initially misplaced records like one of the Sicilian endemic *Astragalus siculus* Biv., reported by Baroni in 1908 for the Elba Island. Floritaly does not list eleven other species from WPB ("Not listed for Tuscany" in Tab. 1): some are cultivated specimens; others, like *Galium cinereum* All., exhibit multiple, inconsistent conditions (such as "present", "excluded", and "doubtful"); others yet are legitimately present records ignored by Floritaly, like, e.g. *Galium pallidum* C.Presl, documented by two specimens by M. Guadagno.

Our results showed that even actively curated databases may suffer quality issues. Cross-checks between Wikiplantbase #Toscana and Floritaly highlighted discrepancies that must be addressed in both systems. Fortunately, the issues can be easily fixed to increase the quality and reliability of floristic data and, in turn, the quality of research built upon them.

3 = Mapping habitats in Nature 2000 sites in Aosta Valley through photo-interpretation of images from drones and field surveys

Simone Eusebio Bergò[1], Maria Consolata Siniscalco[1], Elisa Giaccone[1], Ludovica Oddi[1], Umberto Morra di Cella[2]
[1]Department of Life Science and Biology of System, University of Turin, Viale Pier Andrea Mattioli 25, 10125 Torino, Italy; [2]Environmental Protection Agency of Aosta Valley, Loc. La Maladière 48, 11020 Saint-Christophe, Italy

So far, vegetation monitoring has been conducted mainly in the field, with methods that can take a long time and logistical difficulties. In recent years, however, remote sensing is becoming more and more important and represents a useful tool and a suitable source of data for the classification of vegetation. The most common approaches are currently satellite remote sensing, followed by other images.
Through the interpretation of aerial photos, the Aosta Valley region, in compliance with the European Directive 92/43/EEC (Habitat), has provided for the development of habitat maps of the Nature 2000 sites in the region.
However, these approaches can produce large errors when applied to highly heterogeneous vegetation on a small scale and when the pixel size is not small enough to avoid a combination of different species within it.
To overcome this obstacle, unmanned aerial vehicles (UAVs), which can reach a very high resolution with pixel size of a few centimetres, represent a great opportunity to monitor vegetation dynamics, replace part of the field work, reduce the costs and to acquire images with a high temporal resolution. However, so far few studies have used images acquired by UAVs to classify vegetation.
In this work we updated the habitats cartography of 5 Nature 2000 sites in the Aosta Valley through photo-interpretation of the images acquired by UAVs and comparison with field surveys data. The aim of the work was to define and test a protocol for the detection of images from UAVs (Fig. 1) that took into account the phenology of the vegetation and guaranteed the acquisition of the best images. The design and classification of the polygons were carried out following the surveys in the field aimed at verifying the phytocoenoses, the composition of the layers and the phenological trend. Finally, a comparison was made between the old maps and the updated ones to quantify the increase in detail in the information obtained from the new high-resolution images and the validity of the approach followed.
The work was supplemented by a phytosociological study conducted on the IT1205082 “Stagno Lo Ditor” site with a particular interest in peat bog vegetation types.
The results show an increase in the resolution of the habitat cartography (Fig. 2), the correct definition of the best conditions and times of the year for image acquisition, and the need of verifying the real conditions of the vegetation in the field for its correct classification.

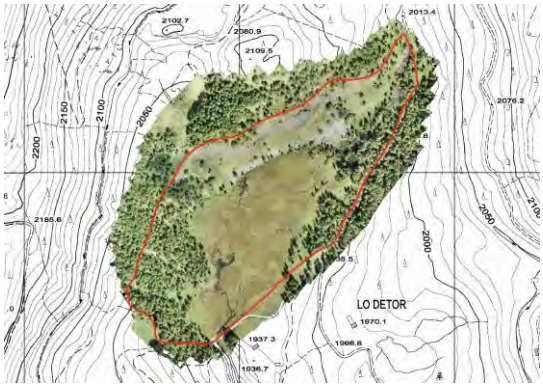


Fig. 1. RGB ortophoto of IT1205082 “Stagno Lo Ditor”

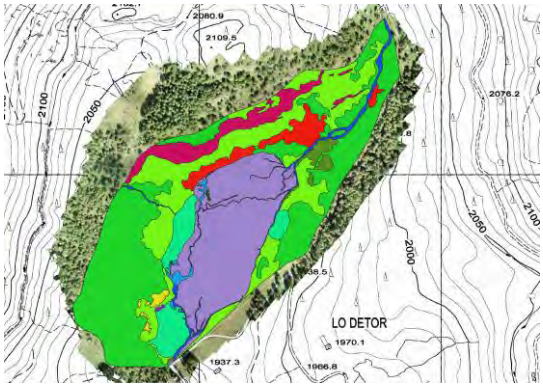


Fig. 2. Habitat maps of IT1205082 “Stagno Lo Ditor”

3 = Marine plastic pollution: how significant is the impact on seagrass ecosystems?

Giuseppe Bonanno[1], Martina Orlando-Bonaca[2]
[1]Department of Biological, Geological and Environmental Sciences, University of Catania, Via Empedocle 58, 95128 Catania, Italy; [2]Marine Biology Station Piran, National Institute of Biology, Fornace 41, 6330 Piran, Slovenia

Plastics have nowadays become the primary marine pollutants accounting for about 80% of sea debris worldwide (Fig. 1). As such, plastic debris is widely recognized as a threat to marine ecosystems and wildlife, and many studies have so far documented the interaction between marine debris and several species of marine wildlife. Seagrasses, in particular, are globally widespread and play numerous and fundamental ecological roles (Fig. 2). However, seagrasses are scarcely mentioned in studies about marine plastic debris. It is therefore of the utmost importance to know the effects of plastics on seagrasses, their associated organisms and food webs. Filling scientific gaps is also pivotal to better guide future research and conservation policies. This study specifically investigated the current state of knowledge regarding the adverse effects of plastic debris on seagrasses, their associated organisms, and the resulting trophic interactions. All the available and relevant published studies on the relationship between seagrasses, plastic debris and environmental policies were consulted until December 2021. These publications were identified through the on-line scientific databases “Scopus”, “ISI Web of Science”, and “ScienceDirect”. The collected information was used to discuss the current state of knowledge about the impact of plastic debris on seagrasses, and the relative policies of management and conservation.
The answer to the title question is that the current impact is not sufficiently known, and tailored conservation policies are lacking. Despite the fundamental ecological importance of seagrasses, current studies are not sufficient to provide a clear picture of the potential consequences of plastic pollution in seagrass ecosystems. Numerous questions are worth further investigating such as the capacity of seagrass ecosystems to act as microplastic sinks, the ability of seagrass species to entrap plastics on their tissues, the impact of microplastic consumption on the health and viability of herbivores, and the alteration of trophic interactions due to macroherbivory as an entry gate for microplastics into the marine food web. In particular, the role of seagrasses in microplastics entering food webs is of particular concern. Without tailored policies, the worldwide quantity of marine litter, especially plastics, is destined to further rise with the increasing global population and industrial production. We are living in an historical period where the pervasiveness of plastics can justify the term *Plasticene*. It is down to all of us – governments, industries, scientists, citizens – to limit our unnecessary levels of plastics consumption before the fragile balance of the marine environment is altered in an irreversible way.

Fig. 1. Marine plastic debris

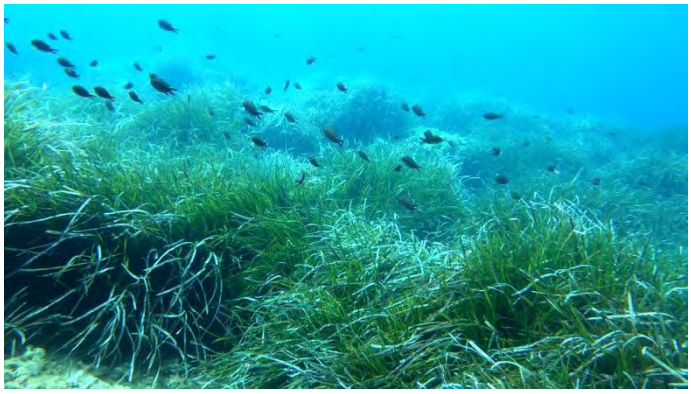


Fig. 2. *Posidonia oceanica* meadows

3 = Annex II-IV (Dir. 92/43/EEC) plant species occurring in Umbria region: distribution maps, demographic studies and conservation actions within the LIFE19 IPE/IT/000015 Project

Federica Bonini[1], Valentina Ferri[1], Laura Cencini[1,2], Simona Ciancaleoni[1], Maurizio Conticelli[3], Francesco Falcinelli[4], Marco Fratoni[4], David Grohmann[1,2], Marco Maovaz[1,2], Maurizio Micucci[1,2], Antonella Montanucci[1,2], Valeria Negri[1], Lorenzo Raggi[1], Giuseppe Ratini[3], Daniela Gigante[1]
[1]Department of Agricultural, Food and Environmental Sciences, University of Perugia, Borgo XX giugno 74, 06121 Perugia, Italy; [2]C.A.M.S.and Botanical Garden, University of Perugia, Borgo XX giugno 74, 06121 Perugia, Italy; [3]GIROS Sezione Umbria, Via della Lepre 11, 05100 Terni, Italy; [4]Carabinieri Biodiversity Department of Assisi, Viale Umberto I 5, 06081 Assisi (PG), Italy

Five Annex II-IV plant species (*Adonis distorta* Ten., *Himantoglossum adriaticum* H. Baumann, *Ionopsidium savianum* (Caruel) Ball ex Arcang., *Iris marsica* I. Ricci & Colas., **Klasea lycopifolia* (Vill.) Á. Löve & D. Löve) are the target of specific conservation actions of the LIFE Integrated Project "IMAGINE" (LIFE19 IPE/IT/000015) in Umbria region.
Their maintenance in a good conservation status is a national duty for each EU country, directly deriving from the Council Directive 92/43/EEC, and requiring a deep knowledge of their ecology, distribution, demography and biological habitats.
A complete survey of these 5 species has been carried out in the first two years of the Project (2020-2021) in the frame of the preliminary action A11 "Development of Action Plans and *ex-situ* conservation protocols for 5 Annex II-IV plant species".
An extensive bibliographic analysis of the plants' biology, ecology, and known distribution, combined with the fieldwork activities carried out in spring-summer 2021, allowed us to: I) implement complete regional distribution maps, adding several new cells to the 10 × 10 km EEA reference grid compared to the 4th Report ex-Art. 17 (Fig. 1), II) obtain a demographic characterization (census) of the populations, III) identify the biological habitats of the target species, IV) detect the main pressures and threats that afflict their conservation status. Noteworthy in the distribution data updating was the support of citizen science and expert contributors, e.g. from the Regional Section of the Orchidologists Association G.I.R.O.S., and the Carabinieri Biodiversity Department of Assisi (PG).
On the ground of the acquired knowledge, specific Action Plans, aimed at improving the *in-situ* conservation of the five target species, and Protocols for germplasm collection, reproduction, and storage (*ex-situ* conservation) have been developed. Germplasm collection and storage are in progress at DSA3 Germplasm Bank (FAO ITA_363).
Additionally, for each species, individuals collected in the most representative regional stations will be conserved *in vivo* in the Botanical Garden of Perugia University (Fig. 2), also in order to produce seeds and seedlings that will be usable for future reinforcements of existing populations.

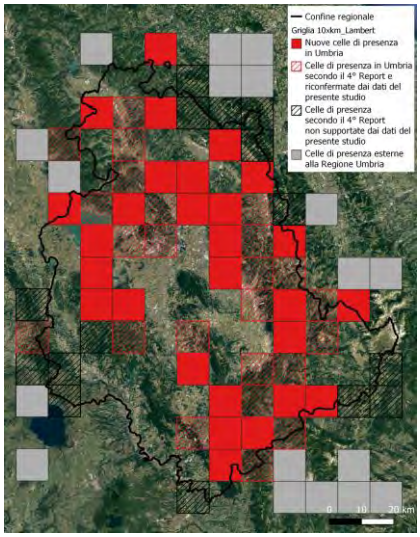


Fig. 1. Updated distribution map of *Himanthoglossum adriaticum* H. Baumann in Umbria (10 x 10 km cells)

Fig. 2. *Iris marsica* I. Ricci & Colas. conserved *in vivo* at the Botanical Garden of Perugia University

3 = Ethnobotany as an effective tool to preserve biodiversity: the case study of Valmalenco (SO, Italy)

Martina Bottoni[1,2], Fabrizia Milani[1,2], Lorenzo Colombo[1,2], Paola Sira Colombo[1,2], Claudia Giuliani[1,2], Piero Bruschi[3], Gelsomina Fico[1,2]

[1]Department of Pharmaceutical Sciences, University of Milan, Via Luigi 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 Agricultural, Environmental, Food and Forestry Science and Technology, University of Florence, Piazzale delle Cascine 18, 50144 Florence, Italy

In recent years, ethnobotanical research has become an essential tool to face one of the most stimulating current challenges: the preservation of plant and cultural biodiversity. Specifically, mountain areas are recognized as a reservoir of traditional plant knowledge, the preservation of which can promote local phytodiversity and the discovery of new bioactive compounds. In this framework, the folk plant uses documented by this ethnobotanical study carried out in Valmalenco (Sondrio, Italy) represented the base to promote an alternative strategy for the sustainable development of the area.

Our survey is part of the European Interreg Italy-Switzerland *B-ICE* project and aimed at investigating the traditional uses of the autochthonous plant species within the municipalities of Chiesa in Valmalenco, Caspoggio, Lanzada, Spriana and Torre di Santa Maria. Open and semi-structured interviews were conducted and archived in a specific database, paying attention to the common and dialectal names of the plants, their traditional purpose, their presence/absence at the study area over time, their past or actual use, together with the employed plant parts, the preparation forms, and the administration methods.

From 2019 to 2022, a total of 401 informants were interviewed, all aged between 10 and 96 years old, providing information on 232 plants, belonging to 79 families, among which Asteraceae (24.2% of the total citations), Rosaceae (13.2%), Ericaceae (8.9%) Pinaceae (7.9%) and Lamiaceae (7.1%) were the most cited ones. Out of the most cited species, the flowering tops of *Arnica montana* L. (5.9% of the total citations) were used in form of ointment in cases of contusions, muscle and joint pain and inflammation. The fruits of *Vaccinium myrtillus* L. (5.6%) were consumed as fresh fruits, country snack or in jams. The decoction of inflorescences of *Achillea moschata* Wulfen (4.8%) was adopted to treat digestive disorders and stomachache. The unripe pinecones of *Pinus mugo* Turra (4.6%) were prepared in form of syrup and administered to treat cough and sore throat. Finally, the infusion obtained from the leaves of *Malva sylvestris* L. (4.4%) was employed for its anti-inflammatory effect to make gargles, or in form of compresses as emollient and soothing.

To preserve this “cultural landscape” the project includes, among others, the design and realization of a high-rise Botanic Garden in Sant’Antonio di Caspoggio, which is being realized with a brave purpose: securing in a “repository without any time and space” the plant and cultural biodiversity of Valmalenco. The Botanic Garden will host the most representative plant species the survey brought out, attracting interest not only for their traditional uses, but also for their conservation value, to strengthen the abilities to manage and safeguard the local natural ecosystems. In addition, the final aim will be to offer a new strategy for a sustainable form of tourism based on the rediscovery of ethnobotanical traditions, which start from people and must return to them in full respect of an *Open Science* policy.

3 = NIR hyperspectral imaging: a tool for visualising the spatial distribution of water in non-vascular epiphytic communities

Giulia Canali[1], Cristina Malegori[2], Paola Malaspina[1], Rodrigo Rocha de Oliveira[2], Monica Casale[2], Paolo Oliveri[2], Luca di Nuzzo[3], Renato Benesperi[3], Juri Nascimbene[4], Paolo Giordani[2]
[1]Department of Earth Sciences, Environment and Life (DISTAV), University of Genoa, Corso Europa 26, 16132 Genova, Italy; [2]Department of Pharmacy, University of Genoa, Viale Cembrano 4, I-16148 Genova, Italy; [3]Department of Biology, University of Florence, Via la Pira 4, 50121 Firenze, Italia; [4]BIOME Lab, Department of Biological, Geological and Environmental Sciences, Alma Mater Studiorum University of Bologna, via Irnerio 42, 40126 Bologna, Italy

Bryophytes and lichens belong to very different taxonomic groups but are very similar in their ecology and ecophysiology. They are poikilohydric organisms, so that their abundance is strongly influenced by atmospheric water availability. Since they lack organs for active water uptake, they absorb water, nutrients, and gases directly from the atmosphere through their entire surface. The effect of their traits on ecosystem functions such as thallus water uptake and release and temperature buffering are upscaled from the level of the individual organism to that of the community, contributing substantially to regulate the microclimate of the whole ecosystem. The response to microclimatic factors, such as water availability during hydration and dehydration cycles, can be very different among these organisms. Indeed, lichens and bryophytes have found different strategies to adapt their water supply, including changes in structure and physiology, to absorb, store and avoid water loss or to tolerate dehydration. The aim of this work is to verify if the composition of the epiphytic community determines favouritism phenomena, prolonging water availability during hydration and dehydration cycles. For achieving this goal, we used near infrared (NIR) hyperspectral imaging to assess the spatial distribution of water at sub-centimeter scales on samples of non-vascular epiphytic communities. Hyperspectral imaging combines the advantages of NIR spectroscopy and imaging to obtain both spectral and the spatial evaluation of the samples. From the images, water maps are developed by applying dedicated chemometric algorithms aimed at understanding the correlation between organisms and water spectral features.

3 = Influence of forest management on microclimate and understorey vegetation in a mixed oak forest

Elisa Carrari, Cristina Gasperini, Marco Cabrucci, Ilaria Santi, Federico Selvi
Department of Agriculture, Food, Environment and Forestry, Laboratories of Applied and Environmental Botany, University of Florence, P. le Cascine 28, I-50144 Firenze, Italy

Forests play an essential role in the mitigation of climate change effects, by limiting temperature extremes and creating microclimatic refuges that can contrast the thermophilization of the biotic communities that live under the tree canopies. Based on recent evidence, forest microclimatic effects are especially pronounced in regions with warm macroclimate, such as the Mediterranean one. Microclimate buffering also depends on forest structure and density, both of which are, in turn, often determined by the type of silvicultural management. Recent findings also supported that the microclimatic alterations caused by wood extraction can determine, in turn, adaptive adjustments in some functional traits of understorey plants, thus also influencing ecosystem functions. Although some of the effects induced by the main forms of silvicultural management on understorey diversity and productivity in some types of broadleaf forests have been already described, their influence on the intraspecific variability of functional traits associated with microclimate changes is still not clear. We therefore started an investigation of these effects in a deciduous mixed oak forest with *Quercus cerris* and *Q. petraea* located in the valley of Mugello, in north Tuscany (Bosco ai Frati). Thanks to its homogeneous site conditions, this semi-planitial forest area represents a suitable model system to compare the effects of two contrasting management types on understory diversity, productivity and leaf functional traits of herbaceous species. To this purpose, in 2021 we installed air and soil temperature dataloggers in three high forest sites and three close coppice-with-standards sites. Forest structural variables and light availability (photosynthetic active radiation, PAR) were determined before surveying understory vegetation in four 5 x 5 m randomly selected quadrats in each of the six sites (24 quadrats). Understory aboveground productivity (dry weight of herbaceous and woody biomass) was determined in 48 0.5 x 0.5 m plots (two per quadrat). Finally, three locally abundant understorey forest specialist herbs (*Physospermum cornubiense*, *Anemone nemorosa*, *Viola reichenbachiana*) and two more generalist species (*Lonicera caprifolium*, *Cruciata glabra*) were selected and sampled following standard protocols to determine variation in major leaf functional traits (Leaf Area, Specific Leaf Area, Leaf Dry Matter Content). Preliminary results showed that, in the high forest plots, extreme values of temperatures were strongly mitigated, especially their maximum during the hottest period of the year (max T in August was 6°C higher in coppice stands). The coppice understory had a higher productivity, associated with higher light availability, while diversity did not show a clear trend. Species composition in the high forest plots revealed a more shade-tolerant and neutrophilic character, as well as a greater abundance of geophytes and a reduction of the small woody species, compared with the coppice stands, which were instead characterized by more numerous generalist species. Effects on leaf traits were clear, including a significant LA reduction in *A. nemorosa* and *P. cornubiense* in the coppice stands. Moreover, increasing PAR induced a decrease in the SLA of generalists, but an increase in the specialists. Building Grime triangles based on leaf traits (using the Stratefy software) we found that forest specialists and generalists were differently affected by management. In fact, forest specialists acquired a more ruderal character in the coppice stands, while generalists were less affected. Present results are still preliminary but seem to support that the coppice-with-standard management, though not reducing overall diversity and increasing understorey productivity, is less effective in buffering macroclimate warming and maintaining microclimatic refuges for forest specialist plants of mixed oak forests. If confirmed, this might help to adopt management strategies that can help to preserve the species composition, ecological profile and optimal functionality of understory communities of our increasingly threatened forests.

3 = Analysis of genetic diversity and phenotypic plasticity in *Phragmites australis* and *Nuphar lutea*. A case study from Northern and Central Italy

Maria Beatrice Castellani[1], Rossano Bolpagni[2], Lorenzo Lastrucci[3], Alice dalla Vecchia[2], Erika Piaser[4], Andrea Coppi[1], Paolo Villa[4]

[1]Department of Biology, University of Florence, via Micheli 1, 50121 Florence, Italy; [2]Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Parma, Italy; [3]Natural History Museum, Botanical Collections, University of Florence, via G. La Pira 4, 50121 Firenze, Italy; [4]Institute for Electromagnetic Sensing of the Environment, National Research Council (CNR-IREA), Milan, Italy

Wetlands are highly productive environments with an increasingly recognized economic, social, and ecological value. The high primary productivity characterizing wetlands is mainly due to a dense macrophytes vegetation, which plays several roles in biological processes and associated ecosystem services. Like terrestrial plants, the persistence and resilience of macrophytes is affected by altering environmental factors such as temperature, salinity, the availability of nutrients and light and by the intraspecific diversity dimension. The intraspecific diversity leads the species' adaptive response to biotic and abiotic environmental factors and plays a crucial role in populations' ecological and evolutionary dynamics. Therefore, understanding patterns and processes of genetic and phenotypic intraspecific diversity become essential for environmental, evolutionary and conservation studies. Moreover, it is crucial to determine the role of selective and neutral processes in driving observed differentiation.

In the last years, several studies have investigated the potential of natural selection on morphological evolution by comparing phenotypic divergence with neutral genetic divergence via a PST/FST approach. In this context, we aimed to assess and compare the genetic differentiation and functional traits variation at the intraspecific level of two macrophyte species, the helophyte *Phragmites australis* and the emergent hydrophyte *Nuphar lutea*. We first analyzed the AFLP-fingerprinting profiles and morphological and biochemical traits in both species sampled across several lakes in north-central Italy. The functional traits were estimated using both proximal measures on the leaves (Leaf Area, Leaf SLA, Chl-a and Chl-a/Chl-b) and inversion of the PROSPECT model (Chl_ab, LMA, LDMC, and Nmesophyll). Then, we investigated the correspondence between the degree of population differentiation in neutral genetic markers and the quantitative traits, as measured by FST and PST indices, respectively. As for *P. australis*, results showed that traits variability was almost entirely shaped by phenotypic plasticity, except for three traits: Leaf SLA for Chiusi, Chl-a for Iseo and the Chl-a/Chl-b ratio for the Pusiano-Annone site. As for *N. lutea*, results were much more complex and heterogeneous. The phenotypic plasticity and genetic diversity appear to have affected the variability of different traits within each site, except for Pusiano-Annone where traits variability was mostly due to genetic diversity (Tab. 1). This work allowed to understand the contribution of genetic variability and phenotypic plasticity to the traits' variability of *P. australis* and *N. lutea* and to propose new considerations on health conditions or response to environmental factors of both species.

Phragmites australis						***Nuphar lutea***				
TRAIT	**CH**	**IS**	**MA**	**MN**	**PA**	**TRAIT**	**CH**	**IS**	**MN**	**PA**
Chl_ab (µg cm-2)	PP	PP-DG	PP*	PP*	PP*	Chl_ab (µg cm-2)	PP*	PP-DG	PP	DG
LMA (g m-2)	PP-DG	PP	PP*	PP*	PP*	LMA (g m-2)	PP	PP	DG	DG
LDMC (g g-1)	PP	PP	PP	PP*	PP*	LDMC (g g-1)	DG	PP	DG	DG
Nmesophyll	PP	PP-DG	PP*	PP*	PP*	Nmesophyll	PP	DG	PP-DG	PP
Leaf area tot (mm2)	PP	PP	PP*	PP	PP*	Leaf area tot (mm2)	PP*	PP*	PP*	DG
Leaf SLA (mm2/mg)	DG	PP-DG	PP*	PP	PP	Leaf SLA (mm2/mg)	PP-DG	DG	PP*	DG
Chl-a (µg g-1)	PP-DG	DG	PP	PP	PP*	Chl-a (µg g-1)	PP	PP	PP*	DG
Chl-a/Chl-b	PP-DG	PP-DG	PP	PP	DG	Chl-a/Chl-b	DG	DG	PP	DG

Tab. 1: Table shows results of PST/FST comparison for each trait within sites (CH= Chiusi; IS= Iseo; MA= Massaciuccoli; MN= Mantova; PA= Pusiano-Annone). PP indicates Phenotypic Plasticity, i.e. PST>FST with c/h^2 <0.63); DG indicates Genetic Diversity, i.e. PST<FST with $c/h^2 \geq 1$); PP-DG indicates that was not possible to discriminate if trait shaped by PP or DG, i.e. PST>FST but c/h^2 is a value between 0.63 and <1). The asterisk (*) shows greater robustness of the result.

3 = Aggregation of occurrence data in FlorItaly, the portal to the flora of Italy

Matteo Conti[1], Michele Lussu[2], Riccardo Testolin[2], David Dolci[2], Nicola Alessi[3], Piero Zannini[2]
[1]Department of Life Sciences, University of Trieste, Via L. Giorgieri 10, 34127 Trieste, Italy; [2]Centro Interuniversitario per la Biodiversità Vegetale Big Data - PLANT DATA, Department of Biological, Geological and Environmental Sciences, Alma Mater Studiorum University of Bologna, Via Irnerio 42, 40126 Bologna, Italy; [3]Italian Institute for Environmental Protection and Research, Via Vitaliano Brancati 48 00144 Roma, Italy

Data collected in floristic and vegetation surveys are essential for botanical and ecological studies. Unfortunately, after serving the specific scope for which they have been collected, these data often remain locked-up and inaccessible to the rest of the scientific community. The aggregation of data collected from multiple sources into a single, freely accessible, database represents a key step to generate further insights. The great advantage of aggregated database systems lies in the ability to retrieve, reuse and summarise information to investigate general patterns.
Despite all fields of botanical and ecological sciences take advantage from data aggregation, studies on the effects of large-scale changes can be primary beneficiaries. Indeed, these changes are altering biodiversity and ecosystems at unprecedented rates, to the point we entered the sixth mass extinction in Earth's history. Occurrence data, which are used for distribution modeling and niche evolution studies, are fundamental to understand the impact of global changes, and assess their magnitude. In Italy, only a fraction of existing occurrence data can be easily accessed and downloaded online. Thus, the use of these data for inferring the impact of global changes on Italian biodiversity and ecosystems is still very limited.
In this study, 497,025 georeferenced occurrences of vascular plants from two national-level vegetation databases, namely Vegetation Plot Database – Sapienza University of Rome and *Alma Mater Studiorum* VegBank – University of Bologna were aggregated, and will become available in the next release of FlorItaly, the portal to the vascular flora of Italy.
The aggregated database includes 7,989 infrageneric taxa. After nomenclatural alignment, by means of the name-match function of FlorItaly, 7,126 taxa (89%) had their names aligned to the Italian checklist, resulting in 5,111 accepted infrageneric taxa. The remaining 863 names could not be resolved. However, they make up only less than 2% (10,005) of the total occurrences in the database.
All the data will be exposed in the taxon pages of FlorItaly, and will be available for download in Darwin Core format. This study, carried out in the framework of the activities of the Inter-University Center Plant Data, is a first step towards the aggregation of several different types of botanical data for the flora of Italy, ranging from functional traits to ethnobotanical data.

3 = Using species distribution models to monitor threatened species

Paola De Giorgi[1], David Dolci[2], Daniela Ciccarelli[1], Gianni Bedini[1]
[1]Department of Biology, University of Pisa, Via Derna 1, 56126 Pisa, Italy; [2]Centro Interuniversitario per la Biodiversità Vegetale Big Data - PLANT DATA, Department of Biological, Geological and Environmental Sciences, Alma Mater Studiorum University of Bologna, Via Irnerio 42, 40126 Bologna, Italy

Species distribution models (SDMs) are statistical tools that estimate the relationship between species' occurrence and environmental variables. They are used for many purposes in ecology, conservation, and evolutionary biology, such as to predict the current potential distribution, to evaluate which environmental variables determine the presence of a species, to assess extinction risks, to understand evolutionary niche dynamics, to predict areas that are suitable for reintroduction, or to forecast distribution under climate change.
In this study we determined the current distribution of *Crocus etruscus* Parl., an endemic geophyte present in deciduous woods and pastures of central Italy. *Crocus etruscus* is included in the National and Global IUCN Red List with the status NT and in Annex IVb of the 92/43/EEC Habitats Directive. Therefore, our goal is to understand the main ecological factors constraining its distribution and use our model to verify new sites of occurrence points for its conservation.
Species distribution models were built with the MaxEnt function (v. 3.4.3) of the R software, using 185 occurrence data of *C. etruscus* taken from Wikiplantbase#Italia and 32 climatic and 22 edaphic variables by Chelsa and Soilgrids, respectively. After running a PCA, we selected the following fourteen variables to include in the models: bulk density of the fine earth fraction, organic carbon density, proportion of sand particles, proportion of clay particles, annual mean temperature, mean diurnal range, temperature seasonality, mean temperature of driest quarter, annual precipitation, precipitation seasonality, precipitation of wettest quarter, elevation, solar radiation in August and in December. All variables were normalized.
We used the ENMval package to optimize the Maxent model. We set the Regularization Multiplier (RM) parameter from 0.8 to 1.6, and selected 4 feature classes (FC) (L, LQ, LQP, H) to test 20 parameter combinations in total.
According to our results, the best model had RM=1.2 and FC=LQ (Fig. 1.a.), with an AUC on the test data of 0.89, reflecting a better ability of the model to discriminate conditions at given occurrence localities compared to those of background localities.
Finally, the habitat suitability map (Fig. 1.a.) was converted into a binary map (Fig. 1.b.) by selecting as threshold equal training sensitivity and specificity. The model suggests that the distribution area of *C. etruscus* might be broader than its current range, as suitable areas without reported occurrence data lie southerly along the coast, between Follonica (GR) and Castiglione della Pescaia (GR) and westerly between Monte Cetona (SI) and Monte San Savino (AR). Interestingly, the model highlights the putative presence of *C. etruscus* on Elba Island where there is the endemic *Crocus ilvensis* Peruzzi & Carta, suggesting that the two species could share the same environmental conditions.

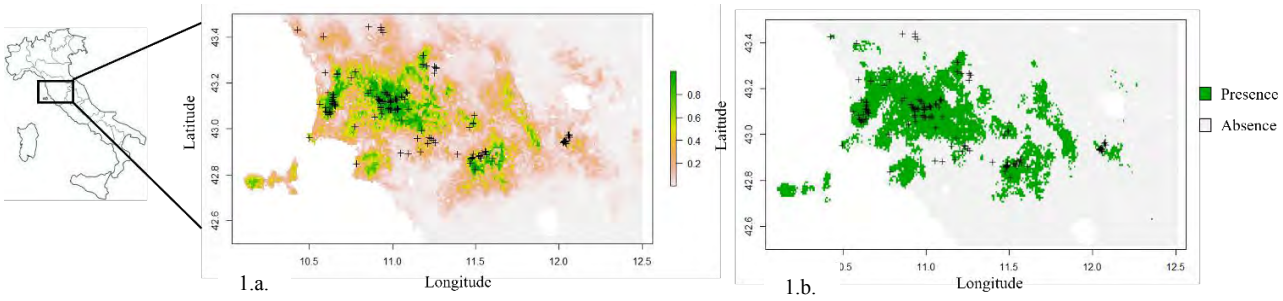


Fig. 1.a. Habitat suitability map of *Crocus etruscus*. 1.b. Binary map of distribution of *Crocus etruscus*. Black crosses represent occurrence points of the species. Green to grey shades identify highly suitable to unsuitable habitats in 1a. Green marks the species potential presence in 1.b.

3 = DNA Barcoding of *Cycas* collections from the Botanical Gardens of Padova and Palermo

Niccolò Forin[1,2], Simone Marcolini[1], Elena Palmieri[1], Sebastiano Nigris[1,2], Manlio Speciale[3], Rosario Schicchi[3], Barbara Baldan[1,2]
[1]Botanical Garden, University of Padova, Via Orto Botanico 15, 35123, Padova, Italy; [2]Department of Biology, University of Padova, Via Ugo Bassi, 58b, 35121, Padova, Italy; [3]Botanical Garden, University of Palermo, Via Lincoln, 2, 90133 Palermo, Italy

Cycas L. is a group of gymnosperms belonging to the order *Cycadales*, it is the only genus of the family *Cycadaceae* and the first for species richness among cycads. *Cycas* species are distributed in tropical and subtropical Asia, Australia, and Africa, with the highest diversity in Southeast Asia and Australia, and they are subdivided into six different sections: *Panzhihuaenses*, *Asiorientales*, *Stangerioides*, *Wadeae*, *Indosinenses* and *Cycas*. Many *Cycas* species are listed in the IUCN (International Union for Conservation of Nature) red list as endangered or critically endangered species. The Botanical Gardens of Padova and Palermo preserve two *Cycas* collections of respectively 22 and 47 specimens comprising about 24 different named species; however, in both collections, some individuals are labelled only as *Cycas* sp. or have multiple possible identifications. Characterize *Cycas* specimens of the two collections from a molecular point of view (DNA barcoding approach) is relevant to define with certainty the identity of the specimens, previously declared only on a morphological basis. This work has two main purposes: i) re-evaluating the *Cycas* collections by correctly classifying individuals that are missing an identification tag or with doubtful identifications; ii) since some species are in danger of extinction in nature, having a certain identification of the different species can have positive consequences in terms of conservation and possible reintroduction in their natural environments.
Preliminary phylogenetic results based on five different chloroplast markers have shown that some individuals are incorrectly named since they fall within sections where they should not be. However, in some cases, these molecular markers do not seem variable enough to define the species. Other molecular analyses will be performed in order to correctly identify all the *Cycas* specimens of the two collections.

3 = Edge effects on the realised soil seed bank along microclimatic gradients in temperate European forests

Cristina Gasperini[1,2], Elisa Carrari[1], Sanne Govaert[2], Camille Meeussen[2], Karen De Pauw[b], Jan Plue[c], Pieter Sanczuk[2], Thomas Vanneste[2], Pieter Vangansbeke[2], Giovanni Jacopetti[1], Pieter De Frenne[2], Federico Selvi[1]
[1]Department of Agriculture, Food, Environment and Forestry, University of Florence, P. le Cascine 18, 50144 Florence, Italy; [2]Forest & Nature Lab, Department of Environment, Faculty of Bioscience Engineering, Ghent University, Geraardsbergsesteenweg 267, 9090 Melle-Gontrode, Belgium; [3]IVL Swedish Environmental Institute, Valhallavägen 81, 114 28 Stockholm, Sweden

The buffering of extreme macroclimatic conditions under tree canopies is a worldwide phenomenon, implying that forests are likely to form temporary local microclimatic refugia under globally rising temperatures and during heat waves. However, this buffering capacity is severely threatened by increasing forest fragmentation that creates more forest edges, whose buffering capacity is lower compared to the forest interior. The ecological alteration caused by the formation of sudden, natural or artificial edges of forest fragments is defined as edge effects. This effect can likely amplify the negative consequences of climate change. In fact, local differences in microclimatic conditions (i.e. incoming radiation, wind speed and direction, temperature and humidity) are especially pronounced near forest edges. The role of the soil seed bank is particularly important in relation to these environmental threats on forest ecosystems (i.e. habitat fragmentation and climate change). Indeed, the realised soil seed bank can produce a new cohort of plants for the re-establishment of populations when environmental conditions are favourable and, as biodiversity reservoirs, can help maintain plant species and genetic diversity by buffering species extinctions and genetic erosion. Despite the crucial role of the seed bank in forest conservation and dynamics, the effects of forest edge microclimate and climate warming on germination responses from the forest seed bank are still almost unknown. We investigated edge effects on the realised seed bank and seedling community in two types of European temperate deciduous forest, one in the Oceanic and one in the Mediterranean climatic region. Responses in terms of seedling density, diversity, species composition and functional type of the seed bank at the forest edge and interior were examined along latitudinal, elevational and stand structural gradients by means of soil translocation experiments. Moreover, we translocated soil samples from high to low elevation forests in the two regions, thus performing a warming simulation. Density, species diversity and mortality of the seedlings varied with region and elevation. Seedling density also differed between forest edge and interior position, while seedling cover mainly depended on forest structure. Both the edge and interior forest seed bank contained a high proportion of generalist species. In Belgium, a more homogeneous seed bank was found at the forest edge and interior, while in Italy compositional and ecological differences were larger: at the forest edge, more light and less moisture demanding seedling communities developed, with a higher proportion of generalists compared to the interior. In both regions, the upland-to-lowland translocation experiment revealed effects of warming on forest seed banks with thermophilization of the realised communities. Moreover, edge conditions shifted the seedling composition towards more light-demanding communities. The establishment of more light and warm-adapted species from the seed bank could in the long term alter the aboveground vegetation composition, with communities becoming progressively richer in light-demanding generalists and poorer in forest specialists.

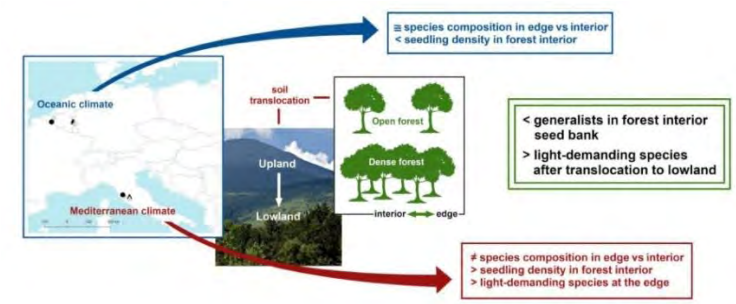


Fig. 1. Experimental design

3 = The challenge of cryptic species for biodiversity conservation: the case study of the morphologically uniform genus *Cetrelia* in Italy

Gabriele Gheza[1], Luca Di Nuzzo[2], Renato Benesperi[2], Elisabetta Bianchi[2], Paolo Giordani[3], Helmut Mayrhofer[4], Pier Luigi Nimis[5], Walter Obermayer[4], Chiara Vallese[1], Juri Nascimbene[1]
[1]BIOME Lab, Department of Biological, Geological and Environmental Sciences, Alma Mater Studiorum – University of Bologna; [2]Department of Biology, University of Firenze; [3]Department of Pharmacy, University of Genova; [4]Institute of Biology, University of Graz; [5] Department of Life Sciences, University of Trieste

Cryptic species are a challenge for conservation since their ambiguous recognition can hinder a reliable evaluation of their distribution and ecology, thus affecting the assessment of their conservation status. *Cetrelia* W.L.Culb. & C.F.Culb. is a foliose chlorolichen genus with four species in Europe, which provides a good case-study on this issue. *Cetrelia*-species are morphologically very similar and apparently have also a similar ecology. They can be identified by chemical characters related to their distinctive secondary metabolites, whose diagnostic value is also supported by molecular data. In addition, they are overall rare, and therefore virtually endangered, although in previous assessments they were evaluated as "data deficient" due to the scarcity of available data. The few, old literature records in Italy refer almost exclusively to one species (*C. olivetorum*) which, however, has been shown to be quite rare in other European countries (i.e. Austria, Belarus, Hungary, Latvia, Lithuania, Poland). To better elucidate the actual situation of the four species in Italy, and potential differences in ecology and distribution between them, we carried out a revision of all the available herbarium specimens and checked several new collections from the main centers of distribution in Italy. Furthermore, occurrence data were used to model species distribution across the country, which can help to plan new explorations and estimate species patterns across time. We analyzed about 250 specimens from about 50 sites, confirming the occurrence in Italy of all the four species reported from Europe, *C. monachorum* being the most widespread, ranging from the Alps to the Apennines. *C. cetrarioides* is less widespread, occurring across the Italian Alps. *C. olivetorum* is actually confined to the Eastern Alps, and *C. chicitae* is the rarest, being found only in three sites in the Central and Eastern Alps. All the four species dwell in old, moist montane forests dominated by beech and/or conifers, but they show different biogeographical patterns, which should be considered for planning conservation actions. Our results highlight the importance of a robust taxonomical backbone to properly address conservation issues.

3 = Seed germination patterns of segetal species collected in North Western Alps

Elisa Giaccone, Martino Adamo, Marco Mucciarelli
Department of Life Sciences and Systems Biology, University of Turin, Viale Mattioli 25, 10125, Torino, Italy

Plants growing in arable lands are almost exclusively annual called "segetal" or "arable" plants. Traditionally, segetal plants have been treated by human as noxious weeds. More recently, many studies have shown that these plants perform many different functions in the agro-ecosystems, for example, providing benefits for many organisms. Therefore, conservation of these species is crucial for preserving the agrobiodiversity (Fanfarillo et al. 2020). With the aim of establishing management strategies and contribute to the *ex situ* conservation of their seeds, in this study, we analyzed the germination response of eight species coming from the segetal flora of Piedmont (North Western Alps). Germination preferences were investigated also to look for functional groups with similar germination patterns according to the type of habitat and/or the cultivated species.

The germination tests were conducted with seeds collected in nature from *Adonis flammea* Jacq. (Ranunculaceae), *Agrostemma githago* L. (Caryophyllaceae), *Bupleurum rotundifolium* L. (Apiaceae), *Consolida regalis* L. (Ranunculaceae), *Orlaya grandiflora* L. (Apiaceae), *Papaver dubium* L. (Papaveraceae), *Petrorhagia prolifera* L. (Caryophyllaceae) and *Xeranthemum inapertum* L. (Asteraceae).

Seeds were sterilized with an aqueous solution of ethanol (70% v/v) for 3 minutes, followed by a passage in an aqueous solution of sodium hypochlorite for 15-20 minutes, according to the size of seeds. 375-960 seeds were tested for each species based on their availability and they were placed on sterile agar medium in Petri dishes of 3 cm diameter. For each species, four different treatments were carried out as reported in Table 1. Winter was simulated by a cold stratification at 4°C in the dark, lasting 0, 30, 60 or 90 days. Petri dishes were then moved to a spring thermoperiod, both at light and dark conditions. Germination at autumn conditions and at 20°C constant were also tested (Table 1). Germination tests lasted from a minimum of 30 to a maximum of 107 days, depending on the species and treatment. Seed response was calculated as final germination percentage (**FG**). Other parameters estimated were: germination delay ($\mathbf{T_1}$), mean germination time ($\mathbf{T_{50}}$), and the mean time to germination (**MTG**) in days. Seed viability at the end of tests was estimated by an overnight treatment of ungerminated seeds with 2,3,5-Triphenyltetrazolium chloride (TTC), 0,1% aqueous solution.

Results showed that a strict germination spectrum is difficult to be envisaged in segetal species because most of them showed different preferences for light and temperature and for the optimal time of germination.

O. grandiflora, for example, had its optimum at warmer temperatures and light conditions (MTG of the entire experiment = 21.7 days). *P. dubium* preferred warm temperatures but dark conditions (MTG = 10.5 days). Instead, *C. regalis* showed the highest percentage of germination after the cold stratification but within a longer incubation period (MTG = 26.8 days) as well as *B. rotundifolium* (MTG = 35 days). *P. prolifera* germinated rapidly and almost completely in all treatments (MTG = 8.4 days) as well as *A. githago* (MTG = 4.4 days). *X. inapertum* germinated completely in all the treatments with a MTG of 10.5 days, on the contrary *A. flammea* did not germinated at all.

Germination tests and statistical analysis are in due course for all the species with the aim to ascertain if germination preferences in this group of plants can be correlated to phenology of the cultivated species or to the habitat of provenience of the seeds.

Table 1. Number of repetitions for each treatment at different climatic conditions.

Treatment	**Thermoperiod**	**Light/dark 12/12h**	**Dark 24h**
Spring	Alternating 25/5°C	5	5
Autumn	Alternating 25/15°C	5	5
Constant	20°C	5	5
Winter	4°C	–	18

3 = The microclimate from a lichen functional diversity perspective

Paolo Giordani[1], Renato Benesperi[2], Luca Di Nuzzo[2], Giulia Canali[1], Elisabetta Bianchi[2], Chiara Vallese[3], Gabriele Gheza[3], Paola Malaspina[4], Philipp Porada[5], Juri Nascimbene[3]
[1]DIFAR, University of Genova, Viale Cembrano 4, 16148 Genova, Italy; [2]Dipartimento di Biologia, University of Florence, Via la Pira 4, 50121 Firenze, Italia; [3]BIOME Lab, Department of Biological, Geological and Environmental Sciences, Alma Mater Studiorum University of Bologna, via Irnerio 42, 40126 Bologna, Italy, [4]DISTAV, University of Genova, Corso Europa 26, 16132 Genova, Italy; [5]Ecological Modelling, University of Hamburg, Ohnhorststraße 18, 22609 Hamburg, Germany

Due to their physiological characteristics, lichens are closely dependent on climate and its changes: they are evolutionarily adapted to conditions of anhydrobiosis, i.e. the ability to survive repeated cycles of hydration/dehydration. This dependence on different climatic aspects can be observed at all levels of biological organisation and shapes the specific and functional diversity patterns of lichens.
In this talk we will present an overview of recent work by our research group that has investigated the relationships between climate and lichen functional diversity, from species to community level.
The effects of light intensity, temperature and different water sources are related to shifts in community composition, population structure and distribution of model species.
Particular emphasis is placed on the need to investigate these interactions at the microscale, in order to obtain more robust general upscaled models capable of predicting possible future alterations in the functional contribution of lichens at the ecosystem scale.

3 = Heat treatment during microsporogenesis triggers distyly disruption in *P. palinuri*

Maurizio Iovane, Giovanna Aronne
Department of Agricultural Sciences, University of Naples Federico II, via Università 100, 80055 Portici, Italy

Pollen functionality plays a key role in seed and fruit formation and both are crucial for long term survival of endangered plant species. Within *Primula* spp., *Primula palinuri* Petagna is the only Mediterranean and maritime species reported in the IUCN RED list as vulnerable. Data on floral morphology proved the occurrence of the fully distylous syndrome in *P. palinuri* due to the reciprocal position of stigma and anthers in the flower as well as the intra-morph incompatibility and the necessity of cross pollination between the two morphs to succeed in seed development. Reproductive traits such as distyly resulted evolutionary successful to enhance outcrossing and increase genetic diversity of the offspring. However, within the scenario of rapid climate changes such traits can result as a constraint to achieve successful reproduction because of the complex interactions with environmental factors. Previous studies highlighted that pollen viability is significantly lowered by increasing temperature and air humidity, envisaging a rising risk of extinction in the ongoing trend of climate changes. Previous studies on pollen functionality clearly showed that pollen from short-styled flowers performed better than that from long-styled flowers in all the temperature and humidity conditions provided at anthesis. Particularly, high temperature and high humidity combination resulted to be the most constraining for the equilibrium between long styled and short styled flower individuals within *P. palinuri* populations. However, data on the effect of different environmental temperatures during microsporogenesis (before anthesis) on the subsequent microgametophyte functionality of the two flower morphs are currently not available. Therefore, we aimed at studying pollen thermo-tolerance in *P. palinuri* comparing the effect of a heat treatment exerted during the earliest stages of flowering with that applied during microgametogenesis.
Specifically, we hypothesized that providing few C° over the mean natural flowering temperature during microsporogenesis may strongly affect the pollen functionality up to the microgametophyte stage with a differential effect between the two flower morphs in terms of pollen viability.
The experiment was performed on two populations 50/50% long and short styled flowers, ensuring that plants were exposed both at the natural flowering temperature of *P. palinuri* and at high controlled temperature during microsporogenesis (before flower buds opening). Thus, we assessed thermotolerance of pollen within each microsporogenesis temperature evaluating pollen viability after 72 hours incubation at 0% RH combined with 5 different temperatures (0, 5, 10, 22, 35 °C). Incubation temperatures ranged from cold to high temperature peaks detected during the long flowering season of *P. palinuri*.
Results showed that in *P. palinuri* higher temperature during microsporogenesis reduced pollen thermotolerance to all the incubation temperature treatments compared to the control treatment. Moreover, higher microsporogenesis temperature enhanced pollen viability difference between long-styled and short-styled flowers. Since dry conditions exposure are generally known to turn pollen into a quiescent status resistant to environmental constraints, the choice of adopting 0% RH enforces reliability of our results highlighting that temperature peaks during the long flowering season of *P. palinuri* can severely target the earliest flowering stages in *P. palinuri* resulting in a drastic decrease of pollen viability up to microgametophyte stage. Further, the enhanced difference in pollen viability detected between long-styled and short-styled flowers in the heat-treated population revealed that temperature occurring during microsporogenesis should be taken into account as a critical phase in pollen ontogenesis of *P. palinuri* and as bottleneck in the equilibrium of the two flower morph ratios in distylous species.

3 = Population structure of *Salvia ceratophylloides* Ard., a very rare endemism of Reggio Calabria (Southern Italy)

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

Understanding how environmental changes interact with the structure and viability of populations of rare and/or endemic species is of crucial importance for their protection and conservation.
In this study, carried out between 2019 and 2021, we analysed the structure and population dynamics of *Salvia ceratophylloides* Ard. (Lamiaceae), a valuable endemic species exclusive to the suburban periphery of the city of Reggio Calabria. The protocol, in accordance with Heglad *et al.* 2001, envisaged the census of the entire population by distinguishing different age classes identified as 'life stage' (S= seedlings; J-I= juvenile - immature; V= vegetative adult; GS= small generative adults; Gl= large generative adults). The collected data were assembled into a matrix and analysed by cluster analysis with the Past 4.10© software. In addition, correlations and the level of significance between pressures and different life stages were examined using Microsoft Excel© 2019 software.
The pressures that affect the demographic viability of the species by influencing its growth, reproduction and distribution across the territory were coded in accordance with the "Reference list Threats, Pressures and Activities" of Salafsky *et al.* 2007, modified by EIONET (European Environment Information and Observation Network) in 2018. The different pressures were estimated in relation to importance and expressed as: 1 - low (L), 2 - medium (M) or 3 - high (H).
The census showed that the population of *Salvia ceratophylloides* is currently fragmented into 17 micro-populations and that over the three years the number of individuals in each micro-population varies, from a minimum of 3 to a maximum of 289 in 2019, from 2 to 373 in 2020, and from a minimum of 1 to a maximum of 424 in 2021.
The distribution of the different life stages also varies from year to year. Life stage S represents 1 % of the population remaining constant in all three survey years, JI constitutes 5 % of the individuals surveyed in 2019-2020, with a 5 % increase in 2021 (10 %). V increases gradually year by year, from 20% in 2019, to 25% in 2020, to 31% in 2021. The adult generative stages SG and GL tend to decrease over time, SG in 2019 representing 43% of the population, in 2020 39% and in 2021 37%. LG is 31% in 2019, 30% in 2020, and 21% in 2021. The total number of individuals varies being 1242 in 2019, 1317 in 2020 and 1555 in 2021.
The aggregation obtained through cluster analysis revealed three groups of micro-population types. The first group, defined as 'Dynamic' in accordance with Heglad *et al.* 2001, is characterised by a high number of non-breeding juveniles and is characterised by a higher ratio [(S+JI+V)/G], with an average of 2.91 and a range of 1.25 to 5.75. The average number of juvenile individuals is very high 71.1% of which 46.6% are represented by life stage V, 19.9% by JI and 4.6% by S. The second group is characterised by micro-populations with fewer non-reproductive juvenile individuals than the previous group, and instead presents a higher number of reproductive individuals. The ratio [(S+JI+V)/G] averages 0.49 and ranges from 0.27 to 0.79. Life stages are represented by 32.1% young, non-breeding individuals and 67.9% breeding individuals, of which 45.6% are SG. This group can be defined as 'Normal' or 'Stable' according to Oostermejer *et al.* 1994. The third group is represented by aged micro-populations, characterised by the high presence of SG, LG breeding individuals, corresponding to 83.1% of the entire micro-population, of which the majority belong to the life stage LG (58%). Very few juvenile non-breeding individuals were found in the micro-populations that make up this group; they make up 16.9% of all the populations, of which 14.7% are V, 1.9% J-I and only 0.3% S. The ratio [(S+JI+V)/G] has a mean value of 0.21 and ranges from 0.07 to 0.30. This group can be defined as 'Regressive' in accordance with Oostermejer *et al.* 1994. Using linear regression analysis, significant correlations were found between the structure of the micro-populations and the pressures exerted on it. The youngest life stages S - JI are positively correlated with mowing and recreational tourism activities (A08 R=0.43, P= 0.03; F07 R2=0.58, P=0.003) and negatively with grazing (A10 R= -0.40, P=0.05). Vegetative stages V are positively correlated with recreational tourism activities (F07 R=0.352, P= 0.08). Reproductive stages SG-LG are positively correlated with grazing (A10 R2=0.395, P= 0.05) and negatively with mowing (A08 R=0.395, P= 0.05).
In order to keep the population of *S. ceratophylloides* balanced and stable over time, measures to conserve the species and its habitat would be useful, with tools such as the establishment of micro-reserves so that good management practices can be adopted, such as regulating grazing, controlling infestations by insects that parasitise stems and seeds, preventing mowing and fires, and monitoring the spread of invasive alien species.

3 = Two years of monitoring within the project Life LETSGO GIGLIO: pre-interventions state of the impacts on biodiversity at Giglio Island

Lorenzo Lazzaro[1], Michele Mugnai[1], Michele Giunti[2], Fabrizio Bartolini[2], Giulio Ferretti[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). We report here the results of the first two years (2020 and 2021) of monitoring of flora and vegetation foreseen in action D1 "Scientific monitoring of flora and fauna". The monitoring is conducted on the components affected by three of the four concrete actions foreseen in the project Life LETSGO GIGLIO, in particular by actions C.1 "Eradication of the mouflon", C.2 "Control of the wild rabbit" and C.3 "Actions on *Carpobrotus* spp. and on *Pinus* spp. plantations". The data showed represent a pre-intervention baseline of data, necessary for the evaluation of the effects of the project actions.
The monitoring of the effects of eradication of mouflon (*Ovis aries*) has been conducted on the habitat of the holm oak woods (Hab. 9340: *Quercus ilex* and *Quercus rotundifolia* forests) and the high and tall scrub and low scrub/garrigue habitats. We surveyed a total of 40 transects, randomly extracted in 2020 and 2021 (20 each year), in the western sector of the Island. In each transect, all individuals of woody species were counted, recording any evidence of grazing according to a simple four-level impact assessment scale (absent, low, medium, and high), and a subdivision between adult individuals, shoots, and seedlings. The results show a greater load of grazing by mouflons in the northwest of the island at the Franco Promontory (where the mouflons were originally introduced to the island) and in particular in holm oak forests and high scrub habitats. In both, the impact is important in particular for the *Quercus ilex* shoots, while in the more southern areas, where the presence of low scrub and garrigue is more significant, we found less impact on the vegetation.
Regarding the actions on wild rabbits (*Oryctolagus cuniculus*), monitoring focuses on impacts on plant diversity and species typical of Mediterranean annual grasslands (Hab. 6220* Pseudo-steppe with grasses and annuals of the *Thero-Brachypodietea*). We surveyed five subplots in each of four transects, listing the species present in each plot and recording any sign of predation, and also assessing the effects on soil and plant community, again according to the above four-level impact assessment scale. The data collected show a generally medium-high impact on soil and plant communities. Moreover, some of the species typical of Habitat 6220* showed a high rate of predation (for example *Brachypodium distachyon*, *Hypochaeris achyrophorus*, *Stipellula capensis*, *Trifolium subterraneum*).
The monitoring of containment actions on *Carpobrotus* spp. focuses on the mosaic of coastal vegetation (most affected by the presence of this species) which includes, in particular, the Habitats 1240: Vegetated sea cliffs of the Mediterranean coasts with endemic *Limonium* spp., 1430: Halo-nitrophilous scrubs (*Pegano-Salsoletea*) and 5320: Low formations of *Euphorbia* close to cliffs. We surveyed the presence and abundance of native species on 44 permanent square plots of 2 meters of side, in invaded and uninvaded areas, in each of the 3 Habitats mentioned. The preliminary results clearly show 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.
Finally, with regard to actions on *Pinus* spp. plantations, the monitoring concerns the pine forests that will be subject to the thinning out operations and seeding of *Quercus ilex*. We monitored 12 squared plots in 2020 and 2021, in which we collected information on the presence and abundance of understory species and on the cover of the canopy layer. The data provide insight of a decrease in species richness and cover at increasing cover of the canopy of the pines.

3 = Study of an endemic plant as a resource for mountain agroecosystems: *Sanguisorba dodecandra* Moretti

Valeria Leoni[1,2,3], Luca Giupponi[1,2], Alberto Tamburini[1,2], Carla Gianoncelli[4], 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, Agroenergy (DISAA), University of Milan, Via Celoria 2, 20133 Milan, Italy; [3]PhD school in Environmental Sciences (PhDES), University of Milan, via Celoria 26, 20133 Milan, Italy; [4]Fondazione Fojanini di Studi Superiori, Via Valeriana 32, 23100 Sondrio, Italy

Sanguisorba dodecandra Moretti is an endemic plant of the Alps of Lombardy region (Northern Italy). Differently from most endemic species, this plant grows in a wide array of environments (avalanche channels, edges of creeks, meadows, and pastures) from the mountain to the sub-alpine belt, and it is often very abundant, besides being a distinctive element of some mountain and sub-alpine agroecosystems. The ecological features and the role of this species in some mountain agricultural activities are poorly investigated.
This article shows the results of a synecological analysis of *S. dodecandra* and the evaluation of its functional strategy. Furthermore, its forage value was investigated and the melissopalynological analysis was used to characterize the honey produced in an area where this species grows.
The ecological analysis defined this plant as euriecious and ruderal/competitive-ruderal strategist (C = 25.14%, S = 8.61%, R = 66.25%), explaining its adaptability to environments characterized by biotic and abiotic disturbance. Bromatological analysis showed a good forage value, confirming the ethnobotanical knowledge concerning this species. In fact, it has good protein content (12.92 ± 1.89%) and non-fiber carbohydrates (47.12 ± 3.62%) in pre-flowering. *S. dodecandra* pollen was identified as a "frequent pollen" in the honey, showing that this plant is attractive for honeybees, that can be then definitely included among its pollinators.
This research allowed a deeper knowledge of *S. dodecandra* ecology and showed that this species is a resource for traditional and sustainable agricultural activities of Lombardy Alps such as pastoralism and beekeeping.

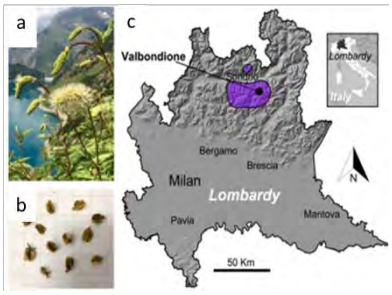


Fig. 1a-1b-1c. *S. dodecandra* whole plant in its environment (a), fruits (achenes) (b) and distribution range (c)

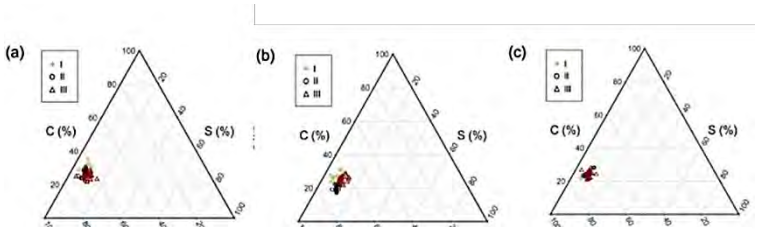


Fig. 2a-2b-2c. CSR triangular plots of the leaf samples of *S. dodecandra* collected in the three sampling areas (a, b, and c) at different phenological stage: I, pre-flowering; II, flowering; III, post-flowering

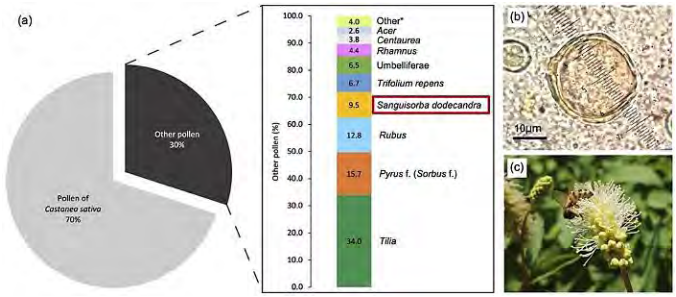


Fig. 3a–3b-3c. presence of pollens in the honey sample (a), microscopic image of pollen of *S. dodecandra* (b) and bee on an inflorescence of *S. dodecandra*. Key: *, sporadic pollen (< 1%), 36 other species identified

Parameter	Phenological stage		
	I	II	III
PC (% DW)	12.92 ± 1.89	11.36 ± 0.13	8.73 ± 0.91
NDF (% DW)	31.33 ± 1.61	31.70 ± 3.88	38.22 ± 3.09
ADF (% DW)	25.40 ± 1.64	26.52 ± 3.33	31.63 ± 3.22
ADL (% DW)	4.36 ± 0.11	5.51 ± 1.52	7.123 ± 1.50
Ash (% DW)	6.55 ± 0.25	5.99 ± 0.06	5.89 ± 0.26
EE (% DW)	2.08 ± 0.10	2.01 ± 0.21	2.18 ± 0.26
NFC (% DW)	47.12 ± 3.62	48.94 ± 3.99	44.99 ± 3.62
DM (% FW)	23.38 ± 2.06	27.95 ± 0.60	33.14 ± 1.73

Fig. 4. Bromatological analysis (forage value) (I, pre-flowering; II, flowering; III, post-flowering). PC, protein content; NDF, neutral detergent fiber; ADF, acid detergent fiber; ADL, acid detergent lignin; EE, ether extract; NFC, non-fiber carbohydrates; DM, dry matter

3 = GLORIA-WALSAT: a new analysis protocol to assess winning and losing alpine plant species in the context of climate change within the international GLORIA network

Silvano Lodetti, Francesco Porro
Department of Department of Earth and Environmental Sciences, University of Pavia, via Ferrata 1, 27100 Pavia, Italy

Climate is a major determinant of ecosystem's habitat suitability, also at local scale, influencing the latitudinal and altitudinal distribution of plant species. Alps are projected to experience a higher rate of warming than most other regions of the world. The GLORIA project (Global Observation Research Initiative in Alpine environments) operates a long-term monitoring network to assess changes of alpine diversity in response to climate change across the main mountain chains of the planet, collecting floristic and climatic data since 2001.
The aim of this work is to define a new standardized analysis protocol, GLORIA WALSAT (Winning and Losing Species Assessment Tool), built on the non-parametric Cliff's 'Delta' measure of 'effect size' indices, which provides both size and direction of alpine vascular plant taxa abundance and frequency changes across time in GLORIA study sites. The advantage of the developed protocol is to be applicable in all the GLORIA Target Regions (TRs) worldwide, and to be particularly suitable for the analysis of the semiquantitative abundance data collected at GLORIA's Summit Area Sections (SAS), that often are disregarded or under-utilized by researcher within the GLORIA network (Fig.1).
We tested the WALSAT with data from the summits of three TRs in the Italian Alps and Apennines, in which resurveys have been carried out with at least 7 interval years. We present an example of the applicability of WALSAT in detecting "winning" and "losing" species within the alpine plant communities considered, and also the average changes of plant functional types classified by growth forms: forbs, graminoids (including grasses, sedges and rushes), shrubs and ferns.
As result, a total of 414 plant taxa have been analyzed. 63 species have been assessed as 'winning', as they colonized new areas and/or were characterized by significant increment in their presence on the summits. On the other hand, 32 species have been assessed as 'losing', as they either disappeared locally, or showed significant abundance decrement trends detected in the study sites. Through the study of plants assemblages' responses across the north to south gradient, we confirmed the tendencies of wider response of plant communities in the Apennines areas to climate change (Fig.2). The southernmost site also showed a higher number of decreasing species in respect of the other two regions, particularly among the graminoids (Fig.2).

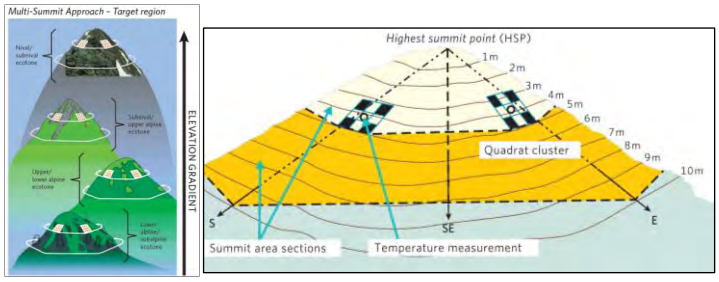


Fig. 1a GLORIA Target Region: suite of four summits of different elevation. 1b Frontal view of summit sampling design

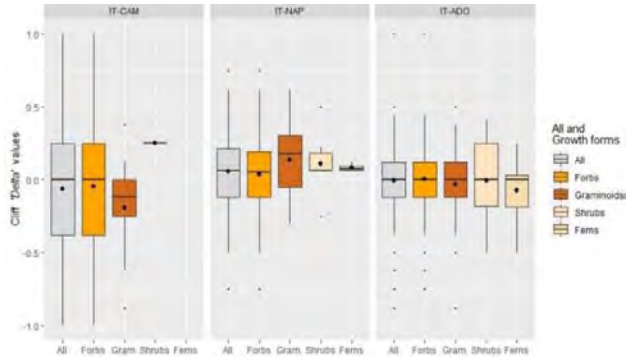


Fig. 2 Boxplots representing distribution of Cliff 'Delta' indices subdivided by study site ('All' of IT-CAM, IT-NAP, IT-ADO) and simplified Growth Forms groups (Forbs, Graminoids, Shrubs and Ferns) for each site

3 = Alteration of plant and invertebrate communities in waterbodies dominated by the invasive alien macrophyte *Lemna minuta* and proposals for its containment

Flaminia Mariani, Emanuele Pelella, Simona Ceschin
[1]Department of Sciences, University of Roma Tre, Viale G. Marconi 446, 00146 Roma, Italy

The American duckweed *Lemna minuta* Kunth is an invasive pleustophyte in freshwater habitats. Currently, in many European countries, including Italy, it is considered one of the alien aquatic species with the highest potential of invasiveness. The wide distribution of *L. minuta* is due to its high reproductive rate, which allows it to rapidly colonize extensive waterbodies and form dense free-floating mats on the water surface. In a study, we investigated the effects of its presence on water quality and communities of waterbodies in Central Italy. Biological sampling (macrophytes, macrobenthos) and water chemico-physical parameters were analyzed out at 17 pairs of aquatic sites, where the two sites of each pair differed in presence or absence of multilayered mats of *L. minuta*. At sites where the alien species was present, environmental factors, such as light, dissolved oxygen, and water temperature were negatively correlated with increased cover and thickness of *L. minuta* mats, creating underwater conditions of near darkness, hypoxia and colder. For these conditions, sites with *L. minuta*, compared with those without, had plant communities characterized by lower total cover and species richness, and the disappearance of many macroalgae and rhizophytes, such as *Veronica anagallis-aquatica*, and *Apium nodiflorum*. Similarly, the macrobenthic communities were characterized by taxa more tolerant hypoxic conditions, while were rare or absent the more sensitive taxa, taxa with winged adult stages and those swimming on the water surface.
Given this strong negative impact on the aquatic ecosystems, it proved necessary to identify a biological control agent able to contain the expansion of *L. minuta* and limiting its growth as much as possible. Classical Biological Control (CBC) is the most recognized and accepted biological control method, and it is based on the introduction of natural enemies co-evolved with the alien species and coming from the same area of origin as the alien species to be controlled. However, understandable concerns about the environmental risks associated with the introduction of an alien biocontrol agent led EU countries to prohibit any introduction of alien species, although potentially useful. Therefore, an attempt was made to identify a native biocontroller able to consume the alien *L. minuta*. The attention was focused on *Cataclysta lemnata* L. 1758, a European moth with an aquatic herbivorous larval phase. Through laboratory studies, larvae of *C. lemnata* were found to utilize *L. minuta* both as food and as material to build anti-predator protective cases. In addition, the larvae consumed effectively and without preference, both the alien species and the native *L. minor*, contrary to the Enemy Release Hypothesis, which predicts greater consumption by the biocontroller of the species with which it co-evolved.
Having ascertained under laboratory conditions the effective trophic interaction between *L. minuta* and *C. lemnata*, it was investigated whether the alien plant and the native herbivore had similar phenological and ecological characteristics, to understand whether the insect could be successfully released into natural sites invaded by *L. minuta*. The phenology and ecology of the two species were found to be similar and compatible, suggesting that the insect is suitable for release into the wild at the same sites where *L. minuta* is present. Through laboratory studies, *C. lemnata* turned out to be multivoltine, i.e. producing many generations per year, allowing this insect to be used year-round for controlling the perennial mats of *L. minuta*. In addition, the species could be continuously reared in the laboratory for mass rearing aimed at periodic releases in the field, as required by an augmentative biological control protocol, when the biocontroller populations are not too abundant in nature.
A host-specificity study of the herbivore was also carried out for predicting possible effects on non-target aquatic plant species both native and alien, which occupy the same ecological niche of the target alien species *L. minuta*. In this study, it was shown that *C. lemnata* preferred the alien *L. minuta* both in terms of trophic consumption and for the performance of its life cycle, in accordance with previous findings obtained.
The laboratory and field experiments carried out led to design a scientific protocol of augmentative biocontrol that has been applied in a natural site of a regional park in Rome, spontaneously invaded by the American duckweed. This outdoor experiment is still in progress, but from preliminary observations, in the experimental site where the larvae were released, the coverage and the thickness of *L. minuta* mats were greatly reduced compared to the control site where no insect larvae was released. The thinner mat of *L. minuta* has allowed the recovery of the oxygenation conditions in the water column, with values of dissolved oxygen six times higher than in the control site. This first test in a natural site demonstrated that *C. lemnata* seems to be suitable for control of *L. minuta* even under natural conditions, although further testing will be necessary to confirm and refine the experimental protocol designed.

3 = Preventing the loss of biocultural diversity in Valle Imagna (BG, Italy): an Ethnobotanical survey to the rescue

Fabrizia Milani [1,2], Martina Bottoni [1,2], Laura Bardelli [1,2], Lorenzo Colombo [1,2], Paola Sira Colombo [1,2], Piero Bruschi [3], Claudia Giuliani [1,2], Gelsomina Fico [1,2]
[1]Department of Pharmaceutical Sciences, University of Milan, Via Luigi Mangiagalli 25, 20133 Milan, Italy; [2]Ghirardi Botanic Garden, Department of Pharmaceutical Sciences, University of Milan, Via Religione 25, 25088 Toscolano Maderno (BS), Italy; [3]Department of AgriFood Production and Environmental Sciences, University of Florence, Piazzale delle Cascine, 18, 50144 Florence, Italy

The project presented hereafter is part of an ongoing collaboration with the *Centro Studi Valle Imagna* (Center of Studies and Research concerning the traditions of Valle Imagna, BG, Italy). This partnership began with the publication of the book '*Raccolta di diversi rimedj a varj mali*', an in-depth ethnobotanical study of an ancient XVIII century manuscript that contains approximately 200 plant-based medicinal remedies. The synergy that originated this work was natural, considering how the goals of Ethnobotany and the *Centro*'s are perfectly aligned on the recovery of lost traditions and the preservation of biocultural diversity of the rural areas. A patent point of contact between these two realities is the precious heritage embodied by the spontaneous plant species of the territory.
In this context, we proposed an ethnobotanical survey to be carried out through interviews of the Valley's inhabitants aimed at investigating the traditional use of autochthonous plants for human and animal health care, in the food sector, in cosmetics, in the craft field, and for ritual purposes. Special attention was paid to the common and dialectal names of the plants, their past or current distribution and use, forms of preparation, and methods of administration, specifically pertaining to therapeutic use.
The following preliminary results concern the first 100 interviews conducted between mid-March and mid-April 2022. More than 100 plant species were mentioned during the field work. *Rosaceae* (with 20 species), *Asteraceae* (n=8), *Lamiaceae* (n=8), and *Fabaceae* (n=5) were the most recurrent botanical families. Out of the most cited plant species, the leaves of *Agrimonia eupatoria* L. ('*Erba del venil'*) were used as anti-inflammatory and wound healing agents for the external treatment of wounds and cuts. The bitter decoction of leaves and aerial parts of *Artemisia absinthium* L. ('*Mistrù'*) was mentioned as a digestive remedy for cattle, while the leaves and flowers of *Malva sylvestris* L. were widely used for the treatment of internal and external inflammation, such as gastrointestinal disorders, gingivitis, toothache, and dermatitis. *Tilia* spp. flower teas ('*Fiur de tej'*) were taken to promote drowsiness and to treat upper airways ailments, whilst the leaves of *Brassica oleracea* L. var. *sabauda* L. ('*Verz'*) were applied on the back and the joints to ease joint pain and inflammation.
Moreover, in order to evaluate the intergenerational knowledge transfer throughout the territory, future investigations within some key-family groups will be conducted, along with a thorough survey among the students at the Comprehensive Institute of Sant'Omobono Terme (Valle Imagna, BG).
This ethnobotanical survey thus represents some concrete first step in a wider project that has the audacious purpose of mitigating the loss of ancient traditions and the plant and cultural biodiversity of Valle Imagna by placing value and giving voice to the testimonies of a world that is at risk of being lost and is desperately crying out to be protected and never forgotten.

3 = Better together? - Freshwater lichens, including three new species in the genera *Aspicilia* and *Verrucaria*, associated with *Lobothallia hydrocharis* (Poelt & Nimis) Sohrabi & Nimis from watercourses of Sardinia

Juri Nascimbene[1], Pier Luigi Nimis[2], Holger Thüs[3]
[1]Biome-Lab, Dept. of Biological, Geological and Environmental Sciences, Alma Mater Studiorum, University of Bologna, Via Irnerio 42, I-40126 Bologna; [2]Dept. of Life Sciences, University of Trieste, Via Giorgieri 10, I-34127 Trieste; [3]Dept. of Botany, State Museum of Natural History Stuttgart, Rosenstein 1, D-270191 Stuttgart

Lichenized fungi have developed their largest diversity in terrestrial habitats, while relatively few taxa exist in permanently or temporarily inundated sites in and along watercourses. Here they have to cope with several limiting factors (e.g. excessive hydration over long periods, repeated submersion/emersion cycles, substrate instability, strong currents). These pressures resulted in the evolution of convergent morphological adaptations even across distantly related taxa, thus making species identification difficult. However, while the freshwater lichen biota of the Alps and most other parts of Europe are being increasingly studied, targeted inventories are lacking for Mediterranean areas, where new freshwater lichens are likely awaiting to be discovered. Mediterranean watercourses have strong fluctuations in the water regime, thus posing extreme conditions both for species from the temporarily inundated zone as well as for fully aquatic species. In this work, we summarize the results of the exploration of freshwater lichen biota on the island of Sardinia associated with the regional flagship species *Lobothallia hydrocharis*, a large-sized placodioid lichen typical of the splash water zone along mountain streams on this island, but so far not known from elsewhere. We used molecular data to confirm its distinctiveness from other taxa and its systematic placement within the genus *Lobothallia.* Our data identified it as a close relative of the mostly terrestrial *L. radiosa*, and only distantly related to the second Italian subaquatic species in the genus, the alpine *L. melanaspis*. Asscociated with *L. hydrocharis* we found a surprisingly species-rich community of other freshwater lichens, including three species new to science in the genera *Aspicilia* and *Verrucaria.* Interestingly, the lichen biota of Sardinian watercourses appears to have specific traits as compared to similar situations in the Alps or Northern Europe, e.g. the scarcity of subgelatinous lichens, except for micro-sites in the permanently submerged zone, while black-brown, heavily cracked to areolate crusts dominate. This situation is likely related to the more stressful conditions in Mediterranean watercourses where lichens have to cope with periods of abundant and impetuous water flow alternating with long periods of complete dryness and warm temperatures. These extreme conditions may also explain the remarkable frequency of apparently parasitic interactions in the splash water zone, as in the case of a two different *Placopyrenium* species, or of *Kuettlingeria atroflava* var. *submersa*. Even *Lobothallia hydrocharis* was found with thalli clearly developing over other *Aspicilia*-lichens, appearing at least as a facultative youth-parasite.

3 = Lichen communities on *Fraxinus* in Aosta Valley: conservation issues

Silvia Ongaro, Deborah Isocrono
Department of Agricultural, Forest and Food Sciences., University of Torino Largo Paolo Braccini 2, 10095 Grugliasco (TO), Italy

European broad-leaved forests have naturalistic, recreational and historical value, and they also serve as significant habitat for many groups of organisms. *Fraxinus* is a native widespread European species linked to socio-economic, cultural, and ecological aspects in many countries but, a significant decline has to be expected, in the coming years, due to ash die-back caused by *Hymenoscyphus fraxineus*. Given that ash dieback threatens the survival of common ash, the many organisms dependent upon *Fraxinus* are also under threat. Among epiphytes, lichens are well known indicators of ecosystem integrity, widely used in habitat protection and conservation, and in the framework of biodiversity programs. Therefore, we planned to explore affiliate epiphytic lichen communities of ash trees in the Italian Alps.
Lichen communities appear to be different in terms of occurrence and frequencies in different sampling sites. The tested environmental parameters contribute in shaping the lichen communities.
The conservation value of lichen species was assessed by comparing distributional data at the national and local level.
Our findings show that, in the Italian Alps, ash trees represent an important substrate for locally, or even nationally, rare lichens: several of the species recorded on ash are not previously known for the area and some are included in the red list of Italian epiphytic lichens.
Out of the 45 species of lichens recorded in this study, 14 species have been recorded for the first time in the Aosta Valley and 2 additional species were no longer reported in the study area for over one century. Moreover, ash hosts some red-listed lichen species like *Rinodina polyspora*, a critically endangered species previously considered regionally extinct and the vulnerable – according to IUCN categories – *Lecanora impudens* for which this finding broadens the known range of occurrence in Italy to the western Alps. Presently, these notable species were only reported on ash in Aosta Valley and are rare in the Italian Alps. As a consequence, the local extinction of ash at the landscape scale in Aosta Valley might have a negative impact on diversity of epiphytic rare or threatened lichen species.

3 = Revisiting seminatural grasslands at Pian Grande di Castelluccio di Norcia to assess patterns of vegetation change

Panichella Tiziana[1], Gentilucci Matteo[2], Tardella Federico Maria[3], Catorci Andrea[4]
[1]School of Advanced Studies, University of Camerino, Via Lili 55, 62032 Camerino, Italy; [2]School of Science and Technology, University of Camerino, Via Gentile III da Varano 27, 62032 Camerino, Italy; [3]Herbarium Universitatis Camerinensis, University of Camerino, Via Pontoni, 5, 62032 Camerino, Italy; [4]School of Biosciences and Veterinary Medicine, University of Camerino, Via Pontoni, 5, 62032 Camerino, Italy

Most grasslands of Europe, defined as secondary grasslands, are the result of human activity (e.g., grazing and mowing) and are important hotspots of biodiversity. However, these ecosystems are highly vulnerable due to changes and intensification of land use (e.g., abandonment of agricultural practices), and afforestation. In addition to anthropogenic activities, another factor that currently influences the maintenance and species richness of secondary grasslands is climate change, which in the last 20 years in the central Apennines has led to a strong reduction in the snow cover period and an increase in minimum annual temperatures. This has led to the occurrence or the increase in coverage of drought- and stress-tolerant chamaephytic species, together with the decrease of more mesophilic, micro-thermophilic and competitive species. Thus, to contrast climate and land use change, it is important to understand the vegetation dynamics.
The study area is the Pian Grande di Castelluccio di Norcia (central Apennines), a vast flat area covered by grasslands managed as grazed and mowed meadows. It is bordered by mountains and in its central part there is a deep incision called “Fosso Mergani” (Fig.1), which ends in a sink-hole, which drains the surface waters. This area has been investigated in 1973 for which have been recognized and mapped different plant communities.
To evaluate the possible changes that have occurred over the last 50 years, we made a diachronic analysis of the vegetation, through the comparison of the relevés dating back to 1973 (116 relevés divided into 9 tables) with those recently carried out (2021-2022) in the same area. We proceeded as follows: 1) digitization and cluster analysis of old phytosociological relevés and updating of grassland species names; 2) digitization of the vegetation map (scale 1: 5,000) and georeferencing of the relevés; 3) acquisition of climatic data from the NASA site; 4) analysis of climatic data; 5) resampling of the study area; 6) analysis of vegetation changes.
The next research steps will be to map again the plant communities and to compare their distribution with that highlighted in 1973.

Fig. 1. The Pian Grande di Castelluccio di Norcia (Umbria, Italy)

3 = Potential climatic and elevational range shifts in the Italian narrow endemic *Bellevalia webbiana* (Asparagaceae) under climate change scenarios

Lorenzo Peruzzi[1,2], David Dolci[3], Alessandro Chiarucci[3]
[1]Dipartimento di Biologia, Università di Pisa, Via Derna 1, 56126 Pisa, Italy; [2]CIRSEC, Centre for Climatic Change Impact, University of Pisa, via del Borghetto 80, 56124 Pisa, Italy; [3]Centro Interuniversitario per la Biodiversità Vegetale Big Data - PLANT DATA, Department of Biological, Geological and Environmental Sciences, Alma Mater Studiorum University of Bologna, Via Irnerio 42, 40126 Bologna, Italy

The Webb's hyacinth (*Bellevalia webbiana* Parl., Asparagaceae) is one of the most relevant Italian narrow endemic species. The range of this bulbous perennial herb is restricted to two disjunct areas of Central Italy, separated by the mountains of Northern Apennine, growing in open fields and meadows, wood margins, olive groves and vineyards. The first is located in Emilia-Romagna (~ Lat. 44.3, Long. 11.6), the second in Tuscany (~ Lat. 43.8, Long. 11.3). Recent studies investigated the population genetics, functional traits and reproductive performance of the five largest populations known for this species. These studies highlighted some differentiation in the population located at the upper N-E limit of the species range, around Faenza (Ravenna). The International Union for Conservation of Nature (IUCN) inserted the Webb's hyacinth into the Red List of Threatened Species, classified as Endangered (EN A2c). During the last century, the main threat for this species was represented by the spread of human settlements, which caused local extinctions from several historical localities. Despite soil consumption is a major concern for plant conservation, climate change caused by human activities can further exasperate conservation problems. Climate change is already impacting biodiversity and is likely to intensify over the next century, so that mitigation efforts are hard to implement.
To evaluate the impact of climate change on *Bellevalia webbiana*, we used ecological niche modeling (ENM) to predict future potential distribution under different scenarios. A total of 42 recent occurrence points were collected from Wikiplantbase #Italia. Mean monthly climatic data for three temporal ranges (1981–2010 'current times', 2041–2070, and 2071–2100) were downloaded from CHELSA and used to calculate three biologically relevant climatic variables (annual mean temperature, annual precipitation, and annual potential evapotranspiration) with dismo and envirem R packages. Future climatic scenarios were derived from IPSL-CM6A-LR climatic models and related to three Shared Socioeconomic Pathways (SSP): SSP1-2.6 (CO_2 emissions cut to net zero around 2075), SSP3-7.0 (CO_2 emissions around current levels until 2050, then falling but not reaching net zero by 2100), and SSP5-8.5 (CO_2 emissions triple by 2075). Maximum entropy algorithm implemented in Maxent software (version 3.3.4) was used to calculate all potential distributions. In order to avoid extrapolation errors, the model was calibrated on the area within longitude 6.2–19.1 E and latitude 35.5–47.3 N. Only three non-collinear and biologically meaningful climatic variables were used as predictors. All habitat suitability maps were converted to binary presence-absence outputs by applying a threshold value based on equal training sensitivity and specificity. Model quality was assessed with two different evaluation metrics: the Area Under the Receptive Curve (AUROC) and the Continuous Boyce Index.
The Maxent model for Webb's hyacinth with the selected environmental variables performed particularly well, achieving high evaluation metric scores (AUC_{Train} = 0.926, AUC_{Test} = 0.929, CBI_{Test} = 0.901). The CBI score provides valuable information of the model's quality (robustness, probability of occurrence and deviation from randomness) and highlights the reliability of the predictions. The estimated potential distribution for multiple temporal range highlights the vulnerability of Webb's hyacinth to the effects of climate change. The current potential Area Of Occupancy (AOO) (732 2×2 Km cells) is forecasted to decrease in the range 2041–2100. The ssp1-2.6 scenario highlights limited potential variations in AOO (2070 = +32, 2100 = −23). On the other hand, both SSP3-7.0 and SSP5-8.5 highlight a dramatic reduction of the AOO (SSP3-7.0: 2070 = −128, 2100 = −510; SSP5: 2070 = −291, 2100 = −711). In all future scenarios the predicted potential distribution tends to shift towards higher elevations, located in between to the two main areas in which this species currently occurs. By admitting that these areas could effectively host *B. webbiana* for their extra-climatic niche features, this would imply an increasing connection between the two currently disjunct population groups, and a potential loss of the differentiation documented for some of them. Considering that the current effective AOO amounts to just 24 2×2 Km cells, concerns about conservation of *Bellevalia webbiana* appear founded.

3 = An overview of invasive *Acacia* species in the Mediterranean Basin: from germination ecophysiology to monitoring and management plans

Lina Podda[1], Marco Porceddu[1,2], Ludovica Dessì[1], Vanessa Lozano[3], Giuseppe Brundu[3], Gianluigi Bacchetta[1,2]

[1]Department of Life and Environmental Sciences, Centre for the Conservation of Biodiversity (CCB), University of Cagliari, Viale Sant'Ignazio da Laconi 11-13, 09123 Cagliari, Italy; [2]Sardinian Germplasm Bank (BG-SAR), Hortus Botanicus Karalitanus (HBK), University of Cagliari, Viale Sant'Ignazio da Laconi 9-11, 09123 Cagliari, Italy; [3]Department of Agricultural Sciences, University of Sassari, Viale Italia 39, 07100 Sassari, Italy

The Mediterranean Basin is one of the 36 mega-hotspots of global biodiversity. However, it is also one of the most disturbed areas, since the very beginning of the human occupation in Europe, mostly in coastal areas. The influence of human presence and land-use changes are important facilitators for the colonization and establishment by invasive species.

The Mediterranean Basin hosts a high percentage of invasive alien plants, including woody species belonging to the *Acacia* genus, which have been introduced for ornamental, afforestation, reforestation, and other uses. Introduced acacias are widespread especially in coastal and riparian habitats, where their impacts are one of the most significant pressures in these extremely sensitive habitats, already affected by anthropogenic perturbations and often degraded and fragmented. *Acacia* species can modify the chemical-physical and biological characteristics of soils, threatening the conservation of habitats and native species, often rare and / or threatened.

In the framework of Interreg project Italia-Francia Marittimo (ALIEM - Action pour Limiter les risques de diffusion des espèces Introduites Envahissantes en Méditerranée - ERDF: European Regional Development Fund) four species of *Acacia* s.l. were selected to study some of their invasive traits in the Mediterranean Basin. Many *Acacia* species reproduce both sexually and vegetatively and produce large quantities of viable seeds forming persistent soil seed banks.

Our research focused on understanding the seed germination traits of three invasive Australian acacias [*Acacia dealbata* Link, *Acacia mearnsii* De Wild. and *Acacia saligna* (Labill.) H.L.Wendl.] and one native to Southern Africa [*Vachellia karroo* (Hayne) Banfi & Galasso] from different Mediterranean areas. The laboratory experiments were carried out at the Sardinian Germplasm Bank (BG-SAR) and allowed to evaluate the germination capacity of scarified and non-scarified seeds in response to different temperatures and photoperiods. Seeds of *A. saligna* and *V. karroo* were also tested under NaCl stress.

Water availability after scarification provided an advantage to germination for seeds of *A. dealbata* and *A. mearnsii*, which can help to explain why they are frequently widespread in riparian habitats. On the other hand, seeds of *A. saligna* and *V. karroo* showed a remarkable ability to germinate in spite of NaCl stress conditions, which is consistent with their capacity to establish in coastal and halophilous habitats. Our findings could help to predict and model the potential ability of each species to successfully colonize via seeds different habitats and provide important baseline information for risk analysis, monitoring, and planning tailored management strategies for alien acacias.

3 = Does floral complexity increase the likelihood of extinction for the Italian flora?

Rosa Ranalli[1], Mauro Fois[2], Marta Galloni[3], Gianluigi Bacchetta[2], Giovanna Aronne[4], Anastasia Stefanaki[5,6], Theodora Petanidou[7], Ziva Fiser[8]

[1]CREA Research Centre for Agriculture and Environment, Via di Corticella 133, 40128 Bologna, Italy; [2]Department of Life and Environmental Sciences University of Cagliari, Viale S. Ignazio da Laconi 13, 09123 Cagliari, Italy; [3]Department of Biological, Geological and Environmental Sciences, University of Bologna, Via Irnerio 42, 40126 Bologna, Italy; [4]Department of Agricultural Sciences, University of Naples Federico II, Via Università 100, 80055 Portici (NA), Italy; [5]Department of Biosystematics, University of Wageningen, 6708 PB Wageningen, The Netherlands; [6]Naturalis Biodiversity Center, Darwinweg 2, 2333 CR, Leiden, The Netherlands; [7]Department of Geography, University of Aegean, 81100 Mytilene, Greece; [8]Department of Biodiversity, University of Primorska, lagoljaška 8, SI-6000 Koper, Slovenia

Italy with approximately 8200 native vascular plant taxa, 21% of which are endemic, is one of the European countries with the highest level of biodiversity, second only to Turkey in the Mediterranean. However, an increasing number of species is at risk of extinction, mainly due to anthropogenic pressures. Currently, about 1/3 of the total taxa have been assessed, but to halt the decline, taxonomic and ecological studies need to be pursued and deepened by implementing monitoring plans aimed at conservation strategies. To increase ecological studies on plant taxa for conservation purposes, the 'Floral Complexity' project was proposed within the COST Action 18201 Conserve Plants. The project considers all threatened European entomophilous flora and is based on the hypothesis that plant species' vulnerability is influenced by several intrinsic and extrinsic factors, such as floral morphological complexity and its possible relationship with plant-pollinator interactions. A Floral Complexity Index (FCI) has been conceived to summarize several parameters, and to compare them with conservation status, morphological, ecological and chorological traits.
Preliminary results are here presented for the 1760 insect-pollinated flowering species listed in the Italian Red Lists and subsequent updates. We considered all Italian assessed taxa, including LC IUCN category to show a complete view of threatened flora. Almost all species flower in spring and summer, are typical of grassland habitats and are mainly represented by Compositae (with 279 taxa), Caryophyllaceae (131), Fabaceae (125), Orchidaceae (119). Fifty percent (871 spp.) of the entomophilous species listed in the Italian Red List are assessed as LC, followed by 20% NT and 13% EN. The FCI (*sensu* Stefanaki et al. 2015) was calculated as a preliminary evaluation: most taxa have medium FCI values included between 2 and 2.5, while we have a minimum value for 148 taxa to a maximum for only 1 taxon.
The Italian assessed plant taxa show in general a moderate risk of extinction, relatively low flower complexity, and preliminary analysis (Figure 1) does not show clear trends in the relationship between FCI and IUCN categories. Nevertheless, as morphological complexity is strictly linked with pollinators, a more in-depth study will follow up on the final European data involving more pollination experts to refine FCI calculation
The preliminary results here presented should be considered a starting point for the development of more comprehensive studies aimed at conservation purposes.

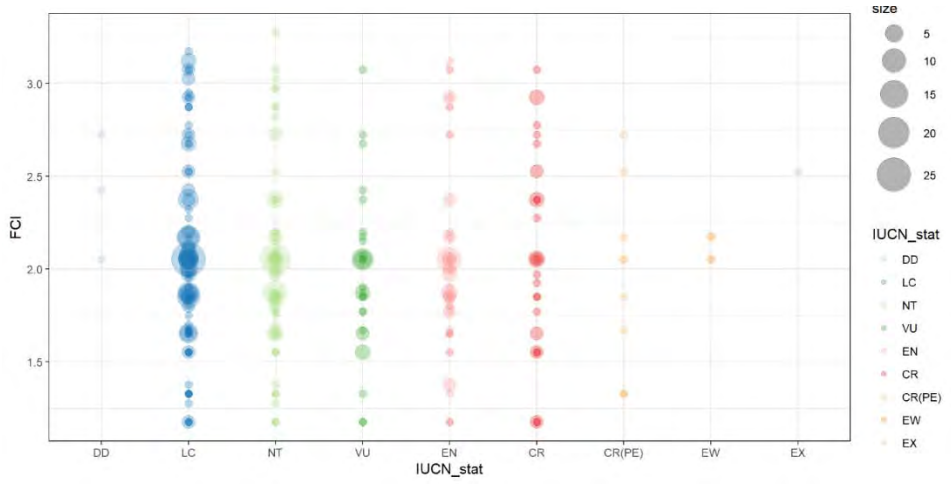


Fig. 1 Correlation between IUCN and FCI assessment for Italian plant taxa

3 = Influence of abiotic factors on tree-level lichen diversity in Mediterranean mountain forests

Sonia Ravera[1], Claudia Cocozza[2], Renato Benesperi[3], Elisabetta Bianchi[3], Luca Di Nuzzo[3]
[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; [3]Department of Biology, University of Florence, Via la Pira 4, 50121 Florence, Italy

Lichens play an important role in a number of forest ecosystem processes (i.e. in water and nutrient cycling, supply of food, cover material and nesting for a variety of birds, mammals, insects, etc.) and their sensitivity to air pollution and disturbance makes them ideal candidates for long-term monitoring of forest health. Conservation of biodiversity and sustainable forestry are essential elements in a multifunctional forest environment.
To specifically ensure the conservation of forest lichens, ecological studies at different scales are needed allowing to define abiotic and biotic factors associated with high diversity. While research has focused more on larger-scale (macroscale) environment and local (mesoscale) scale, less information is available at smaller (microscale) level. In a forest, an example of a microscale is represented by the chemical-physical characteristics belonged and /or related to a single tree: a fundamental habitat for epiphytic lichens. Several tree-level environmental factors (i.e. microclimatic variables and bark properties) are important for the growth of epiphytic lichens and drive the diversity and composition of their communities.
Our aim is to evaluate the effects of environmental variables measured at tree-level on lichen epiphytic communities in the mountain forests of central Italy. The survey was conducted in the Biogenetic State Natural Reserve of Vallombrosa (Fig. 1) and in the Sant'Antonio Forest (Fig. 2) in Tuscany. In these sites, the Lichen Diversity Value (LDV) - a standardized and widely used biomonitoring method for estimating total lichen diversity on trunks - was calculated respectively on 40 silver fir (*Abies alba* Mill.) (Fig. 3) and 40 beech (*Fagus sylvatica* L.) trees, along the trunk of which, at approx. 3 m high, specific experimental devices (TreeTalker) are installed (Fig. 4).
The TreeTalker (TT) is a multisensor device designed for the simultaneously measurement of variables including: water transport in the trunk (sapflow), tree radial growth, stem water content, multispectral signature of light transmitted through the canopy and air temperature. Using the Internet of Things (IoT) technology, they transmit the data acquired through the internet in real time from a continuous monitoring system.
The outcomes from a planned long-term monitoring will make possible to know the responses of forest epiphytic communities to environmental changes in progress and to evaluate the influence on the lichen diversity of the tree-level factors: (i) size and (ii) growth of trunks, (iii) water-holding capacity of bark (iv) light availability and (v) air temperature.
In this paper, the first results relating to 2 years of investigation are presented. They allowed us to identify the differences between the lichen diversity of the 2 sites and the influence exerted by the recorded factors on the LDV. Combining these data, we are able to set up the strategy, also and not only for the purposes of adequate forest management that includes biodiversity conservation among its objectives.

Fig. 1. Vallombrosa

Fig. 2. Sant'Antonio

Fig. 3. Lichens on stem

Fig. 4 TT installation

3 = Future and past of the endemic alpine plants of the Dolomites: response to future climate change and phylogeography

Francesco Rota[1], Gabriele Casazza[2], Peter Schönswetter[3], Camilla Wellstein[1]
[1]Faculty of Science and Technology, Free University of Bozen/Bolzano, Piazza Università 5, 39100 Bozen/Bolzano, Italy; [2]Dipartimento di Scienze della terra, dell'Ambiente e della Vita, Università Di Genova, Corso Europa 26, 16132, Genova, Italy; [3]Department of Botany, University of Innsbruck, Sternwartestraße 15, 6020, Innsbruck, Austria

Climate change is expected to threaten alpine plants in the Alps. Among mountain species, endemic plants are expected to be more affected by climate change due to their small niche-breadths, small population sizes, low genetic diversity, specific habitat requirements and low dispersal ability. The aim of the study was to assess the impact of future and Quaternary climate change on the alpine endemic species of the Dolomites. Firstly, using ensemble species distribution models, we projected the range change under different climate change scenarios with downscaled climatic data at a fine spatial resolution of 50m, as well as topographic factors, to investigate factors that may modulate the responses to climate change of eight alpine endemic plants of the Dolomites at a local scale. Secondly, we investigated the intraspecific genetic patterns of three species, *Campanula morettiana* Rchb., *Primula tyrolensis* Schott ex Rchb. and *Saxifraga facchinii* Koch, to clarify their phylogeographical pattern and to shed light on their populations response to Quaternary glaciation by using SNPs data. The negative impact of future projected climate change ranged from moderate to severe, depending on scenario and species. Generally, range loss occurred at the lowest elevations, while gained and stable areas were located at highest elevations. Our findings supported the role of topographic heterogeneity in maintaining climatic microrefugia, however, the peculiar topography of the Dolomites, characterized by high altitude plateaus, resulted in high climate change velocity in areas of projected future climatic suitability. The molecular RADseq analyses suggested differentiation among eastern and western populations of *C. morettiana* and *P. tyrolensis* on both sides of the Piave valley. Both species showed peripheral glacial survival in the southern and southeastern Dolomites. Differently, the more cold adapted *S. facchinii* showed highly differentiated populations occurring over short distances in the centre of the distributional range, while the northern and northeastern edge populations showed marked genetic isolation, a pattern congruent with nunatak survival. In conclusion, the present study constitutes an original contribution to the knowledge of the endemic flora of Dolomites. In particular, the results of our research can be useful for the elaboration of proactive conservation strategies within this center of endemism to ensure the survival of these unique rare species facing future climate change.

3 = Manual pollinations among individuals of *Abies nebrodensis* (Madonie, Sicily)

Rosario Schicchi[1], Anna Geraci[2], Filippo Amato[1], Giuseppe Di Noto[1], Gaetano La Placa[1], Francesco Sgadari[1], 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

Abies nebrodensis (Lojac.) Mattei is an endemic forest species, critically endangered, whose natural population consists of thirty individuals distributed in a small area of the territory of the municipality of Polizzi Generosa (Madonie Park) between 1375 and 1690 m altitude.
As part of the project "Decisive *in situ* and *ex situ* conservation strategies to secure the critically endangered Sicilian fir, *Abies nebrodensis*," acronym LIFE4FIR (LIFE18 NAT/IT/000164), launched in 2019, one of the most significant actions is aimed at obtaining seeds of secure genetic purity and increasing seedling diversity. This action was initiated in 2020 and will continue until October this year.
It consists of isolating, towards the end of April, young female cones, using special wax paper bags, in order to prevent both potential genetic pollution by pollen grains from other exotic entities of the *Abies* genus and self-fertilization.
Then, by the first decade of May, hand pollination is carried out among the different sexually mature individuals of the natural population (25 individuals), according to an experimental design aimed at maximizing the number of different combinations between unrelated and geographically more distant genotypes. Between the end of July and the first half of August, when the cones have reached full maturity, the protective bags are removed and replaced with a "nonwoven fabric," which is more permeable to light and air, to prevent seed dispersal following cone disarticulation, which normally begins in the first decade of October.
Seeds are collected before disarticulation and used in the nursery after their origin has been confirmed by paternity testing.
An extraordinary production of cones occurred in 2020. A total of 488 female cones were isolated, accounting for nearly 20% of the total female cones produced by sexually mature plants. The length of the cones varies greatly among individuals, taking values between 9 cm (plant No. 27) and 16 cm (plant No. 13), while cone circumference values (measured in the median zone) vary less, being between 13 cm and 16 cm;
In 2021, individuals of *A. nebrodensis* did not produce female and male cones, while this year, at the beginning of May, numerous pollinations have already been carried out, and the monitoring activity of cone development is currently ongoing.
The result of the intensive and complex hand-pollination activity of *A. nebrodensis* cones has enabled us to obtain 4,900 g of seeds so far, equivalent to about 100,000 seeds.
These seeds have been used in nursery sowings to obtain new seedlings with greater genetic diversity to be used for reforestation interventions in ten areas of the Madonie Park and for other conservation and dissemination interventions of the species.

3 = Study of pollen viability in *Corylus avellana* cv. Tonda Gentile

Elisabetta Sgarbi[1,2], Claudio Brandoli[1], Mara Politi[3], Consolata Siniscalco[3]
[1]Centre BIOGEST-SITEIA, University of Modena & Reggio Emilia, Via Giovanni Amendola, 2, 42122, Reggio Emilia, Italy; [2]Department of Life Sciences, University of Modena & Reggio Emilia, Via Giovanni Amendola, 2, 42122, Reggio Emilia, Italy; [3]Department of Life Science and Systems Biology, University of Torino, Viale Mattioli, 25, 10125 Torino, Italy

Hazelnut (*Corylus avellana* L.) is one of the most popular nut crops in the world. Its growing economic importance justifies its diffusion in different geographical areas outside its traditional production habitat. Despite its importance, little is still known about the reproductive biology and in particular on pollen viability, processes that determine the success of the production.
Since the species is characterized by sporophytic self-incompatibility, a process controlled by a single gene locus with multiple alleles that occurs at the level of the stigmatic surface, all hazelnut cultivars need an appropriate pollinizer so that the reproduction leads successfully to the subsequent fruiting phase. With this aim, several studies have recently been conducted on the reproductive processes in *C. avellana* in some geographical areas characterized by different environmental conditions such as South Africa and Chile. It has also been observed that artificial pollination is able to increase the yield of orchards compensating for pollination problems due to poor pollen viability of some cultivars. However, the application of this practice is not fully analyzed and well regarded, due to costs and difficulties by small producers.
In this study we compared the different viability levels of pollen in the cultivar Tonda Gentile (TG) and hazelnut Wild-Type (WT) grown during the flowering season 2021-2022 in the traditional cultivation area of Langhe, Piedmont region, Italy. We used the Ampha© Z32 impedance flow cytometer (https://amphasys.com/), a versatile lab-on-chip technology that allows to analyze the different levels of pollen viability without the use of dyes but exploiting the dielectric characteristics of the cytoplasmic content in pollen grains. The work was based on the recognition of viable, anomalous (sterile) and dead pollen grains during the three phenological phases: the beginning of flowering, full blooming and the end of flowering.
A general average increase in the levels of anomalous (sterile) pollen during the whole period of dispersal was found in TG compared to the WT genotype which, on the contrary, showed very low levels, almost inconsistent, of anomalous (sterile) pollen, never higher than 5%. As regards the concentrations of viable pollen, TG was characterized by rather low levels, never higher than 20% with the exception of a peak of emission during the period of full blooming. On the other hand, WT showed an increasing trend, up to the fourth week in which the pollen percentage viability has reached the 82%; finally, it gradually decreased until the end of the dispersal phase.
The dead pollen levels were generally quite high in both genotypes but with different emission behavior. The TG showed irregular levels of dead pollen throughout the flowering period while the WT genotype was characterized by a definitely high level at the beginning of flowering (79%), followed by a rapid decrease towards full flowering (14%) and return towards the end of the dispersal period, re-settling on values similar to the initial ones.
Phenology did not show significant differences in the time and duration of dispersal of the two genotypes. Only the WT has extended release period by one week compared to the TG. Our results indicate that a high percentage of abnormal (sterile) pollen grains are found in TG. Further analyzes must be carried out on the correlation of this occurrence of limited fruit production in this cultivar compared to others.

4 = The Herbarium of Fr. Gioacchino Russo in San Martino delle Scale (Palermo)

Gianniantonio Domina[1], Giuseppe Venturella[1], Emilio Di Gristina[1], Giulio Barone[2]
[1]Department of Agricultural, Food and Forest Sciences, University of Palermo, Viale delle Scienze Bldg.4, 90128, Palermo, Italy; [2]Department of Architecture, University of Palermo, Viale Delle Scienze Bldg. 14, 90128 Palermo, Italy

The Benedictine monastery of San Martino delle Scale is located at 600 m a.s.l. a few kilometers from Palermo; in its rich library there is a historical herbarium whose initial nucleus dates back to the first half of the 19th century. The herbarium is made up of the collections from the surroundings of Palermo by Father Gioacchino Maria Russo. In addition to the monk's own collections, there are also samples exchanged or donated by other botanists of the time: Giovanni Gussone, Carel Presl, Antonino Bivona-Bernardi, Constantine S. Rafinesque, and Giuseppe Bartolotta.
San Martino delle Scale, was, in fact, a must for all naturalists who went to Palermo. The access road to San Martino coming from Palermo was for Boccadifalco, a village that grew up around a royal hunting reserve of the Bourbons. Inside this reserve Prince Francesco di Borbone had set up a botanical garden in 1817 to direct which he called Giovanni Gussone of the school of Naples. The Monastery was visited by those who went to Monte Occhio to study the natural riches of this area surrounded by woods. Father Gioacchino Russo is mentioned by C. Presl in his travel diary in Sicily and by Francesco Tornabene in the historical picture of Botany in Sicily but few are the news about this botanist. Neither the date of birth nor that of death of the monk are known. In the register of the Monastery is reported that he, born in Palermo, professed his vows on February 25 in 1804. It was customary to celebrate the profession around the age of 18, therefore, at the time he met Gussone, Presl, Bivona and Rafinesque, Father Russo had about thirty years.
The relations of Father Russo with the botanists of the time are testified by the description of *Paeonia russoi* Biv. and *Saxifraga russoi* C. Presl on samples collected by him.
The herbarium currently consists of 1308 sheets of 16 × 21.5 cm, it is composed of native Sicilian plants plus medicinal or ornamental ones cultivated in Sicily, plants collected from North America, probably collected by Rafinesque, and 7 lichens, 1 seewed and 3 bryophytes. Ercole Tedeschini in 1860, on behalf of the abbot Luigi Castelli, reviewed the herbarium and arranged it in 87 folders, according to the De Candolle system. Subsequently the collections were studied by Rosetta Bonomo in 1956 and, more recently by Pietro Mazzola and collaborators in 1994. The herbarium includes the list, in alphabetical order, with the scientific and dialect name of the plants included.
The specimens are fixed to herbarium sheets with paper glued paper strips. Almost all specimens are labeled with the Latin name and in part are provided with detailed notes on the localities and local medical uses. The specimens have names and annotations written with different tool and handwritings. Some plant samples are fixed on the front of the sheet, others on the reverse. This testifies that the specimens have different origins and were annotated by different authors in later times. Actually, the herbarium is included in 23 cardboard boxes recently manufactured; many specimens are damaged by insects or humidity.
Owing to its content the collections of Fr. Russo remain an important testimony for the study of Sicilian botany at the beginning of the 19th century. The revision of these materials, started and suspended several times, is therefore of great interest for systematics and museography. An interesting aspect of the revision of this collection concerns for many taxa the variations of the taxonomic delimitations and of the nomenclature over the centuries.

4 = Phenotypic plasticity is a potential mechanism for responding to novel environmental conditions

Giuseppe Emma[1], Greg M. Walter[2], James Clark[5,4], Delia Terranova[1,3], Simon J. Hiscock[4], Jon R. Bridle[5,6], Salvatore Cozzolino[3], Antonia Cristaudo[1]
[1]Department of Biological, Geological and Environmental Science, University of Catania, Catania, Italy; [2]School of Biological Sciences, Monash University, Melbourne, Australia; [3]Department of Biology, University of Naples Federico II, Naples, Italy, [4]Department of Plant Sciences, University of Oxford, Oxford, UK; [5]School of Biological Sciences, University of Bristol, UK; [6]Department of Genetics, Evolution and Environment, University College London, London, UK

Due to changing climatic conditions and anthropogenic impacts, natural populations face increasingly variable environments. These factors affect the demography of populations which can impact their survival and resilience. Therefore, it is important to investigate the mechanisms that could help species to maintain fitness and avoid extinction. Populations can persist in novel environments through plastic changes in phenotype, enabling fitness to be maintained within a single generation. Where the limits of plasticity are exceeded in novel environments, genetic variation in plasticity could emerge and allow for rapid adaptation.
In this study, we assessed whether differences in fitness in a novel environment in known genotypes of the model species *Senecio chrysanthemifolius* (Asteraceae) are due to genetic differences in phenotypic plasticity and whether this plasticity can lead to an adaptive change that enables population persistence.
A previous study (Walter et al. 2021: https://doi.org/10.1101/2020.10.26.356451) identified genotypes that show high fitness ER (*Evolutionary rescue*) and low fitness HR (*Home range*) in a novel environment, and that genotypic differences were related to plasticity. Interestingly, the ER genotypes showed lower fitness at the native site than HR genotypes, suggesting that they segregate in the natural population. Our study aimed to understand the differences in plasticity between ER and HR genotypes. From the original study, we selected 10 ER and 10 HR genotypes and for each genotype, we transplanted multiple clones (32 to 40) at four elevations on Mount Etna (Sicily), which included the native range for the species (500 and 1,000 m a.s.l.), the edge of its range (1,500 m) and a novel elevation (2,000 m). A total of 3,200 cuttings were placed in the four fields. We included multiple clones to test whether any differences between ER and HR genotypes are due to genetic or non-genetic effects.
We estimated fitness by counting the number of flower heads produced over a 5-month period, for each individual plant/each clone. Estimating and comparing plasticity and fitness for ER and HR genotypes allowed us to test whether plasticity in morpho-physiological traits is associated with the maintenance of fitness within the native and novel environment. We used the cuttings of each genotype to estimate plasticity in leaf morphology and physiology by calculating the Coefficient of Variation. CV captures how much the clones of each genotype varies within and between transplant sites, relative to the mean for that genotype.
By correlating the fitness of the native site with the plasticity (CV) of the site itself, we found that the HR genotypes showed increased plasticity associated with increased fitness for leaf area only, whereas increased plasticity in the other traits was associated with reduced fitness. In contrast, ER genotypes showed higher plasticity associated with higher fitness for all traits. For plastic changes across elevation (i.e. from the native to the novel site), the ER genotypes showed higher plasticity associated with higher fitness for all leaf traits, while the HR genotypes showed this positive association only for the leaf complexity trait and no association for the other traits. We can therefore conclude that the HR genotypes develop a type of maladaptive plasticity within the native site, while in the novel environment the plasticity appears to be neutral. In contrast, ER genotypes develop, both within the native site and across elevations, an adaptive plasticity.
This shows that fitness is associated with different patterns of plasticity, both within and across elevations. Thus, for ER genotypes, we found a positive correlation between fitness and plasticity, both within and across sites, suggesting that plasticity may be more adaptive to varying environmental conditions. In contrast, the HR genotypes showed no such positive correlation but rather a neutral trend with respect to environmental variation.
In conclusion, our study shows that the genotypes that can aid population persistence in novel environments are the genotypes ER that exhibit adaptive plasticity in the novel environment. To understand whether the presence of genotypes that develop such plasticity can allow for the evolutionary rescue of species, further studies on the demography of natural populations are required.

4 = Local adaptation to biotic and abiotic factors in the cliff species *Dianthus rupicola*: a preliminary study

Lucrezia Laccetti, Mariachiara Cangemi, Giovanni Scopece
Department of Biology, University of Naples Federico II, Via Cinthia 26, 80126 Naples, Italy

Geographic variation in plant phenotypic traits is usually assumed to be the consequence of local adaptation to biotic and abiotic selective factors, a process of fundamental importance in evolutionary and conservation biology. Local adaptation is more likely to exist within species whose distribution and dispersal are constrained by natural barriers, hence limiting the homogenizing effect of gene flow. Recently, renewed interest was raised by the study of morphological and physiological adaptations of scrub and wood species in the Mediterranean area. However, little is known about Mediterranean cliff plant species which inhabit environments characterized by strong natural barriers.
Here, we present preliminary results of an ongoing project focusing on local adaptation in *Dianthus rupicola*, a chasmophyte, strictly related to cliff habitat and endemic to central Mediterranean. This species represents a useful model to conduct local adaptation studies, due to its highly fragmented distribution and its mutualistic (pollination) and antagonistic (floral herbivory) interactions which can impose different selective pressures on floral traits. We analyzed eight populations localized in peninsular southern Italy, Sicily and Aeolian islands. For each population, we collected qualitative and quantitative data including population size, floral traits, leaf anatomy, breeding system, pollinator set, floral herbivory, dispersal system, germination and growth ability on different soils.
All the populations showed an adaptation to cliff habitat such as a high germination ability on different soils, an efficient dispersal system through sea water and leaf morpho-anatomical features typical of plants adapted to high solar radiation. However, preliminary analysis showed no differences among the populations suggesting similar abiotic conditions and similar selective pressures in the investigated areas. According to preliminary results biotic factors were instead variable among populations both in terms of pollinator set and floral herbivory degree. These differences reflected variable results in terms of reproductive success and floral traits. In particular, style length and flower number within inflorescences showed significant differences among populations.
These preliminary results suggest that *D. rupicola* populations might be locally adapted to different pollinators and floral herbivores. Our study might provide insights into the patterns and causes of variation in local adaptation of this cliff species, highlighting the importance of considering biotic factors as drivers of adaptation in long-lived plants.

4 = Components of post mating isolation in a genus of Mediterranean orchids

Ilaria Masullo, Salvatore Cozzolino, Maria Rosaria Barone Lumaga, Giovanni Scopece, Donata Cafasso
Department of Biology, University of Naples Federico II, Via Cinthia, 80126, Napoli, Italy

In orchids, the specificity of pollination strategy has been widely considered as the main reproductive isolation mechanism and the main driver of speciation processes. As consequence, most studies focused on the description and estimation of pre-mating isolation mechanisms, paying less attention to the role of post-mating (prezygotic and postzygotic) barriers (Cozzolino and Widmer 2005: https://doi.org/10.2307/25065482). Over the years, however, it has been showed that Mediterranean orchids include both pollinator-specialized and pollinator-generalized groups; the latter attract, in a not selective way, different species of pollinators (bees, butterflies, flies) (Cozzolino et al. 2005: https://doi.org/10.1098/rspb.2005.3069). Among pollinator-generalized orchids, the genus *Anacamptis* includes a range of rewarding (*A. coriophora*) and deceptive species (*A. laxiflora*, *A. morio*, *A. papilionacea*). These orchids share the period of anthesis and pollinating insects, so they are potentially exposed to hybridization. Nevertheless, hybridization is either surprisingly rare between some species pairs or, when common, such as between *A. morio* and *A. papilionacea*, hybrids are mostly unfertile. The lack of evident interspecific gene flow in the face of pollinator sharing suggests the presence of some strong form of post-mating reproductive isolation mechanisms.
Here, we focused on post-mating reproductive isolation in *Anacamptis* trying to dissect between not-competitive gametic incompatibility (i.e. inhibition of the development of the pollen tube, failure to develop the fruit, lack of egg fertilization - GI) and competitive gametic incompatibility (pollen competition or conspecific pollen precedence- CPP, i.e. the offspring generated is preferentially not-hybrid) (Luca et al. 2014: https://doi.org/10.1111/plb.12199).
For this aim, we carried out hand pollinations in which only heterospecific pollen or only conspecific pollen was added on the stigma of a receiving species and pollinations in which both conspecific and heterospecific pollens (double pollination) were simultaneously added on the stigma of the same receiving species. Addition of conspecific pollen to interspecific pollinations (i.e., double pollination) removed the gametic or stigmatic incompatibilities and allowed to evaluate the presence of barriers related to conspecific pollen precedence (CPP). From all crosses, we investigated the development of the pollen tubes using epifluorescence microscopy, and seed paternity by molecular analyses of *in vitro* germinated seeds (protocorms).
We found the four orchid species to be characterized by different degree and type of post-mating barriers. Furthermore, these barriers were different between the pairs of examined species and were often asymmetrical (depending on the direction of the crossing). Specifically, *A. papilionacea* didn't show GI with *A. laxiflora*, *A. morio* and *A. coriophora* while the CPP was complete as none of seeds and protocorms from double pollinations was of hybrid origin. Instead, GI was observed in *A. morio* only when pollinated by *A. coriophora*, while the CPP was not strong in the presence of heterospecific pollen of any of the other two species. Pre-zygotic stigmatic incompatibility (inhibition of the development of the pollen tube) was observed in *A. laxiflora* when pollinated by *A. papilionacea* or *A. morio*. Finally, *A. coriophora* did not show any form of GI post-mating barrier. Overall, our results showed that the strength and type of post-mating barriers depend more on the species' ecology rather than on the common phylogenetic ancestry.

4 = A comparison of supervised classification models in detecting morphospecies: a case study in the *Armeria denticulata* complex (Plumbaginaceae)

Manuel Tiburtini[1], Luca Sandroni[1], Giovanni Astuti[2], Lorenzo Peruzzi[1]
[1]Department of Biology, University of Pisa, Via Derna 1, 56126 Pisa, Italy; [2]Orto e Museo Botanico, Sistema Museale di Ateneo, University of Pisa, Via Ghini 13, 56126 Pisa, Italy

Plants systematics in recent times has seen the steady increase of novel integrated approaches that heavily rely on the use of statistics for hypothesis testing in species circumscription. In this framework, classification models have the duty to assess the reliability of a taxon circumscription, maximizing the percentage of the correct classification (Acc) between alternative taxonomic hypotheses. However, incorrect data preprocessing, violation of model assumption and unbalanced group sizes could influence the results obtained and thus, the conclusions drawn. Among the classification models available, Linear Discriminant Analysis (LDA) is the most used classification model in plant taxonomy, even though its performance depends on several assumptions. Moreover, when testing different taxonomic hypotheses, it is common to have an unbalanced grouping that influences Acc and Cohen's K, since they suffer from what is known as "Accuracy Paradox". More robust ways to compare classification models exist, such as Balanced Accuracy (BAcc) and Area Under the Curve (AUC), that are unsensitive to sample size. Therefore, the aims of this study are to set up a protocol for applying effectively classification models in plant morphometric datasets and to compare classification models to maximize the AUC e BAcc in an unbalanced confusion matrix. To do so, we selected a pair of closely related central Italian endemic species, *Armeria denticulata* Bertol. and *A. saviana* Selvi (Plumbaginaceae), the former more widely distributed in Tuscan serpentine outcrops, the latter occurring in a restricted area of southern Tuscany, close to Monte Amiata. *Armeria denticulata* (n = 59) was sampled in three localities: Monte Ferrato, Brina di Ponzano (type locality), and Poggio Pelato, while *Armeria saviana* Selvi (n = 20) was sampled in its type locality, Stribugliano. A morphometric study based on 54 characters was carried out. All the analyses were done in RStudio 4.0.3. The dataset ($n \times d$) was 79 × 54. For LDA, Box's M and Royston tests were used as multivariate homoscedasticity and normality tests. We choose 2 linear and 2 non-linear supervised classification models and we compared them on the full dataset and with Leave one Out Cross Validation (LOOCV). In the former group, we compared the Binomial Linear Logistic Regression (BLLR) and LDA (DA only on quantitative data previous scaling) whereas in the latter we compared Random Forest (RF) and k Nearest Neighbors (kNN) classifier. Quantitative data violated the multivariate normality assumption and covariance matrix was heteroscedastic. The results showed that BLLR on the full dataset suffered from overparameterization ($n \gtrsim d$) and no β was significant. To deal with this problem, a mixed PCA was used from {PCAmixdata} package. KMO {EFAtools} (0.71) and Bartlett's test {EFAtools} ($p<0.01$) tests were successfully carried out. Characters with the most contribution (>0.40) on PCA axes where selected. Model diagnostics were observed. Stepwise on both direction for model selection using stepAIC {MASS} was performed resulting in 8 quantitative continuous variables selected. For linear models, LOOCV-BLLR obtained an Acc = 0.8861 [IC95 = 0.7947– 0.9466], a BAcc = 0.8411, K = 0.6937 with AUC = 0.94.
LOOCV-LDA on solely quantitative data performed effectively resulting in an Acc = 0.9494 [IC95 = 0.8754– 0.986], BAcc = 0.9230, K = 0.8704 and AUC = 0.99. For non-linear models, LOOCV-kNN on scaled numeric data resulted in an Acc = 0.9747 [IC95 = 0.9115– 0.9969], BAcc = 0.9665, K = 0.9331, AUC = 1, whereas RF on the mixed data obtained 8.86% OOB Error and an overall performance similar to BLLR (Acc = 0.8481 [IC95 = 0.7497– 0.919], BAcc = 0.7000, K = 0.4989, AUC = 0.96). According to RF's Dot chart of variables and mixed PCA variables contribution, only quantitative characters helped to discriminate *Armeria denticulata* from *Armeria saviana*. This could explain why BLLR and RF did not take advantages of their ability to include qualitative data resulting even in a loss of performances for RF. In this scenario, where of LDA assumptions where not met, kNN outperformed LDA. From our result we can see that different models can archive different classification metrics, according to how suited is the model for the data. Thus, the choice of the wrong model for assessing different taxonomic hypothesis could influence the results obtained and so, the conclusions drawn. Lastly, we encourage the combined use of Acc, BAcc and AUC, as more reliable estimators of supervised classification models performances (morphospecies circumscription), especially in imbalanced contingency tables.

Acknowledgements
This work is supported by the "Progetto di Ricerca di Rilevante Interesse Nazionale" (PRIN) "PLAN.T.S. 2.0 - towards a renaissance of PLANt Taxonomy and Systematics" led by the University of Pisa, under the grant number 2017JW4HZK (Principal Investigator: Lorenzo Peruzzi).

5 = Effect of a metabolite produced by the invasive macroalga *Rugulopteryx okamurae* (Phaeophyceae) on unicellular phototrophs

Denise Lenzo[1], Ana Bartual[2], Bárbara Úbeda[2], Eva Zubía[3], Marina Antonia Colangelo[1], Andrea Pasteris[1], Laura Pezzolesi[1]

[1]Department of Biological Geological and Environmental Sciences, University of Bologna, Via Sant'Alberto 163, 48123 Ravenna, Italy; [2]Instituto Universitario de Investigaciones Marinas (INMAR), Campus de Excelencia Internacional del Mar (CEI-MAR), Universidad de Cádiz, Puerto Real, 11510 Cádiz, Spain; [3]Departamento de Química Orgánica, Facultad de Ciencias del Mar y Ambientales, Universidad de Cádiz, 11510-Puerto Real, (Cádiz), Spain

In recent years the presence of non-indigenous macroalgal species (NIS) has been observed, among which are included invasive algae whose negative impact on marine ecosystems could affect the biodiversity and economy of coastal areas. Marine algae, particularly diatoms but also macroalgae, are known to produce allelopathic compounds with high structural variability, that play an important role in species' successions.
Additionally, whether invasive algae could produce allelopathic and toxic compounds, and how they could potentially change the population dynamics along the trophic chain remained unknown.
In the last decade the brown macroalga *Rugulopteryx okamurae* has shown an intensive proliferation in the southwestern coasts of Europe (Strait of Gibraltar). Currently, there is no clue regarding the reasons of such huge invasive potential, although the involvement of chemical defenses has recently been suggested. Chemical study on *R. okamurae* from the Strait of Gibraltar led to the isolation of different secondary metabolites, among which the compound "Dilkamural" stands out because of its high concentration.
In this context, this study was aimed to investigate the potential role of chemical defenses in the invasive success of *R. okamurae* on unicellular phototrophs. In particular, this study was carried out through ecotoxicological tests on two different species of microalgae (the diatoms *Phaeodactylum tricornutum* and *Cyclotella cryptica*) and a cyanobacterium (*Synechococcus elongatus,* PCC 7002) using different concentrations of "Dilkamural". The effects on cells were evaluated for each species after 0, 3, 24, 48, 72 hours, adding "SYTOX green" to mark dead cells and analysing them through image flow cytometry (IFC) (Amnis ImageStream XMkII, Luminex Corporation).
Preliminary results showed an acute effect of Dilkamural, immediately after three hours on all the analysed species; particularly, effects on the viability and cell morphology of *P. tricornutum*, as well as cell integrity and reproduction of *S. elongatus* and *C. cryptica* were observed.
Elaboration of the data is still in process, to better understand the implication that Dilkamural have at the base of the trophic chain and as the production of this compounds could affect either the epiphytic community associated to macroalgae and the biodiversity of coastal areas where this invasive algal species has been introduced.

6 = Botanical studies on the peri-urban system of private historical gardens in the city of Trapani

Enrico Bajona[1], Giampiero Musmeci[2], Francesco Maria Raimondo[1], Filippo Salerno[1] & Giovanni Vito Zizzo[1]
[1]*PLANTA*/Center for Research, Documentation and Training, Palermo, Via Serraglio Vecchio 28, 90123 Palermo (Italy); [2]Studio di Architettura, Via B. Bonaiuto 20, 91100 Trapani (Italy)

Starting from some studies concerning the ornamental plants of Trapani's historical gardens, in the last few years, the dendrological contingent of some gardens inside some ancient villas in which architectural or landscape constraints exist was investigated.
In particular, some gardens placed in the outskirts of Trapani, along the Trapani-Palermo road axis, have been studied; all these gardens were built from the first decade of 19th century to the end of the same century.
Villa Maria's garden, Villa Augugliaro's one and Villa Pampalone's garden jump out among the most representative gardens of Trapani.
Right in the gardens listed above, ornamental masts have been found: they are already known in Sicilian ornamental masts but they stand out for their dendrological and taxonomic unique values.
Among all, Villa Maria's garden, that it is owned by D'Ali family, jumps out not only for the monumentality of so many ficus trees (*Ficus microcarpa* L. fil. and *Ficus macrophylla* Desf. ex Pers. f. *columnaris* (C. Moore) P.S. Green) (*Moraceae*) and *Lagunaria patersonia* (Andrews) G. Don (*Malvaceae*), both are Australian species that were introduced in Sicily in the first half of 19th century, but especially for the presence of a tree species that has never been noticed in European and Italian gardens; it is *Pararchidendron pruinosum* (Benth.) I.C. Nielsen (*Mimosaceae*), native of Australian rain forest that now is reported for the first time in Italy.
It is also relevant the presence of exuberant pecan trees (*Carya illinoinensis* (Wangenh.) K.Koch) and some palm trees, like *Brahea armata* S. Watson, observed at Villa Pampalone and at Villa Augugliaro, respectively.
Among the plants surveyed, some of them still remain unidentified.

6 = The past for the future: Botanical analysis of the lost garden of Villa Montalto (Rome) for new green cultural prospective in the city

Bartoli Flavia[*,1], Nucera Arianna[2], Caneva Giulia[1]
[1]Department of Science, University of Roma Tre, Via Guglielmo Marconi, 446, 00146 Roma, Italy; [2]Department of Architecture, University of Roma Tre, Via Aldo Manuzio 68L, 00153, Roma

Urban regeneration is often regarded as the process of renewal of spaces and places, and this change can have significant impacts on cultural heritage. In this period of innovation and improvement of living conditions, the knowledge of the historical landscapes and the understanding of modification over time can be a fundamental tool in making correct choices for our cities.
Villa Peretti Montalto, a possession of Pope Sixtus V (1585-1590), was the largest roman villa inside the Aurelian walls, which occupied a large trapezoidal-shaped territory of 45 hectares between the Basilicas of *Santa Maria Maggiore* and *Santa Maria degli Angeli*, recognized in the modern Termini's Station area.
This work concerns the physiognomy's, floristic and functional reconstruction of the lost garden of this Villa during the years of its development (1500-1600) because, with the end of the Peretti family, the property passed to different families witnesses of its gradual destruction (1860 - 1888) (Fig. 1). We proceeded with the reconstruction work through the consultation of several bibliographic and iconographic sources, comparing the ancient sources with the modern ones to find a match and to recognize the floristic composition of the garden and the placement of the plant.
We recognized five different areas (Triangular Garden, Secret Gardens, Hanging Garden, Citrus Grove, Vineyard, Hunting Reserve) for the garden of the XVI century and three more (Gardens for Rent, Cavallerizza gardens, Heights) for the changes in the XVII century. We could find 88 plant species, and their placement in the different areas of the garden in relation to their usefulness and symbolic value. The typical elements of such Roman Renaissance Garden were *Cupressus sempervirens* L., *Quercus ilex* L, *Pinus pinea* L. and *Ulmus minor* Mill. as ornamental trees, some of them with high visual values (Fig. 2), together oranges (*Citrus* x *aurantium* L., *Citrus* x *bergamia* (Risso & Poit.); such as *Buxus sempervirens* L., *Myrtus communis* L. and *Viburnum tinus* L. for the *Ars topiaria*. Moreover, it is interesting cite the avenue of mulberries (*Morus nigra* L.) used to produce silk, and ancient lost or neglected varieties of fruit trees in the Vigna area, such as *Pyrus communis* var. *crustumina* described by Plinius and Virgilius, or also *Cornus mas* L., *Cornus sanguinea* L., *Mespilus germanica* L., and *Sorbus domestica* L.
Rediscovering the past urban landscape could be an essential tool for the cultural enhancement of the city and the knowledge of the past flora in historical gardens is an instrument that could be exploited in a planning of urban greenery, which considers the historical values.

Fig. 1. Assessment of Villa's gardens over the time

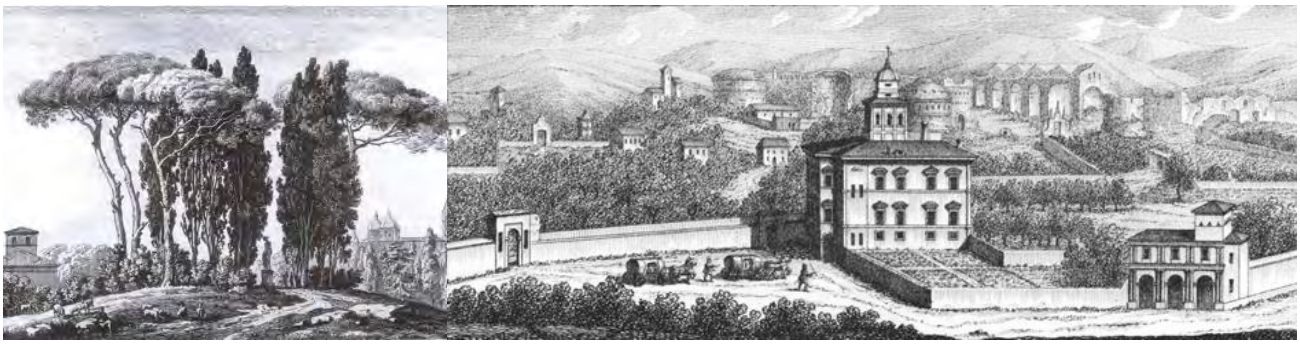


Fig. 2. Views of Justice mountain's up (XVIII century, Bourgeois C.) and of Villa (Cipriani G.B., 1836)

6 = Microclimatic and Environmental Improvement with Nature-Based Solutions in a Mediterranean City

Chiara Calise[1], Katia Perini[2], Paola Castellari[2], Elena Nicosia[3], Enrica Roccotiello[1]
[1]Department of Earth, Environment and Life Sciences (DISTAV), University of Genoa, Corso Europa 26, 16124 Genoa, Italy; [2]Department of Architecture and Design (DAD), University of Genoa, Stradone di Sant'Agostino, 37, 16123 Genoa, Italy; [3]Regione Liguria, Dipartimento Salute e Servizi Sociali, Settore Tutela della Salute negli Ambienti di Vita e di Lavoro Via Fieschi 17, Piano U8, 16121, Genoa, Italy

Most cities today are facing important social, health, environmental and economic challenges resulting in their high vulnerability. Epidemiological studies showed a close correlation between Urban Heat Island (UHI) and mortality among the most vulnerable groups of the population (aged >65). Nature-based Solutions (NbS) can represent an important strategy to mitigate the effects of climate change and to improve urban environmental conditions within the urban areas.
The current case study is supported by 'CCM Climactions' and is part of a regeneration project with Nature-based Solutions in the city of Genoa (Italy), in a neighbourhood characterised by relevant health and well-being issues. The aim of the study is to evaluate the microclimatic effects of NbS on a dense city in the Mediterranean area and to optimise the ecosystem services' provision by means of a targeted selection of plant species.
In order to study the potential benefits on urban microclimate of NbS and light/reflecting surfaces, a widely used approach is to run simulations using the ENVI-met software. The performances, including plant species, selected with a systemic approach, and light pavements, are analysed in terms of improved microclimate by means of the ENVI-met software V4.4.5. As regards the selection of plant species, a checklist of criteria was prepared considering the ecosystem services provided and the urban context, specifically the mitigation of the UHI. Other key aspects considered are the stratification of vegetation layers, to improve local biodiversity and to obtain microclimate benefits. Three scenarios, based on the progressive increase in the biomass percentage and high albedo pavements, are designed and analysed. Specifically, for the tree layer, *Cercis siliquastrum* L. has been selected in the first two scenarios. As for the shrub layer, Mediterranean species, with a strong rusticity and adaptability to various stress conditions, have been selected, such as *Phillyrea angustifolia* L., *Pistacia lentiscus* L., *Viburnum tinus* L. The herbaceous layer, in order to support connectivity and maximize ecosystem services, consisted in wildflowers and other species, such as *Festuca glauca* Vill. Climbers have been also included, covering vertical surfaces, like *Hedera helix* L., that has been chosen as being able to suckle larvae of insects as well as entomophilous pollinators.
The results show different benefits on the microclimate, compared to the current state (no NbS), in all the different scenarios with the selected plant species and light paving. These solutions can play a key role in improving microclimatic conditions during summer in terms of thermal comfort, mitigating air temperature (up to 1.8 °C less), mean radiant temperature (up to 17.3 °C less), and increasing relative humidity (up to 5.6%), resulting in a relevant improvement of UTCI (up to 5.4 °C less), despite the air flow reduction (up to 0.3 m/s less). Those parameters significantly improved in relations with the gradual increase in greenery in the scenarios, except for the air temperature, that mostly decreases in the scenario with less greening (Δt =1.8 °C). It is also worth mentioning that mean radiant temperature in the maximum greening scenario is <5 °C compared to the minimum greening one. Overall, the type of plant layers and related coverage percentage plays a key role on microclimate parameters, except for the relative humidity.
The study allowed selecting the most effective design strategy for the draft of a final design for a pilot redevelopment project and can be applied implying less economic effort for local administration.

6 = School gardens as a resource to be exploited for learning Botany in primary and kindergarten school

Patrizia Campisi[1], Elisa Petrotto[2], Mattia Letizia Marino[3], Maria Giovanna Dia[4]
[1]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies, University of Palermo, via Archirafi 38, I 90123 Palermo, Italy; [3]via dei Cedri n. 40 91100 Trapani, Italy; [4]via Monte Bonifato 191, 91011 Alcamo (Trapani), Italy

As has long been recognized by teachers and pedagogists, a direct relationship with nature has various positive effects on the development and both physical and mental well-being of children. From Jean Jacques Rousseau to Friederich Fröbel to Maria Montessori and Richard Louv, the importance for children to be outside not only playing, but also during educational activities in contexts in which they can come into contact with plants and animals and with different natural elements is underlined. With the awareness of this, a new educational model has been developed for some years now, the "outdoor education", which is implemented in the so-called "outdoor school", as an alternative to the traditional school model in which didactic activities take place within the classrooms. Examples of these new school structures, particularly widespread in Northern Europe and for some years also present in Italy, are agriasili, educational farms, kindergartens in the woods, in which most of the educational activities are designed to be carried out outdoors. Obviously, a teaching method of this type requires the abandonment of habits and of consolidated methodologies, first of all by teachers but also by parents, who often show distrust and reluctance in doing this. There is no doubt that, despite the increasingly widespread awareness of the effectiveness and the need for outdoor teaching, there are also difficulties of a management nature that make a wide dissemination of these structures difficult to implement. Surely a more easily feasible alternative solution could consider school gardens, so far little used for educational purposes. This is a need that also in Italy is beginning to be felt at many levels in the educational environment, as also demonstrated by the implementation of a recent bill, which we hope will see definition as soon as possible, entitled: "Norms for the development of green spaces in school buildings "(Dl S 1764). This paper reports the results of a first survey carried out for a degree thesis in the course of "Didactics of Botany for primary and kindergarten school" of the course of study in Primary Education at the University of Palermo. The survey was aimed to provide a contribution of ideas for a better use of gardens in the primary school for educational purposes with particular reference to the teaching of Botany. The study was conducted through a census of the species cultivated in the gardens of 11 schools, distributed in the provinces of Agrigento, Caltanissetta and Palermo. According to what emerged, certainly one of the aspects that must be considered concerns the choice of the type of plants to be placed in these spaces. This choice must take into account not only grow or aesthetic aspects but also those related to the educational objectives typical of these school levels as, for example knowledge of reproductives structures as well as of typical species of most representatives natural environments. In general, it is observed that the school gardens have so far been used by the teachers themselves mostly as recreational places, in which to carry out motor activities, also because they are designed with more aesthetic than didactic purposes. Frequent are cases of unsuitable plants because toxic, such as *Nerium oleander* L., or of species that do not lend themselves to manipulation or observation through direct contact by children.

6 = New plants in the urban green of the city of Palermo (N-Sicily)

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

Over 30 years of censuses and observations, conducted in particular in the city of Palermo, have made it possible to acquire a huge amount of data on the floristic component of both public and private parks and gardens, as well as squares and streets of the Sicilian capital. It is enough to go through the index of the "Quaderni di Botanica Ambientale e Applicata", since the first issue published in 1990, to get an idea of what has been done and the results achieved. In addition to the articles, there are also numerous contributions presented in the form of cards in the series "Schede per la flora ornamentale siciliana" curated by P. Mazzola and F.M. Raimondo.
In consideration of the scientific, ecological and social value that urban greenery is increasingly assuming, not only for the well-being of citizens, the preparation of a catalogue has been started in which to summarize the taxonomic, phytogeographical and distributive data of arboreal and shrubby plants and succulents registered over the years and still to be registered in the city of Palermo. This contribution is part of this project, in which some new floristic findings are reported to be added to the data already acquired and published.
To date, numerous taxa are unpublished for the city of Palermo and partly for the regional and national territory, including: *Archontophoenix alexandrae* H. Wendl. & Drude [*Arecaceae*], *A. cunninghamiana* (H.Wendl.) H. Wendl. & Drude [*Arecaceae*], *Cordia francisci* Ten. [*Boraginaceae*], *Ficus australis* Willd. [*Moraceae*], *F. lyrata* Warb. [*Moraceae*], *Howea belmoreana* (C.Moore & F.Muell.) Becc. [*Arecaceae*], *Metrosideros spectabilis* Sol. ex Gaertn. [*Myrtaceae*].
The authors report the complete list of unpublished woody taxa together with the corresponding retrieval data in the urban context of the Sicilian capital.

6 = First data from a palynological analyses of urban green area pollen in the city of Reggio Emilia

Elisa Furia[1,2], Anna Maria Mercuri[2], Alessandro Travaglini[3]
[1]Doctorate in Evolutionary Biology and Ecology, Department of Biology, University of Tor Vergata, Via Cracovia, 50, 00133 Rome, Italy; [2]Department of Life Sciences, University of Modena, Via Campi, 287, Modena, Italy; [3]Department of Biology, University of Tor Vergata, Via Cracovia, 50, 00133 Rome, Italy

Given the increased sensitivity to climate change, it is important to consider the effects climate change is having on both vegetation and human health. This is why plants used in urban green areas become interesting, as they are both sensitive to climate change and a possible risk to human health. In fact, pollen can cause allergies and even complications such as asthma. Changes and increases in both pollen and allergens can also be linked to the increase in temperatures and air pollution. Careful considerations should be placed on what species are introduced in urban green areas, especially ornamental species that were not present before, since it can introduce a new possible allergenic pollen and can interact with the present environment in hardly predictable ways. Therefore, it is important to reconstruct changes in urban green areas. The city of Reggio Emilia was selected as a case-study because of the presence of both archeological samples dated from around the foundation of the city and aeropalynological samples (both moss samples and the analyses from the ARPA station located in Reggio Emilia), in order to know the present day pollen rain. This will allow tracking of the arrival of new taxa in the urban area of Reggio Emilia and reconstructing the history of highly allergenic pollen in the city. The archaeopalynological information was obtained from two excavations in the city center, located in Vittoria Park and in San Prospero square respectively. These samples have been prepared through the method used at the Laboratory of Palynology and Paleobotany of the University of Modena. The moss sampling has been carried out by dividing the city with a grid made of 1 km squares for the outskirts and 500 meters squares for the city center. Moss samples have also been treated following the acetolysis method after sieving. The study is still at the beginning, but some archeological samples have already been analyzed, allowing a first look at the history of urban green areas in the city, as well as some moss samples collected in the city to obtain a mean pollen rain of around the last 5 years. The data obtained so far describe a mostly open area since the start of the history of the city, with some interesting arboreal pollen (both for possible cultivation for the edible fruit, like *Castanea* and *Prunus,* and for the known allergenic pollen, like *Alnus*, *Carpinus, Corylus*). For the non arboreal pollen, taxa of interest for pollinosis are Brassicaceae, Asteraceae (with the presence of *Artemisia*), Poaceae and Urticaceae. The data from archaeopalynolgical samples compared with the analysed moss samples allows for a first description of the evolution of urban green areas in Reggio Emilia. The authors would like to thank Dott. A. Capurso as the responsible for the Soprintendenza Archeologia Belle Arti e Paesaggio per la città metropolitana di Bologna e le province di Modena, Reggio Emilia e Ferrara Sabap-BO, for involving us in regard to archaeobotany studies of the excavation in Reggio Emilia and Prof. M. Cremaschi for acquiring the samples and support for geoarchaeology.

6 = The Pollinator Garden: an oasis for urban pollinators and a point of reference for pollinator-friends

Nicola Lothar Herrmann[1], Umberto Mossetti[1], Fortunato Fulvio Bitonto[2], Laura Bortolotti[3], Giovanna Dante[2], Annalisa Managlia[1], Marta Galloni[2]
[1] SMA Botanic Garden and Herbarium, University of Bologna, Via Irnerio 42, 40126 Bologna, Italy; [2]Department of Biological, Geological and Environmental Sciences, University of Bologna, Via Irnerio 42, 40126 Bologna, Italy; [3]CREA Research Centre for Agriculture and Environment, Via di Corticella 133, 40128 Bologna, Italy

Since the end of the 20th century, there has been a decline in insect pollinator populations around the world. Habitat loss, land use change, intensive agriculture, use of pesticides and herbicides, introduction of invasive species and climate change are the main causes of this loss. Pollination is an enormously important ecosystem benefit, that guarantees the stability of natural habitats, but also food security. Up to 75% of major world crops rely on pollination by insects. The overall aim of the project LIFE4Pollinators (LIFE18 GIE/IT/000755), in line with the EU Pollinators initiative, is to improve pollinator conservation by creating a virtuous circle leading to a progressive change in practices across the Mediterranean region. Public awareness and engagement together with conservation measures are necessary to halt pollinator decline. These are the starting points that led to the realization of the Pollinator Garden at the historical Botanic Garden of Bologna University. The Pollinator Garden is thought to be both dedicated to educational activities and to pollinators sustainment. The project involves an area of 43 sqm divided into 8 different sectors, one for each flower type. This structure, emphasizing the "pollination syndrome", will help to understand the diversity and specificity of interactions between pollinators and plants. At the same time, the presence of the 8 different flower types will provide food for different pollinator taxa. The selected species are mainly autochthonous, Except for few because of their high ornamental value and attractiveness for pollinators. Other important criteria for selection have been the flowering phenology, species habit and life cycle. The intention is to have some flowered plants throughout the year. The garden will host around one hundred plant species. All the species (adults and/or seeds) were collected during autumn and winter 2021 from different sites: most of them from natural populations in Bologna's Apennines, while few were transplanted from spontaneous populations growing at the Botanic Garden of Bologna. A nursery and a seedbank have been created, to provide plantlets and seeds both for the garden and for distribution during dissemination events. We are going to create 8 ceramic sculptures representing the 8 flower types. These sculptures, able to withstand the effects of weathering, will be placed to tag the 8 sectors in order to allow a better understanding of flower morphological diversity and coevolution processes with pollinating insects. Moreover, an observation nesting box (Pollinator Hotel) will be placed near the garden. It will be a safe nesting site for different bee and wasp species and, at the same time, an opportunity to observe the entire insect's life cycle. Both these activities are planned to be realized in collaboration with the artistic high school "F. Arcangeli". Connected to the pollinator garden are the "List of (some) entomophilous plants" suggested to help the choice of plants for private and public urban gardens (freely downloadable at www.life4pollinators.eu), and the digital guide of the wild bees of Botanic Garden of Bologna University. The aim of the Pollinator Garden is to make a little part of the Botanic Garden of Bologna University the core of most education, communication, and dissemination actions of LIFE 4 Pollinators project.


Fig. 1. The Garden begins to bloom


Fig. 2. One of the nurseries

6 = Role of leaf surface functional traits in sequestration and retention of particulate matter and polycyclic aromatic hydrocarbons in Mediterranean urban forests

Antonello Prigioniero[1], Daniela Zuzolo[1], Ülo Niinemets[2], Alessia Postiglione[1], Mariano Mercurio[1], Francesco Izzo[3], Marco Trifuoggi[4], Maria Toscanesi[4], Pierpaolo Scarano[1], Maria Tartaglia[1], Maria Maisto[1], Maria Antonietta Ranauda[1], Rosaria Sciarrillo[1], Carmine Guarino[1]

[1]Department of Science and Technology, University of Sannio, via de Sanctis snc, Benevento 82100, Italy; [2]Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Fr. R. Kreutzwaldi 1, Tartu 51006, Estonia; [3]Department of Earth Sciences, Environment and Resources, University of Naples Federico II, via Cintia, Naples 80126, Italy; [4]Department of Chemical Sciences, University of Naples Federico II, via Cintia, Naples 80126, Italy

We explored relationships between particulate matter (PM) and polycyclic aromatic hydrocarbon (PAHs) leaf concentrations, uptake rates and leaf surface functional traits in twenty-eight plant species in the urban forest of Naples city (Southern Italy) during a dry month: *Ailanthus altissima*, *Brachychiton acerifolius*, *Celtis australis*, *Ceratonia siliqua*, *Cercis siliquastrum*, *Chamaerops humilis*, *Cinnamomum camphora*, *Citrus* × *aurantium*, *Dracaena draco*, *Erythrina crista-galli*, *Eucalyptus camaldulensis*, *Ficus retusa*, *Jacaranda mimosifolia*, *Ligustrum japonicum*, *Ligustrum lucidum*, *Liriodendron tulipifera*, *Magnolia grandiflora*, *Melia azedarach*, *Musa basjoo*, *Nerium oleander*, *Olea europaea*, *Phoenix canariensis*, *Phytolacca dioica*, *Pittosporum tobira*, *Platanus* × *hispanica*, *Populus nigra*, *Quercus ilex* and *Robinia pseudoacacia* (Fig. 1).

Leaf material sampling was carried out on two separate days one month apart (1st August and 1st September), taking leaf samples on both dates from individuals in groups or rows near the main city streets.

Particulate matter and PAHs leaf concentration at different dates were quantified and uptake rate were calculated in order to correlate them. We quantified PM by gravimetric analysis and PAHs were extracted from intact and dewaxed leaves and analyzed by GC-MS. Main features of cuticle, stomata and non-glandular trichomes have been chosen as functional traits potentially useful in air phytoremediation. Cuticle thickness, number and surface of stomata and stomatal pore (N_s, S_s and S_{sp}) and trichomes (N_t and S_t) were determined by optical microscopy on both leaves surfaces (upper and lower). Infrared spectroscopy was used to investigate the leaves surfaces composition and assess esterification index (E). Studied species were characterized by unique combinations of functional traits and pollutant uptake capacities. PM_{10} uptake mainly scaled positively with N_t, S_t and cuticle thickness (T_c) across species. $PM_{2.5}$ uptake scaled positively with cuticle associated with greater shares of uptake of hydrophobic PM fractions. Uptakes of different fractions of PAH were mainly related to cuticles chemistry and structure which influenced their translocation in leaf subcuticular tissues. We conclude that both plant surface morphological and chemical leaf traits influence pollutants retention, unveiling their potential role in air phytoremediation.

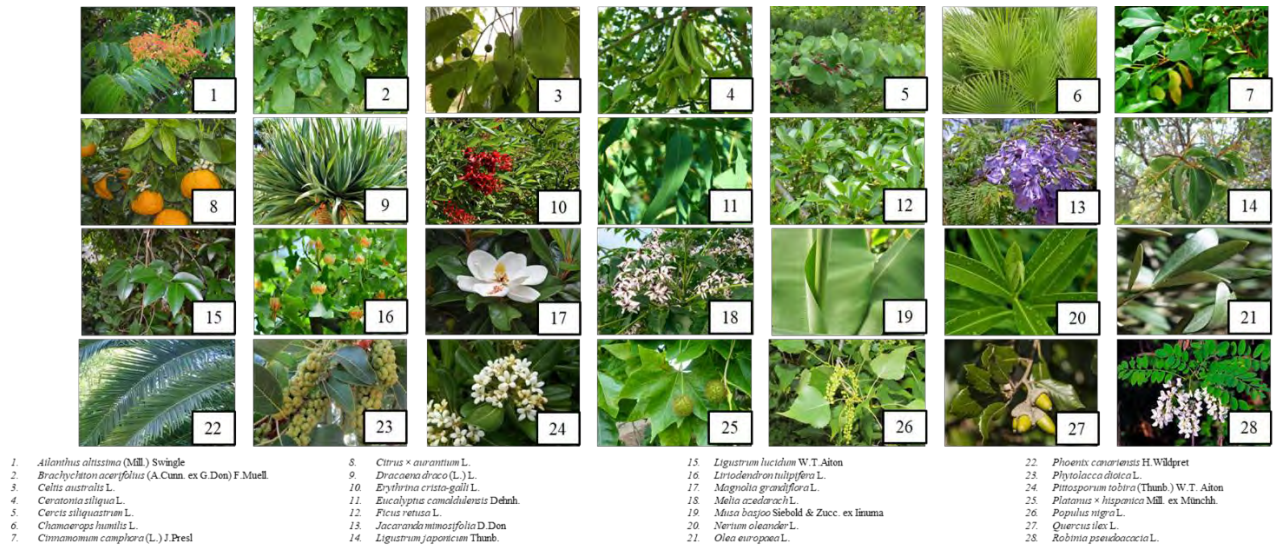


Fig. 1. Table of the twenty-eight tree species analysed in the work in alphabetical order with details of the different leaf morphologies

6 = Active citizen involvement in green management through the LIFE CLIVUT project

Chiara Proietti, Fabio Orlandi, Luigia Ruga, Desirèe Muscas, Silvia Marrapodi, Aldo Ranfa, Marco Fornaciari
Department of Civil and Environmental Engineering, University of Perugia, Borgo XX Giugno,74 Perugia

The LIFE CLIVUT project is an “information and governance” project. The methodologies applied to realise the themes to be developed within the project relate to increasing the awareness and knowledge of citizens on the role of urban greenery and climate change in cities. Within the project, a participative methodology between public administrations and citizenship was developed to improve urban green management practices according to the latest low environmental impact management techniques and to improve the storage of CO2 and the absorption of particulate matter, the main pollutants in urban areas. The main actions of the project therefore involved the use of participatory methodologies to make citizens aware and give them the tools to collaborate in drafting implementation guidelines for better management of the green heritage. In fact, one of the main actions of the project was to draw up and disseminate a strategy for urban green for climate purposes in the four pilot cities, Perugia, Bologna, Thessaloniki and Cascais.
Once these guidelines had been drafted by a working group, public forums were opened in different areas of the city and online formats were set up where people could express their dissent or assent to each of the actions outlined in these guidelines. At the same time, citizens were called upon to be informed and become protagonists in an action of monitoring and census of urban tree greenery, through a web tool that collected bio-morpho-metric data and calculated in real time the species-specific ecosystem performance for each specimen entered in the web portal. They were then asked to enter the data of their private trees into this application, and in doing so it was estimated that approximately 3 out of 4 trees are privately owned and insist on private property bordering public property.
For each plant entered, citizens were able to check the climatic and ecosystem role of their tree and verify the actual benefits to the urban heat island mitigation and adaptation that their property assumes at a complicated time like the present in which climate change is very insistent, especially in urban areas. This has given us the opportunity to verify how citizens are very attentive to public and private green management, even carrying out awareness-raising campaigns to dispel false myths, and instead propose real and possible concrete actions to improve participatory green management in urban areas, with the result that citizens are now genuinely interested in green and its benefits.

Fig. 1 clivut project event

6 = The LIFE CLIVUT Project: Citizen Science through Plant Phenology

Luigia Ruga, Fabio Orlandi, Chiara Proietti, Aldo Ranfa, Silvia Marrapodi, Marco Fornaciari
Department of Civil and Environmental Engineering, University of Perugia, Borgo XX Giugno 74, 06121 Perugia, Italy

LIFE CLIVUT (Climate Value of Urban Trees - LIFE18 GIC/IT/001217) is a Project financed by LIFE, the EU instrument for the environment and climate action. It is an "Information and Governance" Project and therefore foresees information, training and citizen participation actions to improve urban green and contribute to the well-being and quality of life in cities.
Responsible and collaborative communication is an important step to tackle climate change together, but above all to cooperate in the identification of a strategy that can limit the effects of climate change on the urban ecosystem through a greater knowledge of the ecosystem functions of green areas. So, several actions of the Project envisage the involvement of "active citizenship" (Managers and Professionals of Urban Planning and Green Areas, Associations, Schools of every order and degree, Businesses, and citizens all) monitoring green areas, improving their management and care, and generally increasing the awareness of the value of green areas for mankind.
Among the various actions, the project envisaged, in each pilot municipality (Perugia, Bologna, Thessaloniki and Cascais), the creation of Phenological monitoring Areas (PMA), with different plant species, 10 trees and 10 shrubs, whose thermal needs are known in the different phases of life (phenological phases).
Through direct observation of the plant development phases, it is intended to develop, especially in the younger generations of urban citizens, an awareness of the role of Urban Green Areas and to increase knowledge about the potential plant adaptation ant their mitigating climate change effects in cities, and to encourage responsible behaviour towards climate and the environment. Through cognitive walks in the urban phenological areas with citizens and through the application in schools of didactic modules created by the CLIVUT working group, the aim is to bring citizens closer to the themes of climate and climate change, to the knowledge of plants and their functioning and development, and to the importance of the interaction between tree species and climate, highlighting their role in combating climate change. In particular, in schools of all levels, the project provides training courses in which theoretical lessons are followed by educational activities in Phenological Monitoring Areas, where students will learn to observe plants in their developmental stages (Fig. 1). The PMA will represent permanent Teaching Laboratories, for the study and understanding of basic biological phenomena, natural or induced, and as a visual approach to urban plants, to increase young students' knowledge of biology and climate change. PMA will represent biological tools that can also provide information of great interest for the knowledge of the territory, for improving the management of public and private green areas, and for landscape enhancement. Phenology makes it possible to observe the long-term effects of climate change on plants, and in order to build reliable databases, citizens and students can play an important role.

Fig. 1. Students during teaching activities in the Phenological Monitoring Areas

6 = Urban Ecosystem Services assessment: a systemic approach and evaluation framework for Milan

Ilda Vagge[1], Stefano Bocchi[2], Valentina Vaglia[2], Alessio Gosetti Poli[1], Ambrogio Zanzi[2]
[1]Department of Agricultural and Environmental Sciences, University of Milan, Via Celoria 2, 20123 Milan, Italy; [2]Department of Environmental Science and Policy, University of Milan, Via Celoria 2, 20123 Milan, Italy

Since the concept of Ecosystem Services (ES) was enunciated, it became clear the tight bond with urbanization processes. This link has become even more strict during the last decades, due to the continuous migration from rural to urban spaces and the growing urgency of assuring sustainable living conditions to urban inhabitants. Several studies show the importance of urban ecosystems in improving long term life conditions, in terms of human health; social and cultural services; security and environmental parameters. Urban green spaces have a major role in this contribution, being the principal actor in assuring ES in urban environments. This well-known role has been recently acknowledged by several international directives – e.g., EU Biodiversity Strategy, EU Green Deal – and by the United Nation Agenda 2030 and its Sustainable Development Goals (SDGs). However, green urban areas' ES are still an open frontiers for researchers, especially if compared to other ecosystems, like forests, wetland and seas. Most of the available studies are focused on singular ES, implicitly ignoring the high complexity of urban ecosystems which are characterized by heterogeneity and fragmentation. Furthermore, territorial plans seems to fail in applying and promoting modern urban development plans, as land consumption and soil sealing keep on growing at relentless rate. This due to different facts, as economic drivers and a common and widespread misconception of green area values, as it emerges in current decision-making processes: as example, most of Italian municipalities follow a limited and subjective process to evaluate the impact of new building project on urban ecosystems. The process is mainly based on a compensation hypothesis based on estimated economic value of the green areas that is going to leave place to buildings: however, the estimation regards only the trees whose value is determined on their dimension, healthy status and, often, on qualitative and subjective parameters. Several lacks clearly appears in this kind of processes, as different ecosystem components are not taken into consideration - such as soils; herbaceous layer; water cycle; as well as citizens perception and opinion regarding possible change to their beloved environment. All these elements are fundamental to form urban natural capital and should therefore considered during each redevelopment project to have a broad and complete ES quantification and better understand the new building impact on urban environments. Therefore, to better preserve urban green areas and assure more sustainable living conditions to current and future citizens, it appears essential find new evaluation framework available to planners and decision makers as proactive tools to make cites greener. Starting from these assumptions, the present study has the following goals: to develop an alternative framework, with a high level of ES integration, useful for designing, monitoring and assessing green urban areas' ES (Fig. 1); to test and implement the framework in a concrete case study for the city of Milan, therefore assessing ES level in a real scenario where a requalification project is undergoing, thus understanding its impact on urban ecosystems.

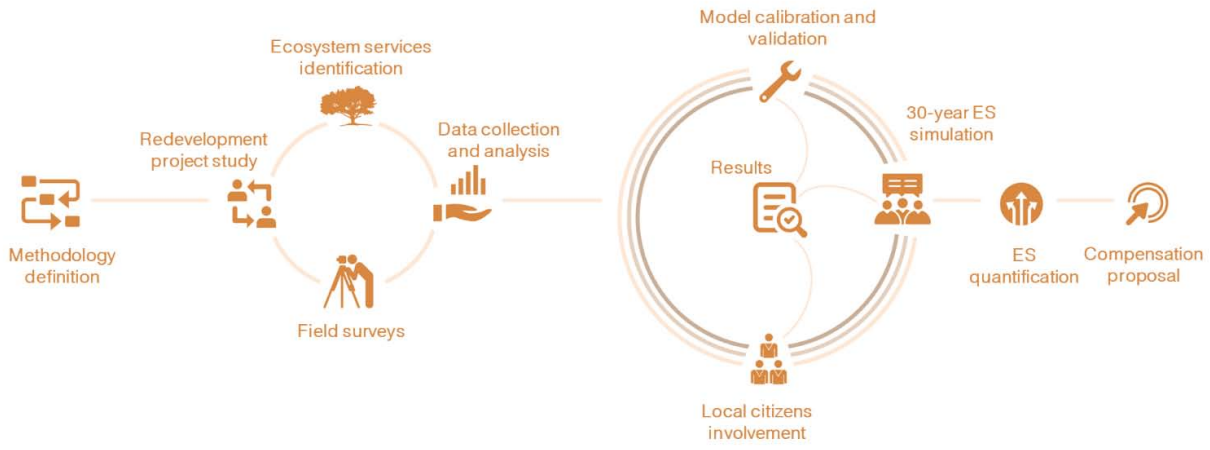


Fig. 1. Methodological scheme to conduct the ES analysis

7 = Differences in gluten protein composition in a historical wheat seed collection: the extent of protein changes in the climate change scenario

Iris Aloisi[1], Giovanni Maria Poggi[1,2], Simona Corneti[1], Stefano Del Duca[1], Francesca Ventura[2]
[1]Department of Biological, Geological and Environmental Sciences, University of Bologna, Via Irnerio 42, 40126 Bologna, Italy; [2]Department of Agricultural and Food Sciences, University of Bologna, Viale G. Fanin 44, 40127 Bologna, Italy

The study of cereals nutritional quality in relation to climate change (CC) is of great importance to predict climatic impacts on food security in the next future; from this perspective, more qualitative studies focused on nutritional and nutraceutical traits would be needed, besides those predicting crop yields. This need stems from evidence about how variations in meteorological parameters, mostly temperature, have huge consequences on plants' phenology, and how these changes can downstream have deep impacts on kernel quality in terms of protein, sugars, fiber and secondary metabolites content. Specifically, the wheat grain-filling stage is the most sensitive for its quality. This mostly depends on gluten proteins, i.e. gliadins and glutenins, which confer the physical and chemical properties to wheat dough and are accumulated from anthesis throughout the whole kernel development phase.
While the various mechanisms by which rising temperatures impact wheat physiology and performance are well-known and described in literature, comprehensive information about CC effects on wheat phenology and its consequences in terms of kernel quality is still poor. Hence, we assessed the effect of CC on bread wheat phenological development, and its consequences on grain gluten quality, through a case study in the Bologna plain (North of Italy). Daily weather data from a mechanical historical series ranging from January 1st 1952 to the present, were analyzed to assess CC impact in the area. The alteration of bread wheat phenological development in the Bologna Plain was assessed comparing phenological data from two 15-years periods (past-period and present-period, respectively), selected before and after the identified breakpoint in temperature increase for the area. Phenological correlations with grain gluten quality were possible thanks to the availability of a precious collection of seeds, harvested in different years in the Bologna Plain, and properly stored in Laras (Seed Research and Analysis Laboratory of the Department of Agricultural and Food Sciences, University of Bologna, Italy). Seed samples from this historical seed collection, covering years from 1951 to 1973, were compared, in terms of gluten composition, to modern seeds belonging to the same accession, i.e. San Pastore cultivar, currently cropped in the area.
The increasing trend in mean annual air temperature in the area was significant, and a significant shortening of the timing necessary to reach the main wheat phenological phases emerged in present-period, compared to past-period, including a shortening of the grain-filling period, finally affecting gluten accumulation. This emerged analyzing gluten composition in the San Pastore cultivar seed samples from both past- and present-period. Proteins in the gliadin and glutenin fractions, extracted according to protein solubility in water-alcohol mixtures, were analyzed by SDS-PAGE. General trends were visible in SDS-PAGE, e.g. an increase in crude protein and glutenin content, mostly high molecular weight glutenins, along the decades under examination, with higher values in recent years. Differently, gliadins showed a decreasing trend.
Proteins were identified within the gluten fractions through LC-MS and relative protein amount was analyzed in relation to the length of the phenological stages, specifically to the days of grain filling in each year under investigation. In general, the shortening of grain filling period correlated with changes in the gluten composition, in particular the shorter the grain-filling stage was, the highest glutenin and the lowest gliadin percentages were.
In conclusion, this case study highlighted the impact of CC on wheat gluten composition. Certainly, more qualitative studies would be needed, on more wheat varieties, and on more quality parameters to fully understand how CC affects wheat seed chemical composition. These types of studies would be desirable, but they are often not possible given the limited availability of historical seed collections. Hence, the importance of studying the effects of CC in controlled experimental conditions, which not only allows comparing the phenological and yield response in numerous varieties at the same time, but also allows the production of sufficient seed material to fully understand the nutritional and nutraceutical changes in wheat kernel in response to phenological changes.

7 = The MICROx2 project: how the morpho-functional traits are needed to define growth requirements of microgreens in Space cultivation systems

Chiara Amitrano, Christophe El-Nakhel, Youssef Rouphael, Roberta Paradiso, Rosanna Caputo, Stefania De Pascale, Veronica De Micco
Department of Agricultural Sciences University of Naples Federico II, Via Università 100, Portici (Napoli), Italy

Microgreens are young seedlings of edible crops and herbs that can be harvested and eaten 7 to 21 days after sowing. Thus, they are very convenient to grow, as they adapt to a variety of locations, including indoors small growth chambers, greenhouses and even windowsill. Despite their small size, they often contain higher nutritional levels in terms of vitamins, antioxidants and minerals if compared to their mature leafy green counterparts. This makes them a good addition to any diet; recently, they are also gaining popularity since they are used to embellish fancy recipes. In Space environment, where astronauts are subjected to harsh environmental conditions due to altered gravity and ionizing radiation, the integration of diet with fresh, nutritional, easy, and fast to grow food, could represent an added value for future exploration missions.
From the '80s, Space crew have been trying to cultivate plants directly on board, however one of the main constraints for production of fresh vegetable food in-orbit is the definition of scientific requirements for a flight apparatus dedicated to cultivation. In this study, part of the project *MICROx2 (microgreens x microgravity)* funded by the Italian Space Agency (ASI), we investigated the effects of the environmental factors on the morpho-anatomical and biochemical composition of two microgreens' species. More specifically, we cultivated *Brassica oleracea* var. sabauda cv. 'Vertus' and *Raphanus raphanistrum* subsp. *sativus* cv. 'Saxa' microgreens in a growth chamber. During the project, using a multidisciplinary approach, we tested two types of substrates (cellulose sponge and coconut fiber), two nutrient solutions (Hoagland quarter strength throughout the cycle vs. half strength for the first half of the cycle followed by osmotic water during the second half), two VPD levels (low VPD of 0.3 KPa and high VPD of 1.2 kPa) and two light intensities (300 µmol photons $m^{-2}s^{-1}$ vs 150 µmol photons $m^{-2}s^{-1}$). The best combination of each environmental factor was chosen step by step by comparing the microgreens growth, biomass, morphological, anatomical, and biochemical traits.
Results suggested that the occurrence of positive outcomes, like increments in antioxidant molecules and biomass production, are strictly dependant on the environment and the microgreens' cultivar. This is a phenomenon that should be carefully considered when designing modules for crop production in Space. The outcomes of this study will also be helpful to optimize microgreens production in controlled environment agriculture systems on Earth.

Fig. 1. Microgreens indoor cultivation

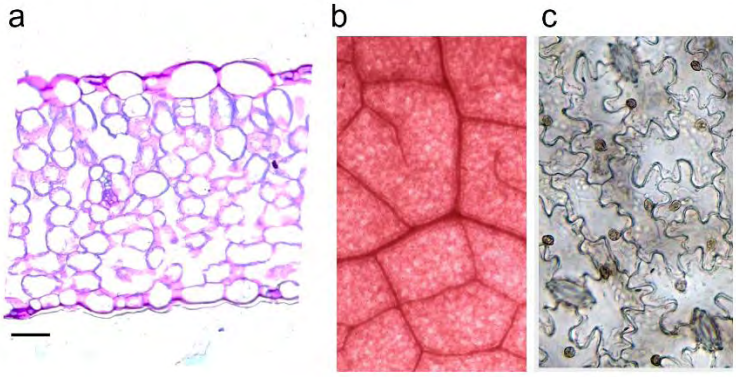


Fig. 2. a) light microscopy images of microgreens leaf lamina cross section, b) veins and c) epidermis with stomata

7 = Exploring the potential use of quinoa seed by-products as biostimulants in *Lactuca sativa* L. seedlings

Fabiana Antognoni[1], Lorenzo Marincich[1], Sara Pipponzi[2], Stefania Biondi[3], Karina Beatriz Ruiz[4]
[1]Department for Life Quality Studies, University of Bologna (QuVi), Rimini Campus, Corso d'Augusto 237, 47921, Rimini, Italy; [2]Department of Life Sciences, University of Modena and Reggio Emilia, via Amendola 2, 42122 Reggio Emilia, Italy; [3] Department of Biological, Geological and Environmental Sciences, University of Bologna, Via Irnerio 42, 40126 Bologna, Italy; [4] Qu.mica y Farmacia, Facultad de Ciencias de la Salud, Universidad Arturo Prat. Av. Arturo Prat (UNAP) – Casa Central, Ch. 2120, Iquique 1100000, Chile

Chenopodium quinoa Willd. is an ancient seed-producing crop native to the Andean regions, which has recently been expanding outside South America, thanks to its exceptional properties, in terms of both nutritional/nutraceutical value and abiotic stress tolerance.
After harvesting, quinoa seeds must be processed to obtain a marketable grain, without saponins. To do this, grains undergo a scarification or abrasive de-hulling process, which removes the outermost hull, rich in the bitter-testing saponins. Thus, this process results in the production of waste, rich in saponins and other bioactive compounds. Depending on the degree of scarification, two types of wastes can be obtained, one deriving from the outermost layers and richer in saponins (QSap), the other one deriving from a deeper abrasion, thus also containing some proteins (QProt).
This work wants to investigate if these two by-products could have a biostimulant effect to improve plant performance under normal or salt-stress conditions in lettuce plants.
As a preliminary experiment, the effect of QSap and QProt extracts, supplied as foliar spray, was assessed under normal conditions, while in the second part the salt stress response of lettuce plants was determined. The extracts were prepared by weighting 0.5 g of dried powder material and adding 10 mL of water. Extraction was carried out using an ultrasound bath and then filtering the suspension under vacuum using a Buchner funnel. Lettuce seeds (*Lactuca sativa* L. var. summerbel) were germinated on blotting paper and transferred into pots filled with perlite after 4 days. Here they were grown using the Hoagland solution full strength in a growth chamber at 25 °C and under a light/dark cycle of 16/8 hrs. After 13 days, approximately 1.25 mL of each by-product extract was sprayed on the plant leaves, and treatment was repeated every 3 days, using water as a negative control. After 10 days, half of the lettuces were harvested and frozen in liquid nitrogen for the chemical analysis (pigment content, phenolic bioactive compounds, proline concentration) and the remaining half was used for measuring morphological parameters (plant height, leaf area, FW, DW, root length). To assess the salt stress response of lettuce plants, salt treatment was applied to 10-day-old plants. Each final salt concentration (50, 100 and 150 mM NaCl, respectively) was reached in 10 days, and plants were kept under salt stress for another 10 days before collecting samples for either chemical or morphological analyses. Negative control was represented by lettuce plants grown in Hoagland solution without salt.
Statistical analysis of obtained results highlights that the leaf surface area is the most relevant morphological parameter to be affected by the extract treatment. Both extracts seem to slightly reduce the plant growth, even if the differences are not significant. Under salt conditions, morphological analyses reveal that 150 mM NaCl concentration was able to significantly reduce the lettuce growth, while no significant effects were observed at lower salt concentrations.
The next task will be the evaluation of the effect of QSap and QProt foliar spray on the plant response to salt treatment, to verify if the two extracts could have a protective role. Moreover, since saponins were known to have antibacterial and antifungal activities, the antagonistic action of the two extracts against *Fusarium oxysporum* f.sp. *lactucae* will also be evaluated *in vitro* with an agar diffusion test.

7 = Studies on metal homeostasis/detoxification mechanisms in the liverwort *Marchantia polymorpha*

Elena Bandoni[1], Erika Bellini[1], Silvia Giardini[1], Carlo Sorce[1], Carmelina Spanò[1], Debora Fontanini[1], Alessandro Saba[2], Andrea Andreucci[1], Monica Ruffini Castiglione[1], Mingai Li[3], Claudio Varotto[3], Luigi Sanità di Toppi[1]

[1]Dipartimento di Biologia, Università di Pisa, Via L. Ghini 13, 56126 Pisa, Italy; [2]Dipartimento di Patologia Chirurgica, Medica, Molecolare e dell'Area Critica, Università di Pisa, Via Roma 67, 56126 Pisa, Italy; [3]Fondazione E. Mach, Via E. Mach, 1, 38098 San Michele All'Adige (TN), Italy

With the colonization of emerged lands, plants faced new challenges in securing and maintaining homeostasis of macro- and micronutrients, and of metal elements in particular, being the latter toxic at supraoptimal concentrations. In this regard, thiol-peptides such as glutathione (GSH) and phytochelatins (PCn) may play a key role both in regulating the homeostatic levels of metal micronutrients – e.g., copper (Cu), iron (Fe) and zinc (Zn) – and in detoxifying cells from non-essential metals – e.g., cadmium (Cd). According to a previously proposed hypothesis (Fig. 1), we focused here on the evolutionary relevance of the phytochelatin synthase enzyme (PCS) and thiol-peptides, which progressively lowers its activity/decrease from the pyramid base (homeostatic needs) towards the middle part (detoxification), reaching the minimum at the top (adaptive tolerance). We tested this hypothesis on the liverwort *Marchantia polymorpha* subsp. *ruderalis* Bischl. and Boisselier-Dubayle (Marchantiophyta), by investigating the role of PCS and thiol-peptides in Cd detoxification and metal micronutrient homeostatic regulation. *In vitro* analysis of the recombinant MpPCS enzyme was performed using Cu, Fe, Zn and Cd, in order to evaluate the enzyme activity response. The results obtained in HPLC-ESI-MS/MS showed that PCS was fully activated by Cd, followed by Zn, Cu and much less by Fe. Moreover, *in vitro* axenic cultures of gametophytes of *M. polymorpha* were set up to carry out *in vivo* studies in the presence of excess of Cu, Fe, Zn (and Cd) and under metal-starvation (Cu-free, Fe-free, Zn-free and Cu-Fe-Zn-free), over different exposure-times (Fig. 2). In these conditions, the *in vivo* response of MpPCS was analyzed by evaluating the intracellular content of GSH and PCn by HPLC-ESI-MS/MS analysis. In the presence of an excess of Cu, Fe, Zn (and Cd), a significant increase in the levels of total PCn was observed already in the first hours of exposure. On the other hand, under metal-starvation, a decrease in total PCn was evidenced, especially in the Cu-Fe-Zn-free samples. Indeed, from the data of quantitative Real Time-PCR, overexpression peaks of the *MpPCS* gene at different times were detected, depending on the metal analyzed in the samples treated with the highest concentrations. This observation suggests that the gene is associated with a transcriptional regulation that varies according to the metal present in excess. In contrast, no variation of *MpPCS* gene expression was observed in the samples under metal-starvation conditions. Overall, our results support the initial hypothesis, thus highlighting the fundamental role of the MpPCS enzyme and thiol-peptides, both in the homeostatic control of metal micronutrient requirements and in metal detoxification processes.

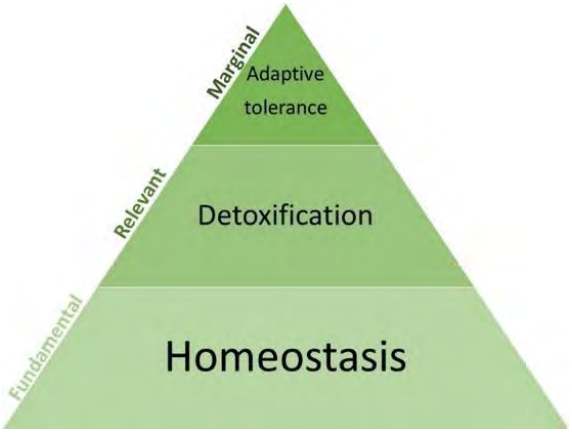


Figure 1. Hypothesis on the evolutionary role of phytochelatin synthase and thiol-peptides in metal homeostasis/detoxification/tolerance responses

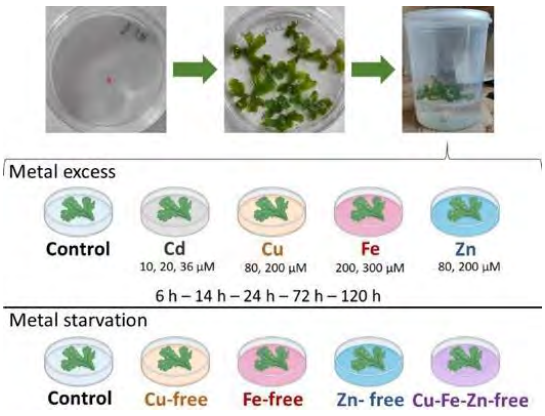


Figure 2. Experimental set up

7 = Unravelling the role of *Mildew Locus O* genes in arbuscular mycorrhizal symbiosis

Filippo Binci, Giacomo Rago, Lorella Navazio, Marco Giovannetti
Department of Biology, University of Padova, Via Ugo Bassi 58/B, 35131 Padova, Italy

Mildew Locus O (MLO) proteins are largely known as susceptibility factors as they provide resistance to infection from biotrophic fungi if mutated and *mlo* mutant plants have been used for decades in agriculture. However, the *MLO* gene family is wide, and this is reflected in the involvement in many plant developmental and cellular processes, such as redirection of root growth, pollen tube growth and programmed cell death. Recently, an *MLO* gene has also been shown to modulate beneficial plant-microbe interactions, in particular the arbuscular mycorrhizal (AM) symbiosis in wheat, barley and *Medicago truncatula*, as well as the association with endophytic bacteria in barley. Despite the broad characterization of mutant phenotypes, the precise biochemical function of MLOs still remains to be elucidated. On the other hand, MLOs are transmembrane proteins with a well-known topology, they contain a calmodulin-binding domain and their mode of action seems to be related to the exocytic machinery. Moreover, the activities of MLOs have been shown to be modulated by ROS and calcium. Thereby, a unifying role for the MLOs has been recently proposed in the perception and transduction of external physical stimuli.
In this work, we focus on the role of *MLO4* in AM symbiosis using *Lotus japonicus* as a model organism. *LjMLO4* belongs to the clade IV of the gene family, which is present only in AM-host species. Indeed, we show that *MLO4* is expressed in mycorrhized roots of *L. japonicus* and that its promoter activity is specifically localized along fungal structures in *L. japonicus* hairy roots. Considering that the perception of diffusible symbiotic fungal signals, such as tetrameric chito-oligosaccharides (CO4) and lipo-chitooligosaccharides (LCO), is mediated by Ca^{2+} as intracellular messenger, we are currently addressing the putative role of MLO4 in the perception of these biotic stimuli. To this aim, we transformed the roots of *mlo4* genetic background with aequorin-based Ca^{2+} reporters targeted to either the cytosol or the nucleus. Knowing that the subcellular localization of MLOs is strictly related to their functions, we ectopically expressed LjMLO4 fused to GFP in *Arabidopsis thaliana* protoplasts.
Altogether this work provides first hints about the role of a specific *L. japonicus MLO* gene in the establishment and development of the AM symbiosis.

7 = Horticultural crops response to salt stress: role of seed priming and acclimation

Ilaria Borromeo[1*], Fabio Domenici[2], Gian Piero Marzano[1], Cinzia Forni[1]
[1]Department of Biology, University of Rome Tor Vergata, Via della Ricerca Scientifica 1, 00133 Rome, Italy; [2]Department of Chemical Sciences and Technology, University of Rome Tor Vergata, Via della Ricerca Scientifica 1, 00133 Rome, Italy; [*]PhD course in Evolutionary Biology and Ecology, Department of Biology, University of Rome Tor Vergata, Via della Ricerca Scientifica 1, 00133 Rome, Italy

Salt accumulation in the soil and water resource scarcity can represent severe threats to agriculture by strongly affecting plant growth and reproduction, as well as reducing the biodiversity and metabolism of soil microorganisms. Soil salinization affects at least 20% of irrigated soil worldwide; this phenomenon involves arid, semi-arid and sub-humid areas, leading to an irreversible degradation of the soil, called desertification. In recent years, several approaches have been used to improve plant tolerance to salt stress, such as acclimation and seed priming. The latter is a pre-sowing treatment, consisting of soaking the seeds in a priming agent, followed by drying the seeds to avoid radicle emergence. A priming agent can provoke abiotic stress to seed, inducing a cross-tolerance to different abiotic stresses. While, an acclimation to stress can be obtained by a gradual exposure of the plant to stressful conditions, leading the plant to an adaptation to stress, thus to a better performance.
The aim of this project is to verify the possibility to ameliorate the growth and reproduction in food crops, i.e. bean (*Phaseolus* spp.) and tomato (*Solanum lycopersicum* L.). Both tomatoes and beans are glycophytes, very sensitive to salt stress, which not only compromises their growth but also their yields, causing huge economic loss. Seed priming protocols with polyamines were applied in beans and tomato.
Priming does not affect seed germination, nevertheless primed and germinated seeds did not survive when sown in saline soil. Therefore, we decided to test if acclimation would provide a better plant performance in saline condition. Since the high level of sensitivity to salt was not overcome by acclimation, we further applied combined treatments, i.e. seed priming followed by acclimation. Plants, either primed or not, were acclimated by watering with saline solution twice a week.
At the end of the experiments the electrical conductivity (EC) of the soils were 0.45 dS/m (not saline soil) and 2.05 dS/m (saline soil). The EC of the latter was above the threshold of salt tolerance of both species. The effects of salinity on primed plants were negligible with respect to not primed ones. Overall, the results suggest the possibility to use both seed priming and acclimation as a tool to improve salt stress tolerance in tomato and beans.

7 = In-field assessment of drought stress effect on the photosynthetic apparatus functionality in three different apple tree (*Malus domestica* Borkh) rootstocks

Andrea Colpo[1], Sara Demaria[1], Marzio Zaccarini[2], Alessandro Forlani[2], Elena Marrocchino[1], Lorenzo Ferroni[1]
[1]Department of Environmental and Prevention Sciences, University of Ferrara, C.so Ercole I d'Este 32, 44121 Ferrara, Italy; [2]Consorzio Italiano Vivaisti, Strada Statale Romea 116 Loc. San Giuseppe, 44022 Comacchio (Ferrara), Italy

The plant lifetime is characterized by a continuous fight-to-survive against environmental stresses, both biotic and abiotic. Within the latter, drought stress has become a hot topic because of its impact on the natural ecosystem fitness as well as on the crop productivity. The prevention of irreversible damages due to such unfavorable environmental conditions depends on the responsiveness of environmental operators, farmers, and scientists, which are constantly in the need for fast and non-invasive screening techniques. Some of these techniques rely on the monitoring of the plant photosynthetic efficiency using field tools, such as portable fluorometers.
In this work, we monitored the progress of drought stress in apple trees (*Malus domestica* Borkh) during the productive season (May-September) using a pocket plant phenotyping approach based on chlorophyll *a* direct fluorescence measurement. To this scope, a portable HandyPEA fluorometer (Hansatech, Norfolk, UK) was used. The comparative study was performed on *M. domestica* cv. Superchief® trees grafted on three different rootstocks: CIVP21^{pbr}, M106, M26. CIVP21^{pbr} is a new rootstock patented by Consorzio Italiano Vivaisti and was selected for its wide root system, potentially supporting a superior resistance to drought stress. The plants were cultivated in water shortage conditions and compared to their control counterparts, which were normally irrigated throughout the season. Soil moisture and temperature were monitored using Watermark (Irrometer, Riverside, USA) sensors. Measurements were performed at intervals of 7-10 days during the whole experiment.
Our results indicate that drought stress had an influence on several fluorometric parameters, with variable severity depending on the rootstock. These fluorometric parameters were positively correlated with seasonal productivity and soil moisture data, and negatively with soil temperature. Undoubtedly, low soil moisture negatively affected the seasonal productivity of all water-stressed rootstocks compared to control plants. However, M26 showed a higher fruit production than M106 and CIVP21^{pbr}, although M26 fruits had a lower average weight compared to those of other rootstocks. Interestingly, fruit production was associated with the availability of electron carriers between photosystem II (PSII) and photosystem I (PSI) and, thus, the efficiency of the PSII-PSI intersystem electron transport chain. Conversely, the average fruit weight correlated with the photochemical efficiency of PSII. Therefore, some specific PSII fluorescence parameters can be proposed as robust indexes to assess the effect of a moderate chronic drought stress in order to agroecosystems preservation and crop management, including predictive issues about fruit production.

This research was developed within the project "*Metodi fluorimetrici innovativi a supporto della selezione varietale di fragola e di portinnesti di melo contro lo stress idrico*" granted to L.F. by the University of Ferrara with the contribution of the Camera di Commercio Industria, Artigianato e Agricoltura di Ferrara (Bando 2019).

7 = Metallophytes colonizing mine wastes as valuable source for environmental technologies

Ilaria Colzi[1], Maria Beatrice Castellani[1], Elisabetta Bianchi[1], Pierluigi Cortis[2], Antonio De Agostini[2], Donatella Fibbi[3], Marco Dainelli[1], Andrea Coppi[1], Cristina Gonnelli[1]
[1]Department of Biology, University of Florence, Via Micheli 1, 50121 Firenze, Italy; [2]Department of Life and Environmental Sciences, University of Cagliari, Via Sant'Ignazio da Laconi 13, 09123 Cagliari, Italy; [3]GIDA S.p.A., via di Baciacavallo, 36, 59100 Prato, Italy

Metallophytes can offer an invaluable source of plant biodiversity to be exploited for environmental technologies. Such plants show the surprising ability to adapt to metalliferous soils under the selective pressure exerted by these unfavorable substrates. Most metallophytes can tolerate a high concentration of metals in the substrate, by physiologically limiting their entry into the root and/or their transport to the shoot (termed "excluders"). On the other hand, a few species are metal accumulators, or even "hyperaccumulators", having extremely specialized biological mechanisms for accumulating metals in their shoots at extraordinarily high concentrations. Both excluders and accumulators/hyperaccumulators can be an optimal choice for soil remediation purposes, and can be exploited in environmental technologies, such as immobilization (phytostabilization) or extraction (phytoextraction) of toxic metals from polluted soils, respectively. Phytoextraction, in particular, uses plants that accumulate metals in harvestable organs and tissues in order to remove the pollutants from the field.
The aim of this work was to explore the valuable resource of spontaneous plants colonizing different mine wastes and to identify suitable accumulating species to be used for metal phytoextraction of sewage sludge. Due to their common use of heavy metals in various anthropogenic activities, wastewaters can be heavily contaminated and, consequently, such elements are found in sewage sludge of wastewater treatment plants (WWTP). The WWTP of Prato, managed by GIDA SpA, treats urban and industrial wastewaters and the sewage sludges may be contaminated by several heavy metal(loid)s, mainly deriving from local textile industries, such as antimony (Sb), cadmium (Cd), and zinc (Zn). Although the sewage sludges are regularly disposed by incineration, phytoextraction may provide an alternative low-cost remediation approach for such substrates. Furthermore, four mine sites located in Sardinia were selected based on the presence of Sb, Cd, or Zn contamination of the tailings: Ingurtosu mine (Arbus), Su Suergiu mine (Villasalto), Monteponi mine (Iglesias), and Barraxiutta mine (Domusnovas). At each site, the plants spontaneously growing in correspondence of the contaminated mine tailings were collected. The substrates were scarcely vegetated, therefore it was possible to collect almost all the plant species present. For each species, 6 whole plants were sampled for the analysis of metals. Moreover, a representative substrate sample was collected from each mine waste. Once brought to the laboratory, all plants were accurately washed with deionized water, separated in roots and shoots and dried in the oven at 60 °C for at least 48h. Soils were dried at room temperature and sieved to 2 mm before drying them again in the oven. Plant and soil materials were then mineralized with HNO_3 through a microwave system and analyzed by ICP for determination of Sb, Cd, and Zn concentrations.
During the field investigation, some of the plant species were found in all the mine sites visited, i.e. *Rumex* sp., *Euphorbia* sp., and *Reseda* sp. The metal(loid) analyses allowed to discover the attitude of each species to exclude or to accumulate the elements present in the mine tailings. This preliminary screening investigation was fundamental for selecting the most suitable species for metal(loid) phytoextraction purposes. In the next step, the plants will be tested by cultivating them directly in the sewage sludges from the GIDA WWTP to confirm the metal tolerance and accumulation capacity.


Fig. 1. Ingurtosu mine


Fig. 2. Su Suergiu mine


Fig. 3. Monteponi mine


Fig. 4. Barraxiutta mine

7 = Towards a Nickel-free tomato production

Clara Conte[1], Mauro Mariotti[1], Micaela Tiso[2], Gabriella Fenoggio[3], Elena Nicosia[4], Enrica Roccotiello[1]
[1]Department of Earth, Environment and Life Sciences, University of Genova, Corso Europa 26, 16132 Genova, Italy; [2]MICAMO srl, Corso Andrea Podestà 12/9 Genova, Italy; [3]Federazione Regionale Coldiretti Liguria, Via XX Settembre 21/5, 16122 Genoa, Italy; [4]Regione Liguria, Dipartimento Salute e Servizi Sociali, Settore Tutela della Salute negli Ambienti di Vita e di Lavoro Via Fieschi 17, Piano U8, 16121, Genoa, Italy

Tomato, *Solanum lycopersicum* L., is one of the main components of the Mediterranean diet and contains key micronutrients able to limit the occurrence of cardiovascular diseases and cancer. Despite its nutritional importance, allergic phenomena are frequent, both cutaneous (allergic contact dermatitis) and extra cutaneous, even severe (systemic nickel allergy syndrome) due to the nickel in the fruit. Nickel (Ni) quantities in tomato may vary depending on the physical-chemical characteristics and the concentration in the soil/substrate, the water used for irrigation or fertirrigation, the cultivar, the degree of fruit ripeness and the seasonality. The allowed concentrations in European agricultural soils range 30-75 mg/kg with pH 6-7. Currently, there are no legal limits for Ni in food, but EFSA considers a daily intake of 13 μg Ni/Kg per body weight to be tolerable and fresh tomatoes declared as 'Ni-free' are commercially available. Since currently there is no product specification that regulates the Ni-free tomato production, this work aims at evaluating how to limit Ni accumulation in fruits with different agricultural practices thanks to the support of the PSR-TOMATO project. Samples of water and fertirrigation solution, soils, amendments, and tomatoes were collected in the involved farms. Tomatoes were randomly selected and collected at different times of the year to assess the concentration of Ni. An initial screening of the soils was directly performed in the field with a portable X-ray spectrometer (X-MET7500 FP-EDXRF Analyser, Oxford Instruments), to obtain information on the chemical composition of the soils. Subsequently, each soil sample was oven-dried at 60°C and then subjected to analysis by ICP-MS (Inductively Coupled PlasmaMass Spectrometry). The tomato samples were fresh-weighed, washed with tap and deionised water and oven-dried at 60°C, then dry-weighed. Each fruit sample was pulverised and analysed by ICP-MS analysis, with the Ni detection limit of 0.01 mg/kg. Even if the Ni detected in fruits is almost acceptable, several field tests are currently running on farms to find solutions for being able to obtain Ni-free fruits for allergic people consumption. This study allows realising a product specification considering current regulation limits, listing all the procedures required to obtain a real Ni-free product, and allowing future safe consumption for allergic people.

7 = Distinct tomato cultivars are characterized by a differential pattern of biochemical responses to drought stress

Veronica Conti[1], Claudio Cantini[2], Marco Romi[1], Maria Michela Cesare[1], Luigi Parrotta[3,4], Stefano Del Duca[3,4], Giampiero Cai[1]

[1]Department of Life Sciences, University of Siena, 53100 Siena, Italy; [2]National Research Council of Italy, Institute for Bioeconomy (CNR-IBE), 58022 Follonica, Italy; [3]Department of Biological, Geological and Environmental Sciences, University of Bologna, 40126 Bologna, Italy; [4]Interdepartmental Centre for Agri-Food Industrial Research, University of Bologna, 47521 Cesena, Italy

Future climate scenarios suggest that crop plants will experience environmental changes capable of affecting their productivity. Among the most harmful environmental stresses is drought, defined as a total or partial lack of water availability. It is essential to study and understand both the damage caused by drought on crop plants and the mechanisms implemented to tolerate the stress. Tomato is common and economically relevant in the whole Mediterranean basin and, in previous studies, the behaviour of 13 distinct tomato cultivars in response to a chronic drought condition at both vegetative and reproductive stages were analysed. In this study, we focused on four cultivars of tomato, which were the most relevant. We investigated the biochemical mechanisms of plant defense against drought by focusing on proteins specifically involved in this stress, such as osmotin, dehydrin, and aquaporin, and on proteins involved in the general stress response, such as HSP70 and cyclophilins. Since sugars are also known to act as osmoprotectants in plant cells, proteins involved in sugar metabolism (such as RuBisCO and sucrose synthase) were also analyzed. The results show crucial differences in biochemical behaviour among the selected cultivars and highlight that the most tolerant tomato cultivars adopt quite specific biochemical strategies such as different accumulations of aquaporins and osmotins. The data set also suggests that RuBisCO isoforms and aquaporins can be used as markers of tolerance/susceptibility to drought stress and to select tomato cultivars within breeding programs.

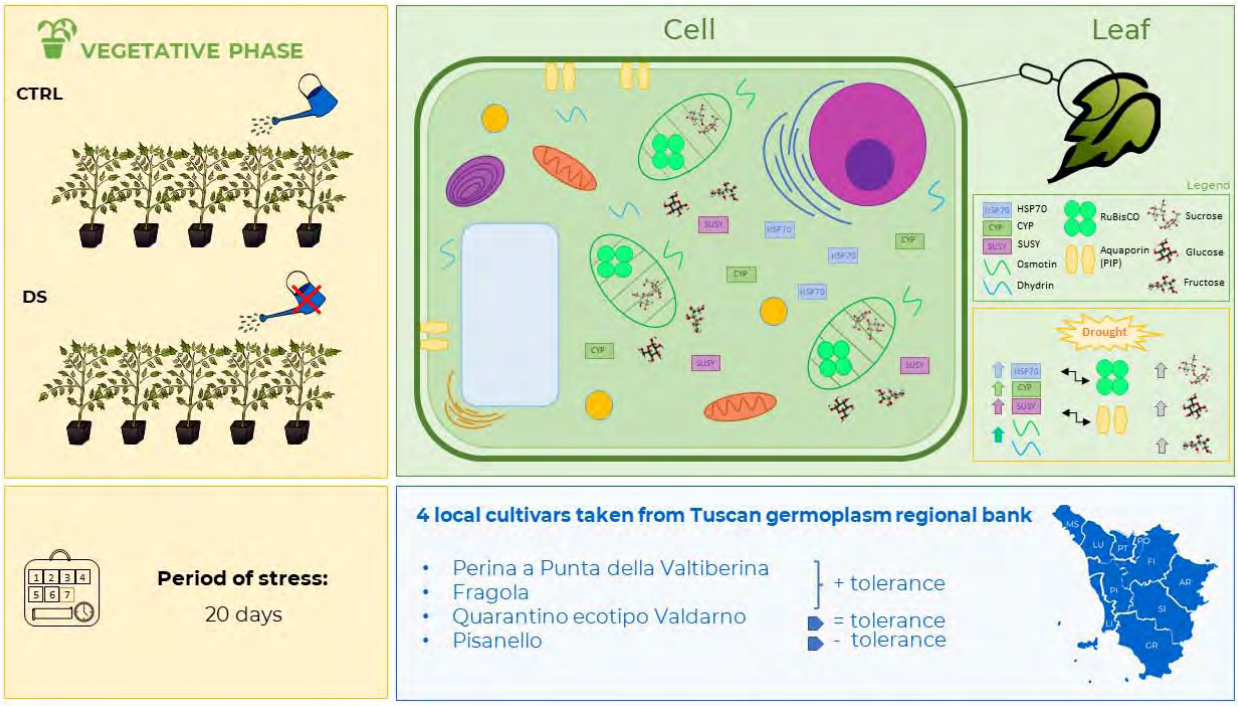


Fig. 1. Graphical Abstract. The yellow section shows the number of plants which were taken in account for the study (5 for the control-CTRL, and 5 for the drought stress-DS). The blue section shows the local Tuscan tomato cultivars chosen for this study. Perina e Fragola are catalogued as the most tolerant to drought stress, Quarantino is the cultivar with a medium tolerance and Pisanello is the most susceptible. Finally, in the green section are shown the proteins that are most involved in the drought stress.

7 = Symbiotic vs asymbiotic seed germination of eight terrestrial orchid species included in the LIFEorchids Project

Laura Cornara[1], Andrea Volante[2], Claudia Turcato[1], Samuele Voyron[3], Martino Adamo[3], Mariangela Girlanda[3], Miriam Bazzicalupo[2]
[1]Department of Earth, Environment and Life Sciences, University of Genova, C.so Europa 26, 16132, Genova, Italy; [2]CREA - Council for Agricultural Research and Economics, Research Centre for Vegetable and Ornamental Crops, Corso Inglesi 508, Sanremo, 18038, IM, Italy; [3]Department of Life Sciences and Systems Biology, University of Turin, Viale Mattioli 25, 10125, Torino, Italy

LIFEorchids (LIFE17NAT / IT / 000596) is an European Project that aims to prevent the decline of wild orchids and their natural habitats, i.e. arid grasslands rich in biodiversity. Project actions include the restoration of habitats and the reinforcement of populations of 9 orchid species by transplanting *in vitro* produced individuals. Previous results from orchid translocation indicate that, before acclimatisation and/or translocation into the field, the inoculation of plant material with compatible symbionts is recommended to increase the survival rate of individuals. Indeed, in nature, orchids intertwine symbioses with mycorrhizal fungi, which are essential for the germination of seeds, for the transfer of nutrients, and for the resistance to drought of established seedlings and adult plants. Accordingly, *in vitro*, the use of compatible fungal strains is known to promote and speed up germination.
In order to obtain plants already mycorrhized to be transferred into the field, in this work we evaluated the effect of different strains of *Rhizoctonia*-like fungi from Tulasnellaceae and Ceratobasidiaceae in stimulating the germination of 8 orchid species included in the project (*Anacamptis morio* (L.) R.M. Bateman, Pridgeon & M.W. Chase; *Serapias neglecta* De Not*; Himantoglossum adriaticum* H. Baumann*; H. robertianum* (Loisel.) P. Delforge*; Ophrys apifera* Huds.*; Ophrys bertolonii* Moretti*; Ophrys holosericea* (Burnm. f.) Greuter*; Orchis anthropophora* (L.) All.).
Mature seeds obtained by hand-pollination were sown in petri dishes containing oatmeal agar and fungal inoculum. Control replicates were also set up by sowing aliquots of the seeds in M551 standard medium for orchid asymbiotic propagation. Fungal strains used for symbiotic growth were previously isolated from fresh roots of *A. morio*, *S. neglecta*, and *O. anthropophora*. In addition, we also tested AL13 and SV-6 strains of *Tulasnella calospora* already deposited at the Mycotheca Universitatis Taurinensis (University of Turin).
For *A. morio, Ceratobasidium sp.* MoCOS5 exerted the best stimulatory effect with respect to the asymbiotic condition (42.15 ± 6.42% vs 6.29 ± 3.09%), while other strains from the *T. calospora* complex also triggered germination of *A. morio* (10-32%).
S. neglecta germinated both asymbiotically (19.42 ± 3.42 %) and symbiotically with different isolates of the *T. calospora* complex, with SV-6 strain as the most effective one (55.53 ± 4.44%).
H. adriaticum germinated only on asymbiotic medium (60.66 ± 6.80%); similarly, *H. robertianum* germinated on asymbiotic substrate (21.83 ± 5.28%), while weak performances have also been recorded under symbiotic growth conditions (5.80 ± 2.29% with the *T. calospora* strain SV-6).
Orchis anthropophora, a species known for being recalcitrant in asymbiotic sowing trials with mature seeds, in our study germinated with *Ceratobasidium sp.* strain OANT 2.4c (16.28 ± 1.71%).
Different isolates of *Tulasnella calospora* promoted the germination of *Ophrys* species; *O. apifera* reached 36.26 ± 3.54% germination with the AL13 strain, while in *O. bertolonii* maximum stimulation was reached with the SV-6 strain (39.83 ± 1.69%). Mature seeds of both these species did not germinate in asymbiotic conditions. Similar results were obtained with other recalcitrant species. *O. holosericea* germinated at very low rates on M551 medium (0.49 ± 0.2%), whereas symbiotic growth with the AL13 strain resulted in 13.71 ± 1.13 % germination, and with *Ceratobasidium sp.* OANT 2.4c resulted in 16.92 ± 2.94 % germination.
The above data improve the knowledge on orchid-fungi interactions during early stages of seed germination, especially for two hard-to-germinate species (*O. anthropophora* and *O. holosericea*). These results are useful for future studies on the *in vitro* propagation of orchids aimed at conservation purposes.

7 = Tuning plant perception of plasma-activated water to foster a more sustainable agriculture

Enrico Cortese[1], Luca Cappellin[2], Alessandro Galenda[3], Alessia Famengo[3], Alessio G. Settimi[4], Manuele Dabalà[4], Diego De Stefani[5], Vanni Antoni[6,7], Lorella Navazio[1*]
[1]Department of Biology, University of Padova, Via U. Bassi 58/B, 35121 Padova, Italy; [2]Department of Chemical Sciences, University of Padova, Via F. Marzolo 1, 35131 Padova, Italy; [3]National Research Council, Institute of Condensed Matter Chemistry and Technologies for Energy (CNR-ICMATE), Corso Stati Uniti 4, 35127 Padova, Italy; [4]Department of Industrial Engineering, University of Padova, Via F. Marzolo 9, 35131 Padova, Italy; [5]Department of Biomedical Sciences, University of Padova, Via U. Bassi 58/B, 35131 Padova, Italy; [6]National Research Council, Institute for Plasma Science and Technology (CNR-ISTP) , Corso Stati Uniti 4, 35127 Padova, Italy; [7]Consorzio RFX, Corso Stati Uniti 4, 35127 Padova, Italy

Cold atmospheric plasmas are weakly ionized gases that can be generated in ambient air. At a relatively low consumption of energy, they constitute a unique delivery system of a rich family of short- and long-lived chemicals, such as reactive oxygen (ROS) and nitrogen (RNS) species. When interacting with a liquid, cold plasmas can generate further new chemical species, as in the case of the so-called plasma-activated water (PAW), which has recently attracted a great deal of interest because of a variety of applications in agriculture and in the food sector, according to the strategy "from Farm to Fork". In plant biology PAW has been shown to increase the seed germination rate and to promote plant growth. Moreover, PAW irrigation of plants has been reported to induce defence gene expression, suggesting a beneficial effect of PAW not only in terms of plant growth promotion but also in plant "priming", a phenomenon defined as the pre-emptive readying of plant defences against potential, subsequent pathogen attacks. PAW may therefore represent an attractive eco-friendly alternative to pesticides, whose administration in bulk quantities represent a matter of growing concern for their impact on the environment. We have recently demonstrated the induction by PAW of rapid and sustained cytosolic Ca^{2+} elevations in the model plant *Arabidopsis thaliana*, providing evidence for the involvement of Ca^{2+} as an intracellular messenger in the signalling pathway(s) triggered by PAW in plants. Our data concerning Ca^{2+}-mediated sensing of PAW by plants suggest the possibility to use Ca^{2+} measurements as a rapid and reliable assay to monitor early plant responses to PAW.
In this work we have thoroughly analyzed the intracellular Ca^{2+} signals triggered in aequorin-expressing *A. thaliana* seedlings by the administration of PAW generated with two different plasma sources, *i.e.* a plasma torch and a dielectric barrier discharge. The rationale is to strictly correlate the different operational conditions of the distinct plasma devices with: i) the unique mixture of chemicals contained in the PAW; ii) the specific intracellular Ca^{2+} signatures triggered in plants; iii) the observed effects in terms of increased plant resilience to environmental stresses of both biotic and abiotic nature. The project is aimed to collect an extensive database concerning PAW-induced Ca^{2+} dynamics, which will allow for a clear-cut understanding of how to finely modulate PAW generation, in order to effectively drive this innovative technology towards the desired outcomes on plant physiology.

7 = Endocytic uptake of biopolymeric PLGA nanoparticles in *Arabidopsis thaliana* cells and roots

Giulia De Angelis[1], Camilla Badiali[1], Marco Possenti[3], Simone D'Angeli[1], Cleofe Palocci[2], Laura Chronopoulou[2], Elisa Brasili[1], Gabriella Pasqua[1]
[1]Department of Environmental Biology, "Sapienza" University of Rome, P.le Aldo Moro 5, 00185 Rome, Italy; [2]Department of Chemistry, "Sapienza" University of Rome, P.le Aldo Moro 5, 00185 Rome, Italy; [3]Council for Agricultural Research and Economics, Research Centre for Genomics and Bioinformatics (CREA-GB), 00185 Rome, Italy

A variety of natural and synthetic polymers has been explored for the preparation of nanoparticles (NPs) to be used in plants. Poly(lactic acid) (PLA), poly(glycolic acid) (PGA), and their copolymer (PLGA) have been extensively investigated due to its proven biocompatibility and biodegradability. In plant cells, the major route for the internalization of PLGA-NPs seems to be clathrin-mediated endocytosis, which starts with the formation of clathrin-coated membrane invaginations, also termed clathrin-coated pit. An essential protein for vesicle detachment is dynamin, a GTPase protein. However, in addition to clathrin-dependent endocytosis pathway, emerging researches have revealed several clathrin-independent pathways in plant cells, not all dependent on dynamin. Further research is needed with the aim to highlight the NPs uptake mechanisms, the interactions of NPs with the plants and the environment. In this study, PLGA-NPs (30 nm) loaded with the high fluorescent probe coumarin-6 have been synthesized by microfluidic technology. The localization of Cu6-PLGA NPs has been studied in suspended cells and roots of *A. thaliana* by confocal microscope apparatus and by fluorescence microscopy at different times. To investigate the mechanisms of NP uptake and internalization, the Dynasore, a specific inhibitor of dynamin was added to cultured cells and roots. Dynasore, whose activity was verified on animal cells, is able to block the GTPasic activity of the dynamin preventing the detachment of the vesicles from the membrane. The PLGA-NPs were rapidly internalized by *A. thaliana* cells, as revealed by the small highly fluorescent round bodies (endosomes) and a low diffuse background observed after 10 min. The treatment with Dynasore at 80 μM or 160 μM for 10 minutes before the addition of NPs did not prevent the formation of spherical fluorescent bodies. It has been also demonstrated that NPs penetrate the root epidermis through the root hairs after 10 minutes. Moreover, the administration of Dynasore at 80, 160 or 320 μM for 30 and 60 minutes before the addition of 15 mg L^{-1} NPs did not prevent the uptake of NPs observed in the epidermis, suggesting that Dynasore was not able to inhibit the uptake of NPs 30 nm loaded with coumarin-6, and that the internalization of NPs could occur by GTPase and dynamin-independent pathway. After the administration of Dynasore (320 μM) for 120 minutes no fluorescence was observed in the root. However, the cytotoxicity test with propidium iodide showed that the roots treated with Dynasore at 320 μM for 120 minutes were not viable. To further investigate the effect of Dynasore on NPs uptake, roots were treated with Dynasore at 80 μM for 120 minutes. In this case, the cytotoxicity test showed that roots were viable and fluorescence was clearly visible in the epidermis, after NPs administration.
The results of this research add new information to the understanding of PLGA-NPs internalization pathways in plants with potential applications in the agronomic field, where NPs could be applied for a sustainable agriculture.

7 = Arsenic effect on the expression of NIP1.1 in *Dittrichia viscosa*: looking for a marker for genetic improvement

Angelo De Paolis[1], Monica De Caroli[2], Makarena Rojas[2], Lorenzo Maria Curci[2], Gabriella Piro[2], Gian-Pietro Di Sansebastiano[2]
[1]Institute of Sciences of Food Production (ISPA-CNR), Via Prov.le Lecce-Monteroni, 73100 Lecce, Italy; [2]Department of Biological and Environmental Sciences and Technologies (DiSTeBA), University of Salento, Via Monteroni, 73100 Lecce, Italy

Dittrichia viscosa (L.) has been attracting attention as a tool for phytoremediation because of its ability to adapt to adverse environmental conditions, including pollution by heavy metals and metalloids. Phytoremediation through phytoextraction appears to be a good ecological solution, especially if we consider the hyperaccumulating capacities of plants. *D. viscosa* is not a hyperaccumulator plant but can grow under very stressful conditions, producing large biomass even in the presence of As[III], As[V] and Cd[II].
Its uptake and translocation of cadmium, copper, iron, nickel, lead, and zinc to the shoots have been characterized but its performance with arsenic is less known and sometimes contradictory. The As tolerance is not related to a reduced uptake, the null mutation of the aquaporin Nip1.1 gene in Arabidopsis confers complete resistance to the metalloid in the plants. This aquaporin, localized in the Endoplasmic Reticulum is responsible for arsenite and antimony (Sb) membrane permeation, however, arsenite uptake also takes place in the null mutant, which is not surprising because it is known that As uptake depends on several mechanisms.
A *D. viscosa* NipIP1.1 homologue showing very high identity with aquaporin Nip1.1 from *Helianthus annuus* was cloned and its expression analyzed. DvNipIP1.1 expression is specifically reduced by As but not by Cd treatments.
Plantlets derived from the wild population DI3 selected on As 50 μM showed a very diverse tolerance to As. Two groups of plants were selected from this population, showing a “strong” and a “weak” phenotype related to tolerance to arsenic. To characterize the expression level of Nip1.1 in these 2 groups of plants an index to avoid the heterogeneity related to genetic variability was established. This index corresponds to the rate of expression between the root and the aerial parts. The plants with higher tolerance to As had the lowest expression level already in control conditions. This experiment supported the hypothesis that tolerance to As may be related to a basic low expression level of Nip1.1.
We can consider the *DvNip1.1* expression rate between root and shoot, as an index of As tolerance and use this knowledge to assist the selection of plants with increased resistance. At the same time, the role of Nip1.1 appears to be conserved and the possibility of genetic improvement can be extended to other plant species.

7 = Afternoon changes in the structure and function of the gigantic chloroplast of *Selaginella martensii* Spring

Lorenzo Ferroni, Sara Demaria, Andrea Colpo, Simonetta Pancaldi
Department of Environmental and Prevention Sciences, University of Ferrara, C.so Ercole I d'Este 32, 44121 Ferrara, Italy

Selaginella martensii Spring is an ancient vascular plant typically found in the shade environment of equatorial rainforests. Constrained in such habitat, the plant deals with light scarcity through major chloroplast adaptations to optimize the light harvesting process. Along with other *Selaginella* species, *S. martensii* presents a unique gigantic and cup-shaped chloroplast in the cells of the upper epidermal layer, which is the major responsible for the leaf photosynthesis; other smaller, lens-shaped chloroplasts can be found in the lower cell layers, i.e., mesophyll and lower epidermis, but their contribute to the photosynthesis is negligible when compared to the gigantic chloroplasts (named "bizonoplast"). The inner structure of the bizonoplast consists of two quite well-defined thylakoid compartments: an upper region characterized by long and appressed lamellae and a lower region with predominant grana structure.
Observation on *S. martensii* plants grown in the warm humid greenhouse of the Botanical Garden of the University of Ferrara evidenced a progressive daily microphyll clearing, in some terminal branches, which turned to light green especially in the late afternoon. The mechanistic reason for the change in the hue of green and its physiological significance are unknown. To investigate the reason behind the progressive diurnal clearing of the microphylls in *S. martensii* we performed structural and functional analyses in plants at different times of the day (11:00, LM; 14:30, EA; 17:30, LA).
In the terminal branches, the light green area increased during the day from 6% in LM to 45% in LA. Observed under a fluorescence microscope, the light green leaves showed that the bizonoplast of the upper epidermal cells flattened against one lateral cell wall, with the same orientation in all neighboring cells. The typical thylakoid organization of the bizonoplast was completely lost, presenting a stroma uniformly filled with appressed thylakoids and almost undistinguishable single grana stacks. In the LA samples, the light or dark green branch portions were analysed separately. Spot-directed analyses of chlorophyll *a* fluorescence indicated that the relocation of the chloroplast and the loss of the thylakoid zonation in the light green samples corresponded to lower minimal fluorescence (Fo) emission. The Photosystem II (PSII) photochemical activity was high in LA samples, without differences between light or dark green portions. However, a marked downsizing of the PSII functional antenna size occurred specifically in the light green portions. The thylakoid function in the light-acclimated state was probed with a multiparametric MultispeQ device and revealed that all samples had a good control of the linear electron flow, although it seemed to have been achieved in contrasting ways in light or dark portions of the LA samples.
Information obtained in this study indicates that the change in shape and thylakoid organization of the gigantic chloroplast of *S. martensii* is accompanied by changes in light harvesting efficiency by PSII. Given the occurrence of the change after the diurnal peak in irradiance, it is speculated that this change might be beneficial to PSII turnover.

This research was developed within the project "*Fenomica vegetale con lo strumento open source MultispeQ e la piattaforma PhotosynQ: fattibilità, efficacia e potenzialità*" granted to L.F. by the University of Ferrara (Fondo per l'Incentivazione alla Ricerca - FIR 2020).

7 = Similarities and differences of functional landscapes underlying the convergent evolution of monocot and dicot isoprene synthase key residues and their implications for poplar genetic improvement

Mingai Li[1], Iuliia Khomenko[2], Silvia Carlin[2], Urska Vrhovsek[2], Franco Biasioli[2], *Claudio Varotto[1]
[1]Biodiversity, Ecology and Environment Area, Research and Innovation Centre, Fondazione Edmund Mach, Via E. Mach 1, 38010 S. Michele all'Adige (TN), Italy; [2]Food and Nutrition Area, Research and Innovation Centre, Fondazione Edmund Mach, Via E. Mach 1, 38010 S. Michele all'Adige (TN), Italy

Angiosperm isoprene synthase (IspS) plays a pivotal role in the global isoprene production from plants, amounting to about half a billion tonnes per year and constituting a phenomenon with great ecological relevance for atmospheric chemistry and the carbon cycle. IspS has been shown to repeatedly originate by convergent evolution from ocimene synthases, and the role of two of the amino acids of the IspS diagnostic tetrad from the monocot *Arundo donax* L. (the two phenylalanine residues in the FSFS tetrad) has been analyzed by saturating mutagenesis.
In this study, we carried out the characterization of the other two amino acids of *A. donax* tetrad as well as of the complete diagnostic tetrad (FSFN) of the dicot *Populus nigra* L. by in planta saturating mutagenesis and high-throughput volatile organic compound analysis by PTR-ToF-MS of the headspace of more than 3000 Arabidopsis transgenic lines from 112 different constructs. Analogously to what observed in *A. donax*, if replaced by small apolar residues, the first phenylalanine of the *P. nigra* diagnostic tetrad constituted the major single-amino acid switch to the production of the monoterpene ocimene. Also the second phenylalanine of the *P. nigra* tetrad showed limited contribution to the switching capacity to ocimene production by contrast to the complete lack of such capacity in *A. donax*. Most interestingly, the other two amino acids of the tetrads displayed a low functional tolerance to substitution, generally leading to a nearly complete absence of activity of the enzyme. Exceptions to this rule showed only a partial overlap between residues.
Taken together, these results corroborate the convergent evolution of monocots and dicots IspS and indicate subfunctionalization in *P. nigra* IspS, further implying possible trade-offs linked to isoprene emission in this species. The implications of these findings for the possible genetic improvement of poplar are discussed.

7 = Genetic transformation of plant cells as a possible strategy for the production of bioactive compounds or biopolymers

Elisa Maricchiolo[1], Michele Bellucci[2], Francesca De Marchis[2], Daniele Fraternale[1], Francesco Paolocci[2], Andrea Zattoni[3], Barbara Roda[3], Valentina Marassi[3,] Andrea Pompa[1]
[1]Department of biomolecular sciences University of Urbino Carlo Bo, Via Aurelio Saffi 2, 61029 Urbino, Italy; [2]Istituto di Bioscienze e Biorisorse, CNR, via Madonna Alta 130, 06128 Perugia, Italy; [3]Department of Chemistry "Giacomo Ciamician" University of Bologna, Via Selmi 2, 40126 Bologna Italy

Biotechnologies are currently applied to a wide range of fields, which span from the production of active compounds for human health to high-tech materials used in industry. Among biotechnologies, genetic engineering can modify gene content and/or expression in different organisms such as bacteria, yeasts, animal cells, and plants. Recently, plant genetic engineering has been mainly focused on conferring beneficial agronomic properties to crops, such as pest or disease resistance, stress tolerance, or improved yield. Here we report on examples of biotechnologies employed for the production of high-added value compounds from plants, which might serve as "green" bioreactors.
Two research lines are currently carried out at the Botanical Garden laboratories of Urbino University, with the support of various external collaborations. The first one aims at inducing the production of anthocyanins, secondary metabolites with marked nutraceutical properties, in apple pulp calli. To reach this goal, apple calli has been transformed via *Agrobacterium tumefaciens* vector with a gene cassette harbouring *Sn,* a maize *helix-loop-helix* (*bHLH*) transcription factor and a marker for kanamycin resistance. Ectopically expression of *Sn* gene can boost the accumulation of anthocyanins in different species and under different environmental conditions. Here we show that the expression of this gene is sufficient to significantly promote the synthesis and accumulation of anthocyanins in apple calli exposed to light compared to control calli transformed with the *Gus* reporter gene.
The second research line uses genetic engineering to modify the major reserve protein accumulated in Phaseolus vulgaris (common bean) seeds, called phaseolin, to produce an environmentally friendly biopolymer. Our idea starts from previous studies that demonstrated the possibility of forming disulfide bridges by a genetically modified version of phaseolin with a single cysteine residue insertion in the c-terminal tail. We thought that expressing this protein in a closed compartment such as *Nicotiana tabacum* chloroplasts facilitated the formation and accumulation of polymerized forms of phaseolin. In this study, the biochemical basis underlying the synthesis, accumulation and purification of polymeric forms based on mutated-phaseolin in the chloroplast of transgenic tobacco plants is described.

7 = Role of zaxinone, a novel growth promoting apocarotenoid metabolite, in shaping rice rhizomicrobiota

Teresa Mazzarella[1], Matteo Chialva[1], Jian You Wang [2], Cristina Votta [1], Patrizia Vaccino[3], Salim Al- Babili [2], Luisa Lanfranco[1], Valentina Fiorilli[1]
[1]Department of Plant Sciences and Systems Biology, University of Turin, Viale Mattioli 25, 10125 Torino, Italy; [2]The BioActives Lab, Center for Desert Agriculture, Biological and Environmental Sciences and Engineering, King Abdullah University of Science and Technology, Thuwal, Saudi Arabia; [3]Cerealicoltura e Colture Industriali, Consiglio per la ricerca in Agricoltura e l'analisi dell'economia agraria, Strada Statale 11 per Torino km 2.5, 13100, 13100 Vercelli VC, Italy

In plants, carotenoid biosynthesis is a vital metabolic pathway, that is the source for hormones and growth regulators (*i.e.* Abscisic Acid and Strigolactones – SLs). Carotenoids are susceptible to oxidation processes that break the carotenoid backbone. This cleavage reaction is catalyzed by carotenoid cleavage dioxygenases (CCDs), which leads to products called apocarotenoids. Zaxinone is a recent discovered apocarotenoid, produced by ZAS (Zaxinone Synthase, belonging to the CCD gene family), which is emerging as a signaling compound involved in plant growth and arbuscular mycorrhiza (AM) establishment. A rice loss-of-function *zas* mutant shows a lower root zaxinone content, a retarded root and shoot growth, and higher SL levels. Zaxinone exogenous application positively regulates rice growth and development reducing SLs content in root tissues and exudates. The impact of zaxinone on plant fitness and on SLs biosynthesis suggests that zaxinone could also influence the recruitment of rhizomicrobial communities.
In this work we analyzed the bacterial and fungal microbial communities comparing wild-type (wt) rice plants with genotypes with reduced level of zaxinone content (*zas1* and *zas4* mutants) in roots. All genotypes were grown in pots containing a native paddy soil in a semi-field condition. For the microbiota assessment a set of plants from each genotype (wt, *zas1* and *zas4*) was sampled at two different developmental stages (tillering and milky stage) while the phenotypic evaluation, considering the major agricultural traits was carried out at the end of the rice life cycle. The microbiota associated with soil and root rhizosphere and endosphere was investigated using a metabarcoding analysis on 16S and ITS rRNA gene amplicons.
Phenotypic analysis confirmed that the lower level of zaxinone impaired rice development and seeds production. Metabarcoding data highlighted that microbiota composition changes during rice developmental stages and that significant changes in the abundance of bacteria and fungi composition were detected in the endosphere of *zas* mutants compared to wt plants, suggesting a role of zaxinone in endophytes recruitment. Furthermore, we observed that both root compartments (endosphere and rhizosphere) of *zas* mutants showed a different relative abundance of AM fungi (Glomeraceae, Claroideoglomeraceae and Acalourosporaceae) compared to wt. With this research, we provide new insights into the role of zaxinone in the interplay between plants and microbes in the rhizosphere.

7 = The globe artichoke landrace “Ortano”: bioactive molecules from by-products cultivation

Ovidi Elisa[1], Laghezza Masci Valentina[1], Alicandri Enrica[1], Paolacci Anna Rita[1], Vinciguerra Vittorio[1], Tomassi William[1], Manni Matteo[1], Garzoli Stefania[2], Del Lungo Alberto[3], Tiezzi Antonio[1], Ciaffi Mario[1], Scarascia-Mugnozza Giuseppe[1]

[1]Department for Innovation in Biological, Agro-food and Forest systems (DIBAF) - University of Tuscia, L.go dell'Università, 01100 Viterbo, Italy; [2]Department of Drug Chemistry and Technology, Sapienza University, 00185 Rome, Italy; [3]No-profit local Organization “Horti in Tiberi”, 01028 Orte, Italy

The globe artichoke [*Cynara cardunculus* L. var. *scolymus* (L.) Fiori] is a diploid species (2n = 2x = 34) of the Asteraceae family, native of the European Mediterranean basin. The economic use of the crop includes mainly the consumption of the edible immature flower heads, highly appreciated for their organoleptic and nutraceutical properties. The artichoke cultivation involves the production of a large volume of "waste" such as leaves, stems and roots, sources of several phytochemical compounds with biological activity such as phenols, flavonoids, sesquiterpene lactones, enzymes and prebiotic dietary fibers. Although the health-promoting properties of artichoke extracts, even from non-edible portions of the plant, have been studied, most of the commercial products of pharmaceutical and medical interest are derived from foreign Countries.

The present study is part of a project financed by the Lazio Region (POR FESR Lazio 2014-2020) focused on the recovery, characterization and conservation of a globe artichoke landrace at high risk of genetic erosion still cultivated in non-specialized small-holdings in the municipality of Orte (VT, Lazio Region) and therefore named “Ortano artichoke”. In particular, the aim of this study is to investigate the landrace at molecular, morphological and ultrastructural level and to isolate and characterize specific pools of biologically active compounds from different plant organs.

Genetic analysis based on SSR (Simple Sequence Repeat) and ISSR (Inter Simple Sequence Repeat) molecular markers confirmed the belonging of “Ortano artichoke” to the “Romanesco” varietal typology, and revealed a high level of genetic variability within this landrace. Indeed, based on 74 plants sampled in the district of Orte, it was possible to distinguish two genetic groups, named Orte 1 and Orte 2. The genotypes belonging to the first group were found closely related to the landraces and clones that represent the varietal platform of the PGI (Protected Geographical Indication) “Romanesco Artichoke of Latium” (Reg. EC n. 2006/2002), while those of the second group were found related to the landrace “Montelupone”, grown in the district of the homonymous municipality in the province of Macerata (MC). The morphological characterization, undertaken using UPOV descriptors, revealed significant differences between the genotypes belonging to the two genetic groups, confirming the complex genetic structure of the “Ortano artichoke” landrace. One representative genotype for each of two distinct genetic groups Orte 1 and Orte 2 was selected for the chemical and biological investigations.

The methanolic extracts obtained from four different plant organs (principal and secondary immature flower heads, stems and leaves) of the representative genotypes belonging to the two genetic groups of "Ortano artichoke" were investigated by HPLC-DAD in order to quantify the polyphenolic contents. The results show that caffeoylquinic acids content is the highest in the stems (waste biomass) of both the genotypes with respect to the other plant organs, whereas the highest content of flavonoids was found in the leaves.

Antiproliferative, cytotoxic, antimicrobial and antioxidant studies were carried out to determine the biological activities. Different human tumor cell lines were treated in time and dose dependent mode and the obtained EC_{50} values showed that stems and leaves possess a higher cytotoxic and antiproliferative activity respect to the immature flower heads. A significant selectivity index was found. Slight bactericidal and bacteriostatic activities were detected against environmental bacterial strains and antioxidant properties were also defined by DPPH and ABTS showing that steams of both genotypes had the highest activities.

Taken together, the present results confirm that artichoke cultivation could be evaluated as a source of raw material containing molecules with valuable biological activities whose properties can be exploited. The issue of safeguarding agricultural biodiversity and the socio-economic development of the territory through the implementation of a short agri-food chain is of relevance both to enhance national production and to support innovation in the field of pharmaceutical biotechnology and food supplements.

7 = Different physio-molecular mechanisms affect water storage strategy and drought tolerance in olive trees

Sara Parri[1], Francesca Cristiana Piritore[1], Marco Romi[1], Maria Celeste Pereira Dias[2], Claudio Cantini[3], Giampiero Cai[1]
[1]Department of Life Sciences, University of Siena, Via Mattioli 4, 53100 Siena, Italy; [2]Department of Life Sciences, University of Coimbra, Calçada Martim de Freitas, 3000-456 Coimbra, Portugal; [3]National Research Council of Italy, Institute for Bioeconomy (CNR-IBE), 58022 Follonica, Italy

Future projections depict a decrease in water availability due to climate change, that cause a serious limit in the quantity and quality of water available for agriculture, especially in a semi-arid area such as the Mediterranean.
In this context, the study of the responses to drought conditions of crop plants is important: Genotypes adapted to drought are a heritage from which to draw information, strategies, and genetic traits useful for stress tolerance. Olive tree is an evergreen plant belonging to the family Oleaceae and the genus Olea. This plant is known, in addition to the fruit-derived oils, to be quite resistant to drought. However, different genotypes do not respond to water stress to the same extent.
In this work, 18-months-old plants of three different olive cultivars (Giarraffa, Leccino and Maurino) were monitored during thirty-five days of drought stress (no irrigation). The experiment was set in a growth chamber.
The aim of the work is to investigate the management of the hydric resource in a condition of water scarcity and its influence on plant health and photosynthesis. The work focused on the distribution of the water content in soil, stem, and leaves. Water content was correlated with the rate of water loss as regulated by stomatal density and conductance. The biochemical mechanism of water content was investigated in stems and leaves by analysing both proteins (such as dehydrins, aquaporins, and osmotin) and osmoprotectants (like proline, mannitol, and glucose).
The three cultivars mainly differ in water compartmentalization by using leaves (Leccino) or soil (Giarraffa) as water tank. Instead, water content in stems during the stress is quite similar among the genotypes, although Maurino has a poor capacity of water storage. Maurino also shows the highest levels of membrane damage in leaves as well as the fastest decrease in photosynthetic efficiency among the cultivars. These results suggest that water management strategy under drought conditions could play a central role in stress tolerance. The early appearance of the protein osmotin and the rapid and significant increase in proline in the cv. Leccino could be a valuable trait to conserve water in leaves, allowing this cultivar to better maintain the photosynthetic activity.
The identification of physio-molecular mechanisms behind an efficient strategy of water storage can be a starting point for selection or breeding of new cultivars in order to support agriculture in anticipation of climate change.

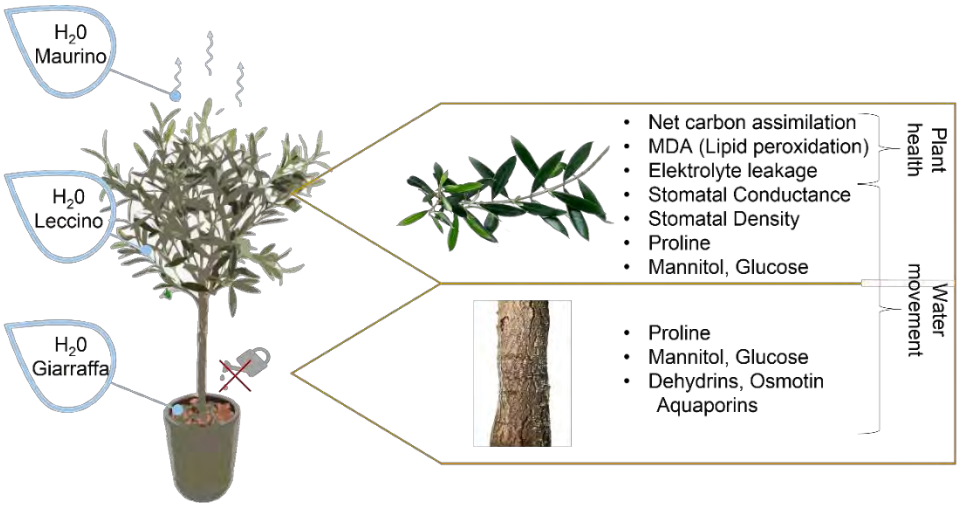


Fig. 1. Synthetic scheme describing the main water storage compartment among the different olive cultivars (on the left) and the parameters used in order to analyse water movement within the plant and plant health (on the right).

7 = Biochemical and cytological interactions between callose synthase and microtubules in the tobacco pollen tube

Luigi.Parrotta[1*], Claudia Faleri[2], Cecilia Del Casino[2], Lavinia Mareri[2], Iris Aloisi[1], Gea Guerriero[3], Jean-Francois Hausman[3], Stefano Del Duca[1], Giampiero Cai[2]
[1]Department of Biological, Geological and Environmental Sciences, University of Bologna, Via Irnerio 42, 40126 Bologna, Italy; [2]Department of Life Sciences, University of Siena, Via P.A. Mattioli 4, 53100 Siena, Italy; [3]Research and Innovation Department, Luxembourg Institute of Science and Technology, 5 Avenue des Hauts-Fourneaux, 4362 Esch/Alzette, Luxembourg

In plant cells, callose is a β-1,3-linked glucose polymer with some 1,6-branches, which accumulates under abiotic and biotic stresses as well as during specific developmental stages such as control of plasmodesmata trafficking and reproduction. Therefore, callose is a multifunctional polymer involved in several aspects of plant growth and development. To gain insight into the deposition pattern of callose, it is important to know how the enzyme callose synthase is regulated through the interaction with the cytoskeleton. Actin filaments likely determine the long-range distribution of callose synthase through transport vesicles but the spatial/biochemical relationships between callose synthase and microtubules are poorly understood, although experimental evidence supports the association between callose synthase and tubulin.
In this research, we investigated the interaction between callose synthase and microtubules as microtubules might control the insertion or activation of callose synthase into the plasma membrane, finally regulating pollen tube growth, where callose is an essential component of the cell wall. We further investigated the connection between callose synthase and microtubules through a spin-down assay. In addition, we analyzed the molecular composition of the callose synthase complex both by native two-dimensional electrophoresis and by isolating the callose synthase complex using spin-trap and anti-callose synthase antibody. Subsequently, we studied the ultrastructural relationship between callose synthase and microtubules by immunogold labeling. Further information on the relationship between callose synthase and microtubules was obtained by far-western blotting while analysis of the association of callose synthase with soluble or insoluble fractions of membrane lipids showed that the enzyme is not uniformly distributed in cell membranes.
Results confirmed that callose synthase is associated with tubulin and can therefore interface with cortical microtubules. In contrast, actin and sucrose synthase were not permanently associated with callose synthase. Overall, the data indicate that pollen tube callose synthase exerts its activity in cooperation with the microtubular cytoskeleton.

7 = Nanotechnology to improve micronutrient uptake in plants

Valerio Petruccelli[1], Domenico Rosa[2], Irene Bavasso[2] Camilla Badiali[1], Matilde Benassi[1], Luca Di Palma[2], Gabriella Pasqua[1], Elisa Brasili[1]
[1]Department of Eenvironmental Biology, University of La Sapienza, Piazzale Aldo Moro5, 00185 Roma, Italy; [2]Department of Chemical Engineering, University of La Sapienza, Via Eudossiana 18 Roma, Italy

According to the Food and Agriculture Organization of the United Nations, world population is expected to reach 10 billion people by 2050 and there is no doubt that it will be necessary to increase food production Farming efficiency relies on the massive use of agrochemicals to maintain crop yields, with a dramatic environmental impact. A drastic change in the methodologies used in agriculture is thus needed. Agroecology aims to obtain the high quality of agricultural products with a low environmental impact through precision agriculture. In this context, nanotechnologies provide a promising new tool to achieve decontamination, fertilization and crop protection. It has been estimated that over 70% of the phytochemicals are released into the environment without having a direct effect on the crops. Nanofertilizers, due to their high surface area to volume ratio, could be more effective than most of the latest conventional fertilizers to deliver substances to targeted organs. Recent studies have shown that iron nanoparticles (nZVI) represent a promising strategy to be used as a carrier for delivery of plant nutrients the effect of nanoparticles in agriculture is still full of controversy and the literature data show unclear results on the efficacy of nanoparticles as nanofertilizers. This research aims to investigate the effect of nanofertilizers, as an alternative to commercial fertilizers, on germination and development of *Solanum lycopersicum* plants. The nanofertilizers composed of Fe^{2+} $Fe^{3+}{}_2O_4$, Fe_2O_3, and FeO, were tested at different concentrations (5-50-100-500-1000 ppm) on tomato seeds and seedlings. Preliminary results showed that seed germination and root growth were not negatively affected by the treatment with different concentrations of nanoparticles. The chlorophyll and carotenoid content in the leaves was also evaluated. No alteration caused by the nanoparticles has been observed.

7 = *Quercus ilex* phyllosphere: intra- and interdomain features in urban and natural Mediterranean context

Alessia Postiglione, Daniela Zuzolo, Antonello Prigioniero, Maria Tartaglia, Pierpaolo Scarano, Mariantonietta Ranauda, Maria Maisto, Rosaria Sciarrillo, Carmine Guarino
Department of Science and Technologies, University of Sannio, Benevento, Italy

The phyllosphere has been defined as the above ground part of plants of which leaf tissue represents the largest surface area providing a unique surface capable of harbouring microorganisms. On a global scale, the surface area offered by the phyllosphere is of considerable importance, as it was estimated that leaf tissue can globally reach a surface comparable in magnitude to the entire land surface of the planet. In recent years, the phyllosphere has become a central topic in various scientific fields, especially urban forestry and environmental microbiology. Leaves surface featured with morphological traits, depictable with cuticle, stomata and trichomes, offers available spaces involved in different ecosystem functioning, acting as a contact interface between plants, external environment and organisms at different trophic levels. Leaves surface, although representing a hostile environment for some species, it represents and offers a micro-environment surface largely colonized by fungal and bacterial microorganisms. Phyllosphere colonization dynamics are strongly influenced by plant species and environmental features, such as season and presence of pollutants, that could differ between urban and natural forest. Epiphytic bacterial and fungal community composition and richness in *Quercus ilex* L. leaves sampled in urban and natural areas, in summer and winter seasons, by microscopy and metagenomic analysis were studied. Two Photon excitation microscopy was used to visualize bacterial and fungal colonization hotspot. A clear signaling was evidenced on the branches of the abaxial stellate trichome and at its base, stomata openings and among lower epidermal cells grooves. Fungal structures were mainly present on upper leaves sides, but hyphae and spores have been even found among the stellate trichomes intricate network, typical of *Q. ilex* leaves. Network analysis was conducted to highlight hub species playing a key role in the microorganism assembly dynamics. Concentrations of particulate matter and polycyclic aromatic hydrocarbons (PAHs) were extracted from sampled leaves to assess variations in the composition of the phyllosphere microbiome. The seasonal factor had a greater influence on phyllosphere biodiversity, while the environmental factor altered the relative abundances of seasonally selected taxa. The microbial network consisted of 146 nodes and analyses identified a core microbiome of *Q. ilex* in urban and natural Mediterranean areas of 150 bacterial and fungal taxa. Microorganism potential PAHs degrading activity has been evaluated identifying 83 genes coding for 5 enzymes (protocatechuate 3,4-dioxygenase, protocatechuate 4,5-dioxygenase, phthalate 4,5-dioxygenase, 3-hydroxybenzoate 4-monooxygenase, 4,5-dihydroxyphthalate decarboxylase involved in PAH degradation pathways. On *Q. ilex* leaves, in the Mediterranean environment, we detected a very stable phyllosphere, mainly regulated by seasonal factors.

7 = Response to metals in the Ni-hyperaccumulator *Alyssoides utriculata* (L.) Medik. in synergy with bacterial and fungal strains

Enrica Roccotiello, Sara Romeo, Silvia Priarone, Stefano Rosatto, Mirca Zotti, Mauro Mariotti
Department of Earth, Environmental and Life Sciences, University of Genoa, Corso Europa 26, 16132, Genoa, Italy

In recent decades, anthropogenic activity caused an exponential input of metals into the soil, causing environmental pollution, posing significant risks to human health and to the environment.
Some metal-hyperaccumulator plants can grow and reproduce successfully on metalliferous soils, accumulating metals in the aboveground biomass. These species are suitable for phytoremediation, an eco-sustainable technique that employs plants to remove contaminants from the soil.
Previous studies have shown that the presence of bacterial or fungal *inocula* at the root level can affect the ability of some plant species to accumulate metals. However, the effect of a joint use of bacterial and fungal *inocula* is almost unexplored. Consequently, the aim of this study was to investigate the ecophysiology, biometry and metal accumulation of the facultative Ni-hyperaccumulator *Alyssoides utriculata* (L.) Medik. inoculated with single or mixed plant growth promoting (PGP) bacterial (*Pseudomonas fluorescens*) and fungal (*Penicillium ochrochloron*) strains, previously selected. Those strains were proved to be able to growth synergically under metal stress.
Soil was sampled from Bric Gippone (Liguria, Italy), sieved, and sterilized at 130°C, then arranged into control and treatments pots (n=30 each treatment), randomized. After 10 months from transplanting, the photosynthetic performance of *A. utriculata* was evaluated by the fluorimeter HANDY PEA (n=3 each plant), and after 12 months from transplanting the biomass production was evaluated weighing the belowground and aboveground biomass. The dried aboveground biomass was also powdered and sent for ICM-MS to evaluate Ni accumulation.
The photosynthetic efficiency (Fv/Fm) and the performance index (PI) are always high and showed no significant differences between the control and the treatment group (T-test, $P>0.05$).
On the contrary, the biomass production was significantly higher in all the plants inoculated compared with the control ($P<0.05$ for above- and belowground biomass). However, no significant differences were recorded among the different treatments (fungi, bacteria, and mix).
The element content in the aboveground biomass showed an increasing ability to uptake Ni by different treatments as followings: Fungi>Bacteria>Mix>Control but with no significant differences among treatments were recorded. However, Mn and Cu accumulation are positively and significantly affected by treatments (mainly bacteria and mix, respectively) compared with control plants.
Co-inoculation seems to alleviate metal stresses and improve plant response in terms of aboveground biomass increase. The accumulation of metals like Mn and Cu, but, surprisingly, not Ni, significantly increased for inoculated treatments.
The plant response to synergic bacterial-fungal consortia required further exploration and provides new insight for a sustainable phytoremediation approach in soils contaminated by different metals.

7 = The barley mutant *happy under the sun 1* (*hus1*): An additional contribution to pale green crops

Lisa Rotasperti[1]†, Luca Tadini[1]†, Matteo Chiara[1], Cristina Crosatti[2], Davide Guerra[2], Andrea Tagliani[1], Sara Forlani[1], Ignacio Ezquer[1], David S. Horner[1], Peter Jahns[3], Katarzyna Gajek[4], Addy García[5], Roxana Savin[5], Laura Rossini[6], Alessandro Tondelli[2], Agnieszka Janiak[4], Paolo Pesaresi[1]*
[1]Department of Bioscience, University of Milan, Via Celoria 26, 20133 Milan, Italy; [2]CREA – Research Centre for Genomics and Bioinformatics, Via S. Protaso 302, 29017 Fiorenzuola d'Arda, Italy; [3]Institute of Plant Biochemistry, Heinrich Heine University, Universitätsstraße, 40225 Düsseldorf, Germany; [4]Institute of Biology, Biotechnology and Environmental Protection, University of Silesia in Katowice, ul. Bankowa 12, 40032 Katowice, Poland; [5]Department of Crop and Forest Sciences, University of Lleida - AGROTECNIO Center, Av. R. Roure 191, 25198 Lleida, Spain; [6]Department of Agricultural and Environmental Sciences - Production, Landscape, Agroenergy, University of Milan, Via Celoria 2, 20133 Milan, Italy

Barley (*Hordeum vulgare*) is the fourth major crop worldwide, with Europe producing the greatest share. Beside grains, barley plants produce an almost equivalent amount of straw that in the past was considered as a secondary product of minimal value. Nevertheless, the increasing demand for renewable materials makes straw, and especially barley straw characterized by the largest content of carbohydrates among cereals, a valuable product for its potential conversion into biofuels and other eco-friendly products. One possible solution could be the realization of a dual-purpose crop ideotype, that produce likewise a large amount of grains and straw. A way to increase the biomass production is tuning the photo-protection mechanism that control photosynthesis. Decreasing the light harvesting chlorophyll antenna size could lead to diminish the excess of light absorption avoiding photo-damage to the plant and boost energy flux for photochemistry. Pale-green leaves appear to perform better under high light conditions or high density cultivation and contribute to reflect a significantly larger proportion of incoming solar radiation, mitigating the local temperature. Decreasing the light harvesting chlorophyll antenna size could lead to diminish the excess of light absorption avoiding photo-damage to the plant and boost energy flux for photochemistry. Pale-green leaves appear to perform better under high light conditions or high density cultivation and contribute to reflect a significantly larger proportion of incoming solar radiation, mitigating the local temperature.
The *hus1* (*happy under the sun 1*) mutant has been isolated by forward genetics approach within the HorTILLUS chemical mutagenize population, searching for plants with improved photosynthetic performance. *hus1* plants are characterised by showing a pale phenotype of the leaves, a reduced antenna size and an improved photosynthetic performance respect to Sebastian control plant. Segregation analysis performed on the F_2 population obtained by crossing *hus1* with Morex indicates that the *hus1* phenotype is caused by a monogenic recessive allele. Using exome capture sequencing, the putative SNP mutation, that causes a premature stop codon, has been identified in the *A. thaliana* homologous *CHAOS* gene (Chloroplast Signal Recognition Particle 43), that codifies a stromal chaperone that transfers the antenna proteins from the chloroplast stroma to the thylakoid membranes. Western Blots analysis of antenna proteins and components of the electron transport chain of the thylakoid membranes were carried out and the results indicate that barley *hus1* and the Arabidopsis *chaos* mutants have an identical level of LHCII and LHCI proteins accumulation. Moreover, the HUS1 gene was able to complement the Arabidopsis *chaos* mutant. Therefore, the *hus1* mutant was renamed *hvchaos*.
In addition to a detailed molecular and physiological characterization of the mutant grown under controlled greenhouse conditions, we demonstrate that the agronomic performance of *hus1* plants, in terms of total biomass production and grain yield under standard field conditions, are comparable to the wildtype. Moreover potential benefits of the *hus1* phenotype and natural allelic variants of the HvcpSRP43 locus, with respect to productivity and climate change mitigation were also investigated.

7 = Does polyploidy enhance plant growth, Ni-accumulation and tolerance in hyperaccumulator plants? Insights from the model species *Odontarrhena bertolonii* (Brassicaceae)

Federico Selvi[1], Ilaria Colzi[2], Cristina Gonnelli[2]
[1]Department of Agriculture, Food, Environment and Forestry, P.le Cascine 28, I-50144 Firenze, Italy; [2]Department of Biology, Via Micheli 1, I-50121 Firenze, Italy

Since its discovery, polyploidy, the whole-genome duplication process, has long been recognized as a major force of plant evolution. Polyploids usually display increased vigor, higher adaptive ability, and competitive advantage over their diploid progenitors, in different functions and environmental contexts. Evidence from many studies supports that these plants can occupy new ecological niches thanks to their higher tolerance to abiotic stresses. Among the various forms of stress that plants can experience, the presence of trace metals in toxic concentrations in the growth substratum is one of the most relevant ones. Most terrestrial plants display severe toxicity symptoms to very low concentrations of metals such as Cr, Co, Zn, Pb, Cu, Ni and others.
A remarkable exception, however, are the metal-tolerant species, that are often found on ultramafic soils with naturally elevated metal levels. These species are provided with adaptive physiological mechanisms which allows either the exclusion of the metal from the root system (excluding species) or its uptake, translocation and accumulation in the shoot tissues (accumulating species).
Despite the large number of studies investigating polyploid fitness, whether the whole-genome duplication can affect growth responses, tolerance and accumulation ability in metal accumulator plants remains obscure. To this purpose, it is necessary to compare conspecific diploid and polyploid cytotypes in controlled conditions, to exclude the effects of species-specific constraints that may influence the responses to the metal. Also, it is required that the cytotypes are found in similar site conditions, especially soil chemical and physical characteristics, in order to also exclude preadaptation to different metal levels.
Metal hyperaccumulation in plants was discovered around the mid of the 20th century in *Odontarrhena bertolonii* (Desv.) Jord. & Fourr. (Brassicaceae), an endemic chamaephyte restricted to serpentine soils in Tuscany and Liguria. Based on recent evidence, this species includes diploid and tetraploid populations with allopatric distribution, which grow on soils with largely the same physical and chemical characteristics and levels of Ni, Cr, Co and Mg. Hence, *O. bertolonii* represents an excellent model system to investigate the relationship between ploidy level and responses to the metal in terms of growth, tolerance and accumulation capacity. Accordingly, we used this species to perform the first explicitly designed experiment to test the polyploid fitness hypothesis in terms of responses to elevated metal concentrations. Three diploid (2n = 2x 16) and three tetraploid (2n = 4x = 32) accessions were compared for Ni concentrations in native soil, roots and shoots. After that, plants were grown from seeds placed in pots with natural serpentine soil, with total Ni concentration of 1870 μg g−1 dw, and with commercial soil as control. At a third step, 6-weeks-old seedlings were transferred to hydroponic culture and exposed for seven days to a series of increasing $NiSO_4$ concentrations (0, 50, 100, 250, 500, 100, 2000, 3000 μM). Preliminary results show that tetraploid plants are characterized by increased growth rate and shoot biomass production, when cultivated on both natural serpentine and commercial soil, and in hydroponics at various metal concentrations. When cultivated on natural serpentine soil, shoot Ni concentration was similar in diploids and tetraploids, though the mean content per plant was higher in tetraploids because of their higher biomass production. Comparable results were obtained in hydroponics, for both roots and shoots. While tolerance to Ni concentration in the culturing solutions was similar, the tetraploid populations displayed higher tolerance to the metal concentration inside the tissues.
If confirmed, these results can help to assist with the selection of the most useful plant accessions in environmental restoration practices and field biotechnological applications, such as phytoremediation and agromining, which are based on the use of metal accumulator plants and are increasingly adopted worldwide.

7 = Evidence of cross-kingdom RNA interference in the arbuscular mycorrhizal symbiosis

Alessandro Silvestri[1], William Ledford[1,4], Valentina Fiorilli[1], Cristina Votta[1], Massimo Turina[2], Raffaella Balestrini[2], Hailing Jin[3], Ignacio Rubio-Somoza[4], Luisa Lanfranco[1]
[1]Department of Life Sciences and Systems Biology, University of Torino, Viale Mattioli 25, 10125 Torino, Italy; [2]Institute for Sustainable Plant Protection – CNR Torino, Strada delle Cacce 73, 10135 Torino, Italy; [3]Department of Microbiology and Plant Pathology, University of California, 900 University Avenue, Riverside, CA 92521, US; [4]Molecular Reprogramming and Evolution (MoRE) Lab, Centre for Research in Agricultural Genomics (CRAG), Carrer de la Vall Moronta, 08193 Cerdanyola del Vallès, Barcelona, Spain

Small RNAs (sRNAs) are short non-coding RNA molecules that regulate gene expression in eukaryotes *via* RNA interference (RNAi). Interest in sRNAs has grown as we have discovered the fundamental roles they play in a wide variety of biological processes, such as developmental regulation and stress responses. sRNAs are also emerging as important signaling molecules in different inter-species, and even inter-kingdom, interactions. Indeed, mobile sRNAs can be transferred from "donor" to "receiver" organisms, where they can regulate host gene expression by exploiting the host's molecular RNAi machinery. While this process, cross-kingdom RNAi, has been principally studied in plant-pathogen interactions, increasing evidence points towards mobile sRNAs as potential contributors to the inter-species molecular dialogue in plant mutualistic associations, including the arbuscular mycorrhizal (AM) symbiosis.
We previously characterized the sRNA population of the AM fungus *Rhizophagus irregularis* during the symbiosis with the host plant *Medicago truncatula* and we reported, based on *in silico* data, that dozens of fungal sRNAs have potential for host gene regulation through cross-kingdom RNAi (Silvestri *et al.* 2019 BMC Genomics). We focused on a fungal sRNA (*Rir*-2216) that was found to have 246 potential target transcripts in the *M. truncatula* transcriptome, including the MtWRKY69 transcription factor. To investigate whether *Rir*-2216 is involved in cross-kingdom RNAi we first demonstrate, using stem-loop RT-PCR, that *Rir*-2216 is present in samples of extraradical mycelium and *M. truncatula* mycorrhizal roots. Next, we observed that the co-expression of *Rir*-2216 with its predicted target transcript MtWRKY69 in *Nicotiana benthamiana* leaves, led to a decrease in the abundance of the target protein, suggesting that *Rir*-2216 can silence plant genes *in planta*. Using laser microdissection followed by RT-qPCR, we were able to quantify relative target transcript levels at cellular resolution. In arbuscule-containing cells, two target transcripts, including MtWRKY69, were down-regulated compared with cortical cells of non-mycorrhizal roots. Finally, we set up small RNA-Ago1 co-immunoprecipitation assays from mycorrhizal roots of *M. truncatula*. After extracting bound sRNA from plant Ago1 proteins, the presence of *Rir*-2216 was confirmed in the immunoprecipitated fraction of colonized roots. These results suggest that the fungal sRNA *Rir*-2216 is transported to the plant root where it associates with plant Ago1 leading to the silencing of plant target genes, and provides first evidence of cross-kingdom RNAi in the AM symbiosis.

7 = Fungal-derived biostimulants boosting *Cichorium intybus*: effects of *Chaetomium globosum* and *Minimedusa polyspora* culture filtrates on growth performance and metabolomic traits

Veronica Spinelli[1], Elisa Brasili [1,2], Fabio Sciubba[1,2], Andrea Ceci[1], Ottavia Giampaoli[1,2], Alfredo Micheli[1,2], Gabriella Pasqua[1,2], Anna Maria Persiani[1]
[1]Department of Environmental Biology, Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy; [2]NMR-Based Metabolomics Laboratory (NMLab), Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy

Implementing environmentally friendly and climate-resilient agronomic solutions to increase food production while addressing modern agriculture's issues is the biggest challenge our society is currently facing. In this context, biostimulants represent a sustainable solution to increase crop resilience and productivity in adverse environmental conditions, while minimizing agrochemicals applications and tackling climate change effects. Among microbial biostimulants, fungi have been reported as particularly effective in promoting plant growth. Moreover, fungi, thanks to a complex extracellular metabolism, exert their influence on plants also through the release in soils of diffusible metabolites in the environment. So, the metabolites released in the culture medium, during growth in controlled conditions, may be applied to simulate these interactions and effectively stimulate plant growth. Indeed, fungal culture filtrates have been consistently reported in several studies to be effective in promoting plant growth by enhancing seed germination, biomass production, and metabolites production. Therefore, this study aimed at investigating the biostimulant effect of fungal culture filtrates of *Chaetomium globosum* Kunze and *Minimedusa polyspora* (Hotson) Weresub & P.M. Le Clair on growth performance and metabolomic traits of *Cichorium intybus* L. (chicory) plants. *C. intybus*, belonging to the *Asteraceae* family, is an alimurgic plant whose popularity is steadily increasing due its rich and complex phytochemical profile, including a great number of bioactive substances, and a high nutritional value which make it a plant of agricultural and medicinal importance. This study was conducted as a pot experiment set up in walk-in chambers. Chicory plants, one month after the transfer of the seedlings in pots, were stimulated by soil drenching with 8 ml/pot (30 ml/kg of soil) of the culture filtrates obtained by a 14-days incubation of the fungal strains in Malt Extract Broth (MEB), or the same amount of uninoculated MEB or distilled water in the control groups. Fourteen days after the stimulation, plant biomasses were recovered to estimate several growth parameters and analyze the metabolomic variations occurred in roots and leaves through ^{1}H-NMR 600 MHz.
We observed for the first time that *M. polyspora* culture filtrate promoted an increase of biomass, both in shoots and roots, and of the leaf area, while no increase was observed in plants treated with *C. globosum* culture filtrate. Based on ^{1}H-NMR metabolomics data, differential metabolites and their related metabolic pathways were highlighted. A common response in *C. intybus* roots involving the synthesis of 3-OH-butyrate through the decrease of the synthesis of fatty acids and sterols, as a mechanism balancing the NADPH/$NADP^{+}$ ratio, was observed in both the treatments with *C. globosum* and *M. polyspora* culture filtrates. The phenylpropanoid pathway was differently triggered by the fungal culture filtrates. *C. globosum* culture filtrate increased phenylalanine and chicoric acid in the roots. Chicoric acid, whose biosynthetic pathway in chicory plant is putative and still not well known, is a very promising natural compound playing an important role in plant defense. Instead, *M. polyspora* culture filtrate interestingly stimulated an increase of 4-OH benzoate, being benzoic acids precursors for a wide variety of essential compounds playing crucial roles in plant fitness and defense response activation. Therefore, both *C. globosum* and *M. polyspora* culture filtrates affected *C. intybus* metabolome and could be considered as fungal bioresources for the development of new biostimulants.

7 = Study of an innovative semi-idroponic Hybrid-flow Constructed Wetland system for the First Flush Stormwater treatment

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

The use of phytoremediation processes for wastewater treatment through the implementation of Constructed Wetlands (CWs) represents, among the green technologies, a sustainable alternative to traditional wastewater purification plants. The case study examined in this research concerns the treatment of First-Flush Stormwater (FFSW), the rainwater that falls in the early stage of a meteoric event. FFSW flushes the waterproofed surfaces of urban areas so it is enriched with the various types of contaminants there accumulated, especially after long periods of drought. In order to avoid eutrophication of the receiving water bodies, FFSW should be intercepted and remediated by sewage treatment plants before discharge. The aim of our study was to remediate FFSW in a cost-effectively way through the use of CWs, but also to protect and maintain the water resources by achieving the standards required by law for water quality reuse.
To this purpose, we designed and tested the performance of an innovative CW pilot plant, consisting in a semi-hydroponic Hybrid-flow Constructed Wetland (HCW), formed by two filter beds placed in series where the wastewater follows first a vertical and then a sub-surface horizontal flow (Fig. 1). The aquatic macrophytes employed in the pilot HCW have been carefully selected between five species commonly used for phytoremediation, by means of in-pot phytotoxicity tests that showed *Arundo donax* L. and *Phragmites australis* (Cav.) Trin. ex Steud as the most suitable species for the treatment of an *ad hoc* synthetic FFSW (Davis et al., 2001). In addition, the HCW was acclimatized for about one week with a solution of compost tea, in order to increase the microorganism biodiversity which could promote plant growth and assist the remediation process. Another technical innovation adopted concerns the use of plastic supports, namely Moving Bed Biofilm Reactors (MBBRs), as one of the filling medium of the HCW multi-layered beds, to support the development of extensive biofilms on their high specific surface. The remediation efficiency of the HCW was evaluated both on the synthetic FFSW and on a FFSW collected downstream of a draining surface of an industrial activity, by analyzing the following parameters: Chemical Oxygen Demand (COD), ammonia, nitrates, nitrites, chlorides, metals and number of colony-forming units (CFU) for mL in the effluents with respect to the inlets.
Significant removals of pollutants (50 – 100%) were observed for all the tested parameters after 96 hours of recirculation in the HCW of both types of FFSW and also a relevant reduction (of at least one order of magnitude) of the total bacterial count was observed after the HCW treatment. Therefore, the technical innovations adopted, including the use of MBBRs as well as the compost tea pretreatment have probably enhanced the effectiveness of the phytoremediation process; in addition, this allowed the reduction of the ratio between the surface area necessary for an optimal water remediation and the equivalent inhabitant (EI), usually up to 10 m^2 EI^{-1}, considered one of the main limiting factors for a wide use of CWs for wastewater treatment. The methods here described could also be adapted to the treatment of other types of wastewater.

Fig. 1. HCW pilot plant

7 = Biotechnological combination for co-contaminated soil remediation: focus on tripartite "meta-enzymatic" activity

Maria Tartaglia[1], Daniela Zuzolo[1]*, Alessia Postiglione[1], Antonello Prigioniero[1], Pierpaolo Scarano[1], Rosaria Sciarrillo[1]*, Carmine Guarino[1]
[1]Department of Science and Technologies, University of Sannio, Benevento, Italy
*Corresponding Authors: dzuzolo@unisannio.it, sciarrillo@unisannio.it

Soil pollution is a pressing problem requiring solutions that can be applied without large-scale side effects directly in the field. Phytoremediation is an effective strategy combining plant and root-associated microbiome to immobilize, degrade and adsorb pollutants from the soil. To improve phytoremediation, it is necessary to think of plants, fungi and bacteria not as individual entities, but as a meta-organism that reacts organically, synergistically and cooperatively to environmental stimuli. Analysing the tripartite enzymatic activity in the rhizosphere is necessary to understand the mechanisms underlying plant-microorganism communication under abiotic stress (such as soil pollution). In this work, the potential of a microbial consortium along with a plant already known for its phytoremediation capabilities, *Schedonorus arundinaceus* (Scheb.) Dumort., was validated in a mesocosm experiment with pluricontaminated soil (heavy metals, PAHs and PCBs). Chemical analyses of the soil at the beginning and end of the experiment confirmed the reduction of the main pollutants. The microscopic observation and chemical analyses confirmed the greater root colonization and pollutant removal following the microbial treatment. To obtain a taxonomic and functional picture, tripartite (plant, fungi and bacteria) enzyme activity was assessed using a metatranscriptomic approach. Total RNA was extracted from a sample of rhizosphere sampled considering 2 centimetres of root and soil attached. From the total reads obtained, mRNAs were filtered, and analysis focused on reads identified as proteins with enzymatic activity. The differential analysis of transcripts identified as enzymes showed that a general increase in potential enzyme activity was observed in the rhizosphere after our biotechnological treatment. Also from a taxonomic perspective, an increase in the activity of some phyla, such as Actinobacteria and Basidiomycota was found in the treated sample compared to the control. An increased abundance of enzymes involved in rhizospheric activities and pollutant removal (such as dehydrogenase, urease, laccase etc.) was found in the treated sample compared to the control at the end of the experiment. Several enzymes expressed by the plant confirmed the increase in metabolic activity and architectural rearrangement of the root following the enhancement of the rhizospheric biome. The study provides new outcomes useful in rhizosphere engineering advancement.

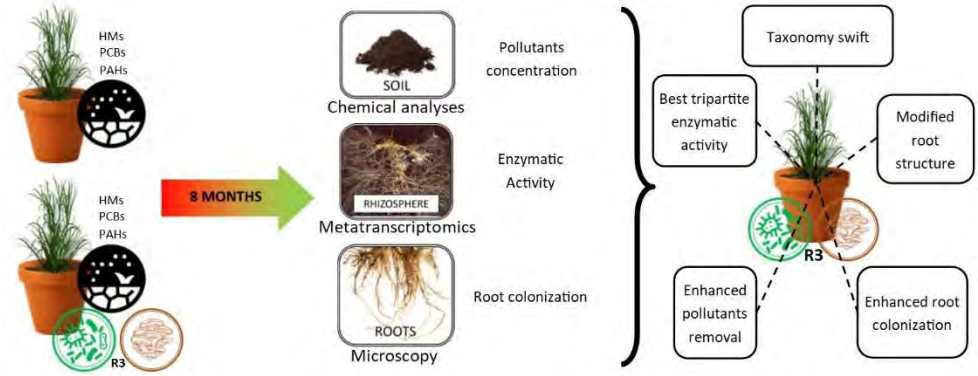


Fig. 1. Experimental workflow

7 = Application of the response surface methodology for the optimization of supercritical CO_2 extraction of oil from pomegranate marc

Riccardo Tornese[1], Miriana Durante[2], Anna Montefusco[1], Marcello Lenucci[1]
[1]Department of Biological and Environmental Sciences and Technologies, University of Salento, Via Lecce-Monteroni, 73100, Italy; [2]Institute of Sciences of Food Production (ISPA), National Research Council (CNR), Via Lecce-Monteroni, 73100, Italy

The industrial technology for pomegranate juice extraction by hydraulic pressing of fruits generates large amounts of pomegranate marc (PM), a by-product constituted of both rinds and kernels extraordinarily rich in lipophilic and hydrophilic health-promoting compounds. It is worth noting that dehydrated PM contains up to 3.5% oil, mainly from the kernels, comprising polyunsaturated fatty acids of the family of conjugated α-linolenic acids (CLnA), as well as large amounts of phenolics, soluble and insoluble dietary fibers from both kernels and rinds. These compounds can be efficiently recovered through biorefining strategies to obtain ingredients of excellence for pharmaceutical, cosmeceutical and/or nutraceutical use, contributing to the exploitation of this agri-food by-product and to the sustainability of pomegranate production/processing chain in a context of circular economy. Supercritical carbon dioxide (SC-CO_2) is a green and environmentally friendly technology for the effective extraction of high-value natural molecules. It is gaining a foothold in industrial production of solvent-free vegetable oils and has also been applied to concentrate oil products of lipophilic micronutrients. Nevertheless, the industrial application of SC-CO_2 technology requires a careful optimization of the operative parameters to make the process efficient from both an economic and a productive point of view. In this context, a response surface methodology approach, based on the Box-Behnken design, was used to model and maximize SC-CO_2 extraction of the oil from PM, while optimizing the main operative parameters (temperature, pressure, CO_2 flow and extraction time). The experimental data were fitted to a second-order polynomial equation by multiple regression analysis [ln(% oil yield) = 3.15 + 0.57 A + 0.29 B + 0.25 C + 0.49 A^2 - 0.30 B^2] and examined using appropriate statistical methods. Temperature (A) and its square (A^2) were the most significant model terms, followed by CO_2 flow (B and B^2) and time (C). Instead pressure and all factor interactions resulted not significant and, consequently, excluded from the equation to improve the model. The 3-D response surface plots (Fig. 1) derived from the mathematical model were applied to determine the optimal extraction parameters, which resulted: temperature 80 °C, pressure 33.4 MPa, expanded CO_2 flux 6 L/min and time 120 min. Under these conditions, the oil experimental yield was 3.9 g·100 g^{-1} dry weight, approximately 64% of the total oil content of the dried PM matrix (as directly estimated by extensive soxhlet extraction with hexane), in good agreement with the model predicted value (73±29%). The extracted oil contained mostly punicic acid and other CLnA, contributing to over 70% of the total identified fatty acids and well known for their anti-inflammatory, immunomodulatory, anti-cancer and anti-oestrogen effects. The oil contained also phytosterols, tocopherols, triterpenes and small amounts of phenol compounds, bioactives with high antioxidant activity and a potential role in the maintenance of human health and in the prevention of degenerative diseases. These results represent the first step towards the optimization of a sequential PM extraction process that will continue with the extraction of the hydrophilic phytocomplex through subcritical water and the obtaining of an exhausted residue rich in insoluble fibers (mostly cellulose), useful by the food/feed industry or as soil conditioner.

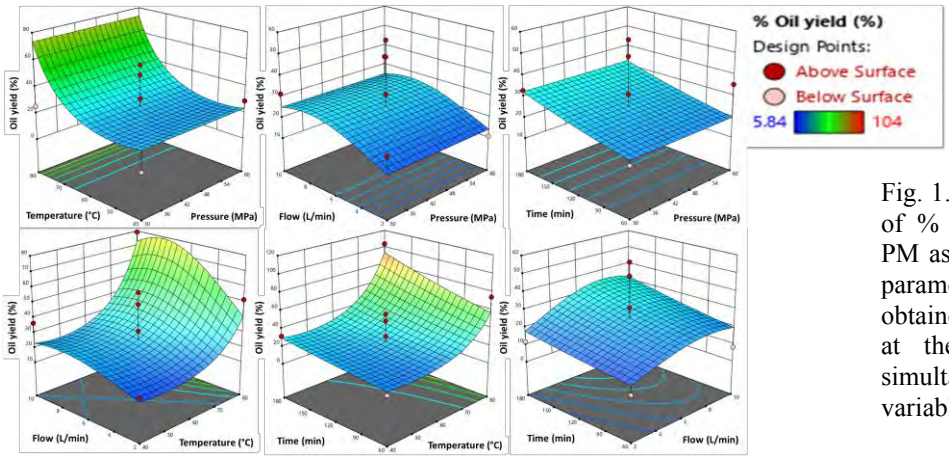


Fig. 1. 3-D response surface plots of % oil yield from dehydrated PM as a function of the different parameters. The plots were obtained by varying two variables at the experiment range and simultaneously fixing the other variables at the central level.

7 = Changes in some bioactive compounds content and growth rate of *Lens culinaris* Medik sprouts as affected by LED light intensity and quality

Alessandra Villani, Benedetta Bottiglione, Linda Mastropasqua, Costantino Paciolla
[1]Department of Biology, University of Bari Aldo Moro, Via E. Orabona 4, 70125 Bari, Italy

Light emitting diodes (LEDs) have the potential to be an effective tool for improving the content of health-promoting phytochemical compounds of sprouts, widely recognized as functional foods in the human diet. The objective of this study was to investigate the effect of different light intensity and spectral composition on the growth, bioactive compound content, and antioxidant metabolism of lentil (*Lens culinaris* Medik) seedlings, after 3 and 5 days of LED treatment. Two light quality × three light intensity treatments were tested: 1) red light (630 nm) and 2) blue light (470 nm) at photosynthetic photon flux density (PPFD) of 100, 300, and 500 μmol m^{-2} $\cdot s^{-1}$. Continuous lighting and temperature of 21±1 °C was used for the above treatments, and dark was used as a control. Both light quality and intensity did not affect germination. At both harvest time, length of seedlings growth under blue light appeared to decrease as PPFD increased, while red light did not affect the growth, compared to the control. The biomass, regardless of light quality, slightly increased with increasing light intensity. The highest chlorophyll and carotenoid content, as well as the ascorbate and total phenols concentration was observed under blue light treatment at 100 μmol m^{-2} $\cdot s^{-1}$. Significant changes in lipid peroxidation and H_2O_2 content also occurred at all tested PPFD. These results suggest that red vs. blue light can promote elongation growth of lentil sprouts, while blue light enhances the bioactive compounds, although the promotion effects vary with light intensities.

7 = Plant-environment interactions: role of leaf functional traits from PTEs air phytoremediation perspective

Daniela Zuzolo[1], Antonello Prigioniero[1], Ülo Niinemets[2], Alessia Postiglione[1], Mariano Mercurio[1], Francesco Izzo[3], Marco Trifuoggi[4], Maria Toscanesi[4], Pierpaolo Scarano[1], Maria Tartaglia[1], Maria Antonietta Ranauda[1], Maria Maisto[1], Rosaria Sciarrillo[1], Carmine Guarino[1]
[1]Department of Science and Technology, University of Sannio, via de Sanctis snc, 82100, Benevento, Italy; [2]Institute of Agricultural and Environmental Sciences, Estonian University of Life Sciences, Fr. R. Kreutzwaldi 1, 51006, Tartu, Estonia; [3]Department of Earth Sciences, Environment and Resources, University of Naples Federico II, via Cintia,80126 Naples, Italy; [4]Department of Chemical Sciences, University of Naples Federico II, via Cintia ,80126 Naples, Italy

Plant-environment interactions can be source of inspiration to provide effective "tool" such as air-phytoremediation. In this context, plant leaves are the main actors in providing this regulation service influenced by environmental factors, pollutant concentrations and specific plant chemical-physical features such as functional traits. However, the combination of these factors is not always considered when the performance (in terms of pollutant removal) of plant species is assessed. Today it appears necessary, given the increasing attention that these nature-based solutions are receiving, to refine both predictive models and to understand which factors may drive the ability of plant species to retain widespread pollutants with high ecosystem-level impacts. This new knowledge could also serve as a blueprint in terms of planning and managing urban reforestation and improving related ecosystem services. In this study the relationships between leaf surface functional traits of tree species commonly used for ornamental purpose in Mediterranean urban forests and Particulate Matter (PM) and Potentially Toxic Elements (PTEs) in leaves (both concentrations and uptake rate) have been explored. We hypothesized that the uptake and concentration of PM and PTEs could be influenced by leaf traits, as well as environmental PTEs concentration levels. To test these hypotheses a double sampling step was carried out in Naples (Southern Italy) for twenty-eight tree species. We estimated the stomatal and trichome surface areas and numbers per leaf area and cuticle thickness by optical microscope. In addition, the degree of cuticle esterification was assessed by Fourier-transform infrared spectroscopy (FTIR) analyses of leaf surface. We quantified PM by gravimetric analysis. The concentrations of PTEs (Al, Sb, As, Be, Cd, Co, Cr, Fe, Hg, Ni, Pb, Cu, Se, Sn, Sr, Tl, V, Zn) in intact leaves and dewaxed leaves were analysed by ICP-MS.
We observed that several analyzed plant species exhibit consistent behaviors. *Olea europaea* L., *Chamaerops humilis* L., *Quercus ilex* L., *Dracaena draco* (L.) L., *Magnolia grandiflora* L., and *Celtis australis* L. tend to be grouped by the functional traits of trichomes, lower stomata, and cuticle thickness that characterize them. Trichome traits correlate primarily with PM_{10} and, in some cases, with PTEs concentrations and accumulations on the leaf surface (e.g., for Sb and Be). Another cluster of species that behave similarly, showing lower concentrations of PTEs in the second sampling than in the first is represented by *Celtis australis* L., *Ceratonia siliqua* L., *Cinnamomum camphora* (L.) J.Presl, *Dracaena draco* (L.) L., *Eucalyptus camaldulensis* Dehnh. and *Liriodendron tulipifera* L.. All these species might be candidates as viable foliar hyperaccumulators.
Overall, existing correlations for As, Sb Cr, Fe, Ni, Pb Cu, Sr and Zn in subcuticular tissues and the stomatal features occur suggesting that in stomata and the surface occupied by them, a breach in the impenetrability of the cuticle that favors the entry of pollutants into tissues located below it may occur.
The correlations between element concentrations in soils, accumulation, and pollutant concentrations in both surface and dewaxed leaf suggest possible root uptake influencing PTE accumulation in leaves as a result of translocation (Al and Fe) or predominance of direct leaf uptake events (As, Ni, Sr, and Zn). Analyses on the elements for which direct foliar uptake was hypothesized show a positive relationship between daily pollutant uptake and degree of hydrophobicity and a negative relationship with cuticle thickness, highlighting the role of this functional traits of leaves as a driver for foliar uptake of PTEs.
Our results showed that atmospheric particulate matter acts as a carrier for PTEs and it is primarily retained by trichomes and cuticle waxes, but uptake is determined by other cuticle features. Overall, this study proves that leaf surface functional traits deserve attention in the study of the interaction between environment and plants from an air phytoremediation perspective.

8 = Does specificity of interactions with mycorrhizal fungi influence the distribution of the Mediterranean orchid, *Orchis italica*?

Marco G. Balducci[1], Jacopo Calevo[1,2], Karl Joseph Duffy[1]
[1]Department of Biology, University of Naples Federico II, Via Cinthia, 80126 Naples, Italy; [2] Department of Ecosystems Stewardship, Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey, UK

Both the distribution and abundance of plant populations may be linked to the availability of mutualists. Orchids depend on particular mycorrhizal (OrM) fungi to germinate from seed and establish new individuals, hence the availability of these fungi may play a key role in explaining their distribution. Previous studies using metabarcoding of OrM fungi associated with the roots of single orchid species over large geographical ranges have shown that OrM communities are taxonomically varied. In this study, we investigated the geographical distribution and diversity of OrM communities associated with the Mediterranean orchid, *Orchis italica* Poir. (Fig. 1) using both fungal isolation from roots and high-throughput sequencing (Illumina NovaSeq). Isolation from adult roots revealed 101 isolates from a total of eight *Orchis* populations in southern Italy. Sequencing of isolated fungi from *O. italica* showed that most isolates are from the *Tulasnella calospora* species complex, a fungal group known to associate with many other orchids (Fig. 2). Indeed, OrM fungal specificity with tulasnelloid fungi has been previously reported in the other *Orchis* species. Metabarcoding, however, revealed a more taxonomically diverse community of OrM fungi, yet the role of these fungi for the life cycle of *O. italica* needs to be better understood. This highlights the importance of quantifying and identifying the distribution of mycorrhizal associates in understanding the current and future distribution of this species.

Fig. 1: An example of a flowering *Orchis italica* in a typical grassland habitat.

Fig. 2: *Tulasnella calospora* isolated from *O. italica* on a petri dish containing Potato Dextrose Agar.

8 = Soil fungal communities under the rare alpine glacial relict *Berardia lanuginosa* with particular reference to the presence of endophyte fungi

Francesco Dovana[1], Erica Lumini[2], Enrico Ercole[1], Marco Mucciarelli[1]
[1]Dipartimento di Scienze della Vita e Biologia dei Sistemi, Università di Torino, Viale P.A. Mattioli 25, Torino, Italy; [2]Istituto per la Protezione Sostenibile delle Piante CNR, Viale P.A. Mattioli 25, Torino, Italy

Berardia lanuginose (Lam.) Fiori is an arctic-alpine paleondemic relictual plant, showing a relatively broad range on the Western Alps. This species exhibits peculiar growth and adaptive traits associated to its habitat, the calcareous scree slopes. The conservation of this plant plays an important role in the conservation of the alpine flora, also in consideration that the climate change in progress could lead to a drastic reduction of the its growing area. A precedent study conducted on fungi associated to *B. lanuginosa* roots highlighted the presence of arbuscular mycorrhizal fungi and dark septate endophytes. In order to better understand the biodiversity of these fungal communities associated with *B. lanuginosa*, in three different sites, we characterized: (I) root endophytic fungi using isolation techniques and subsequently molecular identification (combinate nrITS and partial nrLSU regions) using Sanger sequencing technology, (II) soil fungi associated to *B. lanuginosa* roots detected with NGS (focused on the nrITS region). The NGS analysis revealed the presence of 782 OTUs belonging mainly to the division Ascomycota and Basidiomycota. Four sequences of endophytes isolated from the roots of *B. lanuginosa* (all belonging to the division Ascomycota and the classes Dothideomycetes, Leotiomycetes and Sordariomycetes) were also extensively detected by NGS analysis in all sites considered in this study highlighting that these four species of endophytes are also widely diffused in the soil. Among the Basidiomycota, *Clavaria* and *Hygrocybe* were among the most widespread genera found in soil. Surprisingly, some ectomycorrhizal species, considered typical of Mediterranean environments, were detected in the soil. The presence of ectomycorrhizal fungal species, generally associated with plants also spread at low altitudes or in the Mediterranean area, could indicate an expansion of fungi from land to high altitude Alpine areas. These data could mean that symbiont fungi anticipate the colonization of host plants at higher altitudes due to the global rise in temperatures.

8 = Fungal diversity for bioremediation: tackling co-contaminations in a decommissioned military site

Roberto Giovannini[1], Andrea Ceci[1], Veronica Spinelli[1], Anna Maria Persiani[1]
[1]Department of Environmental Biology, Sapienza University of Rome, Piazzale Aldo Moro,5, 00185 Rome, Italy

Military sites, both active and decommissioned, represent a unique situation in which both biodiversity loss and protection may occur, depending on how those sites are managed. Exclusion zones and good practices such those provided in Natura 2000 report for military sites — can maintain or even increase the detected biodiversity. However, potential and identified contaminations in military zones are soil threats of increasing interest, especially when co-contaminations by organic and inorganic pollutants are established. Remediating such complex contaminations through conventional techniques is not only economically unsustainable but can also impact soil biodiversity. Novel methods to tackle co-contaminations may be found in biotechnological applications of the bioresources isolated from those same contaminated sites, even though there is a knowledge gap to be filled regarding the potentialities of autochthonous microbial communities. Therefore, this study aimed at gaining insight on the culturable fungal community of a decommissioned military site in Italy and the potentialities in fungal bioremediation. To reach the aims of the study, soil samples were collected in six sampling plots and the fungal communities were isolated. Furthermore, the fungal community associated with the rhizosphere of a specimen of *Plantago lanceolata* L., a wild herb largely distributed in the site, was similarly studied for the same purposes. The results showed high differences in species' abundances among samples with *Penicillium*, *Aspergillus* and *Trichoderma* as the most abundant genera. The analysis of alpha diversity and evenness revealed that the samples with the lowest abundance in Colony Forming Units (CFUs) showed the highest values of Shannon's Diversity Index (H') and Simpson Diversity Index (D_1), pointing to a lack of a dominant species among the isolates from those samples, while in samples with higher CFU values a more varied situation arises. In fact, sample S28 had the lowest diversity indexes but also the second highest CFU abundance, pointing to a dominance of few species, especially *Penicillium* S28A5, which was the most abundant species isolated from all samples. The analysis of genera alpha diversity revealed a similar situation, except for sample S22, whose diversity of genera showed to be the lowest among all samples, with a clear dominance of the *Penicillium* genus. Focusing on the rhizosphere sample, the results of alpha revealed a highly diverse community of culturable fungi, in both species and genera. The analysis of beta diversity, using Sørensen's index, showed that while the samples shared few common species, the isolated genera mostly overlapped. To evaluate the potentialities of the isolated species, a set of screenings were performed on a selected group of 30 species, which included the most abundant species and a species for each genus isolated. The assays performed in this study were the Remazol Brilliant Blue R (RBBR) and Fe-Chromeazurol S (Fe-CAS) decolorization assay, which are reported in literature as proxy test to investigate the ability to degrade complex organic compounds and to produce siderophores in response to metallic and non-metallic elements. The species showing the best performances were further tested to assess their tolerance to zinc (Zn), lead (Pb), Polycyclic Aromatic Hydrocarbons (PAHs) and mixtures of organic and inorganic pollutants (Zn-PAH and Pb-PAH) in *in-vitro* assays. The results of the screenings pointed to *Gliomastix* S28RE2 and *Westerdykella* S28RA1 having high capacities of degrading RBBR, while *Acremonium* S76A16, *Aspergillus* S56C4 and the aforementioned *Gliomastix* species showed to be able to produce high quantities of siderophores. Eleven species were chosen to be tested in the tolerance tests on Zn and PAHs, while four strains isolated from a Pb-contaminated sample were exposed to Pb and PAHs. Overall, *Penicillium* S56C6 showed the best results in both test conditions, retaining more than 70% of its growth rate when compared with control, while *Mucor* S56E4 showed no change in growth rate, but suffered a loss in mycelium density. To conclude, several strains isolated from this decommissioned military site showed promising potentialities for possible application in bioremediation and further studies are currently underway to develop microbial consortia to enhance their performances.

8 = Quantitative 1 T MRI Post-Harvest Surveying of Tuber Aestivum Ascomata

Alessia Marino[a], Simone Bianchi[a], Marco Leonardi[a], Alessandra Zambonelli[b], Marcello Alecci[a,c,d], Mirco Iotti[a], Angelo Galante[a,c,d]
[a]Department of Life, Health and Environmental Sciences, University of L'Aquila, 67100 L'Aquila, Italy; [b]Department of Agricultural and Food Sciences, University of Bologna, 40127 Bologna, Italy; [c]National Institute for Nuclear Physics, Gran Sasso National Laboratory, 67100 L'Aquila. Italy; [d]CNR-SPIN Institute, c/o Department of Physical and Chemical Sciences, 67100 L'Aquila, Italy
E-mail: alessia.marino2@graduate.univaq.it

Truffles in the genus *Tuber* are ectomycorrhizal fungi that form hypogeous ascomata of high economic and culinary value. As well as for other mushrooms, water represents the main freshness factor of the truffle and affects all biotic and biochemical activities inside it. Magnetic Resonance Imaging (MRI) is an effective tools for evaluating both water status and water dynamics in many foodstuff without damaging them. The first (and unique) MRI study of truffle morphology was made by Pacioni & colleagues who observed the changes of internal structure of *Tuber melanosporum* ascomata during the post-harvest storage. However, so far, a quantitative analysis of the MRI parameters of truffles has never been carried out. The aim of our work was to map and quantify non-destructively, by means of 1 T quantitative MRI, free water content and its changes over 3 weeks post harvest in injured and unijured *T. aestivum* ascomata stored in two different cooling systems. We also performed an estimation of the volume and residual mass (total mass minus the water mass) of ascomata from the available MRI measurements. The MRI data showed that mass and volume of ascomata followed a logarithmic decrease throughout the 16 days of investigation. The fan forced cooling systems and the presence of wounds in the peridium significantly accelerated the mass and volume losses of ascomata. Free water content decreased linearly (daily loss 1.7 %) over the period investigation and no significant differences were found between uninjured ascomata stored in fan-forced or static fridges. On the contrary, storage of injured ascomata in the fan-forced fridge induced significantly more free water losses (day 11 to day 18) compared to uninjured ones. In conclusion, our results demonstrate that 1 T quantitative MRI is an effective post-harvest monitoring tool for assessing the freshness of valuable truffles without affecting their integrity. The estimated residual mass remained between 3.4 and 4.5 g throughout the period of MRI investigation regarless the experimental conditions. The consistency of the measured residual mass over the period MRI investigation demonstrated that, within the sensitivity of the experimental approach, the ascomata loss in mass and volume is almost exclusively due to dehydration, a process not affected by integrity of peridium and storage conditions. Our results demonstrate that 1 T quantitative MRI is an effective post-harvest monitoring tool for assessing the freshness of valuable truffles without affecting their integrity.

8 = Nigerian rhizospheric *Trichoderma* strains against *Fusarium* phytopathogens: characterization and in vitro antagonistic potential assessment

Lidia Nicola[1], Olumayowa Mary Olowe[1, 2]
[1]Laboratory of Mycology, Department of Earth & Environmental Sciences, University of Pavia, 27100 Pavia, Italy; [2]Food Security and Safety Niche Area, Faculty of Natural and Agricultural Sciences, North-West University, Private Bag X2046, Mmabatho, 2735, South Africa

Since *Trichoderma* species often show powerful antifungal and hyperparasitic activities, seven Nigerian strains were molecularly characterized and their antagonistic potential against pathogenic *Fusarium* strains was investigated. The *Trichoderma* strains were isolated in Nigeria from the rhizosphere of maize (TMZ1, TMZ2), banana (TMS1), and cassava (TC4, TC6. TC7, and TC8). Four *Fusarium* strains were isolated in Italy from diseased oak leaves (F2B and F3) and rice plants (Fus296 and Fus294). The inhibitory effects of *Trichoderma* strains against *Fusarium* species were evaluated using dual and filtrates culture techniques while the mycoparasitic relationships were studied using slide cultures. Moreover, *Trichoderma* and *Fusarium* isolates were characterized using molecular ITS-amplification and sequencing. The results showed *Trichoderma* strains TMZ1 and TMZ2 as the most antagonistic against F2B (inhibition percentage 46.67% and 50.00%), Fus296 (67.34%, 65.38%), Fus294 (59.27%, 55.69%), and F3 (20.09%, 21.84%) respectively. On the other hand, the culture filtrates of *Trichoderma* strains TC6, TC7, and TMS1 had the most significant inhibiting action against the pathogens (in the order F3 > F2B > Fus296 > Fus294). Mycoparasitic activity was observed for all *Trichoderma* isolates in the slide cultures with the *Fusarium* pathogens. Furthermore, the *Trichoderma* species were molecularly identified as *Trichoderma virens* strain 1 (TC4), *T. virens* 2 (TC7), *T. virens* 3 (TMS1), *T. hazanium* 1 (TC6), *T. hazanium* 2 (TC8), *T. erinaceum* (TMZ1), and *T. koningiopsis* (TMZ2), while the *Fusarium* pathogens were identified as *Fusarium proliferatum* (Fus296), *F. proliferatum* (Fus294), and *F. tricinctum* (F2B). In conclusion, all the *Trichoderma* strains analyzed in this work expressed varying levels of antagonism against four different *Fusarium* pathogens *in vitro* and represent very promising candidates as anti-fungal agents. Further studies are needed to explore their interactive potential as broad-spectrum bio-fungicides.

8 = Metabarcoding of fungal assemblages in the *Vaccinium myrtillus* endosphere suggests colonization of above-ground plant organs by some ericoid mycorrhizal and DSE fungi

Silvia Perotto[1], Samuele Voyron[1,2], Elena Martino[1], Stefania Daghino[2]
[1]Department of Life Science and Systems Biology, University of Torino, V. le Mattioli 25, 10125, Torino, Italy; [2]Institute for Sustainable Plant Protection, CNR, Strada delle Cacce 73, 10135 Torino and V. le Mattioli 25, 10125 Torino, Italy

External surfaces and internal tissues of plants are colonized by a highly diverse and finely structured microbial assembly, the microbiota, each plant compartment usually representing a unique ecological niche hosting a distinct microbial community. Niche differentiation, which may mirror distinct functions of a specialized microbiota, has been mainly investigated for bacteria, whereas less is known for the fungal components of the plant-associated microbiota.
Here, we applied a metabarcoding approach to describe the fungal assemblages associated with internal tissues of different organs of *Vaccinium myrtillus* (Ericaceae) plants collected in the field, and identified specific taxa that were enriched in roots, stems, leaves and flowers (Figure 1). We also traced the distribution of some important fungi associated with plants of the family Ericaceae, namely the ericoid mycorrhizal (ErM) fungi and the Dark Septate Endophytes (DSE), both playing important roles in plant growth and health. Operational Taxonomic Units attributed to established ErM fungal species in the genera *Hyaloscypha* and to the DSE species *Phialocephala fortinii* were found in all the plant organs. Although mycorrhizal fungi are thought to be strictly associated with the plant roots, this first observation of ErM fungi in the above-ground organs of the host plant may reflect the evolutionary closeness of these fungi in the genus *Hyaloscypha* with non-mycorrhizal fungal endophytes, also witnessed by the closer similarities of the ErM fungal sequenced genomes with the genomes of plant endophytes than with those of other mycorrhizal fungi, such as arbuscular mycorrhizal fungi.

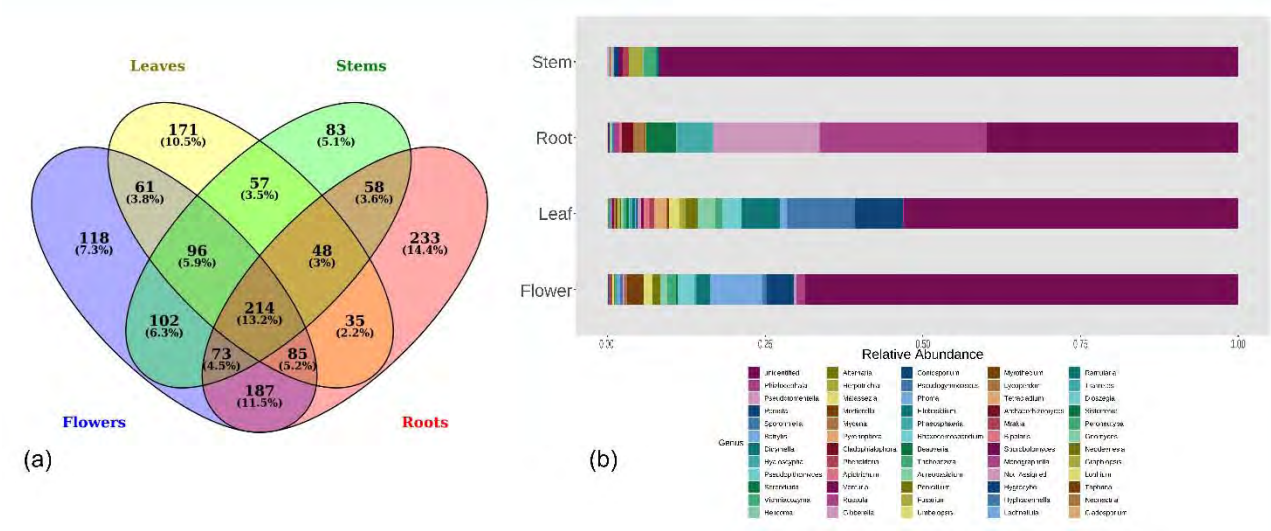


Fig. 1. OTU distribution in the different samples and fungal genera in the core OTUs. a) Venn diagram of the OTUs from the different plant organs; b) relative abundance of the genera within the 214 core OTUs.

8 = Monitoring and innovative approaches applied in *T. magnatum* production areas

Mara Rondolini[1], Nicola Baldoni[1], Leonardo Baciarelli Falini[1], Federica Bonini[1], Daniela Gigante[1], Lara Reale[1], Gilberto Bragato[2], Domizia Donnini[1]
[1]Dipartimento di Scienze Agrarie, Alimentari e Ambientali, Università degli Studi di Perugia, Borgo XX Giugno 74, 06121 Perugia, Italia; [2]CREA - Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Via della Navicella 2/4, 00184 Roma, Italia

In Italy, the areas of natural production of precious truffles are undergoing a strong decline with serious socio-economic and environmental consequences. It is necessary to identify natural truffle management practices capable of encouraging, increasing, and safeguarding the truffle resource and its growing environment, with particular regard to the conservation of germplasm and the proper management of the environment.
The white truffle (*Tuber magnatum* Picco) is mainly found in the bottom of the valleys, along the watercourses, and in the alluvial plains. These are environments involved in an important reduction in the coverage of tree species over time: the symbiont plants have been partially eliminated for other uses of the soil and replaced by non-symbiont shrub species. The singular equilibrium that establishes *T. magnatum* in its natural habitat, together with poor knowledge of its biology, are the reasons why its cultivation is still difficult. This balance requires constant environmental monitoring before any action is taken. Therefore, the aim of this study is to carry out continuous monitoring that allows the analysis and monitoring of natural truffles, identify specific indicators of productive truffles, design interventions to expand the truffle ground, and restore any areas no longer productive. We start, therefore, from an accurate vegetational floristic analysis, from the study of the environmental parameters of climate and soil, and research on the available knowledge about the possible previous and current productivity of each site considered in central Italy. The pedoclimatic data, obtained thanks to the weather stations, the detailed surveys of the soil, the analysis of its microbiome, and also the floristic characteristics are the most important factors involved in the development of carpophores, can be identified, also about previous studies. One of the first interventions to be implemented is clearing the wood underground: a useful practice to promote the development of young symbiont plants and support their vigor. To protect the environmental resource of the habitat of *T. magnatum*, it is necessary to operate through the sustainable use of soil and water, given the increasingly scarce rainfall, carrying out interventions such as the collection and reuse of rainwater, keeping the environment cool and humid. A further aspect of sustainable natural truffle cultivation is guaranteed by the use of local germplasm, both plant, and truffle, for the preparation of truffle seedlings, to be planted on-site, and the inoculum to be distributed. The response to management techniques is estimated by the production of carpophores and the evaluation of the mycelial component in the soil.

8 = Exploitation of fungi in biomining

Vezzola Michele, Solveig Tosi
Department of Earth and Environmental Sciences, University of Pavia, Laboratory of Mycology, via S. Epifanio 14, 27100 Pavia, Italy

The world is facing exponential population growth accompanied by an ever-increasing demand for raw materials, among which metals are of notable importance. The need to extract an ever-increasing amount of metals and the attention to environmental issues that has been developing in recent years has led metallurgical industries to turn to new extraction technologies to maintain the same production levels, access previously untapped reserves, and reduce energy and environmental impacts.
Biomining is one such new technique that is being studied for its industrial and economic relevance and for its low environmental impact. Indeed, this technique uses microorganisms that are involved in metal leaching from ores. These microorganisms have a low energy demand, and the leaching process does not create pollutants typical of pyrometallurgy and hydrometallurgy technologies.
Regarding biomining techniques, bacteria are already being used to extract copper and gold from low-grade ores in some mines in South America. These mines started mining with microorganisms because ores rich in metallic elements were almost finished and intervening on low-grade ores with bacteria proved to be just as effective but cheaper. For this application, chemo-lithotrophic bacteria are involved, but they need ores with metals present in a sulfhydryl form.
Fungi can also play a significant role in metal recovery with a technique that is properly called bioleaching. The leaching process by fungi is possible by acids that some species of fungi produce and the main reaction is called acidolysis. This reaction involves a metal oxide and thanks to the protons that are present in an acid matrix, the binding between metal and oxygen is broken. In this way the metal is solubilized in the solution and water is formed.
Fungal biomining in addition to acidolysis relies on processes such as complexation, bioaccumulation, and bioabsorption. All of these contribute to leaching the metal from the rock and keeping it in solution or sequestering it on the mycelium or in the fungal cell.
Unlike most bacteria so far used in biomining, fungi are heterotrophic organisms that require a source of organic carbon for their activity. This aspect presents both a problem and an opportunity, as it allows to recover metals from non-sulfide ores such as oxide, carbonate, and silicate ores (which is not possible with the current available bacterial leaching technology) but introduces the management of the often expensive or poorly available nutrients that fungi use for their growth.
The use of fungi has also advantages such as: simplicity, low operating costs required, minimal energy input, reduced need for skilled labor, environmental friendliness and minimal capital cost. Therefore, bioleaching produces minimal effluent, greatly reducing pollution and greenhouse gas emissions.
This biomining process, because of its cost-effectiveness, could provide an opportunity to extract metallic elements from rocks and other substrates from human activity (such as solid waste, city wastewater, electronical waste, ash produced by waste disposal activities, and so on) that to date are not used and are discarded because they do not contain sufficient elements for traditional metallurgical extraction.
Finally, fungi are not yet exploited on an industrial scale, although several laboratory studies demonstrate the effectiveness of the fungal acidolysis process. The main problem with the exploitation of fungi is related to the need to provide sufficient organic carbon and the careful control of growth conditions, so that these microorganisms maintain a high production of organic acid over time.
To overcome these problems and take advantage of biomining-based extraction, future challenges will be to find out (or genetically engineer) fungi capable of producing organic acids under different growing conditions and to find a carbon source that is adequate to sustain the microorganisms, but at the same time low-cost, locally available and with minimal environmental impact. The use of agricultural wastes is encouraged because it recycles organic wastes thus reducing the environmental pollution and minimizing the cost at the same time; the best substrates suitable for commercial application should be characterized by high efficiency in metal recovery, low cost of raw material, and constant supply for industrial demand.
Our biomining laboratory has very recently launched a research study to test the feasibility of the biomining process. the purpose of the research work consists of selecting new fungal strains capable of producing acids for the bioleaching process and, at the same time, investigating new culture media that are suitable to produce acids by fungi but are cheap and environmentally friendly. With a focus on innovation, biomining work has been started on simulants of Martian rocks.

9 = Different seasonal collections of *Ficus carica* L. leaves diversely modulates lipid metabolism and adipogenesis in 3T3-L1 adipocytes

Giulia Abate[1*], Manuela Mandrone[2*], Mariachiara Pucci[1], Ilaria Chiocchio[2], Eileen Mac Sweeney[1], Emanuela Tirelli[1], Daniela Uberti[1], Maurizio Memo[1] Ferruccio Poli[2#], Andrea Mastinu[1#]
[1]Department of Molecular and Translational Medicine, Division of Pharmacology, University of Brescia, 25123 Brescia, Italy; [2]University of Bologna, Department of Pharmacy and Biotechnology (FaBiT), Via Irnerio 42, 40126, Bologna, Italy

Background: Due to the high prevalence of obesity and type 2 diabetes, adipogenesis dysfunction and metabolic disorders are common features in the elderly population. Thus, the identification of novel compounds with anti-adipogenic and lipolytic effects is highly desired to reduce the complications of diabetes. Plants are an important source of bioactive compounds. To date, the antidiabetic potential of several traditional plants has been reported, among which *Ficus carica* L. is one of the most promising. Considering that the plant metabolome varies in response to a number of factors, the purpose of this study was to evaluate whether seasonality could influence the biological potential of *Ficus carica* L. extracts.
For this purpose, *Ficus carica* leaves were collected in autumn (FCa) and spring (FCs), and their extracts were characterised by NMR and then tested on 3T3-L1 adipocytes to test their effects on lipid content and modulation of key adipogenic genes.
Results: ^{1}H-NMR profiling of the extracts showed FCs had a higher content of caffeic acid derivatives, glucose, and sucrose than FCa. In contrast, FCa showed higher content of malic acid and furanocoumarins, identified as psoralen and bergapten. *In vitro* assays showed that only FCa treatments were able to negatively regulate lipid content, adipogenesis transcriptional pathway, and insulin sensitivity in 3T3-L1 adipocytes.
Conclusions: This study further supports *F. carica* leaf extract as an optimal source of bioactive compounds to be included in personalised nutritional interventions for targeting obesity and diabetes. However, seasonality was found to have an opposite impact on the effects of *F. carica*, strongly supporting the need for phytochemical-based quality control on products based on *F. carica* leaves and used for medicinal purposes.

9 = Phenolic content and potential health promoting properties of *Eriobotrya japonica* (Thunb.) Lindl. (Rosaceae) extracts

Rosaria Acquaviva[1,2], Barbara Tomasello[1,2], Claudia Di Giacomo[1,2], Simone Bianchi[1], Alfonsina La Mantia[1], Francesco Maria Raimondo[3], Salvatore Ragusa[3], Giulia Ciarcià[1], Giuseppe Antonio Malfa[1,2]
[1]Dept. of Drug and Health Sciences, Section of Biochemistry, University of Catania, Italy; [2]Research Centre on Nutraceuticals and Health Products (CERNUT), University of Catania, 95125 Catania, Italy; [3]PLANTA/Center for Research Documentation and Training, Palermo, Italy

Eriobotrya japonica (Thunb.) Lindl., known as "loquat", belongs to the Rosaceae family. It is a small evergreen shrub or small tree native to China, which can reach 5-6 m in height. It has dark bark, sinuous branches, and a rounded crown, often open like an umbrella. The leaves are very large, 25-30 cm long and 8-10 cm broad, oval, leathery, dark green on the upper surface and have a lighter color under the surface. It has been cultivated in more than 30 countries around the world, including Japan, Turkey, Brazil, Spain, India, Pakistan, Israel, and Italy. This species is grown commercially for its yellow or orange fruit, where the different amounts of carotenoids cause the fruit color and the nutritional differences.
Loquat is a plant with high medicinal value and several organs of this plant have historically been used as folk medicines; in fact, it has been recorded in Chinese history for thousands of years.
Several studies report that the extracts of *E. japonica*, rich in bioactive compounds such as vitamins, polyphenols and terpenes, possess significant biological activities that can counteract inflammation, diabetes, cancer, bacterial infection, aging, pain, allergy and other health problems.
In Sicily there are three main cultivars of loquat: *Red loquat, Trabia loquat* and *Sanfilipparo loquat*.
Since little is known about the biological activities of Sicilian loquat, the present research aimed to evaluate the qualitative and quantitative phytochemical profile and some biological effects of *Eriobotrya japonica* (Thunb.) Lindl. leaf and unripe fruit hydroalcoholic extracts, collected at the feet of Volcano Etna in the area of Viagrande (Catania).
In the present research, we evaluated the total phenolic and flavonoid content of the extracts; afterwards we assessed antioxidant activity in a free cell system–and cytotoxicity effect on human colorectal epithelial adenocarcinoma (Caco-2) and fibroblasts (HFF-1) cells.
Total phenolic and flavonoid contents of leaf and unripe fruits extracts were determined spectrophotometrically. Leaves extract showed the higher content of total phenols (174.10 ± 1.03 mg GAE/g), compared to the unripe fruits (101 ± 2.06 mg GAE/g). The same trend was observed for flavonoids with 82.5 mg and 76 mg CA equivalents/g extract for leaves and fruits, respectively. Antioxidant capacity of *Eriobotrya japonica* of leaf and unripe fruits extracts was performed by DPPH test and measuring SOD-like activity.
The extracts showed a good quenching property (DPPH test, IC50=40.32±1.01 µg/ml and 138±2.11 µg/ml for leaf and unripe fruits, respectively) and radical scavenger activity (SOD-like, IC50 = 4.52 ± 0.09 µg/ml and 0.9 ±0.04 for leaf and unripe fruits, respectively).
Leaf and unripe fruit extracts induced a dose-dependent decrease in cell viability of CaCo-2, after 72 h treatment; no toxicity was found for the HFF-1, used as non-cancer, control cells. The cytotoxic activity of the extracts may be due to the altered oxidative/antioxidant imbalance.
The results obtained showed that the extracts have both *in vitro* antioxidant activity and cytotoxic capacity. The data obtained in this study suggest that biological activity is related to differences in active compounds present in the extracts and to oxidative/antioxidant imbalance they can cause in the cellular system.
These results confirm the health-promoting properties of *Eriobotrya japonica* and its potential as a nutraceutical source and chemotherapeutic/chemopreventive agent.

9 = Chemical composition, agronomic traits of *Allium sativum* and *Allium ampeloprasum* leaves and bulbs, and their action against *Listeria monocytogenes* and other food pathogens

Giuseppe Amato[2], Flavio Polito[1], Lucia Caputo[1], Vincenzo De Feo[1,2], Florinda Fratianni[2], Vincenzo Candido[3], Filomena Nazzaro[2]

[1]Department of Pharmacy, University of Salerno, Via Giovanni Paolo II, 132, 84084 Fisciano (Salerno), Italy; fpolito@unisa.it (F.P.); lcaputo@unisa.it (L. C.); defeo@unisa.it (V.D.F.); [2]Institute of Food Science, National Research Council, Via Roma 64, 83100, Avellino, Italy. Email: filomena.nazzaro@isa.cnr.it (F.N.); florinda.fratianni@isa.cnr.it (F.F.) giuseppe.amato119@gmail.com (G.A.) ; [3]Department of European and Mediterranean Culture, University of Basilicata, Via San Biagio, 75100 Matera, Italy; Email: vincenzo.candido@unibas.it

The term *Allium* identifies a very large genus of monocotyledonous plants, including about 700 species, organized into 15 subgenera and 72 sections. The subgenus *Allium* is the largest, including economically important species, such as garlic (*A. sativum* L.) and leek (*A. ampeloprasum* L.). They are employed firstly as a condiment, but also for therapeutic purposes, due to the properties attributed to them by scientific investigation, such as antihypertensive, anthelminthic, antioxidant, antithrombotic, antibiotic, antiseptic, and balsamic. In folk medicine they are used to promote digestion and treat malfunctioning of the intestines, reduce blood pressure, help in dissolving kidney stones, prevent cramps and colds, help lowering cholesterol, and decongest the respiratory tract. Over the past 50 years, intense research evaluated the biological activities of the essential oils (EOs) of the genus *Allium*, due to the presence of organosulfur compounds, including allicin. In the last years, the rising occurrence of foodborne diseases has been correlated to an expansion of the presence, in foods, of some pathogenic bacteria, such as *Listeria monocytogenes*, *Escherichia coli*, *Pseudomonas aeruginosa* and the emergent pathogen *Acinobacter baumannii*, often with the capacity to exhibit the multiple-drug resistant phenotype and the ability to form biofilms which have an important function in infection and bacterial resistance. The chemical composition of EOs of both bulbs and aerial parts of two cultivars of *A. sativum* and two cultivars of *A. ampeloprasum* was studied by GC and GC-MS. Results agree with the literature which showed that sulfur compounds, first allicine, diallyl disulfide, dipropyl disulfide, diallyl trisulfide, and dipropyl trisulfide, were the main constituents. Qualitative and quantitative differences between the components are evident both between the species and between the cultivars of the same species and between the studied part of the same plant. The capacity of the EOs to inhibit the bacterial biofilm formation and the metabolism of the bacterial cells within biofilm was assessed through the crystal violet and MTT tests, respectively. Crystal violet test showed that all the EOs from *A. sativum* were generally able to inhibit the formation of biofilm of almost all bacterial strains considered, particularly *A. baumannii* and *L. monocytogenes*. The two cultivars of *A. ampeloprasum* were able to inhibit the formation of bacterial biofilms with varying effectiveness, particularly on *S. aureus*. Instead, the MTT test showed how the EOs from *A. ampeloprasum* were overall able to act on the metabolism of the microbial cells present within the biofilm. The two EOs of *A. ampeloprasum* were able to fight the pathogenicity of these four microbial strains, either by acting on the formation of the biofilm or by inhibiting bacterial metabolism. This was also observed with the EOs of *A. sativum* but with less intensity. The EOs, even if in different efficacy, demonstrated their capability to act against the formation of a new biofilm that is a key step in the increase of virulence for pathogenic bacteria, mainly for *L. monocytogenes*. The results suggest the possibility to use these EOs as potential preserving agents in food manufacturing, for example in fermented meats, where the taste and smell of both *Allium* EOs, used as ingredients at the right concentrations, do not have a negative effect from a sensorial point of view and can safeguard the products without affecting their quality.

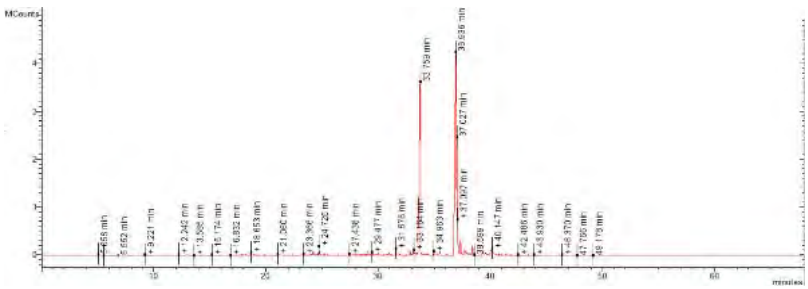


Fig.1. Chromatogram of EOs of *Allium*

9 = Effect of *Gentiana lutea* L. bitter compounds on enteroendocrine secretion as novel strategy against obesity

Nadia Benedetto[1], Maria Ponticelli[1], Ludovica Lela[1], Immacolata Faraone[1,2], Daniela Russo[1,2], Mariapia Moles[1], Luigi Milella[1]
[1]Department of Sciences, 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

Gentiana lutea L. has antioxidant, antifungal, anti-inflammatory, and immunomodulatory properties, but its roots are a significant source of bitter molecules, mainly secoiridoid glycosides, as amarogentin and gentiopicrin.
Overweight and obesity are considered as an abnormal or excessive fat accumulation and nowadays, it is becoming a serious global health problem, among children and adolescents too. It is often associated with inflammatory state and metabolic disorders as hyperlipidemia, diabetes, and cardiovascular diseases.
Recently, natural compounds have received increasing attention due to their capacity to reduce food intake and improve human health. It was reported that bitter substances stimulate the gastrointestinal (GI) hormone secretion and slow gastric emptying by interaction with bitter taste receptors located in the GI tract. Gastrointestinal satiety hormones, secreted from enteroendocrine cells, such as cholecystokinin (CCK), glucagon-like peptide 1 (GLP-1), and peptide YY (PYY) play an important role in suppressing hunger and food intake reduction. This novel approach could contribute to the prevention and management of obesity and related co-morbidities in association with the existing pharmacological therapies.
The intestinal secretin tumor cell line (STC-1) with many features of native intestinal enteroendocrine cells are commonly used for the screening of foods or compounds that modulate the secretion of gastrointestinal hormones by *in vitro* system.
In this study, bitter phytocomplex from *G. lutea* roots reported no toxic effect on STC-1 cell line after 2 and 24 h measured by 3-[4,5-dimethylthiazol-2-yl]-2,5 diphenyl tetrazolium bromide (MTT) assay (10-500 µg/mL). ELISA assay was used for determining the release of gastrointestinal satiety hormones in STC-1 cells and preliminary results showed that the bitter *G. lutea* extract significantly increased GLP-1 release in dose (10, 100, 200 µg/mL) and time (30, 60, 120 min)-dependent manner, but not PYY release was observed. At the highest concentration, *G. lutea* increased about 6 times the GPL-1 release in STC-1 cells, contributing to the decrease in gastric emptying and food intake.
The phytochemical profile of *G. lutea* extract was investigated by HPLC-DAD analysis and no significant loss of bitter compounds was observed after *in vitro* digestion assay simulating the digestive process in human beings.
These results confirmed a promising antiobesity effect of *G. lutea* bitter compounds by the increase of the secretion of gastrointestinal satiety hormones.

9 = Biological activities and phytochemical characterization of *Origanum majorana* L. (Lamiaceae) aerial parts extract rich in rosmarinic acid

Simone Bianchi[1], Giuseppe Antonio Malfa[1,2], Barbara Tomasello[1,2], Claudia Di Giacomo[1,2], Alfonsina La Mantia[1], Francesco Maria Raimondo[3], Rosaria Acquaviva[1,2]
[1]Dept. of Drug and Health Sciences, Section of Biochemistry, University of Catania, Italy; [2]Research Centre on Nutraceuticals and Health Products (CERNUT), University of Catania, 95125 Catania, Italy [3]PLANTA/Center for Research Documentation and Training, Palermo, Italy

The Lamiaceae is a large family, composed of more than 240 genera, made up of mostly herbaceous or suffruticose plants of Mediterranean origin and characterized by external glandular structures secreting essential oils, which make these plants widely used as cooking spices and medicinal herbs from ancient times. Varieties in the Lamiaceae family are characterized by a specific group of secondary metabolites, such as terpenes and polyphenols, and their composition and concentration vary greatly among species. Although many studies have been conducted, a comprehensive bio-systematic and phytochemical survey of wild plant in the Mediterranean, in particular in Sicily, and a screening of the potential biological activities of their secondary metabolites are still scarce.
The characteristic essential oils of Lamiaceae are mainly constituted of terpenes and are endowed with various activities. The therapeutic potential of these plants, however, is not only due to the presence of essential oils, but also to their polyphenol content, with rosmarinic acid as the most responsible for the beneficial effects on human health. This compound was identified by two Italian researchers in 1958 and took its name from the plant from which it was isolated. Chemically, it is an ester between caffeic acid and 3-(3,4- dihydroxyphenyl)lactic acid and its biosynthesis takes place starting from the amino acids L-phenylalanine and L-tyrosine, with the involvement of eight enzymes. In plants, this molecule has defensive activity, and this is highlighted by the fact that its production increases following exposure to fungal elicitors.
Rosmarinic acid has many interesting activities useful to human health: antioxidant, anti-inflammatory by the inhibition of lipoxygenase, cyclooxygenase, the complementary cascade and pro-inflammatory mediators, antiviral, antibacterial, astringent, analgesic, antidiabetic by inhibiting sugar digestive enzymes and increasing insulin sensitivity, hepatoprotective by inhibiting fibrosis and scavenging different reactive radical species, immunomodulatory by the stimulation of the immune system cells, anticancer, cardioprotective and neuroprotective.
The aim of this research was to evaluate the content of rosmarinic acid in the main Lamiaceae species used for food purposes, in order to find the one with the highest amount of this compound. From data obtained with HPLC-DAD, we found that Marjoram (*Origanum majorana* L.) was the richest in rosmarinic acid compared to the other plants examined. Then, we prepared a hydroalcoholic extract from Marjoram and we dosed its polyphenolic (245,47±5,95 mg of gallic acid equivalents/g of extract) and flavonoid (159,26±6,84 mg of catechin equivalents/g of extract) content, respectively, with the Folin-Ciocalteu and aluminium chloride methods. To test the health properties of the extract, we evaluated its antioxidant power in an *in vitro* cell-free system and its cytotoxic activity on tumour (CaCo-2) and normal (HFF-1) human cell lines treated with different concentrations (from 10 µg/mL to 1 mg/mL) for 48 and 72 h. We used synthetic rosmarinic acid as a control.
The obtained results demonstrated that the extract is rich in phenolic compounds which can exert a good antioxidant activity as shown by DPPH test and SOD-like activity assay, confirming the strong antioxidant power of both rosmarinic acid (standard) and extract. In fact, the IC_{50} were 30,11±3,46 µg/mL (DPPH) and 0,71±0,06 µg/mL (SOD-Like) for the extract and 14,8±3,33 µM (DPPH) and 0,34±0,04 µM (SOD-Like) for synthetic rosmarinic acid.
In addition, the extract and the standard induced a significant cytotoxic effect on CaCo-2 cells, with IC_{50} 326±21 µg/mL (72h) and 560±18 µg/mL (48h) for the extract and 243±14 µM (72h) and 321±21 µM (48h) for synthetic rosmarinic acid. Conversely, on non-tumoral cells (IC_{50}= N.D.) only a slight toxic effect was detected on cell viability.

9 = Chemical and biological studies of fruit extracts from different Italian varieties of *Prunus domestica* L. (Rosaceae)

Anna Cacciola[1,2], Emily Cioni[3], Marinella De Leo[3], Maria Paola Germanò[2], Valeria D'Angelo[2], Salvatore Mirabile[1,2], Nunziatina De Tommasi[4], Alessandra Braca[3]
[1]Foundation Prof. Antonio Imbesi, University of Messina, Piazza Pugliatti 1, 98122 Messina, Italy; [2]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Italy, Viale Ferdinando Stagno d'Alcontres 31, 98166 Messina, Italy; [3]Department of Pharmacy, University of Pisa, Via Bonanno 33, 56126 Pisa, Italy; [4]Department of Pharmacy, University of Salerno, Via Giovanni Paolo II 132, 84084 Fisciano (SA), Italy

Prunus domestica L. (Rosaceae), also known as the European plum, is a very ancient tree that was discovered near the Caspian Sea about 2000 years ago. Nowadays, *P. domestica* is one of the most important species for its fruit market reaching more than 10 million tons a year in the world. The plums are drupaceous fruits that are consumed fresh, canned, or dried (prunes), as well as for jam, jelly, and juice production. Recently, plum production has increased due to the growing interest in the health benefits related with the phytochemical content, predominated by flavonoids and phenolic acids showing antioxidant properties and potential activity in the prevention of cancer and chronic diseases. Therefore, as the plums are considered promising healthy agents, the aim of the present work was the chemical and biological investigation of some typical Italian *P. domestica* varieties through comparison with the commercial variety Sangue di Drago.
The fruits of varieties Rossa Casa Velasco (RCV), Formosa (F), Rusticano (R), and Sangue di Drago (SD) were selected and collected at the ripening stage. Then, fresh fruits were extracted with ethanol-water (70% v/v) by dynamic maceration for three consecutive days at room temperature, changing the solvent every 24 h. The dried extracts were firstly screened for the determination of their polyphenolic content and successively their chemical composition was investigated by means of ultra-high performance liquid chromatography coupled to a high resolution Orbitrap-based mass spectrometer equipped with an electrospray ionization source (UHPLC-HR-ESI-Orbitrap/MS). Results evidenced that among the extracts, RCV has the highest content of polyphenols (110.28 mg GAE/g of extract). The UHPLC-MS analyses showed the presence of hydroxycinnamic acids, with chlorogenic acid the most represented, flavonol glycosides, mainly quercetin derivatives, and procyanidins. Interestingly, some components are more expressed in the local varieties rather than in the commercial ones. Among anthocyanins, cyanidin, petunidin, and delphinidin derivatives were found with delphinidin glucoside the most represented, especially in the RCV variety. Successively, the biological activity of the extracts, evaluating their *in vivo* effects on angiogenesis using the chick embryo chorioallantoic membrane (CAM) model, was explored. The extracts were also investigated for their *in vitro* activities by DPPH and ABTS scavenging, ferric reducing (FRAP), and denaturation of bovine serum albumin (BSA). Results showed that all the extracts exhibited a significant antiangiogenic response inducing a marked reduction of the microvasculature in the CAMs (34.44-50.55% of inhibition at 100 and 200 µg/egg) as compared to control. Regarding the antioxidant properties, the extracts showed a strong concentration-dependent activity. Interestingly, RCV has the most notable efficacy in all the antioxidant assays. Furthermore, the extracts demonstrated a promising anti-inflammatory activity (96.01-98.73% of inhibition at 500 µg/mL) due to their high protective effects on thermally induced denaturation of BSA.
In conclusion, the plum extracts presented here can be considered a source of potential nutraceutical agents or functional food components that could reduce the risks of oxidative stress-related disorders.

9= Ethnobotany and wound healing: from tradition to integrated models for the study of poorly investigated species

Giorgio Cappellucci, Giulia Baini, Federica Vaccaro, Elisabetta Miraldi, Marco Biagi
Department of Physical Sciences, Earth and Environment, University of Siena, Via Laterina 8, 53100 Siena, Italy

Ethnobotany and traditional uses of medicinal plants have always been an important starting point and a great source of inspiration for approaching the study of new species, but to date, it is still difficult to translate information from an empirical science such as ethnobotany to clinical practice. Therefore, methods to fill this gap are needed.
The aim of one large project most recently started in our research group is to connect ethnobotanical survey to a rapid and effective pre-clinical experimental protocol that could comprise phytochemical investigations, bio-guided extracts selection, and/or active molecules purification, assisted by rapid *in vitro* cell-free assays and network pharmacology analysis, pharmacokinetic predictions and, finally, cell-based tests consisting in the evaluation of a panel of molecular targets.
In the very first part of the project, starting from ethnobotanical investigations in Tuscany (Italy), we are developing and validating methods to test the skin healing activity of three few investigated botanical species, namely *Calluna vulgaris* (L.) Hull. "CVE", *Arum italicum* Mill. "ARI" and *Sedum telephium* L. "SED". As a result of a survey in collaboration with the University of São Paulo (Brazil) we have also included in the first set of candidates green propolis from *Baccharis* spp., (known as Brazilian green propolis) "PGB".
Combining colorimetric and HPLC-DAD methods, the first phytochemical characterization has shown that CVE flowering tops and SED leaves are enriched in soluble polysaccharides and glycosylated flavonols; CVE also contains hydroxycinnamic derivatives, whereas SED glycoproteins; ARI leaves contain lignans, flavonoids, cyanogenetic compounds and mineral salts. PGB is enriched in terpenes, flavonoids, artepillin C and other prenylphenols. Preliminary network pharmacology analyses have shown that a plethora of targets related to skin diseases could be triggered in different extent by polyphenols of species under investigation suggesting at first to test the entire phytocomplex.
In vitro tests, using human immortalized keratinocytes (HaCaT) and fibroblasts (HFF-1) as cell lines models, have confirmed that the fresh juice of SED and the water extract of CVE, where polysaccharides and polyphenols are combined as well as the ethanolic extract of PGB, that provides the highest yield of polyphenols and terpenes, exert the highest activity in ameliorating cell viability, both at rapid and long times of exposure. ARI has shown a low safety index, as effective concentrations in improving cell viability and toxic ones are close.
Knowing that wound healing is promoted by growth factors such as EGF (epidermal growth factor), FGF (fibroblast growth factor) and KGF (keratinocytes growth factor), and a non-specific increase of cell metabolism, ELISA dosages have been assessed in order to better investigate the healing mechanism. SED fresh juice has shown to mainly increase collagen I synthesis in human fibroblasts, whereas CVE water extract and PGB ethanolic extract mainly act by regulating cytokines release in human keratinocytes.
These data, obtained in a short time, are already moving us towards the formulation of prototypes that could be more deeply investigated.
These first data obtained on the study of wound healing activity of some selected natural products lead us to confirm the importance of the application of a rational and multi-step experimental protocol in the screening and study of species from ethnobotany tradition.

9 = Chemical composition, antibiofilm, cytotoxic, and anti-acetylcholinesterase activities of *Myrtus communis* L. leaves essential oil

Lucia Caputo[1], Francesca Capozzolo[1], Giuseppe Amato[1], Vincenzo De Feo[1], Florinda Fratianni[2], Giovanni Vivenzio[1], Laura De Martino[1], Filomena Nazzaro[2]
[1]Department of Pharmacy, University of Salerno, Via Giovanni Paolo II 139,84084 Fisciano (Salerno), Italy; [2]Institute of Food Science, CNR-ISA, via Roma 64,83100 Avellino, Italy

The potential of essential oils (EOs) and their principal constituents for eradication of biofilm and at the same time the research of new potential acetylcholinesterase inhibitors is gaining increasing interest in recent years. The aims of this study were to determine the chemical composition and to evaluate the antibacterial activity, the human neuroblastoma cells growth inhibition, and anti-acetylcholinesterase property of *Myrtus communis* L. (myrtle) leaves essential oil and its main constituents. The essential oil was obtained by hydrodistillation of *M. communis* L. leaves and was analysed by GC and GC–MS. The antimicrobial activity was carried out against both gram-negative and gram-positive bacteria. The microdilution method was used to estimate the minimum inhibitory concentrations (MICs). The capacity of the EOs and its main constituent to block or limit the formation of biofilm (using the crystal violet assay) and the effect of the EOs on the bacterial cell metabolism (observed by the MTT test) were also evaluated. Moreover, the potential human neuroblastoma cells (SH-SY5Y) growth inhibition (with MTT assay) and the anti-acetylcholinesterase activity (using Ellman's assay) were studied. Myrtenyl-acetate, 1,8 cineole, α-pinene, and linalool were the main components in the EO. The myrtle EO, at the minimum tested dose (0.4 mg/ml), inhibited the formation of *S. aureus* biofilm by 42.1% and the biofilm cell metabolism in all tested strains, except *Staphylococcus aureus*. Moreover, the EO showed good SH-SY5Y cells growth inhibition and anti-acetylcholinesterase activities, with IC_{50} of 209.1 and 32.8 μg/ml, respectively. The results suggest that myrtle EO and its main constituents could be used as possible products that could act against the resistant pathogenic species *E. coli*, *P. aeruginosa*, *L. monocytogenes,* and *S. aureus*, on the other hand, as possible coadjutants in the treatment of neurological diseases.

9 = Qualitative Chemical Characterization and Multidisciplinary Biological Investigation of Different Root and Aerial Parts Extracts from *Epilobium hirsutum*

Simonetta Cristina Di Simone, Alessandra Acquaviva, Maria Loreta Libero, Nilofar, Claudio Ferrante, Luigi Menghini
Department of Pharmacy, Medicinal Plant Unit (MPU), Botanic Garden "Giardino dei Semplici", "G. d'Annunzio" University of Chieti-Pescara, Via dei Vestini, 66100 Chieti, Italy

Medicinal plants have long been used in traditional medicine to treat or prevent diseases in humans and animals. Today, this medicinal use of plants can play a central role to ensure healthy lives and promote well-being. *Epilobium hirsutum* is a widely used plant in phytotherapy, especially as a remedy against prostate inflammation. This multidisciplinary study examines the relationship between chemical profile and biological effects of different extracts (ethyl acetate, methanol, methanol/water, and infusion) from two parts (aerial parts and roots) of *E. hirsutum*.
The chemical characterization of the extracts under study was evaluated through colorimetric assays and high-performance liquid chromatography with electrospray ionization mass spectrometric detection (HPLC-ESI-MS^n) technique. The intrinsic scavenging and enzyme inhibitory properties were investigated through different test systems including free radical scavenging assays (DPPH and ABTS) and phosphomolybdenum, metal chelation, and reducing power approaches (FRAP and CUPRAC). The biocompatibility limits of the extracts under study were determined by the *Artemia salina* (Brine Shrimp) Lethality Test and human prostate cancer PC3 cell line was exposed to the same extracts to assess antiproliferative effects. Finally, after a bioinformatics analysis, the anti-inflammatory effects were studied through the evaluation of the gene expression of different pro-inflammatory biomarkers (TNF-α, COX-2, VEGFA, IL-6, IL-8, NFkB, and TIMP1).
Concerning the quantification of phytochemicals, the compounds found as prominent in the extracts were oenothein B and myricetin. In the *in vitro* antioxidant capacity investigation, the methanol, infusion, and methanol/water extracts exhibited comparable effects. The biological assessment of potential antiproliferative effects on PC3 cells showed a reduction of viability at the highest tested concentration (300 µg/ml) of methanol and methanol/water roots and aerial parts extracts from *E. hirsutum*. In agreement with the bioinformatics analysis, myricetins were predicted to interact with COX-2 and several cytokines, such as TNF-α. The interaction between TNF-α and oxo-dihydroxy-octadecenoic acid was also predicted. The gene expression of COX-2 and TNF-α was significantly inhibited in PC3 cells after exposure to methanol and methanol/water extracts of aerial parts and the methanol/water root extract. The gene expression of IL-8 and NFkB was also decreased, whereas the PGE2 release by PC3 cells, under the same experimental conditions, resulted inhibited.
Therefore, the present findings demonstrate the anti-inflammatory effects of *E. hirsutum* suggesting its potential use as a medicinal plant for the treatment of lower urinary tract diseases, including prostatitis.

9 = Valorization of the essential oils from two Italian hemp crops: a phytochemical and pharmacological evaluation

Antonella Di Sotto[1], Marco Gullì[1], Alessandra Acquaviva[2,3], Massimo Tacchini[4], Simonetta Cristina Di Simone[2], Annalisa Chiavaroli[2], Lucia Recinella[2], Sheila Leone[2], Luigi Brunetti[2], Giustino Orlando[2], Giancarlo Angeles Flores[5], Roberto Venanzoni[5], Paola Angelini[5], Luigi Menghini[2], Claudio Ferrante[2]
[1]Department of Physiology and Pharmacology "V. Erspamer", Sapienza University of Rome, P.le Aldo Moro 5, 00185 Rome, Italy; [2]Department of Pharmacy, Botanic Garden "Giardino dei Semplici", "Gabriele d'Annunzio" University, via dei Vestini 31, 66100 Chieti, Italy; [3]Veridia Italia Srl, Via Raiale 285, 65100 Pescara, Italy; [4]Department of Life Sciences and Biotechnology (SVeB), UR7 Terra&Acqua Tech, University of Ferrara, 44121 Ferrara, Italy; [5]Department of Chemistry, Biology and Biotechnology, University of Perugia, 06100 Perugia, Italy

Industrial hemp (*Cannabis sativa* L.) female inflorescences have long been considered waste material, in the hemp production chain. However, past studies pointed to the valorization of female inflorescences as high-quality byproducts with promising applications as a pharmacological tool. Therefore, in the present study, we investigated the phytochemical and pharmacological properties of the essential oils (EOs) from the inflorescences of two industrial hemp varieties, namely Kompolti and Tisza. The essential oils composition in terpenes and terpenophenols was determined, and the EOs effects on cancer [A549 (non-small cell lung cancer cells), MDA-MB-468 (triple negative breast cancer cells), SHSY-5Y (neuroblastoma cells), HepG2 (hepatoblastoma cells), Mz-ChA-1 (extrahepatic cholangiocarcinoma cells) and Bx-PC3 (pancreas adenocarcinoma cells] and noncancerous [HypoE22 (hypothalamus), BEAS-2B (human bronchial epithelial cells) and H69 (intrahepatic cholangiocytes)] cell viability were investigated. In hypothalamic HypoE22 cells, neurotransmitter levels were also measured, while the EO mycostatic properties were explored towards different dermatophytes species. The prominent terpenes were β-caryophyllene, α-humulene and β-caryophyllene oxide in both Kompolti and Tisza EOs, whereas cannabidiol was the main terpenophenol, in Kompolti. Both essential oils inhibited the viability of different cancer cells; particularly, the essential oil of Tisza variety resulted more tolerable in noncancerous H69 cholangiocytes, despite a marked cytotoxicity in cholangiocarcinoma cells. A possible role of both terpenophenols and caryophyllane sesquiterpenes as bioactive anticancer compounds has been hypothesized. The essential oils also produced antimycotic effects, for which β-caryophyllene oxide could be partly responsible, while cannabidiol could contribute to the stimulation of hypothalamic serotonin release by Kompolti EO.
Overall, the present findings highlight the pharmacological properties of Kompolti and Tisza EOs, which deserve further investigation and strengthen the interest in industrial hemp inflorescences as a valuable source of bioactive extracts and biomolecules.

9 = The microencapsulation of *Calendula arvensis* florets and leaves: a strategy for bioactive compound's preservation

Marika Fiorentino[1*], Claudia Gravina, Marialuisa Formato, Simona Piccolella, Assunta Esposito, Severina Pacifico
[1]Department of Environmental Biological and Pharmaceutical Sciences and Technologies, University of Campania "Luigi Vanvitelli", Via Vivaldi 43, 81100 Caserta, Italy

The Wild Edible Plants (WEPs), defined by the Food and Agriculture Organization as plants growing spontaneously in natural and semi-natural ecosystems without human intervention, for decades have constituted an essential resource of food ingredients for the rural communities, by suppling micro- and macro-nutrients to the dietary requirements as well as bioactive molecules.
Among these, *Calendula arvensis* (Vaill.) L. occupies a relevant place in folk tradition due to the pleasant sensory properties; in fact, their capitula and leaves were employed in salad or soup of local cuisine.
Moreover, previous studies reported the presence of biologically active compounds (e.g. polyphenols) potentially useful for the treatment of diseases affecting gastrointestinal tract.
Unfortunately, phenolic compounds are susceptible to environmental factors (e.g. light, heat, oxygen degradation) and to digestion processes, resulting in a reduction of their antioxidant activity. Thus, the microencapsulation of *Calendula arvensis* polar extracts represents a valuable strategy for the preservation of the integrity and bioactivity of their specialized compounds.
In this study, aerial parts of *Calendula arvensis* (Vaill.) L., harvested in Cilento, Vallo di Diano, Alburni National Park, have been studied. Florets and leaves, divided at harvesting time, immediately placed in liquid nitrogen and subsequently cryo-dried, were extracted in methanol by ultrasound assisted maceration (UAM). Obtained extracts have been encapsulated by freeze dried process in maltodextrin (MD) using the ratio plant extract:coating materials equal to 1:2. The encapsulated extracts were subjected to *in vitro* digestion process. The microstructural analysis of the plant-based materials, the biological activity of encapsulated extracts before and after *in vitro* digestion in cell-free and Caco-2 cell-line systems as well as the analysis of bioavailability with UHPLC-HRMS/MS tools have been carried out to to understand the possible employment of this process to preserve polar extracts *C. arvensis* and their future application in food industry supplements.

9 = Nutraceutical perspectives of industrial hemp: focusing on CBDA

Marialuisa Formato, Maria Tommasina Pecoraro, Simona Piccolella, Severina Pacifico
Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, University of Campania "Luigi Vanvitelli", Via Vivaldi 43, 81100 Caserta, Italy
Email: marialuisa.formato@unicampania.it

Cannabis sativa L. is a plant long used for its textile fibers and seed oil. The renewal interest in industrial hemp arouses deep attention among the scientific community for its therapeutic, cosmeceutical, nutraceutical, and food applications. The potential of hemp, including those parts that appear to be waste from the hemp spinneret, is intrinsic to phytochemistry.
In fact, hemp phytochemistry is very complex, consisting in phytocannabinoids, terpenoids, alkaloids, and polyphenols.
Among the phytocannabinoids, cannabidiolic acid, despite it being the main phytocannabinoid in fiber and seed-oil hemp, is an understudied compound right now. This compound could be easily recovered by industrial hemp processing and by its by-products and wastes, fitting in a scenario of a circular economy. Although for many years cannabidiolic acid (CBDA) has been overshadowed by its neutral derivative cannabidiol (CBD), its anti-inflammatory, anti-emetic, anti-convulsant and anti-cancerogenic activities have been recently investigated. Herein, the recovery of this highly bioactive compound from hemp waste was carried through a very simple, fast, and inexpensive approach employing normal and/or reverse phase thin layer chromatography. In addition to spectroscopic analyses (NMR, UV, IR), electrospray ionization (ESI)-QqTOF-MS/MS tools appeared to provide its rapid discrimination from its constitutional isomers.
CBDA cytotoxicity was evaluated towards keratinocyte HaCaT cells by means MTT, SRB, and LDH release assays. No-toxic CBDA concentrations were screened for their anti-inflammatory efficacy finding that the compound differentially modulated the release of proinflammatory cytokines and chemokines mediators. Furthermore, the antimicrobial activity against Gram (+) and Gram (-) was tested, and the inhibition of biofilm formation was proved against *Staphylococcus aureus* and *Pseudomonas aeruginosa*. Data acquired highlight the unexplored effectiveness of CBDA and other constituents, which make industrial hemp a valuable source of compounds with health properties.

9 = Investigation and valorization of methanolic plant-extracts in cosmeceuticals: a case study of *Lavandula austroapennina* N.G. Passal., Tundis & Upson

Claudia Gravina[1*], Marialuisa Formato, Adriano Stinca, Marika Fiorentino, Simona Piccolella, Severina Pacifico, Assunta Esposito
[1]Department of Environmental Biological and Pharmaceutical Sciences and Technologies, University of Campania Luigi Vanvitelli, Via Vivaldi 43, 81100 Caserta, Italy
* Corresponding author: Claudia Gravina (claudia.gravina@unicampania.it)

Since ancient times, traditional herbal medicine has been used to preserve health or prevent and treat diseases. In fact, Medicinal and Aromatic Plants (MAPs) represent the human's first medicines and still supply more than 80% of the world population, exhibiting potential resources to be used in this field but still little explored today due to their applicative value. In this context, the cosmetic research field actually finds great interest in formulating plant-based products. This is due partly to the growing general concern about the potentially harmful effects of synthetic compounds, and partly to the awareness of responsible consumption of these sustainable products. Thus, there is an increase in demand for plant natural products, and MAPs appear a valuable resource mainly due to their diversity in specialized metabolites with antioxidant, anti-inflammatory, antibacterial, and antiviral activities. Several species belonging to Lamiaceae family are used in traditional as well as complementary medicine, especially in phytotherapy. The therapeutic potential of their EOs can be widely used in perfumery, cosmetics, food processing, and aromatherapy thanks to their antifungal, immune-modulatory and antioxidant activities. However, there is only little information about the medicinal effect of no-EOs extracts. Herein, our attention was focused on *Lavandula austroapennina* N.G. Passal., Tundis & Upson from Cilento, Vallo di Diano and Alburni National Park, a remarkable new lavender of southern Italy that has long been treated as *Lavandula angustifolia* L.. Locally *L. austroapennina*, was named *"spicaddossa",* and known for its long traditional ethnobotanical uses. Our aim was to analyse the chemical composition of the species, especially focusing on polyphenolic compounds in all parts of the plant, and to assess compounds' bioactivity to be exploited in the cosmeceutical research field.
L. austroapennina was harvested, and dissected into corolla, bracts, leaves, stems, and roots. Each plant part was lyophilized, and the obtainment of enriched extracts of polar bioactive compounds was achieved by applying sequential ultrasound-assisted maceration in *n*-hexane and methanol. The chemical composition of the investigated organs was unravelled by Ultra-High-Performance liquid chromatography hyphenated with high-resolution mass spectrometry (UHPLC-HRMS). Subsequentially, a cytotoxic screening of the alcoholic extracts on human keratinocytes (HaCaT) and epithelial (Vero-CCL-81) cells line, in a different treatment time, was achieved. The latter cell line, was also used to assess the antiviral activity, estimating the inhibition of HSV-1 replication, responsible of the common herpes labialis. This research may represent a sustainable approach for the proper use of these unexplored no-EOs plant extracts for cosmeceutical application.

9 = The almond blanching process generates two interesting waste products to be used in the nutraceutical and cosmeceutical field

Mariarosaria Ingegneri, Antonella Smeriglio, Giuseppina Mandalari, Domenico Trombetta
Department of Chemical, Biological, Pharmaceutical and Enviromental Sciences (ChiBioFarAm), University of Messina, Viale Ferdinando Stagno d'Alcontres 31 - 98166 Messina, Italy

This study is part of an innovative PSR project that aims to test and apply a process and product innovation in the almond production chain, through cooperation between agri-food and agricultural companies and the world of research. Specifically, the project aims to enhance the blanched skin (BS) of the Avola almond, which ceases to be a waste material to become a new product to be used in the food and nutraceutical field. Furthermore, the project provides for the recovery of the polyphenols present in the blanching water (BW), which can be used in the nutraceutical and cosmeceutical fields. The recovery of these two waste products will take place through the development and validation of a specific prototype capable of peeling the almond, drying the BS, and treating the BW for the extraction of polyphenols in a standardized way.
Considering this, including the Avola almond (*Prunus dulcis* Mill.) three cultivars (Fascionello, Pizzuta, and Romana), we investigated in the present study, how the peeling process conventionally applied in Sicily, influences the quali-quantitative composition in polyphenols of the two waste products obtained by the Fascionello cultivar, by comparing their phytochemical profile with that of natural almond skin (NS). Preliminary phytochemical screening has already shown a loss in terms of total phenols and flavonoids of the BS (7.29 g GAE/100 g and 35.98 g RE/100 g, respectively) compared to the NS (9.95 g GAE/100 g and 51.04 g RE/100 g, respectively) with part of them found in the BW (2.43 g GAE/100 g and 12.68 g RE/100 g). These results were confirmed by LC-DAD-FLU-ESI-MS analysis, which showed not only a decrease in the total polyphenols content passing from NS (1.11 g/100 g) to BS (0.30 g/100 g) and BW (0.05 g/100 g), but also a different distribution of the different classes of polyphenols identified and quantified, resulting BS the richest one in flavonols and flavanols (40.35% and 36.06%, respectively), and BW in flavonols and phenolic acids (49.97% and 46.40%, respectively). Furthermore, despite NS and BS preserve the same phytochemical profile in terms of the most representative compounds with catechin (0.39 g/100 g and 0.084 g/100 g, respectively) and isoramnethin-3-*O*-glucoside (0.38 g/100 g and 0.12 g/100 g) as the most abundant compounds, BW showed a completely different behaviour with isoramnethin-3-*O*-glucoside as the most abundant compound (0.024 g/100 g), followed by vanillic and protocatechuic acids (0.02 g/100 g and 0.005 g/100 g, respectively). These differences are also reflected in antioxidant activity. In fact, the set of antioxidant assays (ORAC, TEAC, FRAP, and DPPH) that we have carried out confirmed what was observed in the phytochemical studies, decreasing, the antioxidant activity, in a statistically significant manner passing from NS (18.80-113.62 g TE/100 g) to BS (5.65-35.67 g TE/100 g) and BW (3.32-20.81 g TE/100 g), with an activity ratio of about 3:1 for NS/BS and 2:1 for BS/BW.
Data obtained in this study confirm that the two waste products still contain a fair amount of polyphenols and that they could be used as they are if properly treated, or processed for use in the nutraceutical and cosmeceutical fields. However, the standardization of the conventionally used peeling process will certainly allow obtaining waste products with a higher content of polyphenols, thus maximizing production yields.

Fig. 1. *Prunus dulcis* Mill. (a) and particular of almond seed cultivar Fascionello (b)

9= Anthocyanins profile and genotoxic activities of *Myrtus communis* L. (Myrtaceae) berries extract

Alfonsina La Mantia[1], Rosaria Acquaviva[1,2], Barbara Tomasello[1,2], Claudia Di Giacomo[1,2], Giuseppe Carlo Zimbalatti[1], Simone Bianchi[1], Francesco Maria Raimondo[3], Irina Naletova[1], Giuseppe Antonio Malfa[1,2]
[1]Dept. of Drug and Health Sciences, Section of Biochemistry, University of Catania, Italy; [2]Research Centre on Nutraceuticals and Health Products (CERNUT), University of Catania, 95125 Catania, Italy; [3]PLANTA/Center for Research Documentation and Training, Palermo, Italy

Myrtus communis L. is an aromatic plant endemic to the Mediterranean area. It belongs to the Myrtaceae family, that includes around 100 genera and 3000 species. It is an evergreen scrub from 1.5 to 3 m tall, with small, dark green and leathery leaves and fissured bark.
The flowers (Fig. 1) are solitary and axillary, white in color; they are fragrant and their diameter reaches up to 3 cm. They are followed by a dark, oval-shaped berry (Fig. 2), with a blue skin and a small amount of sour, resinous white pulp. *M. communis* L. grows spontaneously in coastal areas and in inland hills across the Mediterranean; knowledge of its therapeutic properties precedes the ancient Greeks.
The extracts obtained from the aerial parts and from the leaves are used as stimulants, antiseptics and hypoglycemic agents; in respiratory and skin diseases; and as analgesics.
It is known that the plant contains numerous biologically active constituents (tannins, flavonoids, glycosides, phenolic acids and terpenes) with antioxidant, anti-inflammatory and anticancer action.
Our previous studies have shown that the alcoholic extract of *M. communis* L. berries, collected on the south coast of Sicily, near Siracusa (SR, Italy), induces apoptotic cell death and is able to significantly reduce ROS levels and to increase GSH content in human breast cancer cell line (MCF-7) treated for 24 h.
Therefore, in this study, to better understand the phytochemical characteristics and the biological effects of the berries extract, we performed the complete anthocyanins profile through high-performance liquid chromatography (HPLC-DAD) analysis. Moreover, we also evaluated the genotoxic activity of the extract, in order to better understand its mechanism of action. We examined the presence of genomic DNA damage by COMET assay, and cell survival by clonogenic test, under the same experimental conditions. In addition, we determined the expression of proteins (PARP-1 and yH2Ax) involved in various DNA damage response processes.
The results obtained showed that the berry extract reduces cell survival of MCF-7 at the highest concentrations (100-150 µM), while at the lowest concentrations (50 µM) no action is evident.
The COMET assay demonstrates the genotoxic effect of the extract in that at the lowest concentrations there is significant DNA fragmentation. The cytotoxic effect of the extract was also confirmed by the decrease in the levels of PARP-1 and yH2Ax proteins involved in DNA damage.
The results obtained in our study are supported by the particular phytochemical composition of the extract, which has a high content of phytochemical compounds, including anthocyanins.
These interesting results encourage further investigation of the chemotherapeutic properties of *M. communis* L. berry extract in the MCF-7 cell line.

Fig. 1. *Myrtus communis* in bloom

Fig. 2. Red and white myrtle berries

9 = From primitive to modern crops: bioactive compound characterization of fifty durum wheat germplasms from Global Durum wheat Panel (GDP)

Mariacaterina Lianza[1], Riccardo Gasparini[1], Lorenzo Marincich[1], Giovanni Maria Poggi[2], Simona Corneti[2], Iris Aloisi[2], Fabiana Antognoni[1]
[1]Department for Life Quality Studies, University of Bologna, Corso d'Augusto 237, 47921 Rimini, Italy; [2]Department of Biological, Geological and Environmental Sciences, University of Bologna, Via Irnerio 42, 40126 Bologna, Italy

Triticum turgidum L. ssp. *durum* (Desf.) Husn., commonly known as durum wheat, is one of the most important food crops in the world, with an annual production of around 40 million tons. Despite accounting only 8% of global wheat production, it represents the most important staple food in the Mediterranean basin. The modern durum wheat currently on the market is a tetraploid species (BBAA genome) deriving from a human-driven evolution process lasting thousands of years, in which wild emmer was domesticated to tamed emmer, then to durum wheat landraces and finally to the modern cultivars. The nutritional and economical importance of this cereal led several research teams around the world to create the Global Durum wheat Panel (GDP), whose aim is to explore the genetic diversity among cultivated crops and wild progenitors and to identify useful alleles for breeding to obtain cultivars with improved characteristics. The GDP collects 1011 genotypes of modern and ancient wheat germplasms, together with wild emmer and primitive sub-species, capturing 94-97% of the original diversity.
In this work, fifty germplasms of the GDP collection, including the most widespread and economically important modern wheat varieties, some accessions of landraces, and some wild emmer progenitors, were characterized for their phytochemical profile, in particular for their anthocyanin, phenolic acid, and carotenoid composition. As concerns anthocyanins, these were extracted from samples using a mixture of methanol and acidified water and refrigerating the extracts at 4 °C to promote protein precipitation, then a spectrophotometric determination was carried out. Results indicate significant differences among the germplasms. For the analysis of phenolic acids, samples were subjected to an extraction process resulting in two fractions, one enriched in free phenolic acids, and the other one in insoluble-bound phenolics. On both fractions, nine compounds of nutraceutical value were targeted and quantified by HPLC-DAD analysis. The main metabolites of the free phenolic fractions were *p*-hydroxybenzoic acid and *p*-coumaric acid, the latter being found in all germplasms, with a concentration range between 18.24 and 30.47 μg /g DW. *p*-hydroxybenzoic acid was found in most of the accessions in widely varying quantities, ranging from 8.77 to 163.2 μg/g DW. Metabolites in the insoluble-bound fractions were considerably more abundant than the free form. Ferulic acid was the most abundant phenolic acid, varying from 413.80 to 1132.74 μg /g DW, followed by *p*-coumaric acid. Interestingly, the wild emmer showed a *p*-coumaric acid concentration markedly higher than that present in all landraces and modern cultivars, being the difference more than ten-fold, suggesting that domestication led to a considerable loss of this bioactive compound. A grain color analysis was also carried out using a stereo microscope, but no significant differences emerged among the accessions. Carotenoid extraction and analysis are in progress to complete the bioactive compound characterization. A multivariate analysis will also be carried out to better understand the variations into the phytochemical levels among the different accessions.

9 = From tradition to ethnopharmacology: a new ethnobotanical approach in the use of antidiabetic medicinal plants in Italy

Maria Maisto, Daniela De Nigris, Daniela Zuzolo, Maria Tartaglia, Maria Antonietta Ranauda, Alessandra Falzarano, Alessia Postiglione, Antonello Prigioniero, Pierpaolo Scarano, Rosaria Sciarrillo, Carmine Guarino
Department of Science and Technology, University of Sannio, Via de Sanctis Snc, 82100, Benevento, Italy

The use of plants for health purposes has been pursued since ancient times. Even today, more than half of the world's population turns to traditional herbal remedies as first aid in case of need, especially in developing countries. Ethnobotany is the study of interrelations between humans and plants. The current use of the term also implies the study of indigenous or traditional knowledge of plants, their classification, cultivation, and use as food or medicine. The conscious and safe use of these plants and their formulations implies environmental and economic sustainability of these remedies, often characterized by a confined use to regions with limited economic availability. This is where ethnopharmacology comes in: it is a highly interdisciplinary field of study, which requires expertise in pharmacological, medical, botanical, chemical, but also historical, anthropological, and environmental fields.
The flora of the Mediterranean Basin is rich in medicinal plants that are widely used in traditional medicine for the treatment of various diseases, including diabetes, one of the most life-threatening chronic metabolic disorders, considered a global health problem. The rapid increase in the incidence of diabetes mellitus is becoming a serious health threat in all parts of the world, and despite the great efforts invested in research, its prevalence continues to grow, while current medications do not cover all symptoms and complications of the disease. Plants possess several classes of bioactive molecules that belong to different chemical families, such as terpenes, phenolic acids, flavonoids, and alkaloids, and through these molecules, they exert their antidiabetic and hypoglycemic potential. The richest families in molecules with anti-diabetic potential are the *Lamiaceae*, *Asteraceae*, *Apiaceae,* and the *Fabaceae*. Many of the species belonging to these families, not surprisingly, are edible and are widely cultivated for human consumption. The aim of this study was to provide useful documentation that can contribute to preserve the knowledge about the use of medicinal plants in the Mediterranean region, to explore their phytochemical potential, and to extend this knowledge to the Italian Flora so that this knowledge can be applied to research and phytotherapeutic remedies in our Country.

9 = Wild cardoon [*Cynara cardunculus* (L.) subsp. *sylvestris* Lam. (Asteraceae)] leaves extract, phenolic profile, antioxidant properties and hepatoprotective activities

Giuseppe Antonio Malfa[1,2], Barbara Tomasello[1,2], Claudia Di Giacomo[1,2], Alfonsina La Mantia[1], Simone Bianchi[1], Francesco Pappalardo[1], Francesco Maria Raimondo[3], Rossella Tundis[4], Rosaria Acquaviva[1,2]
[1]Dept. of Drug and Health Sciences, Section of Biochemistry, University of Catania, Italy; [2]Research Centre on Nutraceuticals and Health Products (CERNUT), University of Catania, 95125 Catania, Italy; [3]PLANTA/Center for Research Documentation and Training, Palermo, Italy; [4]Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, 87036 Rende (Cosenza)

Cynara is a small genus belonging to the Asteraceae family, native to the Mediterranean area. Only a few taxa are in it, including wild cardoon (*Cynara cardunculus* (L.) subsp. *sylvestris* Lam.), considered the wild progenitor of globe artichoke (*Cynara cardunculus* (L.) subsp. *scolymus* (L.) Hegi) and the leafy or cultivated cardoon (*Cynara cardunculus* (L.) var. *altilis* DC). Wild cardoon is a robust perennial plant with wide spiny leaves forming a large characteristic rosette in winter and showing a branched flowering stem in late spring. It is widely present in Sicily, where it is used as food and medicine for centuries; decoctions of the aerial parts of the aforementioned variety (*C. sylvestris*), have been employed in traditional medicine as hepatoprotective, choleretic, and lipid-lowering agents and as a remedy for hepatic diseases. Recently, the Committee on Herbal Medicinal Products (HMPC) of the European Medicines Agency (EMA) recognized preparation from *Cynara cardunculus* L. leaf as an herbal remedy for the symptomatic relief of digestive disorders.
In this study, we evaluated the phytochemical profile and antioxidant characteristics of an aqueous extract of *C. sylvestris* leaves, grown wild on the sea coast of southeast Sicily (Syracuse).
Furthermore, the obtained aqueous extract was tested *in vitro* for its potential hepatoprotective effects. Human HepG2 cells were treated with Free Fatty Acids (FFAs) oleic acid (OA) and palmitic acid (PA) (OA:PA at 2:1) at the total concentration of 1.5 mM resembling the features of moderate steatosis in humans. To evaluate the protective effects of the extract, 24 h prior to FFA addition, cells were pre-treated with *C. sylvestris* extract (10-25-50 μg/ml)
The phytochemical profile by HPLC-DAD revealed the presence of several phenolic acids (caffeoylquinic acids) and flavonoids such as luteolin and apigenin derivatives.
The total phenolic and total flavonoid contents, determined spectrophotometrically were as follow (185.21 ± 1.97 mg GAE/g and 50.32 ± 1.62 mg QE/g) and the antioxidant characteristic of the extract, determined by DPPH, showed an IC50 = 20.04 ± 2.52 μg/mL. In HepG2 cells, no cytotoxicity has been observed at different concentrations of *C. sylvestris*; instead, the extract was able to counteract the injury induced by the co-administration of FFA, restoring cell viability to the level of control cells. The determination of mRNA expression levels of the markers involved in antioxidant defence (Nrf2, Gpx, and SOD) showed that the extract increased all the examined genes, with significant results with respect to FFA treated cells. The determination of reactive oxygen species (ROS) and thiols groups (RSH) levels in HepG2 cells, showed that the extract was able to significantly counteract ROS production promoted by FFA treatment, and, interestingly, RSH contents were not only restored by pretreatment with the extract but increased to levels higher than untreated control cells. Lastly, the increased mRNA expression levels of inflammatory cytokines (IL-6, TNF-α, and IL-1β), induced by FFA treatment, were partially counteracted by the pretreatment with the extract. In conclusion, results obtained in these *in vitro* experiments showed that the extract is able to act with different mechanisms of action, effectively counteracting oxidative stress induced by FFA, both by increasing RSH levels and turning off ROS production and also by mitigating the negative actions of inflammatory cytokines (IL-6, TNF-α and IL-1β). These results suggest that the total phytocomplex contained in *C. sylvestris* extract is effective in modulating the biomarkers evaluated in this *in vitro* model of hepatic distress.

9 = Fiber-Type *Cannabis sativa* L.: Phytochemical Characterization and Biological Activity of the Aerial Parts at Different Growth Stages

Giulia Mastellone[1], Arianna Marengo[1], Gaia Bechis[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 hemp varieties were proposed, the most important refers to drug-type (richer in Δ^9-THC) and fiber-type (richer in CBD or related compounds). Currently, there is a renewed interest in cannabis-related products in different fields because of the rich phytocomplex of this plant, together with its fiber and agricultural features. In this context, the current study aimed to chemically characterize the aerial parts of different samples of fiber-type *Cannabis sativa* L. grown in Italy as a potential health-promoting source. A set of ultrasound-assisted extraction methods were first developed and optimized to obtain a fingerprinting of the investigated plant. Analyses were carried out through an ultra high performance liquid chromatography equipped with a photodiode array detector in series with a triple quadrupole system with an electrospray ionization interface (UHPLC-UV-ESI-MS/MS) and showed that the phytocomplex mainly includes flavonoids and non-psychotomimetic cannabinoids. The method was then applied to characterize and compare 24 samples of fiber-type *Cannabis sativa* L. aerial parts (mainly stems and leaves), which differed in the growth stages (from mid-vegetative to early flowering), growth land plots, and methods of drying (forced-draft oven or freeze-drying). The quali-quantitative analysis showed that a freeze-drying method seems to better preserve the chemical composition of the samples, while the location of the land plot and the growth stage of the plant (which did not comprise inflorescences) had minor influences on the chemical pattern. In particular, the higher temperature of the oven (65°C) for a prolonged time, may have led to partial degradation of the glycosides in the corresponding aglycones. The same degradation probably occurred for the acid form of cannabinoids which are thermally unstable. These results were also supported by spectrophotometric *in-vitro* assays (scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH•) and 2,2′-azinobis-3-ethyl-benzthiazoline-6-sulphonate (ABTS+•) radicals and inhibitory activity against tyrosinase and elastase enzymes) to investigate the potential biological activity of these samples. Cannabinoids showed to contribute to the total antioxidant activity of the extracts, even if flavonoids had the major contribution. Tyrosinase and elastase are among the enzymes involved in skin aging and several natural inhibitors have already been proposed as alternative ingredients for anti-aging products. All the extracts showed slight activity against the action of tyrosinase, suggesting that, although to a minor extent, both flavonoids and non-psychotomimetic cannabinoids negatively affect tyrosinase activity. No inhibition was observed against elastase enzyme. On the contrary, the extracts seemed to slightly increase the reaction initial velocities, but further experiments are needed to evaluate the presence of potential elastase activators within the investigated extracts. These results suggested that fiber-type *Cannabis sativa* L. aerial parts can be considered a valuable natural ingredient, considering that their chemical composition seems to be independent of the growth stage of harvesting, provided that they are collected before the flowering stage.

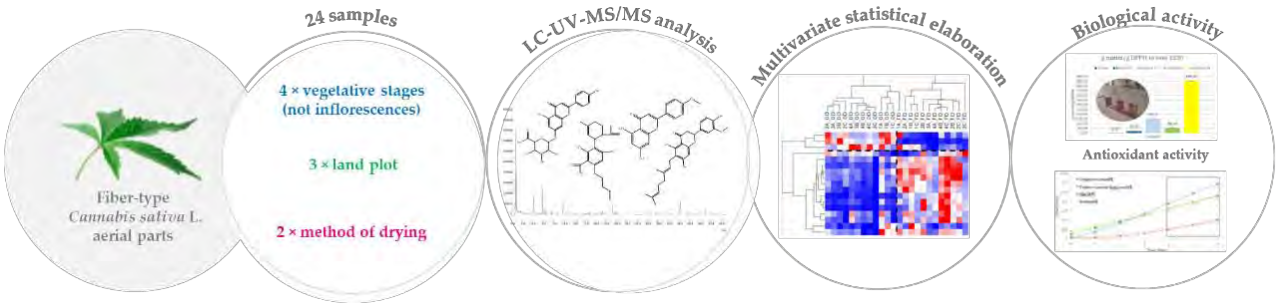


Fig. 1. Flowchart of the main steps of the characterization of fiber-type *Cannabis sativa* L. aerial parts

9 = Phytochemical and biological investigations on the pollen from industrial hemp male inflorescences

Luigi Menghini[2], Alessandra Acquaviva[1,2], Simonetta Cristina Di Simone[2], Antonella Canini[3], Roberto Braglia[3], Gabriele Di Marco3, Claudia Campana[2], Paola Angelini[4], Giancarlo Angeles Flores[4], Roberto Venanzoni[4], Maria Loreta Libero[2], Bruno Tirillini[5], Gokhan Zengin[6], Annalisa Chiavaroli[2], Lucia Recinella[2], Sheila Leone[2], Nilofar[2], Luigi Brunetti[2], Giustino Orlando[2], Claudio Ferrante[2]

[1]Veridia Italia Srl, Via Raiale 285, 65100 Pescara, Italy: [2]Botanic Garden "Giardino dei Semplici", Department of Pharmacy, "Gabriele d'Annunzio" University, Via dei Vestini 31, 66100 Chieti, Italy; [3]Department of Biology, University of Rome "Tor Vergata", via della Ricerca Scientifica 1, 00133, Rome, Italy; [4]Department of Chemistry, Biology and Biotechnology, University of Perugia, 06122 Perugia, Italy; [5]Department of Biomolecular Sciences, University of Urbino, 61029 Urbino, Italy; [6]Physiology and Biochemistry Laboratory, Department of Biology, Science Faculty, Selcuk University, Campus, 42103 Konya, Turkey

Cannabis sativa L. belongs to the Cannabaceae family and includes annual, robust, fast-growing, and generally dioecious plants. Industrial hemp, and particularlythe inflorescences, has been recently recognized as an important source of bioactive extracts with antioxidant and antimicrobial effects. The goal of the present study was to explore the botanical, phytochemical, and biological properties of water and hydroalcoholic hemp pollen extracts from male inflorescences. The extracts from hemp pollen were found particularly rich in phenolic compounds, such as hydroxytyrosol, coumaric acid, and hesperitin. The phenolic profile was also consistent with the observed scavenging/reducing, enzyme inhibitory, and antimicrobial properties of the extracts. Regarding the antimicrobial effects, *Escherichia coli*, *Trycophyton rubrum*, and *T. tonsurans* were the most sensitive to the growth inhibition induced by the extracts (MIC values: 9.92-79.37 µg/mL). Whereas null effects on prostate PC3 and myocyte C2C12 cell viability, in the range 1-1000 µg/mL, are consistent with MIC values and suggest extracts' biocompatibility. The experimental data obtained, which are not reflected in the literature as the topic of hemp pollen is almost completely unexplored, confirm the innovativeness of a product obtained directly from bees, which in the face of greater variability and complexity can reserve promising applications in food, pharmaceutical, and cosmetic sectors.

9 = Radiomodulating effect of an alcoholic extract from leaves *Olea europaea* L. cv. Caiazzana

Hamid Mushtaq[1*], Simona Piccolella[1], Assunta Esposito[1], Lorenzo Manti[2], Severina Pacifico[1]
[1]Department of Environmental Biological and Pharmaceutical Sciences and Technologies, University of Campania Luigi Vanvitelli, Via Vivaldi 43, 81100 Caserta, Italy; [2]Istituto Nazionale di Fisica Nucleare, Sezione di Napoli, Monte S. Angelo, Via Cinthia, 80126, Napoli, Italy; [3]Dipartimento di Fisica, Università Federico II, Napoli, Monte S. Angelo, Via Cinthia, 80126, Napoli, Italy
* Corresponding author: Hamid Mushtaq (hamid.mushtaq@unicampania.it)

Olives (*Olea europaea* L.) and olive oil have been widely studied for their flavor and health benefits but the olive leaf and its chemical composition has only recently attracted interest. This is because the studies on olive leaf chemical composition have revealed the presence of a high number of phenolic compounds mainly phenolic secoiridoids and flavonoids. These compounds are worthy because of their diverse usage as anti-inflammatory and antioxidants in the nutraceutical and food industry. Moreover, olive leaves by the pruning and harvesting of olive trees were one of the by-products of the olive oil industry with negative environmental impact. In this context, it is of interest to explore the potential resource of olive leaf and mainly their cultivar biodiversity.
Herein, the leaves from *Olea europaea* L. cv. Caiazzana, an autochthonous cultivar of Southern Italy, were collected after pruning, extracted by ultrasound-assisted extraction (UAE) technology, as a green treatment solution for olive leaf utilization, using ethanol as extracting solvent. The extract obtained, named OLE, was chemically analyzed by UHPLC-ESI-QqTOF-MS/MS techniques. An antiradical and antioxidant screening in cell-free systems was carried out, as well as the radiomodulating activity was investigated towards two cancer cell lines (DU-145 prostate and PANC-1 pancreatic cells) and two non-cancer ones (HUVEC endothelial and MCF-10A epithelial cells). In particular, the frequency of radiation-induced micronuclei (MN) was assessed. Interestingly, data acquired showed an increase of MN frequency in the cancer cells, while an equally significant radioprotective effect was induced in the tested non-cancer cell lines. Thus, OLE appeared to modulate the radiation effect in a differential manner, leading to simultaneous protection in normal cells and sensitization in cancer cells. Based on the chemical composition analysis, the dual effect may arise from the presence of different classes of specialized metabolites in OLE, such as oleuropein derivatives, flavone glycosides, and oleanolic acid.

9 = *Salvia rosmarinus* Spenn. (Lamiaceae) ethanol extract: phytochemical analysis, antioxidant activity and *in vitro* evaluation of fatty acid accumulation

Vincenzo Musolino[1], Roberta Macrì[2], Jessica Maiuolo[1], Antonio Cardamone[2], Saverio Nucera[2], Luigi Tucci[1], Carmine Lupia[1], Rosamaria Caminiti[2], Mariangela Marrelli[3], Vincenzo Mollace[2]
[1]Department of Health Sciences, Institute of Research for Food Safety&Health (IRC-FSH); Laboratory of Pharmaceutical Biology, University "Magna Græcia" of Catanzaro, 88100 Catanzaro, Italy; [2]Department of Health Sciences, Institute of Research for Food Safety & Health IRC-FSH, University "Magna Græcia" of Catanzaro, 88100 Catanzaro, Italy; [3]Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, I-87036 Rende, (CS), Italy

Salvia rosmarinus Spenn. (Lamiaceae) is one of the oldest Mediterranean shrubs with powerful aroma, dark green elongated leaves and whitish, bluish-, or bluish-purple flowers. The most used name, *Rosmarinus officinalis* L., is considered a synonym of the actual name, *Salvia rosmarinus* Spenn., because recent investigations, based upon molecular phylogenetic, taxonomic, morphological, and practical considerations, underlined the necessity to consider the genus *Rosmarinus* as *Salvia* species. *Salvia rosmarinus* Spenn. is cultivated worldwide but it is native to the Mediterranean region. *S. rosmarinus* Spenn. has drawn attention due to its biological activities, due particularly to the presence of polyphenols, including carnosic acid and rosmarinic acid, and phenolic diterpenes as carnosol. Here, we assessed the cytotoxicity, the radical-scavenging activity, and the effect on lipid accumulation of a *S. rosmarinus* Spenn. phytocomplex obtained by ethanol extraction using the maceration method together with sonication. The total phenolic content (TPC) and total flavonoid content (TFC) were also determined. Among the active constituents of *S. rosmarinus* Spenn., the identification of rosmarinic acid, carnosol, and carnosic acid was performed by HPLC (Fig.1). The aerial parts of *S. rosmarinus* Spenn. were harvested in June, in the geographical area of Trebisacce (Cosenza, Calabria) and, placed in the dark to dry at +40°C for 72 h, then grinded in liquid nitrogen with a mortar and pestle. The minced aerial parts were macerated in absolute ethanol at room temperature in the dark for 3 h. Subsequently, the solution was sonicated in ice for 20 min, then centrifuged and filtered through a filter paper. The extract was evaporated under vacuum, using a rotary evaporator, to obtain a greenish dry crude extract. The major component in the alcoholic extract was carnosol (7.2%), followed by rosmarinic acid (2%) and carnosic acid (1.9%). The extract showed a good radical scavenging activity in DPPH test (IC50 = 3.322 ± 0.16 mg/mL) (Fig.2), whereas TPC and TFC where 45.52 ± 8 mg gallic acid/g and 220.8 ± 12.5 mg rutin/g, respectively. *S. rosmarinus* Spenn. alcoholic extract did not show cytotoxicity at 24 h and at any concentration on the hepatocyte cell line Mcarh-7777, as shown by the MTT assay (Fig.3). Furthermore, hepatocyte cell line Mcarh-7777 treated with Oleic Acid (OA) 100 µM showed an intracellular lipid droplets accumulation, underlined by an intense red coloration under visible light or strong red fluorescence, that was significantly reduced by 50 µg/ml of the phytocomplex, as shown by Nile Red and Oil Red O staining (Fig.4). Finally, besides improving lipids accumulation, *S. rosmarinus* Spenn. alcoholic extract exerts a free radical scavenging activity on Mcarh-7777 cells as a decrease in lipid peroxidation has been observed.

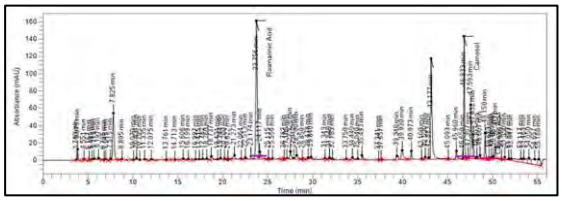


Fig 1. Chromatogram of HPLC analysis

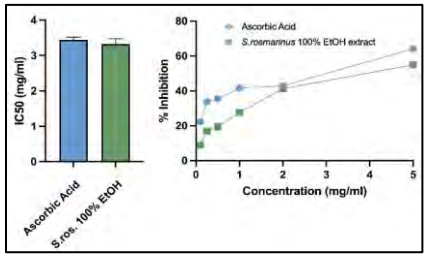


Fig 2. DPPH Assay: IC50 value and %

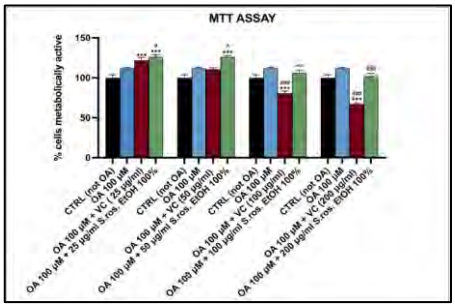


Fig 3. MTT Assay

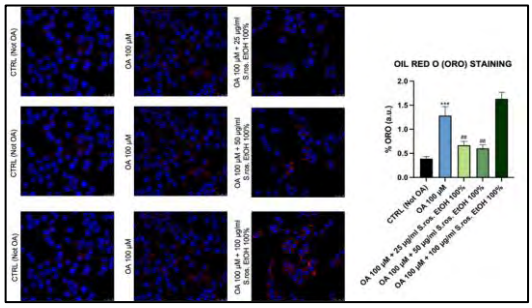


Fig 4. Oil Red O Staining

9 = From the ethnobotanical tradition of Italy to the scientific approach: medicinal plants and functional foods in the treatment and prevention of cardiovascular diseases

Maria Antonietta Ranauda, Daniela Zuzolo, Maria Tartaglia, Antonello Prigioniero, Pierpaolo Scarano, Alessia Postiglione, Maria Maisto, Alessandra Falzarano, Rossella Clemente, Rosaria Sciarrillo, Carmine Guarino
Department of Science and Technology, University of Sannio, Via Francesco de Sanctis, 82100 Benevento BN, Italy

This study analyzed medicinal plants occurring in Italy that are used in the treatment and prevention of cardiovascular diseases. We started from the country's ethnobotanical traditions to the most recent scientific literature. The purpose was to give scientific validation to the therapeutic efficacy of plants traditionally known locally. Ninety-six species, 41 families and 84 genera were identified in Italy. They are known in the ethnobotanical cultural heritage for their role in cardiovascular disease treatment and prevention. Many pharmacological properties characterize the different species studied, like antihypertensive, hypolipidemic, cardioprotective, cardiotonic, antiarrhythmic, antiaggregant, and antithrombotic activities. Many of them are also food plants typical of the Mediterranean diet and of considerable interest for possible nutraceutical applications. We have given particular attention to the phytochemical characteristics of the plant species and to the presence, localization, and extraction techniques of specific bioactive compounds. This information will be useful in understanding the molecular mechanisms upstream of their pharmacological effects. *In vivo*, *in vitro*, and clinical studies were considered to compare, therefore, traditional uses and information derived from a scientific and experimental approach. Finally, in this review, a focus on several case studies of some species particularly interesting for their medicinal characteristics was provided (*Allium sativum* L., *Crocus sativus* L., *Morus alba* L., *Rosmarinus officinalis* L, *Taraxacum officinale* L.). Future prospects will be directed toward investigating the clinical efficacy of medicinal plants and evaluating possible adverse effects. This will be helpful in proposing herbal remedies as viable therapeutic alternatives or complementary to synthetic drugs in the treatment and prevention of cardiovascular disease.

9 = Antidiabetic potential of *Ptilostemon casabonae* (L.) Greuter, a little-known Mediterranean plant

Cinzia Sanna[1,2], Arianna Marengo[3], Antonio De Agostini[1], Giovanna Lucia Delogu[4], Roberta Lai[1], Patrizia Rubiolo[3], Benedetta Era[5], Sonia Floris[5], Antonella Fais[5], Francesca Pintus[5]
[1]Department of Life and Environmental Sciences, Botany division, University of Cagliari, Via Sant'Ignazio da Laconi 13, 09123 Cagliari, Italy; [2]Co.S.Me.Se, Consorzio per lo studio dei Metaboliti Secondari, University of Cagliari, Via Sant'Ignazio da Laconi 13, 09123 Cagliari, Italy; [3]Department of Drug Science and Technology, University of Turin, Via Pietro Giuria 9, 10125 Turin, Italy; [4]Department of Life and Environmental Sciences, Drug Sciences division, University of Cagliari, SP 8, 09042 Monserrato, Cagliari, Italy; [5]Department of Life and Environmental Sciences, Biomedical Sciences division, University of Cagliari, SP 8, 09042 Monserrato, Cagliari, Italy

Diabetes mellitus is a widespread metabolic disease. The most common is type 2 diabetes (T2D), primarily characterized by insulin resistance or β-cell dysfunction, which represents one of the most prevalent health problems with a high rate of incidence and mortality. T2D is associated with postprandial hyperglycemia occurring because of the high level of blood glucose after consuming a meal. Therefore, one of the therapeutic approaches to treat diabetes is to decrease postprandial hyperglycemia by the inhibition of carbohydrate hydrolyzing enzymes like *α*-amylase and *α*-glucosidase that break down starch and disaccharides to glucose. The synthetic enzyme inhibitors currently in use, such as acarbose and voglibose, are known to produce side effects, due to an abnormal bacterial fermentation of undigested carbohydrates in the colon. Thus, there is a need for novel *α*-glucosidase and α-amylase inhibitors without side effects.
In this work, aimed at identifying natural products endowed with antidiabetic potential, we focused on *Ptilostemon casabonae* (L.) Greuter, a little-known endemic species of Sardinia (Italy), Corse, and Hyères islands (France), belonging to Asteraceae family. The aerial parts of this species are traditionally used in Sardinia as intestinal antispasmodic. Additionally, the boiled sprouts and immature flower heads are considered edible. We evaluated *in vitro* the *α*-glucosidase inhibitory activity of the hydroalcoholic extract obtained from its leaves. Moreover, since it has been widely documented that antioxidant compounds reduce the occurrence of several diseases, such as diabetes, the radical scavenging activity and total phenolic and flavonoid contents of the extract were also determined.
Our preliminary results highlighted a marked *α*-glucosidase inhibitory activity of the extract, being more effective if compared with the standard inhibitor (acarbose). Indeed, *α*-glucosidase was totally inhibited (98 % inhibition) at the extract concentration of 90 μg/mL, which correspond to the IC_{50} value of acarbose. The ABTS test revealed a good antioxidant capacity, which could be related to the presence of high content of phenolics and flavonoids detected in the extract. Our phytochemical analysis, carried out by HPLC-PDA-MS/MS, revealed, according to a previous study, a remarkable abundance of flavonoids and phenolic acid derivatives: quercetin, luteolin, kaempferol, apigenin, and diosmetin *O*-glycosides, and caffeoylquinic acid derivatives were found as the main components.
Further studies are ongoing in aiming to evaluate the *α*-glucosidase inhibitory activity of the identified metabolites. In conclusion, this preliminary study contributes to improving the knowledge of this understudied species and, importantly, may pave the way for further investigations addressed to understand the mechanisms underlying the antidiabetic activity of *P. casabonae* and its phytochemical constituents.

9 = Extraction, characterization, and *in vitro* evaluation of the anthelmintic efficacy of pasteur plant species for biological control of sheep gastrointestinal nematodes (GIN)

Pierpaolo Scarano[1], Antonio Bosco[2], Antonello Prigioniero[1], Stefania Iervolino[1], Daniela Zuzolo[1], Maria Tartaglia[1], Alessia Postiglione[1], Maria Maisto[1], Maria Antonietta Ranauda[1], Alessandra Falzarano[1], Giuseppe Cringoli[2], Carmine Guarino[1] and Rosaria Sciarrillo[1]
[1]Department of Science and Technology, University of Sannio, Via F. de Sanctis snc - 82100 Benevento (BN), Italy; [2]Department of Veterinary Medicine and Animal Production, University of Naples Federico II, CREMOPAR, Strada Statale 18 (Località Cioffi) - 84025 Eboli (SA), Italy

Gastrointestinal nematode (GIN) infection jeopardizes the health and welfare of livestock and is commonly associated with economic losses, especially due to subclinical diseases. In recent years, important advances have been made in the biological control of GIN in ruminants. Although these measures are still relatively little used in practice, interest is growing due to drug resistance, increased demand for organic products and legislation regulating and restricting the use of anthelmintic drugs.
The purpose of this study was to extract, characterize and evaluate the *in vitro* anthelmintic activity of aqueous, alcoholic and hydroalcoholic extracts of 28 plants sampled on pastures in Southern Italy.
Extracts were obtained by the conventional maceration technique. The total phenolic content (TPC, Folin-Ciocâlteu assay) of all extracts was preliminarily characterised. The egg hatching test (EHT) was performed to estimate the *in vitro* anthelmintic efficacy of plant extracts using GIN eggs from sheep naturally infected with the species *Haemonchus contortus*, *Teladorsagia circumcincta* and *Trichostrongylus colubriformis*. Finally, extracts showing significant anthelmintic efficacy *in vitro* were characterised by LC-MS. Each extract with high inhibition of egg hatching was tested *in vitro*: MDBK cell lines (bovine kidney cells) were used.
All extracts showed significant TPC. Each extract was tested with EHT at decreasing concentrations of 28.0, 98.0 and 40.0 $mg \cdot mL^{-1}$ for aqueous, ethanolic and hydroalcoholic extracts, respectively. The results indicated that, in particular, the extracts of *Borago officinalis* L. and *Malva sylvestris* L. caused a high inhibition of egg hatching within 48 hours of exposure, showing efficacy (≥98%) at the first two highest concentrations. The first experiments on *in vitro* cell cultures were tested at different concentrations and times: 10, 100, 500 and 1000 $g \cdot mL^{-1}$ for times of 24, 48 and 72h.
All extracts were cytocompatible, except for a few points where the highest concentration was used: for *B. officinalis* 100, 500 $g \cdot mL^{-1}$ at 72 h and for *M. sylvestris* 1000 $g \cdot mL^{-1}$ at 72 h [Fig. 1(a) and (b)]. Furthermore, the results obtained using the *M. sylvestris* extract suggest a proliferative effect.
The characterisation of the extracts showed the presence of several classes of compounds: tannins, flavonoids, glycosides, alkaloids and terpenoids.
Field application with *in vivo* studies would lead to confirm the *in vitro* results, evaluating the therapeutic potential and future applicability with specific plant crops in pastures to achieve year-round "helminth-free" sheep flocks.

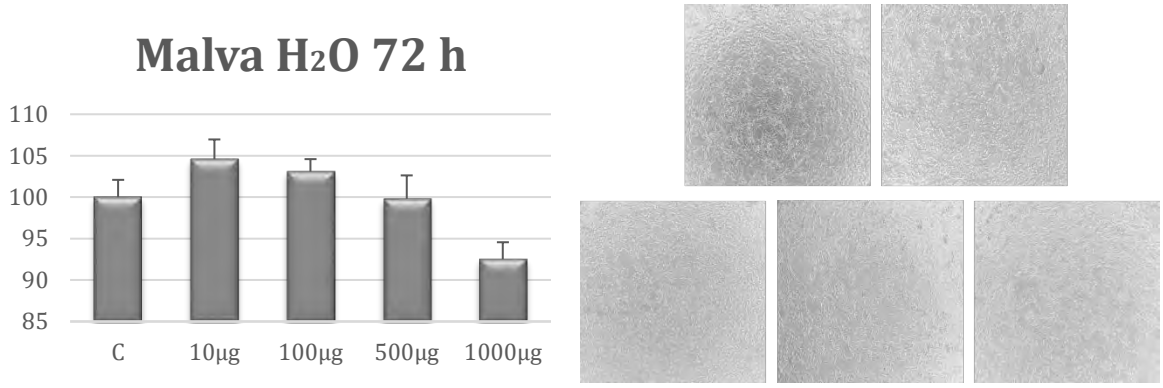


Fig. 1. (a) Histogram and (b) MDBK cultures at 72h of *M. sylvestris* sample in H_2O.

9 = Analysis of Sicilian populations of the genus *Capparis* L. for the determination of quercetin content

Francesco Sgadari[b], Rosario Schicchi[b], Natale Badalamenti[a,], Maurizio Bruno[a,c]
[a]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), Università degli Studi di Palermo. Viale delle Scienze, ed. 17, 90128 Palermo, Italy; [b]Department of Agricultural, Food and Forest Sciences (SAAF), Università degli Studi di Palermo, Viale delle Scienze, ed. 4, 90128 Palermo, Italy; [c]Centro Interdipartimentale di Ricerca "Riutilizzo bio-based degli scarti da matrici agroalimentari" (RIVIVE), Università di Palermo

The caper is a xerophytic shrub of considerable interest and economic value for its medicinal properties, culinary uses, and cultivation characteristics.
C. spinosa L. is a taxon of difficult delimitation because of its wide heterogeneity, extreme phenotypic diversity and the presence of intermediate forms related to hybridization phenomena. In Europe, the genus *Capparis* is represented by a polymorphic complex now referred to a single species, *Capparis spinosa* L., which exhibits high ecological and morphological variability, occurring with subspecies and local varieties in widely different habitats.
The Sicilian territory hosts numerous natural and cultivated populations, well distinguished ecologically and morphologically, and the presence of two intraspecific taxa has been: *Capparis spinosa* subsp. *spinosa* and *Capparis spinosa* subsp. *rupestris*.
Based on studies on wild and cultivated populations of the genus *Capparis*, the objective of this research aimed to quantify the amount of quercitin, a metabolite with interesting biological properties, in the various epigean parts of the plant through the use of high-performance liquid chromatography (HPLC).
Specifically, the study was conducted on twenty-two stations falling over a wide area of Sicilian territory extending from the provinces of Trapani and Palermo through the "Gessoso-Solfifera" series to the Agrigento area, the Ibleo plateau and the province of Messina, including the Pelagie Islands, Pantelleria, Favignana, Marettimo, Ustica and Salina, so as to include in the analysis a substantial part of the genetic diversity existing in Sicily within both natural and cultivated populations.
The study area extending over much of the Sicilian territory includes populations present on various substrates: volcanic, calcareous, chalky and clayey.
With this aim, sampling was carried out under different growth conditions and at various phenological stages, acquiring information on the taxa collected, their distribution and possible cultivation techniques.
The chemical analyses showed that quercetin is mainly present in flower buttons and leaves. Its occurrence will be largely discussed.
The data acquired may provide a good basis for further scientific investigation to support the identification of ecotypes to be introduced in cultivation for pharmaceutical purposes in order to extract quercetin.
This could be a significant boost for the socio-economic development of rural areas in arid or semi-arid regions of Mediterranean environments. Indeed, the caper is a species with remarkable adaptability to hostile environments and poor soils, and this characteristic makes the caper grove a sustainable crop to cope with the impacts of climate change.

9 = *Eragrostis tef* (Zucc.) Trotter (Poaceae) from Ethiopia, a valuable source of nutrients and dietary flavones

Massimo Tacchini[1], Annalisa Maietti[2], Alessandro Grandini[1], Nicola Marchetti[2], Immacolata Maresca[1], Gianni Sacchetti[1], Alessandra Guerrini[1]
[1]Department of Life Sciences and Biotechnology, University of Ferrara, Piazzale Luciano Chiappini, 3, 44123, Malborghetto di Boara, Ferrara, Italy; [2]Department of Chemical, Pharmaceutical and Agricultural Sciences, University of Ferrara, Via Borsari, 46, 44121 Ferrara, Italy

Eragrostis tef (Zucc.) Trotter (Poaceae), commonly known as "teff", is an indigenous Ethiopian cereal mainly cultivated for its small grains which are used in many traditional preparations. It is preferred by farmers and consumers over many other common Eritrean and Ethiopian grain crops because it is stress-tolerant, it grows in different environmental conditions and its caryopsis is also resistant to storage pests. Thanks to these peculiarities and its high nutritional properties, teff cultivation is also spreading to European countries.
This study, part of a cooperation project (Semi di futuro – Intervento integrato di lotta alla malnutrizione, AID code 011880) funded by the Italian Agency for Development Cooperation, considered seven teff varieties (Red, Sergegna, Debrezeit, Quncho, Kora, Woloye, Dagim Teff) and compared them in terms of nutritional characteristics and phenolic content. From the nutritional point of view, this cereal is highly valued for its high protein content and the absence of gluten, which makes it suitable for the preparation of foods for coeliacs. Moreover, it has a high satiating power, a high fiber content and a low glycemic index. Laboratory analyses carried out on the various types of teff showed a lipid content ranging from 3.39 to 4.34%, a protein content ranging from 8.24 to 11.58%, and a total fiber content ranging from 15.47 to 29.75%. Furthermore, the comparative mineral content analysis of teff grains showed that Cu, Zn, and Mn are present in higher concentrations in these caryopses than in other common cereals like maize and wheat.
Teff is rich not only in macro and micronutrients, but it also contains bioactive nonessential but functional metabolites such as phenolic compounds. The optimization of extraction methods has led to the conclusion that the phenolic component is mainly composed of flavones, which are present in greater amount than reported in other cereals or other foodstuffs. Spectrophotometric quantification showed that, among the studied samples, Sergegna appears to be the richest in polyphenols and flavonoids. The thin-layer chromatographic and HPLC-DAD analysis enabled the identification of orientin (luteolin derivative) in red Teff, Sergegna, Debrezeit and Woloye, while vitexin (apigenin derivative) was detected in the remaining samples. Other flavonoids, also derivatives of luteolin and apigenin, are being further investigated to better define their structure. The HPLC-DAD quantification of flavones indicated the Debrezeit variety to be the richest in orientin (23.0±0.1 mg/100g dry drug), while the Dagim one was the richest in vitexin (24.9 ± 1.0 mg/100g dry drug) and apigenin 7-O-glucoside (9.9 ± 0.3 mg/100g dry drug).
The antioxidant activity, valuated through DPPH, ABTS and FRAP tests, indicated a trend quite in line with the total polyphenol content: Teff Debrezeit and Teff Sergegna samples have the highest antioxidant activity and polyphenol content, although the order is reversed. Teff samples containing predominantly luteolin derivatives exhibited higher antioxidant activity.
The preliminary results of this study indicate that the seven teff varieties examined are valuable sources of dietary flavones (glycosylated derivatives of apigenin and luteolin), but also point to these samples as excellent models for studying the bioactive properties of cereal flavones in a natural food resource.

9 = Biochemical characterization of seven Italian medicinal plant species

Annalisa Tassoni, Mirko Salinitro, Maura Ferri, Stefania Monari
Department of Biological, Geological and Environmental Sciences, Via Irnerio 42, University of Bologna, Italy

During famine and war periods wild food plants represented the main source of maintenance for most people. Nowadays these species have an important role in the Mediterranean diet, both for their easy availability and for their nutritional and therapeutic values.
The aim of the present research was to extract and characterize biochemical compounds by different organs of selected Italian alimurgic plant species traditionally consumed and used for therapeutic purposes in 3 regions: Liguria, Toscana and Puglia, which were selected on the basis of the number of mentions in all analysed papers (max 4 citations) and of their geographical North-South East-West positioning in the Italian peninsula.
After a wide and detailed bibliography research of all the published papers related to Italian alimurgic plants also reported to have medicinal effects, 7 species were selected as follows. A "use-index" was specifically created to select the species used for the highest number of different pathological groups. To calculate the index, for each species the total number of categories in which they were used for, was divided by the total number of the pathology categories (16). Next, the "c-index" [a citation index in which the total number of citations of each species was divided by the number of selected papers (56)] was calculated. To select the most cited species with a high number of uses, a final S-INDEX was obtained by averaging the "use-index" and "c-index". The following species were selected (Fig.1): *Borago officinalis* L., *Cynodon dactylon* (L.) Pers., *Foeniculum vulgare* Mill., *Hypericum perforatum* L., *Malva sylvestris* L., *Sambucus nigra* L., *Urtica dioica* L. Plants were sampled and separated into different organs (flowers, leaves, stems, roots and bark). Samples were extracted with methanol and water and analysed using spectrophotometric techniques. Total amount of proteins, polyphenols, reducing sugars, and antioxidant activity were assessed. *H. perforatum* showed the highest values of all analysed compounds and of antioxidant activity, followed by *S. nigra*. Flowers and leaves resulted to be the organs with highest levels of analysed components. All the regions showed the same trend. On the basis of the previous analyses, the most active samples were subjected to an *in vitro* gastric and duodenal digestion to detect how the plant material differs from the raw one after a human digestion.

Fig. 2. Selected Italian medicinal plant species

9 = Phenolic profile, antioxidant and cytotoxic activity of the phenolic-rich fraction obtained from the leaves of *Brassica fruticulosa* subsp. *fruticulosa* (Brassicaceae) grown wild in Sicily

Maria Fernanda Taviano[1], Federica Davì[1,2], Rosaria Acquaviva[3], Giuseppe Antonio Malfa[3], Barbara Tomasello[3], Paola Arena[1,2], Salvatore Ragusa[4], Francesco Cacciola[5], Yassine Oulad El Majdoub[1], Luigi Mondello[1,6,7], Natalizia Miceli[1]

[1]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Viale F. Stagno d'Alcontres 31, 98166 Messina, Italy; [2]Foundation "Prof. Antonio Imbesi", University of Messina, Piazza Pugliatti 1, 98122 Messina, Italy; [3]Department of Drug and Health Sciences, Section of Biochemistry, University of Catania, Viale Andrea Doria 6, 95123 Catania, Italy; [4]*PLANTA*/Research, Documentation and Training Center, Via Serraglio Vecchio 28, 90123 Palermo, Italy; [5]Department of Biomedical, Dental, Morphological and Functional Imaging Sciences, University of Messina, Via Consolare Valeria, 98125 Messina, Italy; [6]Chromaleont s.r.l., c/o Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Viale Palatucci, 98168 Messina, Italy; [7]Department of Sciences and Technologies for Human and Environment, University Campus Bio-Medico of Rome, via Àlvaro del Portillo 21, 00128 Rome, Italy

In the last years, the taxa comprised in the Brassicaceae family belonging to the spontaneous flora of Sicily (Italy) are being investigated by our research team to find new valuable sources of bioactive compounds.
Brassica fruticulosa Cyr. subsp. *fruticulosa* (Mediterranean cabbage) is an herbaceous species, usually biennial to perennial with basal leaves long-petiolate, arranged to form a rosette and cauline leaves smaller, pinnate-lobed to entire. The flowers are gathered in racemes and the fruit is a siliqua constricted at intervals with a beak. It is a species with Mediterranean distribution widespread in Southern Italy, that grows in untilled lands, on walls and debris from 0 to 1200 m above the sea level. *Brassica fruticulosa* subsp. *fruticulosa* is widely diffused in Sicily, where its use in traditional medicine is reported as an antidiabetic, to raise blood pressure, and in the treatment of respiratory and gastric ailments. Furthermore, young shoots and leaves have been eaten since ancient times both raw and cooked, and in Southern Italy, especially in Sicily, they are utilized to prepare traditional dishes. Despite its use in traditional medicine and as an edible plant, *B. fruticulosa* subsp. *fruticulosa* is little studied; in fact, only a few literature data can be found about the phytochemical characterization, while biological properties have never been investigated. Based on these premises, a study was recently undertaken by our research team to evaluate the bioactive potential of a hydroalcoholic extract (MeOH 70%) from *B. fruticulosa* subsp. *fruticulosa* leaves. The study highlighted the *in vitro* antioxidant properties of the extract and led to the characterization of phenolic and volatile compounds. As a continuation of the ongoing research, this work was designed to investigate the biological properties of the phenolic compounds contained in the extract. For this purpose, a phenolic-rich ethyl acetate fraction (*Bff*-EAF) was obtained from the MeOH 70% leaf extract by solvent-solvent partitioning. The total phenolic and total flavonoid contents were determined by spectrophotometric methods (107.44 ± 1.85 mg GAE/g and 34.26 ± 0.52 mg QE/g). A total of 20 phenolic compounds (11 flavonoids and 9 phenolic acids) were characterized by HPLC-PDA/ESI-MS analysis, being kaempferol-derivatives the most abundant ones. The antioxidant potential of *Bff*-EAF was investigated by three *in vitro* methods based on different mechanisms: DPPH, reducing power and ferrous ion chelating activity assays. The fraction exhibited good radical scavenging activity in the DPPH test (IC_{50} = 0.81 ± 0.02 mg/mL), mild reducing power (ASE/mL = 13.10 ± 0.94), and moderate chelating properties (IC_{50} = 2.27 ± 0.18 mg/mL). Considering the *in vitro* antioxidant activity highlighted for *Bff*-EAF, it seemed interesting to evaluate its cytotoxic properties on two different human cell lines, namely colorectal epithelial adenocarcinoma cells (Caco-2) and fibroblasts (HFF-1). The MTT test showed a significant dose-dependent cytotoxic activity on Caco-2 cells after 72 hours of treatment, while no cytotoxic effect was observed on HFF-1 fibroblasts, used as a control cell line. Furthermore, despite its antioxidant activity confirmed by the lower dosages, *Bff*-EAF showed a pro-oxidative activity in Caco-2 cells at concentrations starting from 0.5 mg/mL. In fact, phenolic compounds may show a pro-oxidative potential also, because they can be converted into more reactive radicals or indirectly induce ROS overproduction through interaction with different molecular pathways.
The obtained results provide a substantial contribution to the knowledge of *B. fruticulosa* subsp. *fruticulosa*, so far little studied, indicating this edible species that grows wild in Sicily as a new valuable source of bioactive compounds with potential health-promoting effects.

Acknowledgements
The authors wish to thank the Foundation "Prof. Antonio Imbesi" for financial support.

9 = Metabolomic study of *Cynara cardunculus* L. collected in two different seasons in Sardinia

Simona Trincia[1], Giovanni Pigati [1], Manuela Mandrone [1], Ilaria Chiocchio [1], Cinzia Sanna [2], 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

Cynara cardunculus L. (Asteraceae), commonly named 'cardoon', is a perennial plant with Mediterranean distribution. *C. cardunculus* is a plant with numerous biological activities, it is used in traditional Sardinian medicine to treat liver disease and digestive conditions. In addition, a recent study demonstrated the *in vitro* antiproliferative activity of *C. cardunculus* on human osteosarcoma cells, U_2OS.
According to the promising results regarding the bioactivity of *Cynara* extracts, the aim of this work was to investigate how the metabolome of this plant varies according to seasonality.
In this work *C. cardunculus* (aerial parts) was harvested in five different habitats of Sardinia and in two different seasons (spring and summer). Samples were dried, grounded, and 30 mg of dried plant material were extracted with 1 ml of solution (1:1) of CD_3OD and a phosphate buffer in D_2O (containing TMSP 0.01%). After centrifugation the supernatants were analyzed by ^1H NMR.
The ^1H NMR spectra of the different samples were compared through Principal Component Analysis (PCA) in which bucketed spectra were used as *x* variables. This analysis showed a separation between samples collected in spring and samples collected in summer. In addition, also the altitude seemed to influence the distribution of the samples. Therefore, a supervised analysis (OPLS-DA) was also built, using seasons as discriminant classes, and the most representative metabolites for each class were found by the Biplot and the consequent spectra interpretation. The influence of altitude and other abiotic factors on the metabolome of *C. cardunculus* was investigated building different OPLS models, in which each factor was in turn used as *y* variable. Only the OPLS model built using altitude as *y* variable was validated, highlighting the importance of this variable on the metabolome variations of the samples.
Based on the evident contribution of seasonality and altitude, a SUS-Plot was built combining the results of the two models (OPLS-DA and OPLS with *y*=altitude).
The SUS-Plot showed that cynaropicrin, one of the most peculiar metabolites of the cardoon, together with fatty acids and some flavonoids, were mainly produced in summer, while valine, proline and flavonoids (other than those produced in the summer) were produced more abundantly in spring. Alanine, malic acid and asparagine were found highly concentrated in samples at high altitudes, sucrose, on the other hand, was subjected to a double influence, in fact its production was stimulated by the low altitude only in samples collected in spring.
The concentration of all the detected metabolites was estimated by semi-quantitative analysis based on the internal standard TMSP.
The *in vitro* antioxidant activity was also tested by DPPH assay (resulting between 120 and 140 μg Trolox equivalents /mg dry extract) and the content of polyphenols and flavonoids, natural compounds notoriously important for this activity, were evaluated. However, no significant differences emerged between the various samples.
In conclusion, this study provided useful information about *C. cardunculus*, its metabolites and their variation across seasons and in relation to altitude. These results lay the basis to assess the most suitable period for harvesting the plant to pharmaceutical or herbal purposes. Further studies aimed at the characterization of the different flavonoids found in the samples and to understand the relationship with the seasonal variation are ongoing.

9 = Potential of *Olea europaea* L. leaf extract from Central and Southern Italy in neuronal stress containment

Federica Vaccaro, Giulia Baini, Giorgio Cappellucci, Marco Biagi, Elisabetta Miraldi
Department of Physical Sciences, Earth and Environment, University of Siena, Strada Laterina 8, 53100 Siena, Italy

Olive tree (*Olea europaea* L., 1753, fam. Oleaceae) is one of the most important plant species for the nutrition of Mediterranean basin populations, due to the production of olive oil and, to a lesser extent, edible fruits (olives). During the production process of olive oil, a large amount of leaves is produced as by-product, both from agricultural practices (especially pruning) and mill wastes, which has high disposal cost and ecological impact. Olive leaves have been extensively studied due to their content in polyphenols, among these caffeic acid, tyrosol, hydroxytyrosol and the secoiridoid derivative oleuropein; olive leaves also contain a large amount of triterpenes and sugars. Since olive leaves are an abundant, renewable, economical source of compounds of high biological value, they are an ideal raw material that deserves to be exploited.
Several important biological activities have been reported for olive polyphenols, in particular those related to oxidative stress counteraction: anti-aging, cardiovascular protection, chemo-preventive. Triterpenes such as oleanolic acid have been investigated for their role in endothelium antioxidant protection. Recently, the attention of scientific research on olive leaves specialized metabolites has also focused on neuroprotection but, to the best of our knowledge, no studies have been done yet with the aim of investigating the role of olive leaves phytocomplex in the context of neuronal stress, that is not only related to oxidative impairment.
Therefore, the purpose of the project here shown is to characterize the relationship between different olive leaves matrices and biological activities through innovative *in vitro* models of cellular stress.
In this study we started from pruning wastes of different varieties of *O. europaea* representative of the Italian Central and Southern territory (Sicily, Campania, Basilicata, Lazio and Tuscany). All samples were extracted with different food-grade solvents by means of different methods (ultrasound, maceration, automatic Naviglio Estrattore, decoction) in order to obtain a large dataset of samples with different phytochemical profiles. Currently, all samples and pure isolated compounds are undergoing to cell-free enzymatic assays such as sirtuin-1 activation (direct stress response), fatty acid amide hydrolase inhibition (endocannabinoid system) and neutrophil elastase inhibition (inflammatory response) and are evaluated through electrochemical measures as well (red/ox properties). *In vitro* digestion simulation combined with microfluidic and computational tools are used to predict pharmacokinetic features of different olive leaves compounds. At the same time, cell viability of digested samples is evaluated on SH-SY5Y human neuroblastoma cell line. Further studies will investigate olive leaves extracts and fractions for their ability of modulation of stress markers such as heat shock proteins, NPY, neurotrophins, Nrf-2, in different conditions of stress stimuli: glutamate, microbial lipopolysaccharide, serum deprivation.
First results suggest that, as expected, the antioxidant and antiradical capacity of tested samples depends on polyphenol content, but different phytochemical profiles lead to a variable polyphenol bioavailability and to different activity on considered enzymatic markers and neuroblasts viability.

9 = A rapid metabolomics-guided identification of potential anticancer specialized metabolites active against drug-resistant cancer cell lines from Asteraceae plants

Giovanna Valentino, Adriano Stinca, Assunta Esposito, Antonio Fiorentino
Department of Environmental Biological and Pharmaceutical Sciences and Technologies, University of Campania "Luigi Vanvitelli", via Vivaldi 43, 81100 Caserta, Italy

Chemotherapy resistance and molecularly targeted therapies pose significant challenges to cancer treatment. Plant specialized metabolites have proven to be an invaluable source of therapeutic drugs in recent years. Their high species diversity, structural complexity, and wide range of metabolites can also be used in the search for chemicals that act against resistant cancer cell lines. However, the complexity of the plant metabolome makes separation and characterization of these compounds challenging.
Natural product discovery has been significantly improved with the advent of NMR-based metabolomics. High-throughput identification and quantification of metabolites are achieved by this technique, allowing a large number of samples to be analyzed in a short period of time and observing and distinguishing a wide range of metabolites from different chemical classes within the crude extracts.
Asteraceae species and subspecies, belonging to one of the most numerous plant families, are known to be abundant sources of natural lead compounds used in medicine, pharmaceuticals, and food.
Combined with this approach, twelve Asteraceae species from the Mediterranean region have been screened for their biological activity against a panel of cancer cell lines, in the search of new potential anticancer compound.
Multivariate data analysis of the results allowed the rapid selection of *Anthemis maritima* L., *Centaurea deusta* Ten. subsp. *deusta*, and *Xanthium italicum* Moretti as source of bioactive extracts against resistant cancer cell lines. Furthermore, from 1D and 2D NMR analyses of the extract it is possible to predict the nature of potential active compounds.
With this preliminary knowledge, *ad hoc* procedures could be designed for their targeted isolation and few chromatographic steps were used to achieve the pure compounds. All of them were sesquiterpene lactones, which have already shown anticancer potential.
However, further work needs to be done to validate the biological activity of pure compounds and to understand the molecular mechanism of isolated compounds.

10 = Make it complex: floral nectar as a multifunctional crossroads

Marta Barberis[1], Massimo Nepi[2], Marta Galloni[1]
[1]Dipartimento di Scienze Biologiche, Geologiche e Ambientali, Università di Bologna, Italy; [2]Dipartimento di Scienze della Vita, Università di Siena, Siena, Italy
* Corresponding author: Marta Barberis, e-mail: marta.barberis2@unibo.it

Floral nectar is nowadays known to be the central interface of three parties, namely: the plant, the floral visitors and the microbial communities inhabiting nectar. Seen the most recent findings that show how floral nectar undergoes several post-secretory changes as well as an enrichment in its microbial communities, with unavoidable repercussions on visitors' behavior, here we claim that the traditional definition of floral nectar is insufficient for its precise ecological framing, and thus propose a new definition, which cannot circumvent the fact that reciprocal selective pressure is constantly exerted by the three parties on each other.

10 = Survivability of *Xanthoria parietina* in simulated Mars conditions for 30 days

Elisabetta Bianchi[1], Christian Lorenz[1], Giovanni Poggiali[2,3], Giulia Alemanno[4], Renato Benesperi[1], John Robert Brucato[3], Stephen Garland[4], Jörn Helbert[4], Andreas Lorek[4], Alessandro Maturilli[4], Alessio Papini[1], Jean-Pierre de Vera[5], Mickael Baqué[4]

[1]Department of Biology, University of Florence, Via G. La Pira 4, 50121 Florence, Italy; [2]LESIA-Observatoire de Paris, Université PSL, CNRS, Sorbonne Université, Université de Paris, 5 place Jules Janssen, 92190 Meudon, France; [3]INAF-Astrophysical Observatory of Arcetri, Largo E. Fermi, 5, 50125 Florence, Italy; [4]Planetary Laboratories Department, German Aerospace Center (DLR), Institute of Planetary Research, Ruthefordstraße 2, 12489 Berlin, Germany; [5]MUSC, German Aerospace Center (DLR), Space Operations and Astronaut Training, Linder H. he, 51147 Cologne, Germany

Xanthoria parietina (L.) Th. Fr. is a cosmopolitan foliose lichen that grows on barks and rocks. This species shows a high tolerance to atmospheric pollutants, heavy metals, and UV radiation thanks to the shielding properties of the secondary metabolite parietin. Several astrobiological studies involving lichens demonstrated the ability of these organisms to be able to survive in extreme environments such as space and simulated Mars surface conditions. The experiment aims were (i) evaluate the survival of *X. parietina* in simulated Mars conditions for 30 days, monitoring the viability of the lichen through the fluorescence of chlorophyll a (F_V/F_M), carrying out analyzes in situ and after treatment; (ii) evaluate oxidative stress due to extreme conditions, highlighting any changes in the carotenoid peaks detected by Raman spectroscopy; (iii) verify the peak shifting of the IR spectrum bands of the lichen possibly correlated to the effects of photodegradation given by ultraviolet radiation and (iv) verify possible variations in the ultrastructure of the lichen through electron microscopy (TEM). Eight samples were exposed to Mars atmospheric conditions, four of which were irradiated with a UV lamp with day-night cycles (FM, Full-Mars) and the other four kept in the dark (DM, Dark-Mars). As a best approximation of Mars atmospheric conditions, a mixture of three gases composed of 95% CO_2, 4% N_2 and 1% O_2 was used, with a constant pressure of 600 Pa. Temperature and humidity were subjected to day-night cycles, reaching during the day 15 ° C and 0% RH, and during the night -55 ° C and 100% RH according to the Mars thermophysical conditions in the middle latitudes. UV radiation for the FM samples was simulated using a xenon UV lamp (spectral range 200nm - 2200nm) which turned on automatically for 16 hours (day) and turned off for 8 hours (night) per day. The mean total radiation dose for FM samples was 2452.32 J/cm^2. Four other samples were kept under control conditions during the experiment, at a constant temperature of 25 ° C, wetted daily and exposed to 12 hours of darkness and 12 hours of light (approximately 50 μmol m^{-2} s^{-1} PAR photons). The results showed significant differences between the photosynthetic efficiency parameters of the FM and DM samples during exposure, exhibiting F_V/F_M values correlated with cycles of temperature and humidity. F_V/F_M recovery values also showed significant differences between treatments, highlighting that FM conditions caused stronger effects on fluorescence values. Further analyzes show possible changes in the Raman and IR spectra of the irradiated samples. Overall, *X. parietina* was able to survive FM conditions, and for this reason it can be considered an excellent candidate for long spatial exposures and further evaluation of parietin photodegradability under extreme conditions.

10 = *Prodromus Florae Bononiensis ex horto sicco Ulyxis Aldrovandi*: a flora ahead of its time from a Renaissance Italian herbarium

Fabrizio Buldrini[1 2], Alessandro Alessandrini[3], Alessandro Chiarucci[1], Umberto Mossetti[2], Giovanna Pezzi[1], Adriano Soldano[4], Juri Nascimbene[1]
[1]BIOME Lab – Dip. di Scienze Biologiche, Geologiche e Ambientali, Università di Bologna. Via Irnerio 42, 40126 Bologna, Italy; [2]Sistema Museale di Ateneo, Università di Bologna. Via Irnerio 42, 40126 Bologna, Italy; [3]Independent researcher. Via G. Pilati 19, 40018 San Pietro in Casale (BO), Italy; [4] Largo Brigata Cagliari 6, 13100 Vercelli, Italy

Ulisse Aldrovandi (1522-1605) is one of the major Italian scientists of the Renaissance, author of the richest herbarium of his epoch (more than 5000 specimens), that he put together between 1551 and 1586. In contrast to what was normal among contemporary scholars, he noted the collection places of his samples, not rarely with a precision similar to what is used today, thus his herbarium can be used in a modern sense for diachronical analyses of biodiversity. The entire collection was studied two decades ago, with identification of the specimens and, whenever possible, attribution of the collection place as indicated in Aldrovandi's manuscripts. 91% of all specimens was collected in the Italian territory and about 40% came from the territory of Bologna: this 40% corresponds to 980 species currently recognized, of which 819 can be considered as native to the territory explored. This area was deeply studied in the XIX century by Antonio and Giuseppe Bertoloni and Girolamo Cocconi (the latter published a complete Flora of the province in 1883) and Emilia-Romagna region is provided with a floristic database (which was built up at the former *Istituto beni culturali* of the region) spanning from 1790 to present days, thus we analysed Aldrovandi's floristic data for the province of Bologna and compared them with Cocconi's and modern ones. To better understand the differences occurred during the centuries, we studied in depth the plain portion of the provincial territory, that surely is the most impacted by man's activities.
The differences detected are worthy of note if we look at the autochthonous species and even outstanding when considering exotic ones. The life form spectrum for native species shows some variations in the presence of phanerophytes (in the XIX century they were half the values of Aldrovandi's and present times), hydrophytes (in the XIX century they were more than twice the values of Aldrovandi's and present times), helophytes (same trend as hydrophytes) and chamaephytes (today they are 2,8%, versus 1,4% at Cocconi's epoch and 0,2% in Aldrovandi's times). The chorological spectrum, instead, shows appreciable differences only for boreal species, that in Cocconi's data are 14,3%, whereas in Aldrovandi's and modern ones they do not reach 11%; Eurasian, Mediterranean and cosmopolite species are practically constant, with values oscillating at around 40, 26 and 17%, respectively. The differences detected can be explained in various ways. First of all, the climatic variations: Cocconi's data depict the situation of the late Little Ice Age, whereas Aldrovandi's and modern ones show totally different scenarios from a climate viewpoint, since the Renaissance marked the end of the Middle Ages climatic *optimum* and now we are in a phase of global climatic warming and drying. Second, the exploration effort: we can speculate that Aldrovandi explored the plain territory moving by boat, since many parts were marshy or periodically flooded, whereas Cocconi probably had easier conditions and now there are various persons who dedicate themselves to the floristic exploration of the area. Third, the numeric differences in the species list: Aldrovandi registers 539 species for the plain, Cocconi only 289, today they are 838; anyway, we must note that Aldrovandi's indications of the collection places are sometimes quite generic, therefore the inevitable interpretation to make his data analysable can notably affect the results.
In most cases, allochthonous species in Aldrovandi's data come from different parts of Italy or from the Mediterranean basin, with a marginal presence of species native to Asia and none coming from Africa, Americas or Australia, since we were at the very beginning of their colonisation history in Europe. By contrast, in Cocconi's and modern data the allochthonous often come from other continents, principally Asia and Americas: the transition from an exotic flora dominated by Old World species to a flora with prevalent presence of species coming from the New World is particularly evident considering these two sources of data, since in modern times American species compose 38,9% of the allochthonous spectrum.
To summarize, our study allowed to outline the floristic scenario of an Italian province during the Renaissance thanks to one of the oldest known herbaria. Such a possibility is due to Aldrovandi's extraordinary scientific acumen, because, unlike his contemporary scholars, he noted the collection places of his specimens and explored the territory with a modern perspective, since he intended to collect all plant species existing in the area studied. At least at the present state of knowledge, this case is a true *unicum* at a world level and permits to illustrate the floristic differences experienced by a temperate area in a timespan of *circa* 500 years, i.e. twice the longest interval normally considered in diachronical analyses of biodiversity.

10 = Unusual underwater flowering of *Utricularia australis* population: a botanical enigma

Simona Ceschin[1], Neil T.W. Ellwood[1], Mattia M. Azzella[2], Flaminia Mariani[1], Emanuele Pelella[1]
[1]Department of Sciences, University of Roma Tre, Viale G. Marconi 446, 00146 Roma, Italy; [2]Department of Planning, Design and Technology of Architecture, University of Rome "La Sapienza", Via Emanuele Gianturco 2, 00196 Roma, Italy

The aquatic carnivorous plant *Utricularia australis* R.Br. is a free-floating macrophyte that typically produces subsurface shoots and subaerial inflorescences with showy yellow flowers (Fig. 1a). Surprisingly, we found it growing with large populations among benthic *Chara* stands at 4-6 m depth in two lakes of the Central Italy (Lake Bracciano, Lake Bolsena), which is a phenomenon that was never previously reported for this species. Even more unusual and intriguing, these benthic stands were able to produce chasmogamous flowers similar to the usual subaerial (Fig. 1b). We compared morphological characters from a quantitative-qualitative standpoint between underwater and subaerial flowers, these latter taken from diverse aquatic sites in Central Italy. We used environmental data both to delineate differences between underwater and superficial habitats where flowering *U. australis* populations were found, and to help explain these extraordinary findings. We found significant differences between underwater and subaerial flowers: particularly, the flower scapes of underwater individuals were longer and thicker than the subaerial ones. The elongation of the underwater flower scapes is especially noteworthy, since they were around three times longer than those subaerial. This might be a typical phototropic-response or a failed attempt to get flowers above water surface for allowing entomophilous pollination, or a redundant morpho-physiological trait because of a recent ecological move. In addition, floral elements of the underwater flowers were generally smaller, the exception being the length of the style, which was greater. Moreover, underwater flowers had less obvious nectar signaling, thinner petals, and mucous-coated pollen, whereas pollen in subaerial flowers is typically dry and grainy. Underwater flowers even had fruits and seeds but similarly to subaerial flowers they were sterile, producing abortive fruits. Among the hypothesis, the possibility of underwater ecological conditions causing stress-induced flowering is proposed. The underwater habitat is obviously very different from the one where *U. australis* normally occurs, but it could offer better conditions for *U. australis* growth. For example, the ecological requirement of *U. australis* for still or very slow-moving water is better met at the depth compared to the surface along shoreline where water level changes, wind-generated waves and anthropogenic disturbance could destabilize any *U. australis* population.

Fig. 1. Subaerial (pond near Lake Bracciano) (a) and underwater flowering (6 m depth, Lake Bracciano) (b) in *U. australis*.

10 = Preliminary investigations on Mediterranean temporary ponds: study cases from Sardinia, Corsica, and inland Central Italy

[1]Annalena Cogoni, [2]Daniela Gigante, [1]Silvia Poponessi
[1]Dipartimento di Scienze della vita e dell'ambiente, Via Sant'Ignazio da Laconi, 13 - 09123 Cagliari; [2]Dipartimento di Scienze Agrarie, Alimentari e Ambientali, Borgo XX Giugno, 74 - 06121 Perugia

This work reports the data of a preliminary study about the knowledge of habitat 3170* from a bryophytic point of view, presenting outcomes of botanical surveys carried out in three Mediterranean temporary ponds (MTPs) from different climatic contexts of Sardinia (Pauli in the Giara), Corsica (Padule of Suartone), and inland Central Italy (Piana di Ferretto). The bryophytic element has often been neglected although representing an emblematic feature of the whole habitat type and a very important indicator, especially with reference to habitat monitoring and management. Nevertheless, it represents a key feature of the biotic domain of the MTPs, and its better understanding may provide a significant step forward for this habitat conservation. Interestingly, in the surveyed MTPs, the bryophytic taxa tend to have asynchronous phenology dynamics than vascular taxa, appearing earlier or latterly than the vascular companions. The rainfall distribution plays a pivotal role in this interaction that should be further investigated to identify interdependencies between bryophytic and vascular communities and predict ecological trends. From a bioclimatic standpoint, the Pauli site can be included in the meso-Mediterranean thermotype, whereas Suartone falls in the thermo-Mediterranean one and Ferretto in the Submediterranean Variant of the meso-Temperate. The floristic richness for each site is as follows: *Padule*, Suartone 30 taxa (9 liverworts, 21 mosses), *Pauli,* Giara 56 taxa (6 liverworts, 50 mosses), and Piana di Ferretto 44 taxa (13 liverworts, 31 mosses). A common share of bryophytes is observed in the various studied areas, in particular, the liverworts of the genus Riccia L. (Marchantiophyta, Ricciaceae) find here a preferred biotope with 9 species: 4 are common to all three sites: *R. beyrichiana, R. canaliculata, R. sorocarpa, R. crozalsii*. In the case Pauli of Giara, the distribution of the most frequent life strategies, life forms, and ecological characters of bryophytes was analyzed in particular with reference to the humidity gradient within a Mediterranean temporary pond. The distribution pattern of the species follows the distribution between three defined belts: external (O), central (C) and internal (I). It would be necessary to monitor the frequency and intensity of the rains to prevent changes in these fragile ecosystems, in order to avoid the loss of their functions as temporary wetlands and their associated flora and fauna.

10 = Quantitative analysis of secondary metabolites in *Eruca sativa* under salt stress

Emilio Corti[1], Giuseppe Pieraccini[3], Besiana Nako[1], Cristina Gonnelli[2], Alessio Papini[1], Sara Falsini[1]
[1]Department of Biology, University of Florence, Via Micheli 1, 50121 Firenze, Italy; [2]Department of Biology, University of Florence, Via Micheli 3, 50121 Firenze, Italy; [3]Department of Health Science, University of Florence, Viale Pieraccini, 6 - 50139 Firenze, Italy

Plants are able to synthesize a wide range of chemical compounds, called secondary metabolites, through cell metabolic pathways deriving from the primary metabolism. Secondary metabolites play the main role in providing adaptation and protection against biotic and abiotic stresses, such as herbivores, pathogens, or drought and their accumulation seems to be affected precisely by the adjustments on plant morphology, anatomy, and physiology. Based on their biochemical origin, plants secondary metabolites are divided into three major groups: terpenoids, phenolics, and nitrogen-based compounds. Within the latter category, glucosinolates and their derivates are a wide group of molecules, containing sulfur beyond nitrogen, that are typically detected inside the Brassicaceae family and that are relevant for human health since they showed antioxidant, anti-inflammatory and likely antitumoral effects.
The aim of our study was to (i) understand if salt stress-induced a rising in the amount of glucosinolate-derivates called isothiocyanates (ITCs) in *Eruca sativa* (Brassicaceae), and (ii) identify the NaCl concentration able to enrich the plant in these chemical compounds without impairing its growth and development. In this preliminary study, we investigated the effect of three concentrations of salt (32.8 mM, 65.6 mM and 133,2 mM NaCl) on *Eruca sativa* evaluating the plant morphology modifications and the variation in concentration of three isothiocyanates (Erucin, Sulforaphane and Ibervirin) after 21 days of treatment in hydroponic solutions. The amount of Isothiocianates was detected by Ultra High Performance Liquid Cromatography-Mass Spectrometry analysis. The concentration of Sulforaphane increased in salt-treated plants in comparison to control plants. The highest value was observed in plants grown in 65.6mM saline solution. The same pattern was observed for Erucin. Conversely, Ibervirin amount had the greatest enhancement in plants treated with the highest salt concentration (131.2 mM). Regarding the morphology, plants treated with the lowest salt treatment (32,8 mM) did not show a significant reduction in the surface of the areal part, in the length of root and stem, and in fresh weight and dry weight with respect to control plants. Instead, the two highest salt concentrations significantly affected the growth parameters in a negative way. Thus, our results showed that 32,8mM salt solution was the best concentration to guarantee a significant increase of isothiocyanates concentration without impairing plant development.

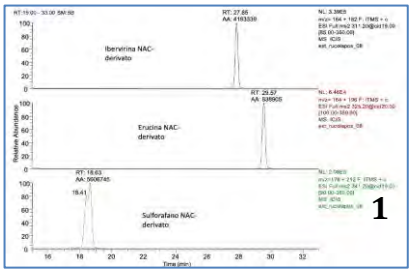


Fig. 1 ITCs chromatographic profile (MS/MS) of E.sativa Fig. 2-3 E.sativa plants (32,8 and 131,2mM salt solution)

10 = Plants do not like it coloured: PET micro/nanoplastics with various colours impact differently on *Arabidopsis thaliana* growth

Marco Dainelli[1], Sara Falsini[1], Massimo del Bubba[2], Lorenzo Venturini[2], Alessio Papini[1], Ilaria Colzi[1], Sandra Ristori[2], Cristina Gonnelli[1], Andrea Coppi[1]
[1]Department of Biology, University of Florence, Via Micheli 1, 50121 Firenze, Italy; [2]Department of Chemistry, University of Florence, Via della Lastruccia 3, Sesto Fiorentino, 50019 Firenze, Italy

Due to its convenient use, plastic is so widely employed that its debris are found all over our planet. Plastic increasing presence in the environment and its global distribution make pollution deriving from polymeric materials among the most important environmental problems that our society must face, progressively reaching even nature sanctuaries and remote areas. Research focused on very fine particles i.e. microplastics (0.1 µm to 5 mm) and nanoplastics (< 0.1µm) involved in the contamination of atmosphere, water bodies and soils, evidencing related possible risks to the various organisms populating these environments. In any case, even though negative impacts of micro/nanoplastics (MNPs) on the whole ecosystem are expected, information about the potential threat of MNPs on organisms other than animals is extremely limited. In this context, plants are fundamental primary producers and contribute to regulate the global climate, therefore their response to plastic particles claims to be better investigated.
The aim of this work was to test if MNPs of polyethylene terephthalate (PET), one of the most widespread environmental pollutants, can impact negatively on plant growth. Specifically, MNPs were produced directly in solution from bottles of different colours (colourless, blue, green, yellow, orange, and red) through reiterated cycles of homogenization in a blender. Their dimension was characterised by DLS (around 200-300 nm) and their composition in metals by mineralization and ICP. The obtained MNPs were used to prepare Hoagland solutions with environmentally realistic concentrations of plastic particles for contaminated waters (around 0.1 g L^{-1}) and were thus administered to ten-day old plantlets of *Arabidopsis thaliana*. Plants were cultivated for two weeks in a growth chamber and at the end of the treatment their biometric traits were analysed together with their photosynthetic indexes (F_v/F_m and P_{index}) and ionome.
The presence of MNPs in the culture medium induced a significant decrease in root length increment, at a different extent for the different colours used (Fig. 1 and 2). Particularly, colourless PET resulted the less toxic material, while blue PET was the one with the greatest negative impact on plant growth. The variations in leaf area resembled those in root growth, although this parameter was generally less influenced by MNPs. Similar results were obtained also for the production of fresh and dry plant, with the root being the most affected organ compared to the shoot and the blue PET being the most toxic material. The variation in plant growth was explained, at least in part, by a negative effect of the MNPs on the photosynthetic performance of the samples. The analysis of the plant ionome suggested the accumulation of some non-essential elements as an additive cause of toxicity for the coloured MPNs. The chemical analysis of the plastics revealed the presence of trace metals like antimony (Sb), hafnium (Hf) and cobalt (Co) in the coloured materials and the same elements were found in the plant body at toxic concentrations.
Our results reported a negative impact of PET MNPs on plants and, for the first time, demonstrated that the production of coloured materials is not only useless but also more dangerous.

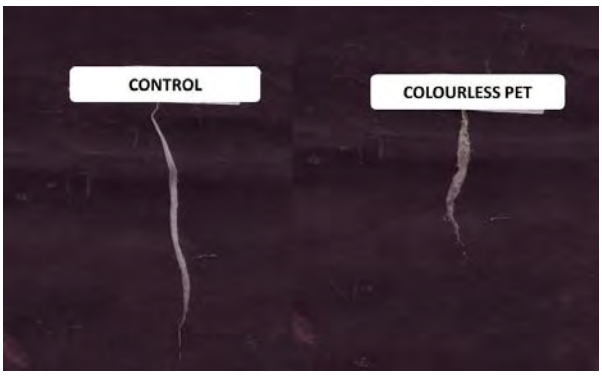


Fig. 1. Roots of *A. thaliana* plants exposed to MNPs for two weeks

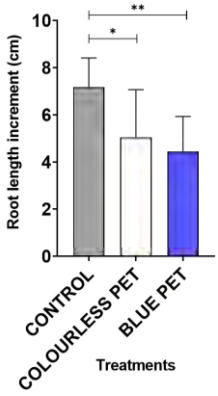


Fig. 2. Increment in root length of *A. thaliana* plants exposed to MNPs for two weeks

10 = Biodiversity informatics and Museums: from the documentation of collections to dissemination at the Botanic Garden and Museum of Pisa

Marco D'Antraccoli[1], Francesco Roma-Marzio[1], Leonardo Cocchi[1], Nóra Weiger[1], Lucia Amadei[1], Giovanni Astuti[1], Roberta Vangelisti[1], Lorenzo Peruzzi[1,2]
[1]Pisa Botanic Garden and Museum, University of Pisa, Via Luca Ghini 13, 56126 Pisa, Italy; [2]Department of Biology, University of Pisa, Via Derna 1, 56126 Pisa, Italy

Botanic Gardens and Herbaria are traditionally recognised as knowledge repositories about the world of plants. The study, management, and conservation of specimens has represented a cornerstone for the advance of botanical disciplines along centuries. In recent decades the spread of informatic tools has opened new opportunities to further enhance these collections, posing new challenges to adequately disseminate the botanical culture among visitors and society. Indeed, Botanic Gardens and Museums have a clear and recognised role in placing educational, social, and environmental responsibilities at the heart of their mission. Accordingly, the Botanic Garden and Museum of Pisa activated a path for updating and implementing the digitization of its collections (over 2,000 cultivated taxa and about 350,000 specimens stored in the Herbarium, international code PI). In November 2017, a digitization process of herbarium specimens was started by joining the project JACQ-Virtual Herbaria (http://jacq.org/#database), an international database project coordinated by the University of Wien. This database allows for metadata acquisition and specimens georeferencing, providing an automatic link connecting data to digital images. To make the digitized specimens accessible to non-expert users, in January 2020 we launched the Virtual Herbarium of the University of Pisa (https://erbario.unipi.it; Fig.1), where all the images and data entered in JACQ are automatically and user-friendly accessible. At 26th May 2022, 37,810 specimens (ca. 11% of the Herbarium) have been made accessible on the Virtual Herbarium through mobile devices, personal computers and a dedicated workstation installed at the Botanic Museum. In November 2021, we launched the portal U-plant DISCOVER (https://uplantdiscover.sma.unipi.it/; Fig. 2): this tool extracts in real-time a pool of data from the living plant documentation system in use by the Botanic Garden staff, namely U-plant. In this way, users can freely access a range of plant information related to each accession, including a photographic gallery of the specimen in cultivation. At 26th May 2022, 2,810 photos have been stored and are available online. This tool is a very useful support for the visit of the garden, for example allowing to observe plants and their details also when not visible due to the phenology. In addition, the portal provides a range of thematic queries, implementing the chance to customise the garden visit. We can also track the accesses to our digital platforms through Google Analytics. Since its launch to the public, the Virtual Herbarium counted 11,952 users (with 2,627 returning visitors) for 17,640 sessions, and an average session duration of around 4 minutes. Besides Italian users (81%), the three most represented user's countries are: United States (3%), China (1%), and Germany (0.9%). The Virtual Herbarium is mainly reached from direct link (84%) and social networks (7%), by means of desktop computers (65%), smartphones (33%), and tablets (2%). U-plant DISCOVER counted so far 351 users (with 4 returning visitors), and an average session duration of 1 minute. Besides Italian users (69%), the three most represented user's countries are: United States (10%), Germany (5%), and France (3%). U-plant DISCOVER is mainly reached from direct link (76%) and organic search (17%), by means of smartphones (78%), desktop computers (21%), and tablets (1%). These data emphasize the positive response obtained by these informatic tools, and their ability to increase the usability of botanical collections by both non-expert visitors and professional users.

Fig. 1. Homepage of PI Virtual Herbarium

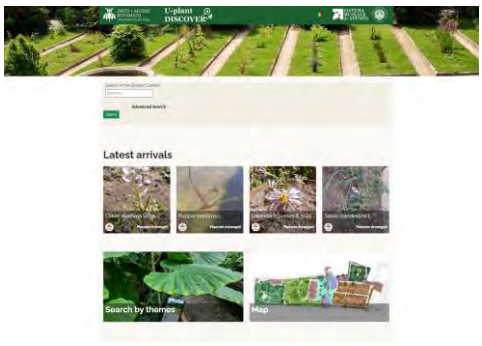


Fig. 2 Homepage of U-plant DISCOVER

10 = Pollination success of the deceptive orchid *Anacamptis morio*: benefit from proximity or similarity?

Anna D'aria[1], Giampiero Ciaschetti[2], Salvatore Cozzolino[1], Giovanni Scopece[1]
[1]Department of Biology, University of Naples Federico II, Via Cinthia, 80126, Napoli, Italy; [2]Majella National Park, Chieti, Italy

An interesting aspect within angiosperms is the ability of a plant to undergo pollination without offering a putative pollinator any form of floral reward. Although deceptive pollination seldom occurs in other groups of angiosperms, it is particularly prevalent within the family Orchidaceae, with approximately one-third of species offering no floral reward. Deceptive orchids are predominantly insect pollinated with high rates of visitation largely dependent on the attraction of mostly naive pollinators through mimicry of rewarding plants. Deceptive orchid flowers often exhibit a high degree of colour or scent polymorphism which aims to delay the associative abilities of the insect and, therefore, make it more challenging to discover the deception (Gumbert and Kunze, 2001). One of the most widespread forms of mimicry is food deception, which consists of the mimesis of a nectariferous plant or typical characteristics of the latter; examples are present in species within the genus *Anacamptis*. In more specialized cases, some species of orchids mimic only a specific nectar plant model (i.e., Batesian mimicry; see Dafni, 1984; Johnson 2000), and natural selection pushes the orchid flower to be almost indistinguishable from its nectar partner, making the pollinator unable to distinguish the mimic from the partner. Pollination success in deceptive orchids can vary based on some species' traits and depending on the environmental context and the presence of other species, which can affect the pollinator community and, consequently, fitness. In our study, we aimed to evaluate whether the pollination success of the food deceptive orchid, *Anacamptis morio*, is related to floral traits of the plant itself, such as density, height, and the number of flowers and / or to the presence of sympatric rewarding plants (also known as the 'magnet species' effect). In particular we tested whether the pollination success of *A. morio* is dependent on the presence and density of the surrounding nectar species, such as *Lotus corniculatus*, *Anthyllis vulneraria*, *Polygala major* and *Rhinanthus wettsteinii*. For all these species, the number of fruits produced relative to the number of flowers was recorded. This value is widely used as proxy to evaluate the pollination success. Our results showed that the pollination success of *A. morio* is positively correlated with both plant height and number of flowers. Interestingly, the majority of fruits formed in the lower section of the inflorescence, confirming the pollinator's typical mechanism of avoidance learning. The density of sympatric nectar species did not significantly affect orchid fitness however pollination success of the deceptive *A. morio* was positively correlated to the pollination success of the co-occurring rewarding *Polygala major*. This may suggest a magnet species effect between *A. morio* and *Polygala* or some an alternative form of Batesian mimicry mechanism may be present that subsequent experiments should confirm.

10 = Biogenic Volatile Organic Compounds (BVOCs) around the world: does geography shapes BVOCs profiles in bryophytes?

Antonio De Agostini[1], Maitane Juarez[2], Marina López-Pozo[3], Javier Martínez-Abaigar[3], Encarnación Núñez-Olivera[3], Unai Artexte[2], Marco Michelozzi[4], Gabriele Cencetti[4], Francesco Loreto[5,6], Susanna Pollastri[6], Daniel Stanton[7], Annalena Cogoni[1], Pierluigi Cortis[1], Alicia V. Perera-Castro[8], Hong Truong Luu[9], Buu Thach Le[9], Thanh Luc Nguyen[9], José Ignacio García-Plazaola[2], Antonio Hernandez[2], Raquel Esteban[2]
[1]Department of Life and Environmental Sciences, University of Cagliari, Botany Section, Cagliari, Italy; [2]Department of Plant Biology and Ecology, University of Basque Country (UPV/ EHU), Bilbao, Spain; [3]Science and Technology Faculty, University of La Rioja, Logroño, Spain; [4]Institute of Biosciences and Bioresources National Research Council of Italy (CNR), Sesto Fiorentino, Florence, Italy; [5]Department of Biology, Agriculture and Food Sciences, National Research Council of Italy (CNR), Rome, Italy; [6]Institute for Sustainable Plant Protection, National Research Council of Italy (CNR), Sesto Fiorentino, Florence, Italy; [7]University of Minnesota, Saint Paul, Minnesota, USA; [8]Departament of Botany, Ecology and Plant Physiology, Universidade de La Laguna, Islas Canarias, Spain; [9]Southern Institute of Ecology, Vietnam Academy of Science and Technology, Ho Chi Minh City, Vietnam

Concomitantly with their emancipation from water, bryophytes faced a variety of stressors linked to the newly discovered terrestrial niche. Such an evolutionary path led to the development of a variety of protective mechanisms that allow plants to thrive despite suboptimal or even extreme environmental conditions. Among the vast array of plant functional and defensive compounds, Biogenic Volatile Organic Compounds (BVOCs) are low-boiling-point secondary metabolites synthesized to address a variety of ecological/physiological challenges (e.g., resistance to abiotic and biotic stress, communication with other organisms, plant to plant interaction and competition, defense against herbivores and microbes, allelopathic activity, resistance to UV-B radiation). Genetics shape plant BVOC content, blend and emission, but the influence of environmental factors in determining plants' BVOC profile is becoming increasingly evident. Here, we investigated the variability of BVOC profiles in bryophytes (mosses and liverworts) in relation to their distribution around the world. A total of 15 species (belonging to 12 genera and 10 families), were sampled in Antarctica, Asia (Vietnam), Europe (Italy and Spain) and North America (Minnesota, US), as representative of the Polar, Subtropical, Warm temperate (mild summer and oceanic), and Hemiboreal climates, respectively, according to Köppen climate classification. The content of BVOCs was determined by gas chromatography-mass spectrometry (GC-MS). Multivariate statistical approaches allowed us grouping species based on similar BVOC profiles and relating them to different geographical and climatic distributions. Our results show that climate shaped BVOC profiles in bryophytes, with bryophytes of different climates showing different BVOC patterns. This is, for example, the case of Vietnam where bryophytes thriving in a tropical climate, showed a very peculiar BVOC profile, whereas species from temperate regions (e.g., Italy and Spain) resulted similar in their BVOC profiles. With this work, an initial BVOC database in bryophytes is setting up, making it possible future exploration of the relationships between BVOC profiles and ecological characteristics of this evolutive important group of plants.

Funding: RE received a research project from AEET, the Spanish Association of Terrestrial Ecology (www.aeet.org).

10 = Quantitative anatomical traits and stable isotopes in wood rings of Greco grapevine reveal acclimation to environmental variability and cultivation factors: the GREASE Project

Veronica De Micco[1], Alessia D'Auria[1], Francesca Petracca[1], Sara De Francesco[1], Francesco Niccoli[2], Simona Altieri[2], Arturo Pacheco[2], Antonello Bonfante[3], Chiara Cirillo[1], Arturo Erbaggio[4], Pierpaolo Sirch[5], Giovanna Battipaglia[2]

[1]Dipartimento di Agraria, Università degli Studi di Napoli Federico II, Via Università 100-80055, Portici (Napoli); [2]Dipartimento di Scienze e Tecnologie Ambientali, Biologiche e Farmaceutiche, Università della Campania Luigi Vanvitelli Via Vivaldi, 43- 81100, Caserta; [3]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); [4]Libero professionista; [5]Feudi di San Gregorio Società Agricola S.p.A., Contrada Cerza Grossa, 83050, Sorbo Serpico (Avellino)

Climate change is threatening grapevine growth and productivity in the Mediterranean region, due to significant increase in temperature and changes in precipitation patterns leading to more frequent and severe drought events. Vineyard productivity and grape quality strictly depend on cultivar, pedoclimatic characteristics (e.g., soil and climate) and cultivation techniques (e.g., canopy and soil management, plant nutrition, training and watering regime). The Italian legislation imposes rainfed cultivation for grapevine whose musts are devoted to the production of wines with quality and/or geographical indication labels. However, the need for irrigation introduction and management in the vineyard is becoming more and more evident. Although supplemental irrigation is already allowed, an efficient and sustainable strategy for irrigation management needs to be designed on the cultivar-specific hydraulic behaviour and vine morpho-anatomical and eco-physiological plasticity that are linked with vine water use.

In the GREASE project we borrowed the dendro-sciences approach of forest sciences and applied it to viticulture to gain knowledge on past hydraulic history of *Vitis vinifera* subsp. *vinifera* 'Greco' vines growing in an experimental vineyard in southern Italy. The GREASE project ("Modelli sostenibili di coltivazione del vitigno Greco: efficienza d'uso delle risorse ed applicazione di indicatori della Footprint family") was funded by the Campania Region through the Rural Development Programme 2014-2020, in the framework of improving grapevine productivity, resource use efficiency and resilience for the sustainable management of vineyards. The study was conducted in a Greco vineyard of the Feudi di San Gregorio farm where vines of two age classes were subjected to a change in pruning technique in a given year. More specifically the vines were converted to the pruning technique according to the Simonit & Sirch method which is based on oriented pruning cuts to drive vine natural tendency in branching, also reducing the resistance to water flow. The aim of the study was to evaluate whether the change in the pruning technique was visible in the tree-ring series of the vine trunk as anatomical or isotopic signals. Therefore, wood cores and stem disks were sampled, tree-rings were dated, and cross sections of the tree-ring series were obtained through a sliding microtome. Sections were analysed through light and fluorescence microscopy; wood anatomical traits linked with hydraulic conductivity and vulnerability to embolism were quantified. The tree-ring series were dissected to for the determination of carbon and oxygen stable isotopes to evaluate intrinsic water use efficiency. The overall wood anatomical and stable isotope parameters were analysed together with climatic data through multivariate statistical analysis.

Results indicated that the change in pruning technique has a different effect depending on the age of the vine at the time of conversion. Understanding how the vine has reacted to past environmental variability and changes in cultivation factors through dendro-sciences can help forecasting how it will behave under the different climate change scenarios.

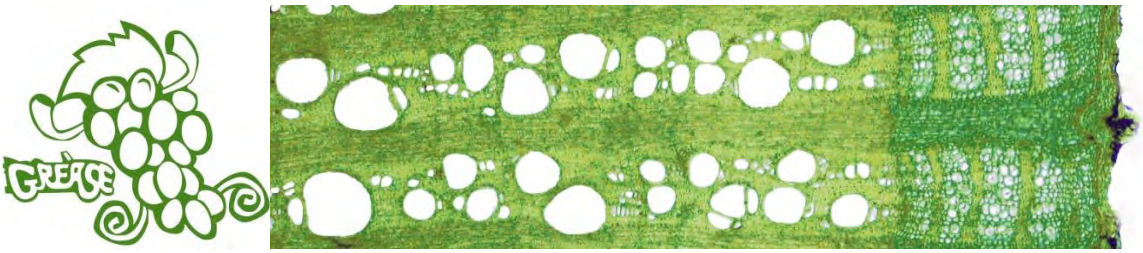


Fig. 1. Logo of the PSR-GREASE Project and microscopy view of grapevine wood rings in stem cross section

10 = Plastic germination response of plants living in Mediterranean temporary ponds (3170* habitat)

Mario Di Stefano[1], Francesca Carruggio[1], Giuseppe Diego Puglia[2], Antonia Cristaudo[1]
[1]Department of Biological, Geological and Environmental Sciences, Germplasm Bank (BGS-CT) University of Catania, Via Empedocle 58, 95128 - Catania, Italy; [2]Consiglio Nazionale delle Ricerche, Istituto per i Sistemi Agricoli e Forestali del Mediterraneo (CNR-ISAFOM) U.O.S. Catania, Via Empedocle 58, 95128 - Catania, Italy

Mediterranean temporary ponds (MTPs) are mostly shallow water bodies, which undergo cycles of flooding and drought according to rainfall seasonality. They have a high conservation value for both their ecological function and high biodiversity richness and are considered as a priority habitat according to the 'Habitats' Directive (3170* Natura 2000 code). Unfortunately, their status is rapidly declining mainly due to anthropogenic factors such as overgrazing, hydrological perturbations, and climate changes.
Seed germination and dormancy of the plant species living in MTPs are quite poorly investigated, whereas studying their germination behavior may allow to highlight species' plastic potential and possible triggers of vegetation dynamics, further considering the high seasonal variability of these habitats.
In our study, we analyzed and compared the germination patterns of two ephemeral key plants from MTPs: *Ranunculus lateriflorus* DC. and *R. ophioglossifolius* Vill., the latter is reported as vulnerable for the Italian territory (Orsenigo et al., 2021). Both species show a pronounced phenotypic plasticity going successively from an aquatic to a terrestrial morphotype. During their aquatic phase, the plants have long petioles and laminar leaves floating on the water surface, whereas during the terrestrial one they develop self-supporting stems and shorter petioles. Both species produce achenes.
The seeds of the two study species were collected in some MTPs of the Hyblean plateau (south-eastern Sicily, Italy), where rich populations occur. Germination experiments were performed using fresh as well as after-ripened seeds to evaluate the effect of temperature (both constant and alternating regimes), light (light/dark - 12/12 h, and full darkness - 0/24 h), after-ripening (Ar) duration, seed position on the receptacle (if basal or apical), and source population. Seed germination was monitored for 30 days, at the end of which the ungerminated seeds from dark experiments were transferred to optimal conditions (15°C in light/dark; recovery conditions) for two additional weeks.
For both species, light always had an enhancing effect on germination, whereas the dark treatment significantly lowered it. Specifically, regardless of constant or alternating temperature regimes, seeds germinated to a high extent in the light. Moreover, Ar improved final germination percentages (> 90%), widened optimal temperatures including warmer values (25°C and 25/20°C), and increased germination rate. As a whole, these findings provide evidences for the Type 1 non-deep physiological dormancy with conditional dormancy (CD). The seeds coming from low temperatures (5°C, 10°C and 15/10°C) and transferred to recovery conditions were still unable to complete germination, indicating the induction of skotodormancy (Baskin & Baskin, 2014). Conversely, the seeds coming from warmer temperatures (≥ 20°C) showed high germination percentages under recovery conditions, implying that thermoinhibition has established during the imbibition in the dark. In addition, in *R. lateriflorus* basal seeds germinated less and more slowly than apical ones and source population did not influence germination behavior.
Our results suggest that a mixed strategy works in the two *Rancunculus* species to meet MTPs variability. On one hand, seeds plastically germinate over a wide temperature range, possibly enhancing seedlings' establishment opportunities, once CD has been released and the ponds have filled up. On the other hand, the inhibiting effect of darkness together with skotodormancy may allow the establishment of a large soil seed bank that could remain viable and germinable at least until the next favorable season.
Considering the high environmental variability of MTPs and the germination needs highlighted by our experiments, it could be interesting to investigate on how the plant community composition of the study sites could be further affected by delayed and reduced rainfalls as well as increased temperature values that are predicted to occur in the Mediterranean region with the ongoing climate change.

10 = Light and electron microscopy observations of *Rhamnus alaternus* L. infected by *Xylella fastidiosa* subsp. *multiplex*

Sara Falsini[1], Corrado Tani[1], Valentina Rech[1], Alessio Papini[1], Paola Faraoni[2], Sara Campigli[3], Luisa Ghelardini[3], Gianluca Bleve[4], Domenico Rizzo[5], Emanuele Marcucci[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 economically important crops, such as grapevine, olive, and citrus. Following introduction in Europe, its spread through xylem-feeding hemipteran insect vectors has been facilitated by the presence of a wide range of suitable hosts, some of which are indigenous species of the Mediterranean landscape like *Rhamnus alaternus*.
In this work, we compared the response of *R. alaternus* plants exposed to natural inoculum in Monte Argentario (Tuscany, Italy), with that of plants that were artificially inoculated by needle puncture with either Xf subsp. *multiplex* strain ST87 or phosphate buffer saline (PBS), and maintained in a growth chamber at the Phytosanitary service laboratory of Orbetello (Tuscany, Italy). Fresh vegetative materials (shoots and petiole) were included and stained with Toluidine Blue (metachromatic staining), Naturstoffreagenz-A (to detect flavonoids) and Safranin staining (to evidence Gram-Negative bacteria).
In the colonized vessels of both naturally infected and artificially inoculated plants, the bacterium was often found to be associated to a pink/red stained gel matrix, which seems to be secreted by the adjacent parenchyma cells (Fig. A). In particular, SEM micrographs revealed the presence of Xf in the petiole of naturally infected plants as several colonies were observed in adjacent vessels (Fig. B). On the contrary, Xf cells were not readily evident in the vessels of artificially inoculated plants (Fig. C). In light of these observations, we speculate that the matrix is involved in the attempt of the host to limit bacterial spread inside the vessel lumen, which stained blue when intact bacterial cells could no longer be seen (Fig. D). Furthermore, the ability of *R. alaternus* to produce flavonoids was also detected as a yellow matrix which was evidenced in some vessels by Naturstoffreagenz-A (Fig D).
Because both matrices seem to trap bacterial cells and are absent in the non-inoculated wounded plants used as negative controls, they appear to be a specific response of the host to limit *X. fastidiosa* progression.

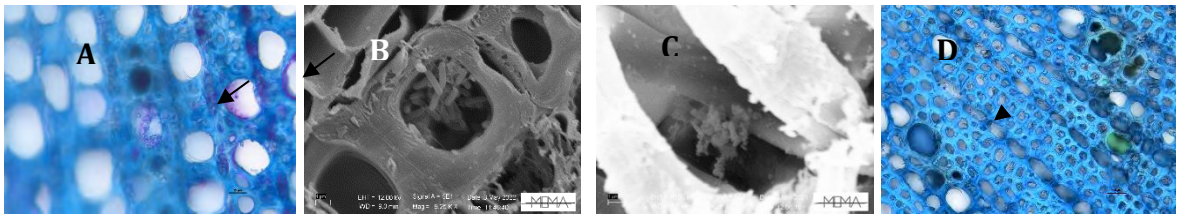


Fig. A: LM image of a naturally infected *R. alaternus* plant. Cross-section of a shoot (pink/violet indicated by arrows) stained with Toluidine Blue. Fig. B: SEM image of petiole transversal section of a naturally infected *R. alaternus* plant. Bacteria are evident in the vessel elements. Fig. C: SEM image of shoot cross-section of an artificially inoculated *R. alaternus* plant. Fig. D: LM image of an artificially inoculated *R. alaternus* plant. Blue (black triangle) and yellow (white arrows) matrices stained with Toluidine blue

10 = *Toscolea* project: enhancing the local *cultivars* of *Olea europaea* L. in Toscolano Maderno (Brescia, Italy)

Claudia Giuliani[1,2], Martina Bottoni[1,2], Fabrizia Milani[1,2], Debora Tura[3], Remo Chiozzotto[3], Irina Baccichet[3], Davide Boni[4], Sergio Cozzaglio[5], Laura Santagostini[6], Alessio Papini[7], Marco Cirilli[3], Gelsomina Fico [1,2]
[1]Department of Pharmaceutical Sciences, University of Milan, Via Luigi Mangiagalli 25, 20133 Milan, Italy; [2]Ghirardi Botanic Garden, Department of Pharmaceutical Sciences, University of Milan, Via Religione 25, 25088 Toscolano Maderno (BS), Italy; [3]Department of Agricultural and Environmental Sciences - Production, Landscape, Agroenergy, University of Milan, Via Celoria 2, 20133 Milan, Italy; [4]Cooperativa Sociale Onlus "L'Albero", Via Galvani 49, 25088 Toscolano Maderno (BS), Italy; [5]Azienda Agricola "La Zadruga", Via Trento 102, 25088, Toscolano Maderno (BS), Italy; [6]Department of Chemistry, University of Milan, Via Golgi 19, 20133 Milan, Italy; [7]Department of Biology, University of Florence, Via La Pira 4, 50133 Florence, Italy

The contribution reported hereafter is included within the biennial project *Toscolea - Censimento, caratterizzazione, conservazione e valorizzazione di accessioni di* Olea europea *L. nell'area del Garda a Toscolano Maderno e territorio contermine,* funded by the Lombardy Region within the Call PSR 2014-2020.
The aim of the project is to complete the *census*, identification and characterization of the native olive *cultivars* located in the municipality of Toscolano Maderno (Brescia) and neighboring areas. Specifically, the most representative ones of the local biodiversity include 'Minìol', 'Gargnà', 'Casaliva', 'Raza', 'Negrèl' and special attention was paid to a *cultivar* considered native, but not yet investigated, known as 'Villa Romana'. The morphological, agronomic, genetic, and phytochemical features will be comparatively evaluated to ensure the *in situ* and *ex situ* conservation of the genetic heritage.
In particular, the following study areas of municipal relevance have been selected: Primary School, Viale Marconi, and Parco Fossati, in addition to the Ghirardi Botanical Garden of the University of Milan, located in Toscolano Maderno, which is configured as an ancient olive grove.
The project includes the subsequent complementary phases:

1. Macro- and microscopic morphological investigation (by means of light and scanning electron microscopy) on the vegetative and reproductive organs, during different phenological phases (pre-anthesis, anthesis, fruit production), with special reference to covering trichome density on leaves, micromorphology of pollen grains, and epicuticular waxes of the drupes.
2. Agronomic observations in relation to some of the main biological and agronomic aspects for the drafting of elaiographic cards.
3. Analysis of DNA markers to verify and support the varietal identification.
4. Phytochemical characterization of the essential oil profiles obtained from leaves and inflorescences to assess their potential taxonomic value in the process of varietal recognition.

After the accurate screening of the varietal panorama and its phytosanitary evaluation, special attention will be paid to propagation and nursery multiplication practices. In the context of an Open Science policy, it is also intended to develop integrated educational-dissemination actions, mediated by the Ghirardi Botanical Garden and addressed to selected local olive farms. This approach will ensure the transmission to the farmers of information and the sustainability and future persistence of the precious genetic material. They will thus enhance it, making it an element of excellence for their companies and guaranteeing its active protection and constant propagation.

10 = Functional strategy and leaf shape of *Campanula elatinoides* Moretti: results of an intraspecific analysis

Luca Giupponi[1,2], Valeria Leoni[1], Marco Zuccolo[1], Davide Pedrali[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

Recent research has shown that the intraspecific variability of plant competitor–stress tolerator–ruderal (CSR) functional strategy can represent an adaptive response to climate. In particular, few authors observed that, in areas with higher temperatures, some species are more stress tolerant while they are more ruderal in cooler areas. This research analysed the CSR strategy of *Campanula elatinoides* Moretti, a little studied species endemic to the Lombardy pre-Alps (Northern Italy) (Fig. 1) that lives in few canyons and rocks with differing climatic conditions, at an intraspecific level. Furthermore, the shape of its leaf blades was analyzed. The results of functional strategy and leaf shape analysis were related to the environmental characteristics of the various sampling areas in order to highlight the intraspecific variation and the adaptation of this species to the environment and to climate in particular.
CSR strategy analysis was evaluated (with "StrateFy" tool) considering seven sampling areas located in the Alps of Lombardy where this species grows (Fig. 1). In each sampling areas were collected 12-30 fully expanded leaves. The analysis of leaf blade shape was carried out on the same leaves used for the analysis of the CSR strategy, using "Momocs" package of R software. The environmental characteristics of the sampling areas were evaluated by synecological analysis (ecological indexes of Landolt).
Campanula elatinoides presents more stress tolerant and competitive individuals (C = 46.6 %, S = 31.4 %, R = 22.0 %) with cordate leaf blades wider at the base in the sampling area at the lowest elevation where it grows with more thermophilic, heliophilous and xerophilous species. These characteristics represent adaptations of this species to the climatic conditions of that area.
This research has shown that the intraspecific variability of CSR strategy could be greater than previously thought and that functional strategy analysis can be useful to understand the adaptation of plants to climate. In addition, this study defined a morpho-ecotype of *Campanula elatinoides* that allows assumptions to be made as to how this endemic species might behave if atmospheric temperature increase.

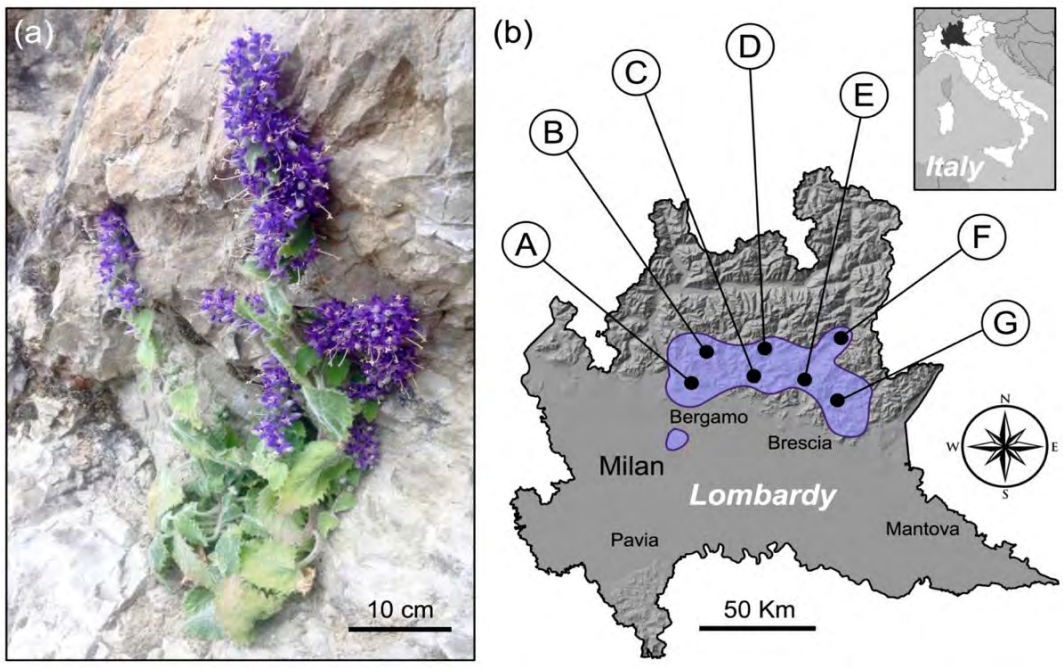


Fig. 1a-1b. *Campanula elatinoides* (a) and location of the seven sampling areas (b): A, Imagna Valley; B, Taleggio Valley; C, Vertova Valley; D, Nossana Valley; E, Iseo Lake; F, Camonica Valley; G, Trompia Valley. The distribution range of *Campanula elatinoides* is highlighted in violet

10 = Effects of several rare earth elements on the growth, photosynthetic pigments, ascorbate content, and lipid peroxidation level of *Lemna minor* L.

Isidora Gjata, Costantino Paciolla, Alessandra Villani, Silvana De Leonardis, Franca Tommasi
Department of Biology, University of Bari Aldo Moro, Via E. Orabona 4, 70125 Bari, Italy

Over the last decade, the global demand for rare earth elements (REEs) has grown significantly, due to their wider application in several critical technologies, including electronics, agriculture, and medicine. Consequently, the increasing release of these elements into the environment has generated a global concern because they may pose a risk to soil and human health. Indeed, the greater availability of REEs for potential uptake by plants and consecutive introduction into the food chain, could influence their intake and metabolism in the living organisms. Although some data reported that REEs might affect the germination, root and shoot development, as well as the function and nutritional quality of plants, there are few studies on the environmental behaviors of REEs. Therefore, further efforts are needed to generate more data on REEs and evaluate the toxicological effects on both ecosystems and organisms, caused by these potential contaminants. In this study, the morphological and biochemical effects of seven REEs, such as cerium (Ce), neodymium (Nd), gadolinium (Gd), dysprosium (Dy), holmium (Ho), ytterbium (Yb), and lutetium (Lu) on *Lemna minor* L., commonly known as duckweed, have been investigated. This species, widely used as a model system of aquatic plants and ecotoxicological bioassays, was grown under standard procedures according to ISO 20079 (2004), exposed to two different concentrations of all REEs individually (0,1 and 1 mM). After 3, 7, and 12 days of treatment, the possible toxicological effects based on the growth rate and changes in morphology, photosynthetic pigments and ascorbate content, and lipid peroxidation level were measured. The results showed that Ho, Lu, and Nd exerted the most relevant toxic effect on the growth rate and morphology, while Ce and Yb, at a lower concentration, did not show any adverse effect. At low concentration, all the REEs had no or slight effect on the chlorophyll and carotenoid contents, while at 1 mM, they showed a significant decrease in the pigments content mainly at longer exposure times. Changes in ascorbate and lipid peroxidation also occurred.

10 = Preliminary results on the geographical traceability of extra virgin olive oil from Valdichiana Senese

Martina Grattacaso[1], Giulia Canali[1], Andrea Vannini[1], Giuseppe Protano[2], Francesco Nannoni[2], Vivien De Lucia[2], Luigi Antonello Di Lella[2], Stefano Biagiotti[3] and Stefano Loppi[1]
[1] Department of Life Sciences, University of Siena, via Pier Andrea Mattioli 4, 53100 Siena, Italy; [2]Department of Physical, Earth and Environmental Sciences, University of Siena, Strada Laterina 8, 53100 Siena, Italy; [3]Pegaso Online University, Piazza Grande 7, 53045 Montepulciano, Italy

Extra Virgin Olive Oil (EVOO) is one of the best known and most appreciated Tuscan products worldwide. Indeed, with its bitter and spicy flavour, it is under the Protected Geographical Indication since 1998. Valdichiana Senese is one of the most appreciated areas of Tuscany for the quality of its food. The clayey soil and the gentle undulating slopes of this territory allow the production of high-quality food, in particular an EVOO rich in polyphenols and with a special taste. Since EVOO is among the most counterfeit foods on the market, methods have been developed in recent years for identifying the geographical origin and authenticity of EVOOs. Therefore, geographical traceability can be a very useful tool to guarantee the authenticity of EVOO to consumers and prevent unfair competition that can possibly harm the local agricultural sector.
The aim of this study is to determine the geographical traceability of EVOO from Valdichiana Senese using chemical elements as specific markers (i.e. multielemental fingerprinting). The concentration of chemical elements in olive oil is mainly determined by the physical, geochemical, and biological features of olive grove soils, the characteristics of the olive tree cultivars and the element translocation from roots to olives. For this purpose, soil samples were collected from different farms located in 9 municipalities within the area of Valdichiana Senese. The EVOO samples were directly provided by the producers involved in the project. In order to verify the geographical traceability of EVOO using the chemical elements, the total concentrations of Ca, Mg, Na, K, P, S, Fe, Mn, Cu, Zn, Rb, Sr, Ba, V, Cr, Ni, As, Cd, Sb, Tl, Pb, U and REEs (Rare Earth Elements) in the EVOO and soil samples were determined by Inductively Coupled Plasma Mass Spectroscopy (ICP-MS).
The next step will be the selection of a set of functional trace elements and REEs and establishing a relationship between EVOO and the geo-lithological features of the soils for determining the geographical traceability and authenticity of EVOO from Valdichiana Senese.

10 = Drivers of plant community (in)stability on coastal dunes

Greta La Bella[1], Marta Carboni[1], Marta Gaia Sperandii[2], Alicia T.R. Acosta[1]
[1]Department of Science, University of Roma Tre, Viale Guglielmo Marconi 446, 00146 Roma, Italy; [2]Centro de Investigaciones sobre Desertificación – CIDE - (CSIC/UV/GV), Ctra Moncada - Náquera km4.5, 46113 Moncada, (Valencia), Spain

With biodiversity extensively declining worldwide, one of the main challenges in ecology today is understanding biotic and abiotic mechanisms steering temporal stability. So far, a large body of evidence supports plant diversity to ensure community stability by increasing species asynchrony. However, most studies focused on species richness, often neglecting other facets of diversity which might better explain how species respond to environmental fluctuations. Also, since diversity patterns are themselves regulated by environmental conditions, it is fundamental to investigate how abiotic stress influences ecosystem stability, whether directly or indirectly through changes in biotic drivers. In this light, we aim to examine the intricate environment-diversity-stability relationship in coastal dune ecosystems, dominated by a strong sea-inland environmental gradient defining plant community assemblages.
To this aim, we analysed vegetation time series of Mediterranean coastal dunes, recorded in 84 permanent plots over the last 12-15 years within the Long-Term Ecological Research (LTER) program in Central Italy. We applied piecewise structural equation modelling to investigate how sea distance, species richness, functional-phylogenetic diversity, dominant species' traits, weighted average population stability, and asynchrony influence community stability, as well as the interplay between these drivers.
Surprisingly, while species and functional-phylogenetic diversity contribute little to stability, dominance of perennials was a key community trait in regulating community stability by enhancing population stability and asynchrony between species. Yet, the sea-inland gradient appears as the main force regulating mechanisms behind temporal stability: sea stress alters the complex diversity-stability relationship by shaping community diversity and composition as well as making species less stable in time and less asynchronous. Overall, our results highlight the importance of examining temporal stability within the environmental context.

10 = Competition and allelopathy triggering the exclusion of the native *Xanthium strumarium* L. by the American *X. orientale* L.

Eleonora Manzo, Justus Müller-Kiefer, Salvatore Tomasello
Department of Systematics, Biodiversity and Evolution of Plants (with Herbarium), George-August University of Göttingen, Untere Karspüle 2, 37077, Göttingen

Xanthium is a genus of the Asteraceae family, present in Europe with three species: *X. strumarium* L. *X. orientale* L. and *X. spinosum*. L. The former two species are similar both in morphology and ecological requirements and are often confused in the field. *Xanthium strumarium* has small burs (female capitula) up to 1 cm long and bearing straight apical beaks; whereas *X. orientale* has bigger burs, fully covered by spines and with differently shaped apical beaks. The former is native to Europe, North Africa and Asia, whereas the latter originated from America and has become widespread due to human trade and human-mediated dispersal.
Naturalized populations of *X. orientale* [i.e., *X. orientale* subsp. *italicum* (Moretti) Greuter] have been observed in Italy starting from 1820 and have spread within a century to the whole Italian territory and most of the Mediterranean basin. On the other hand, *X. strumarium*, has suffered a strong decline in the last 100 years, and (to the best of our knowledge) its last certain herbarium record in Italy is dated 1973. However, the reasons of this decline are fully unknown, and it is especially questionable if (and to which extent) the presence of the American congeneric has contributed to it. *Xanthium* species are also well-known for producing chemicals inhibiting germination and growth of other plant species.
To address these questions, we carried out competition experiments to test the effect of the interspecific competition on the fitness of *X. strumarium* plants. Moreover, we used germination experiments to figure out if *X. orientale* exudates have an allelopathic effect on the germination and growth of *X. strumarium* seeds.
We used dried biomass of plants, number of burrs and bur biomass as measures of fitness. ANOVA analyses showed a significant effect of the interspecific competition of the invasive species over *X. strumarium*. An intraspecific competition effect was also observed albeit to a much lesser extent.

Fig. 4. Seedlings of *X. orientale* (tall one) and *X. strumarium* (small one) in a pot for the competition experiments

Fig. 3. Fruiting plants of *X. strumarium* (in front) and *X. orientale* (still sterile; behind) cultivated in the botanical garden

10 = Multi-Omic approach to investigate phytochemical and gene expression changes in *Prunus persica* L. cultivars during post-harvest ripening

Antonella Muto[1], Leonardo Bruno[1], Innocenzo Muzzalupo[2], Adriana Ada Ceverista Chiappetta[1], Carsten Muller[3], Hilary J. Rogers[3], Maria Beatrice Bitonti[1], Natasha Damiana Spadafora[1,4]
[1]Department of Biology, Ecology and Earth Sciences, University of Calabria, Via Ponte P. Bucci 6b, 87036 Arcavacata di Rende Cosenza, Italy; [2]Centro di ricerca Olivicoltura, Frutticoltura e Agrumicoltura, Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, 87036 Rende Cosenza, Italy; [3]School of Biosciences, Cardiff University, Sir Martin Evans Building, Museum Avenue, Cardiff, United Kingdom; [4]Department of chemical, pharmaceutical and agricultural sciences, University of Ferrara, Via Borsari 46, 44121 Ferrara, Italy

Fruit post-harvest ripening is a complex developmental process controlled by a plethora of genetic, epigenetic, and metabolic factors which leads eventually to cell death through the degradation of proteins, lipids, and nucleic acids. Peach (*Prunus persica* (L. Batsch)) produces fruits of excellent quality but that are highly perishable during postharvest. Thousands of cultivars differing in fruit flavour, texture, skin colour, and other sensorial and nutritional parameters have been selected worldwide. The commercial production of peach fruits is highest in warm, temperate regions such as Southern Europe and exportation to Northern hemisphere countries is very frequent. To delay post-harvest ripening and spoilage peach fruits are transported cold, but long periods of cold storage can result in loss of quality. In the present study, we explored, how cold storage and genotype affect post-harvest ripening and consequently fruit quality in peach and nectarine [*P. persica* (L.) Batsch, var. nectarina] cultivars grown in southern Italy. A multi-omics approach was used for this evaluation: it included volatilome fingerprinting combined with gene expression profiling, intrinsic quality parameter characterisation and sensorial tasting. For analysis of the volatile organic compounds (VOCs) contributing to the volatilome, we applied two-dimensional gas chromatography (GC×GC) combined with time-of-flight mass spectrometry. RNA-sequencing and real time PCR were applied to identify differentially expressed genes during post-harvest storage. Peach fruits were analysed at harvest and during 14 days of cold storage at 1°C. Weighted Correlation Network analysis (WCNA) was used to determine variables statistically important to sensory traits. Significant correlations were observed between samples and sensory parameters for 64 VOCs and seven intrinsic characters. WCNA revealed strong, negative correlations amongst 10 VOCs, two intrinsic quality parameters and three sensory traits. Positive correlations were also highlighted amongst six VOCs, two intrinsic quality parameters and two sensory traits. Furthermore, multivariate statistical analyses of VOCs and gene expression showed significant correlations between the expression profiles of VOC-related genes and VOC abundance. This multi-omics approach highlighted some molecular and metabolic changes that peach fruits go through during post-harvest ripening under cold conditions, correlated to sensorial traits. These results can be of potential use to peach breeders for improving cold storage resilience at the molecular level in relation to sensory changes and could form the basis for markers of use in assessing fruit quality through the supply chain.

10 = Effect of ants' visits on nectary and nectar chemistry in *Prunus avium* L.

Massimo Nepi[1], Claudia Faleri[1], Emanuele Giordano[1], Daniele Calabrese[1], Valeria De Carlo[1], Massimo Guarnieri[1], Daniele Giannetti[2], Donato A. Grasso[2], Stefano Mancuso[3]
[1]Department of Environmental Sciences, University of Siena, Via P.A-. Mattioli 4, 53100 Siena, Italy; [2]Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Parco Area della Scienza 11/A, 43124 Parma, Italy; [3]Department of of Agriculture, Food, Environment and Forestry, University of Florence, Piazzale delle Cascine 18, 50144 Florence, Italy

Extrafloral nectar is quite common in angiosperms being produced by more than 4,000 plant species, which are distributed among 457 independent lineages. The nectar produced is collected by a variety of insects, but in most cases, these are ants that defend plants from herbivorous insects in a kind of mutualism called indirect defense. Although this relationship is widespread in nature, the intimate relationships between nectar-producing plants and insects are poorly understood. Here we examine the relationship between the extrafloral nectaries of *Prunus avium* and the ant *Crematogaster scutellaris* (Fig. 1b). *P. avium* has extrafloral nectaries located at the base of the leaves and on the petiole (Fig. 1a). The nectary is parenchymatous with two layers of epidermal cells covered by a thick cuticle and a mass of parenchymatous cells containing vascular bundles. Nectar accumulates under the cuticle, which stretches and eventually breaks off. Ants were observed biting into the surface of the nectary promoting the release of nectar beneath. Microscopic observation revealed that the ant's bite not only affects the cuticle but also removes epidermal layers. Experiments in which ants were excluded from visiting plants, disclosed several differences in the internal structure of the nectary: starch grains were less abundant as were phenolic droplets. The surface of nectaries not visited by ants was rich in fungal hyphae, whereas these were almost absent on the surface of nectaries visited by ants. In the same experiments, we collected and analyzed nectar that was more abundant if the plants visited by ants. The chemical profile of nectar also varied depending on the presence/absence of ants: extrafloral nectar from plants visited by ants showed a higher proportion of sucrose and a greater variety of amino acids. The results are of particular interest because they revealed for the first time a strong interaction between ants and nectars that is not limited just to nectar feeding, and that this particular relationship influences nectar production and composition.

Fig. 1. Extrafloral nectaries of *Prunus avium* on the terminal part of the petiole (a). *Crematogaster scutellaris* visiting extrafloral nectaries of *Prunus avium* (b).

10 = Contrasting responses of forest growth and carbon sequestration to heat and drought in the Alps

Ludovica Oddi[1], Mirco Migliavacca[2,3], Edoardo Cremonese[4], Gianluca Filippa[4], Giorgio Vacchiano[5], Consolata Siniscalco[1], Umberto Morra di Cella[4], Marta Galvagno[4]
[1]University of Torino, Department of Life Sciences and Systems Biology, Torino, Italy; [2]Max Planck Institute for Biogeochemistry, Jena, Germany; [3]European Commission, Joint Research Center, Ispra, Italy; [4]Environmental Protection Agency of Aosta Valley, Climate Change Unit, ARPA VdA, Aosta, Italy; [5]Università Degli Studi di Milano, Department of Agricultural and Environmental Sciences, Milano, Italy

Climate change is expected to increase both the frequency and the intensity of climate extremes, consequently increasing the risk of forest role transition from carbon sequestration to carbon emission. These changes are occurring more rapidly in the Alps, with important consequences for tree species adapted to strong climate seasonality and short growing season.
In this study, we aimed at investigating the responses of a high-altitude *Larix decidua* Mill. forest (Torgnon, IT-Trf, LTER site: LTER_EU_IT_078) to heat and drought, by coupling ecosystem- and tree-level measurements. From 2012 to 2018, ecosystem carbon and water fluxes (i.e., gross primary production, net ecosystem exchange, and evapotranspiration) were measured by means of the eddy covariance technique, together with the monitoring of canopy development (i.e., larch phenology and normalized difference vegetation index). From 2015 to 2017 we carried out additional observations at the tree level, including stem growth and its duration, direct phenological observations, sap flow, and tree water deficit.
Results showed that the warm spells in 2015 and 2017 caused an advance of the phenological development and, thus, of the seasonal trajectories of many processes, at both tree and ecosystem level. However, we did not observe any significant quantitative changes regarding ecosystem gas exchanges during extreme years. In contrast, in 2017 we found a reduction of 17% in larch stem growth and a contraction of 45% of the stem growth period. The growing season in 2017 was indeed characterized by different drought events and by the highest water deficit during the study years. Due to its multi-level approach, our study provided evidence of the independence between C-source (i.e. photosynthesis) and C-sink (i.e. tree stem growth) processes in a subalpine larch forest.

10 = The importance of technical assistance in the return of rye (*Secale Cereale* L.) in Camonica Valley (north Italy)

Davide Pedrali[1*], Valeria Leoni[1], Luca Giupponi[1], Tiziana Lazzarina Zendrini[1], Marco Zuccolo[1], Alessia Maria Bernardi[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
*Correspondence: davide.pedrali@unimi.it

Recent agricultural policies, like the European Green Deal, move towards the encouragement of a more sustainable agriculture maintaining biodiversity and soil health. There is a new awareness towards agriculture by the non-professional public, especially in Alpine valleys; nowadays people returning to mountains are often newcomers (sometimes hobby-farmers). This tendency makes the training in agriculture fundamental to contrast the lower productivity of organic farming, and/or their higher exposure to environmental contamination, such as bacteria and fungi.
The aims were evaluating the incidence of public workshops/seminars (organized by the Ge.S.Di.Mont. Research Centre of the University of Milan in Camonica Valley) on cereal cultivation between 2016 and 2021 and secondly, analyse the rye produced in Camonica Valley comparing it with commercial products.
An increase in participation along the considered period and a wider use of the streaming service were perceived. The percentage of participants not from an agricultural background had always remained about 50%, but decreased to 15.17% in 2021, in contrast to the increase of farmers and Foresters and Agronomists that increased from 0 to 17.39% in 2019 and to 67.57% in 2021, respectively. The necessity of continuing education for agronomists and foresters, could also explain the inversion of the trend in the age of participants.
Rye produced in Camonica valley from new entrants was phytochemically and nutritionally similar to commercial rye, although within the local samples there was higher variability: a greater diversity was observed for the total content of carotenoid, phenolic, and flavonoids, with local rye samples showing a larger unevenness than commercial ones. Generally, the last ones were higher in anthocyanin content than Camonica valley samples. These results were confirmed by PCA biplot wherein the commercial samples were situated in the middle of the graph where protein and anthocyanin were positioned, while the local rye was more spread in the biplot region. Both commercial and rye produced in Camonica valley samples show similar HPLC chromatographic profiles. All ergot extract and ergocristine solution were used as a standard; no signals associated with ergot alkaloids have been observed in all the rye extracts.
In conclusion, locally produced rye proved to be comparable to the commercial one; however, it showed a remarkable unevenness in secondary metabolite content and productivity, due to environmental differences and diverse agro-techniques. Some traditional cereals (like the rye) could be strategic for the recovery of some marginal areas, triggering sustainable processes of develop the mountain territories.

10 = Foliar distribution of basalt dust in Falanghina grapevine modulates leaf anatomical and photosynthetic traits to improve tolerance to drought

Francesca Petracca[1], Chiara Cirillo[1], Carmen Arena[2], Antonello Bonfante[3], Marco Giulioli[4], Arturo Erbaggio[5], Nicola Damiano[1], Rosanna Caputo[1], and Veronica De Micco[1]
[1]Department of Agricultural Sciences, University of Naples Federico II, Via Università 100, 80055 Portici (Naples), Italy; [2]Department of Biology, University of Naples Federico II, Via Vicinale Cupa Cintia 21, 80126 Napoli, Italy; [3]Institute for Mediterranean Agricultural and Forest Systems, ISAFOM, National Research Council of Italy (CNR), P.le Enrico Fermi 1, 80055 Portici (Naples), Italy; [4]La Guardiense Cooperative farm, Località Santa Lucia 104/105, 82034 Guardia Sanframondi (Benevento), Italy; [5]Freelance

Climate change is creating limitations for viticulture in Southern Italy due to increase in the frequency and severity of drought periods. Such stressful conditions are reducing yield and quality of grapes. Italian legislation imposes rainfed cultivation as a requirement for origin and quality labels of wines, but in the next future the irrigation and/or the introduction of cultivation practice to improve vines resistance to drought would become pivotal to achieve economic and environmental sustainability.
The aim of this work was to evaluate the possibility to mitigate the effects of water deficit on *Vitis vinifera* subsp. *vinifera* 'Falanghina' through the distribution of shielding dusts.
Therefore, we set up an experiment with four treatments combining two main factors, basalt dust distribution (two levels: presence/absence) and irrigation management (two levels: reintegration of water lost by evapotranspiration/rainfed).
The basalt dust was distributed through the foliar spray application. The irrigation plan was defined weekly to reintegrate the water lost by transpiration, applying a model considering precipitation and evapotranspiration, based on data collected through a meteorological station installed in the vineyard. The experimental area is located in Guardia Sanframondi (BN) at the Cantina Sociale La Guardiense premises, selected after soil and geophysical analyses. Plant growth was monitored by biometric and ecophysiological analyses (including gas-exchanges, chlorophyll fluorescence and leaf water potential) (Fig. 1). For gas exchanges and chlorophyll fluorescence analyses, we set up the methodology to check whether the interference of dusts would introduce bias in the results. Leaf functional anatomical traits (e. g. lamina thickness, localization of phenolics, stomatal size and frequency) were quantified through light and epi-fluorescence microscopy and digital image analysis. At the harvest, the grapes were characterized in terms of yield and quality parameters of must and wine for each treatment.
Results showed that the distribution of basalt dust reduces the leaf temperature and transpiration rate, and suggest that vines growing rainfed with basalt dust allocate photosynthates towards reserves with possible positive effects in the long term. The use basalt dust in rainfed condition will be investigated in the next years to unravel both the interference with climatic variability and the long-term effects.

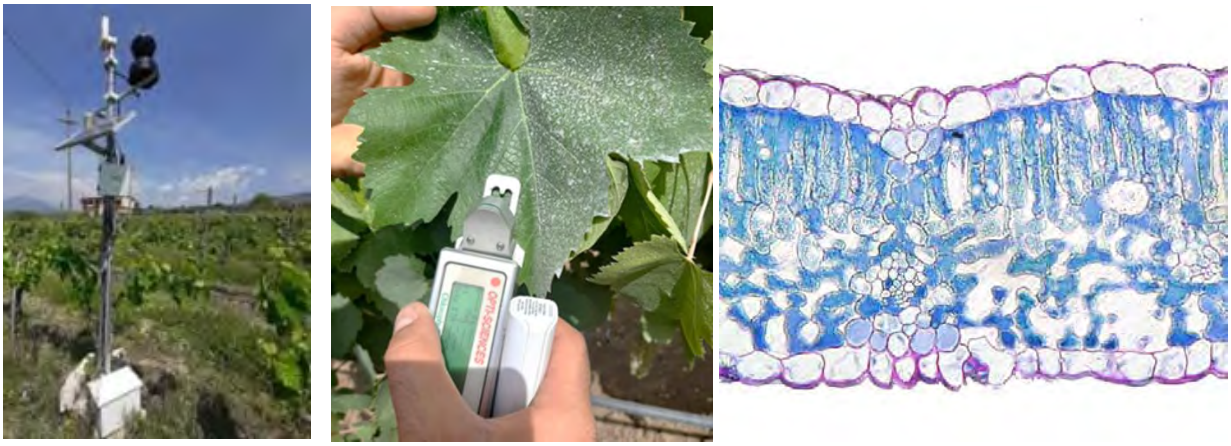


Fig. 1. Study site, chlorophyll fluorescence analysis and microphotograph of vine leaf cross section.

10 = Digitization of the Herbarium Guadagno. 3. Results from a four-year project

Francesco Roma-Marzio[1], Lucia Amadei[1], Giovanni Astuti[1], David Dolci[2], Simonetta Maccioni[1], Nicoletta Magrini[1], Federica Pierotti[1], Roberta Vangelisti[1], Lorenzo Peruzzi[3]
[1]Sistema Museale di Ateneo dell'Università di Pisa, Orto e Museo Botanico, Via Luca Ghini 13, 56126 Pisa, Italy; [2]Centro Interuniversitario per la Biodiversità Vegetale Big Data - PLANT DATA, Department of Biological, Geological and Environmental Sciences, Alma Mater Studiorum University of Bologna, Via Irnerio 42, 40126 Bologna, Italy; [3]Department of Biology, University of Pisa, Via Derna 1 56126 Pisa, Italy

The Herbarium Guadagno (PI-GUAD), is one of the richest among the collections preserved at the *Herbarium Horti Botanici Pisani*. (PI), 547 packages of vascular plants and 9 packages of non-vascular plants, fungi, and lichens are included. This herbarium was assembled by Michele Guadagno (1878-1930) between 1900 and 1925, and most of the specimens were collected by himself in Central and Southern Italy, albeit many were obtained from foreign countries and collectors through exchanges. The Herbarium Guadagno arrived in Pisa in 1939, bought under the direction of Alberto Chiarugi (1901-1960). At the end of 2018, the Botanic Museum of the University of Pisa started a project in collaboration with the Department of Biology, focused on the digitization of all the vascular plant specimens of the Herbarium of the Michele Guadagno. The project has been financially supported by the Fondazione Pisa and Banca d'Italia. The digitization procedure followed two distinct steps: 1) acquisition of high-resolution digital images (600 dpi, 24-bit color depth) using a Bookeye® 4 Professional planetary scanner and 2) label data (metadata) acquisition and entry into the Virtual Herbaria JACQ online database. After four years, the acquisition of the images of all the specimens of vascular plants has been completed. This allowed us to quantify the true number of sheets preserved in the Herbarium, which amount to 44,342. However, in several cases a specimen is composed by two or more sheets or, otherwise, on a single sheet two or more specimens were mounted. Accordingly, we estimated that the Herbarium is composed by about 42,000 specimens, 6,000 more than previously reported by Chiarugi in 1950. Concerning the acquisition of metadata, at May 26th 2022, 28,870 specimens have been completely digitized, corresponding to 68% of the entire collection. Pteridophytes (758 specimens), gymnosperms (176), monocots (6,488), early-branching angiosperms (170) were completely digitized. As concerns the eudicots, Brassicaceae (1,930 scanned sheets), Apiaceae (1732), Plantaginaceae (1,447), Rubiaceae (857), and Boraginaceae (741) are the five richest families, not yet digitized along with several other less represented families (e.g., Campanulaceae, Gentianaceae, Orobanchaceae, Primulaceae, Violaceae). Among the fully digitized families, the richest in specimens are: Asteraceae (5,766), Fabaceae (3,720), Poaceae (3,122), Lamiaceae (2,164), Caryophyllaceae (1,846), Rosaceae (1,302), and Ranunculaceae (1,142). *Hieracium* is the most represented genus (690), followed by *Carex* (662), *Centaurea* (642), *Trifolium* (616), *Silene* (520), and *Vicia* (512). Apart from Guadagno, who collected about ~~the~~ 40% of the specimens, other 957 collectors contributed to the establishment of the herbarium. Among them, the most represented are A. Noblet (France), C. Marchesetti (Italy), W. Behrendsen (Russia), C. Bicknell (Italy), S. Sommier (Italy), and G. Rigo (Italy). Many specimens are linked to series of exsiccata as *Flora Italica Exsiccata* by A. Fiori, A. Béguinot & R. Pampanini (1,685 digitized specimens), *Herbarium Graecum Normale* by T. von De Heldreich (188), *Plantes d'Espagne* by F. Sennen (176), *Plantes d'Espagne* by Elisée Réverchon (102), or *Herbarium Normale* by I. Dörfler (54). All the digitized specimens cover a time span of 89 years, from 1837 (41 years before the birth of Guadagno) to 1926. The specimens collected before his birth, such as a specimen of *Hydrangea arborescens* L. collected by Antonio Bertoloni (1775–1869), or those collected by Giovanni Gussone (1787–1866), Wilhelm Spruner (1805–1874), and Giuseppe Antonio Pasquale (1820–1893), attest for possible exchanges/loans with institutional Herbaria. From a geographical perspective, 58% of the specimens were collected in Italy and, among these, the most represented regions are Campania (51%), Abruzzo (11%), Calabria (8%), and Veneto (5%). Outside Italy, all the continents are represented and France (7%), Spain (4%), Germany and Greece (3% each) are the country's richest in specimens. Finally, 32 type specimens have been detected, 20 of which are syntypes (e.g., *Helianthemum jonium* Lacaita & Grosser), 10 isolectotypes (e.g., *Dianthus tarentinus* Lacaita), 8 isotypes (e.g., *Ranunculus ficaria* var. *gynodioicus* Bég.), 4 lectotypes (e.g., *Hieracium sartorianum* Boiss. & Heldr. var. *lucanicum* Arv.-Touv.), 1 isoepitype (*Cirsium lobelii* Ten.), while 30 further specimens can be considered original material (e.g.,for *Fumaria bicolor* Sommier f. *linosana* Sommier).

10 = May crops be affected by nanoplastics? Current evidence of nano-polystyrene toxicity, internalization, and translocation in plant tissues

Monica Ruffini Castiglione[1], Carmelina Spanò[1], Lucia Giorgetti[2], Lorenza Bellani[2-3], Stefania Bottega[1], Luigi Sanità di Toppi[1], Simonetta Muccifora[3]
[1]Department of Biology, University of Pisa, Via Ghini 13, 56126 Pisa, Italy; [2]Institute of Agricultural Biology and Biotechnology, National Research Council, Via Moruzzi 1, 56124 Pisa, Italy; [3]Department of Life Sciences, University of Siena, Via A. Moro 2, 53100 Siena, Italy.

Plastic contamination represents one of the most pervasive and sturdy types of pollution of specific concern for all ecosystems. Agroecosystems represent more and more a huge long-term sink for plastic compounds which inevitably undergo fragmentation, generating micro- and nano-plastics, the smaller fractions being of specific concern, with potentially adverse effects on soil chemistry and living organisms. In addition, the possible entry of micro- and nano-plastics into the food chain through plants may represent a real problem to be urgently considered in order to improve the quality of the environment and food/feed plant products for human and animal nutrition. To investigate the interactions between nano-plastics and plants, polystyrene nanoparticles (n-PS) were chosen as model particles, representing one of the most found polymers in terrestrial and agroecosystems, while *Allium cepa* L. and *Oryza sativa* L. were the model crops studied from seed germination to their early developmental stages, under different n-PS concentrations in the growing medium. Through an experimental design based on an integrated approach, our work has shown that n-PS, being able to overcome plant cell barriers, can be taken up from root, move in different cellular compartments and tissues and be translocated to the shoot, even in short-term treatment experiments. The presence of n-PS was associated with ultrastructural alterations, limited to the root cells, inhibition of mitotic activity and impairment of the germination process, and seedling growth. In addition, n-PS were able to induce cyto-genotoxicity, not necessarily related to oxidative stress, as recorded in rice, in which, an adequate antioxidant response (APX, POX) was documented. In both plant systems, however, different patterns in the localization of oxidative stress markers have been recorded, highlighting that n-PS could impair homeostasis/diffusion of H_2O_2 and cause localized membrane damage in both plant species, as also evidenced by cell ultrastructural analysis. These damages could be induced not only by changes in the production/diffusion of ROS at the tissue and cellular level but also by direct action of n-PS, taken up and translocated by the plant. Indeed, n-PS might trigger multiple signaling pathways, not only ROS dependent, but able to affect cell proliferation/metabolism and genome functioning. Further studies are needed to clarify n-PS intracellular interactions as well as to investigate their effects in long-term exposition and potential generational transfer. Considering the ability of food plants to internalize these nanoparticles, with the consequent possible entry into the food chain, the problem of pollution of agricultural land by nano PS is to be urgently taken into account.

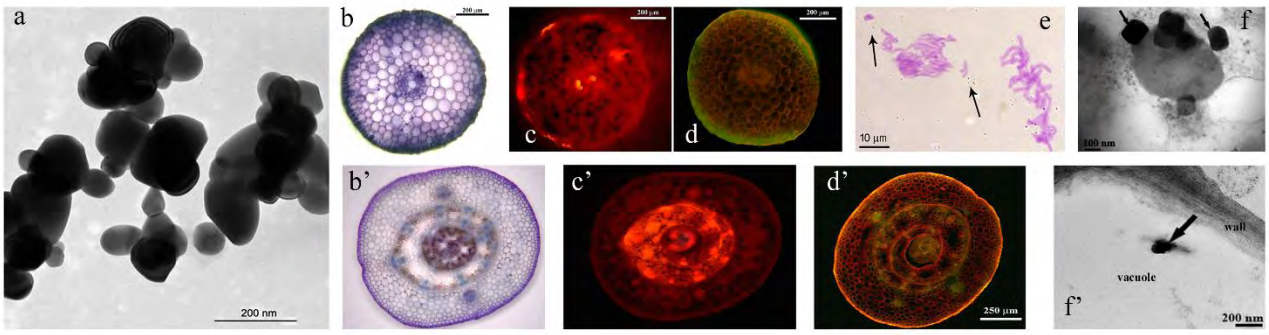


Fig. 1. **a**: polystyrene nanoplastics (n-PS). **b-f** and **b'-f'**: adverse effects and internalisation of n-PS in roots (*A. cepa*) and shoots (*O. sativa*) respectively, in samples treated with 1g/L n-PS, 50 nm diameter.

10 = Towards the application of the Habitats Directive to an EU non-member country: the San Marino case study

Francesco Santi, Fabrizio Buldrini, Alessandro Chiarucci
Department of Biological, Geological and Environmental Sciences, University of Bologna, via Irnerio 42, 40126 Bologna, Italy

In the European continent, biological conservation is driven by several legal instruments, with the Habitats Directive being the key element for countries of the European Union. This Directive was promulgated as a response to the Bern Directive, the Council of Europe's tool to protect biodiversity.The Republic of San Marino is a microstate (61.19 km^2), occupying an enclave position in central Italy. With nearly 60% of the territory occupied by artificial surface or cropland, natural habitats occupy residual small patches and are fragmented. San Marino is one of the few European countries which has not adopted any of the above mentioned legal instruments to protects its biodiversity. With this study we aim to investigate the possible application of the Habitats Directive to San Marino, focusing on the occurrence of habitats. This step is indeed seen as a valuable tool for San Marino, to align its biodiversity conservation measures to the European context.A list of habitat categories potentially present in the Republic was created and then vegetation was sampled in the 2019-2022 period. Surveys included pioneer vegetation on calcareous outcrops, xeric grasslands, and woods; some other microhabitats were actively searched in the field.Collected data were analysed and compared with previous studies and the Italian Interpretation Manual of the 92/43 EEC Directive. Six habitat types were identified as surely occurring in San Marino (3140, 6110*, 6210, 6430, 91AA*, 9340) and additional two habitat types were indicated as very likely occurring (7220*, 8310). A draft of the national habitat map was also realised.Some of the recorded habitats are common in surrounding zones, given the ecological similarity of the Republic territory with them, whereas others are remarkable for their rarity (e.g. 9340 - "*Quercus ilex* and *Quercus rotundifolia* forests"). Despite this study cannot be considered as exhaustive and field studies are still ongoing, we show that some natural or semi-natural habitat types occurring in the Republic of San Marino are worth of protection in the European context, despite the high level of transformation of the territory. Moreover, this study can offer a reference for the adoption of the Habitats Directive by an EU non-member country.

10 = Use of drones for environmental monitoring and rare plants conservation status: a new tool for botanical research

Gianmarco Tavilla[1], Alessandro Crisafulli[2], Arthur Lamoliere[3], Veronica Ranno[1], Giulia Bacilliere[1], Salvatore Cambria[1], Pietro Minissale[1], Gianpietro Giusso del Galdo[1], Saverio Sciandrello[1]
[1]Department of Biological, Geological and Environmental Sciences, University of Catania, Via A. Longo 19, 95125 Catania, Italy; [2]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Via Stagno d'Alcontres, 98100 Messina, Italy; [3]Institute of Earth Systems, University of Malta, Msida, Malta

The use of aerial images offer an efficient and cost-effective support for assessing the conservation status and monitoring the distribution range of rare and isolated plant species, as well as Invasive Alien Plants (IAPs). The development of remote sensing is no longer limited to satellite imagery. In the last decade, consumer-grade Unmanned Aerial Vehicles (UAVs) extended the scope and accuracy of vegetation mapping and native plant investigations. UAVs are versatile and cost-effective tools for environmental monitoring. In particular, they allow to evaluate the occurrence of endemic/rare species on inaccessibile growing sites and monitoring plant cover changes over time.
Such new botanical research tool has two main advantages: 1) it allows to have more reliable data on alien and rare/narrow endemic plants, and 2) data sampling does not affect the natural populations or endangers the field researchers. Besides, these concrete actions are useful in implementing any decision involved in the management of a protected area. An accurate census is essential both for habitat management and for monitoring rare/narrow endemic species distribution, as well as to evaluate invasion and invasiveness of alien plants. Nevertheless, site accessibility may represent a significant obstacle, particularly for those species exclusively occurring on fragile and inaccessibile habitats.
Currently, we are carrying out drone field surveys to improve knowledge about rare/narrow endemic species no longer recorded, especially in inaccessibile areas, poorly affected by grazing and human impacts. In addition, we are implementing a database of orthophotos processed from drone aerial surveys both in Sicily and Malta (Figs. 1 and 2). One of the project's target is the identification of the spectral signatures of IAPs. Moreover, this work aims to facilitate the census of rare/narrow endemic plant species with peculiar morphology and the real or potential competition with IAPs.
This research is supported by the FAST Project "Fight Alien Species Transborder" Programme INTERREG V-A Italia-Malta 2014-2020.

Fig. 1. UAV image of rupicolous species at Rocca Salvatesta Cliff, Peloritani Mounts, Sicily

Fig. 2. UAV image of *Carpobrotus* sp. at San Lawrenz Cliff, Dwejra, Gozo

10 = Substrate type drives species richness and functional traits of alpine terricolous cryptogam communities in the Alps

Chiara Vallese[1], Luca Di Nuzzo[2], Paolo Giordani[3], Renato Benesperi[2], Gabriele Gheza[1], Petra Mair[4], Daniel Spitale[4], Juri Nascimbene[1]
[1]BIOME Lab, Dipartimento di Scienze Biologiche, Geologiche e Ambientali, Alma Mater Studiorum – Università di Bologna; [2]Dipartimento di Biologia, Università degli Studi di Firenze; [3]Dipartimento di Farmacia, Università di Genova; [4]Museo di Scienze Naturali dell'Alto Adige

The alpine soils above the treeline are among the best-known environments in which cryptogams play several ecological functions. Despite its importance, the role of substrate type as a driver of community assembly remains poorly studied. This study aims to explore the relationships in taxonomic and functional richness of terricolous cryptogam communities growing on two substrate types (carbonatic and siliceous) along different elevation gradients in the Eastern Alps. The sampling design included 98 belts surveyed within 12 independent transects, ranging from 2100 to 3000 m of altitude. We established six transects on siliceous soils and six on carbonatic ones. Besides the two soil types, we also considered the cover of vascular plants and the climatic features as explanatory variables. Our results confirmed that a "trade-off" occurs between stress tolerance and the competitive response of cryptogam communities growing above siliceous and carbonatic soils. We also found contrasting responses across the types of diversity analyzed. Our findings contribute to clarify the response of lichens and bryophytes' diversity to a climate change scenario, where rapid changes in temperature and rainfall may interplay with an increase in soil instability and erosion.

10 = Pollen as a tool to reconstruct environmental transformations and land use: case study from a cult area from the Copper and Bronze Age at Gardolo di Mezzo (TN)

Jessica Zappa[1], Paola Torri[1], Anna Maria Mercuri[1], Mauro Rottoli[2], Elisabetta Castiglioni[2], Michele Bassetti[3], Elisabetta Mottes[4]
[1]Laboratorio di Palinologia e Paleobotanica, Dipartimento Scienze Vita, Università degli Studi di Modena e Reggio Emilia, Modena, Italy; [2]Laboratorio di Archeobiologia dei Musei Civici di Como, Piazza Medaglie d'Oro 1, 22100 Como (CO); [3]Cora Società Archeologica S.r.l., Via Salisburgo 16, I-38121, Trento (TN); [4]Provincia autonoma di Trento – Soprintendenza per i beni culturali – Ufficio beni archeologici, Via Mantova 67, I-38122, Trento (TN)

Pollen is an invaluable tool to measure changes of flora, vegetation, ecological conditions and climate over a millennial to decadal scale.
The palynological analyses carried out in the Copper and Bronze Age site in Gardolo di Mezzo (TN) allowed to highlight the vegetation changes from Copper to Recent Bronze Age and the differentiated land use adopted by local population. The archaeological site of Gardolo di Mezzo is particularly important because it represents an example of the funeral and ritual practises in use during the Copper and Bronze Age, that are proved to be quite different from the previous ones, dated back to the Neolithic period. In fact, the research carried out by the Archaeological Heritage Office of the Autonomous Province of Trento brought to light an extensive multi-layered site and, close to the settlement area, a cult area (Garm 2) has also been documented, in particular two monumental tumuli.
The palynological analyses were performed on a trench taken from the cult area. The main aim is to study the vegetation cover surrounding the site during the selected period and to try to highlight the differentiated land use that must be in use during the Copper and Bronze Age.
The results obtained show a major change in the vegetation cover quite at mid sequence. Here, the AP (Arboreal Pollen), prevalent in the 2 more ancient pollen zones obtained (GDL 1 and GDL 2), decreases progressively in GDL 3 and drastically in GDL 4 (the last pollen zone identified). In particular, in GDL 1 the forest cover mainly composed by arboreal plants was characterized by the high presence of *Betula*, showing a cool environment during the Copper Age. Then, in GDL 2, *Betula* is still present but starts decreasing in favour of the increasing of components of the mixed oakwood that are the more present at the beginning of the Late Bronze Age, showing a change in the forest cover towards more temperate conditions. In GDL 3, a drastic reduction of the forest cover took place in favour of the NAP (Non Arboreal Pollen), reflecting an open environment during the Middle Bronze Age. In the upper pollen zone GDL 4, the strong presence of API (Anthropogenic Pollen Indicators), LPPI (Local Pastoral Pollen Indicators) and ruderal plants suggest the higher presence of human activities and their differentiated land use both for cultivation and pastoral activities.
These results are at present in preparation for publication on a Special Issue of Quaternary, titled: *Vegetation changes and land-use history at Gardolo, Copper and Bronze Age site in Trentino (N Italy)*. Zappa J., Torri P., Clò E., Rottoli M., Castiglioni E., Bassetti M., Mottes E., Mercuri A.M.

ELENCO COMPLETO DEGLI ABSTRACT IN ORDINE ALFABETICO