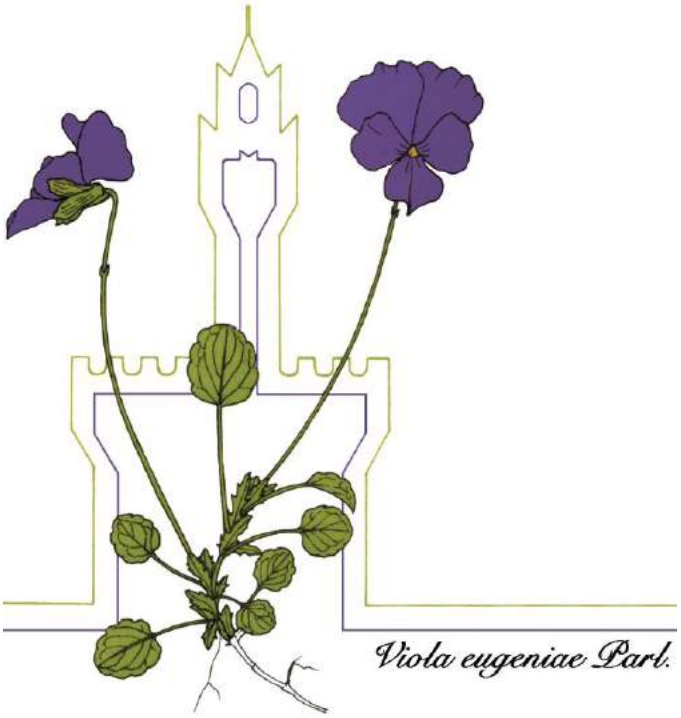
114° Congresso della Società Botanica Italiana

VI INTERNATIONAL PLANT SCIENCE CONFERENCE (IPSC)

Padova, 4 - 7 September 2019



ABSTRACTS

KEYNOTE LECTURES, COMMUNICATIONS, POSTERS

ISBN 978-88-85915-23-7

Scientific Committee

Consolata Siniscalco (Torino) (President)
Maria Maddalena Altamura (Roma)
Stefania Biondi (Bologna)
Alessandro Chiarucci (Bologna)
Salvatore Cozzolino (Napoli)
Lorenzo Peruzzi (Pisa)
Ferruccio Poli (Bologna)
Barbara Baldan (Università di Padova)
Lorella Navazio (Università di Padova)
Livio Trainotti (Università di Padova)
Francesca Dalla Vecchia (Università di Padova)
Isabella Moro (Università di Padova)
Antonella Miola (Università di Padova)
Caniato Rosamaria (Università di Padova)
Filippini Raffaella (Università di Padova)
Piovan Anna (Università di Padova)

Local Committee

Barbara Baldan (Dipartimento di Biologia, Università di Padova)
Lorella Navazio (Dipartimento di Biologia, Università di Padova)
Livio Trainotti (Dipartimento di Biologia, Università di Padova)
Francesca Dalla Vecchia (Dipartimento di Biologia, Università di Padova)
Isabella Moro (Dipartimento di Biologia, Università di Padova)
Antonella Miola (Dipartimento di Biologia, Università di Padova)
Caniato Rosamaria (Dipartimento di Scienze del Farmaco, Università di Padova)
Filippini Raffaella (Dipartimento di Scienze del Farmaco, Università di Padova)
Piovan Anna (Dipartimento di Scienze del Farmaco, Università di Padova)
Tomas Morosinotto (Dipartimento di Biologia, Università di Padova)
Fiorella Lo Schiavo (Dipartimento di Biologia, Università di Padova)
Nicoletta La Rocca (Dipartimento di Biologia, Università di Padova)
Michela Zottini (Dipartimento di Biologia, Università di Padova)
Elide Formentin (Dipartimento di Biologia, Università di Padova)
Alessandro Alboresi (Dipartimento di Biologia, Università di Padova)
Ildikò Szabò (Dipartimento di Biologia, Università di Padova)

114° Congresso della Società Botanica Italiana onlus

VI INTERNATIONAL PLANT SCIENCE CONFERENCE (IPSC)

XI CONGRESSO DELLA SOCIETÀ ITALIANA DI BIOLOGIA VEGETALE

Padova, Orto Botanico, 4 - 7 September 2019

Programme

Wednesday 4 September 2019

12.00-13.30 Registration

12.30-13.30 Welcome lunch

13.30-14.00 Opening ceremony

Symposium 1

JOINT SESSION SBI - SIBV

"PLANTS IN AND FOR THE FUTURE"

(Chairpersons: B. Baldan and T. Morosinotto)

Key words: cell biology, signal transduction, plant development, stress responses, biotechnology

14.00-14.40 **Christine Raines** University of Essex, Colchester, United Kingdom **(SIBV)**
"Improving future plant productivity through manipulation of photosynthesis"

Four communications SIBV

16.00-16.30 Coffee Break

16.30-17.10 **Christian Hardtke**, University of Lausanne Switzerland
"Developing phloem - orchestrating plant organ formation"

Communications SBI

- **Gian Pietro Di Sansebastiano**, Fabrizio Barozzi
  "Plant cell downstream control of conventional and unconventional membrane traffic contribution to the tonoplast"
- **Cristina Votta**, Valentina Fiorilli, Jorge Gómez-Ariza, Fabio Fornara, Luisa Lanfranco
  "The heterologous expression of the *Rhizophagus irregularis* RiPEIP1 gene in rice plants leads to enhanced growth and mycorrhizal colonization"
- **Antonella Muto**, Ernesto Picardi, Leonardo Bruno, Hilary J. Rogers, Antonio Ferrante, Adriana Ada Ceverista Chiappetta, Maria Beatrice Bitonti, Natasha Damiana Spadafora
  "Comparative transcriptomic profiling of peach and nectarine cultivars to elucidate chilling injury mechanisms"
- **Claudio Varotto**, Mingai Li, Luca Stragliati, Erika Bellini, Ada Ricci, Luigi Sanità di Toppi
  "Evolution and functional differentiation of recently diverged phytochelatin synthase genes from *Arundo donax* L."

18.30-20.30 Drinks and appetizers (possibility of visiting the Botanical Garden)

Thursday 5 September 2019

Symposium 2 SBI

“FOREST BIODIVERSITY AND FUNCTIONING IN A CHANGING WORLD: CHALLENGES AND OPPORTUNITIES”

(Chairpersons: S. Casavecchia and G. Filibeck)

Key words: global change, rewilding, conservation, functional responses, vegetation dynamics, vegetation classification

9.00-9.35 **Jean-Luc Dupouey**, INRA – France
“Forest ecological history for the future”

9.35-10.35 **Communications**

- **Gabriele Casazza**, Michele Brunetti, Fabio Malfatti, Valentina Simonetti, Andrew S. Mathews
  “Combining historical ecology, species distribution modelling and instrumental climate reconstruction to infer causes of change in forest distribution: the case of Monte Pisano”
- **Andrea Coppi**, Lorenzo Lazzaro, Evy Ampoorter, Lander Baeten, Kris Verheyen, Federico Selvi
  “Tree species diversity versus identity effects on understorey phylogenetic diversity in thermophilous deciduous forests”
- **Chiara Lelli**, Juri Nascimbene, Davide Alberti, Nevio Agostini, Alessandro Chiarucci
  “Signs from the past: long-term changes in Italian mountain forests under changing management”

10.35-11.00 Coffee Break

11.00-11.35 **Tommaso Anfodillo**, University of Padova
“The simple structure of forests: an allometric approach for assessing the degree of old-growthness”

11.35-12.35 **Communications**

- **Claudia Angiolini**, Bruno Foggi, Antonio Gabellini, Matilde Gennai, Paolo Castagnini, Michele Mugnai, Simona Sarmati, Daniele Viciani, Simona Maccherini
  “Are the effects of the overstory on herb and shrub layers important in forest habitat monitoring? The case of *Quercus suber* woodlands”
- Daniela Gigante, Selvaggi A., Acosta A.T.R., Adorni M., Allegrezza M., Angiolini C., Armiraglio S., **Silvia Assini**, Attorre F., Bagella S., Barcella M., Bazan G., Bertacchi A., Bolpagni R., Bonari G., Buffa G., Caccianiga M., Cacciatori C., Caria M.C., Casavecchia S., Casella L., Cerabolini B.E.L., Ciaschetti G., Ciccarelli D., Cogoni A., Cutini M., De Sanctis M., De Simone W., Del Vecchio S., Di Cecco V., Di Martino L., Di Musciano M., Fantinato E., Filesi L., Foggi B., Forte L., Frattaroli A.R., Galdenzi D., Gangale C., Gianguzzi L., Giusso Del Galdo G., Grignetti A., Guarino R., Lasen C., Maneli F., Marcenò C., Mariotti M.G., Oriolo G., Paura B., Perrino E., Pesaresi S., Pezzi G., Pisanu S., Poponessi S., Prisco I., Puglisi M., Rivieccio G., Sciandrello S., Spampinato G., Stinca A., Strumia S., Taffetani F., Tesei G., Tomaselli V., Venanzoni R., Viciani D., Villani M., Wagensommer R., Zanatta K., Angelini P.
  “An overview of the Italian forest biodiversity and its conservation level, based on the first outcomes of the 4th Habitat Report ex-Art. 17”
- **Simone Pesaresi**, Adriano Mancini
  “NDVI temporal pattern as functional responses of Mediterranean forest plant associations”

12.35-14.00 Lunch and posters

Symposium 3 SBI

“HERBARIA: STILL RELEVANT IN THE 21TH CENTURY?”

(Chairpersons: L. Peruzzi and S. Martellos)

Key words: biogeography, herbarium collections, herbarium digitization, nomenclatural types, systematics

14.00-14.35 **Alexey P. Seregin**, M.V. Lomonosov Moscow State University, Russia
“Moscow Digital Herbarium: global approach through regional actions”

14.35-15.35 **Communications**

- **Maria Zardini**, Raffaella Trabucco, Juri Nascimbene, Stefano Martellos
  “Digitization of the *Lichenotheca Veneta*”
- **Simone Orsenigo**, Graziano Rossi, Thomas Abeli
  “Plant de-extinction: the importance of herbaria for recovering genetic diversity back to the wild”
- **Moreno Clementi**, Antonella Miola
  “A cross-disciplinary study of the work and collections by Roberto de Visiani (1800-1878)”

15.35-16.10 **Charles Davis**, University of of Harvard, USA
“Leveraging a global online herbarium to understand plant phenological response to climate”

16.10-17.10 **Communications**

- Marco D’Antraccoli, **David Dolci**, Francesco Roma-Marzio, Lorenzo Peruzzi
  “*‘Back to the future’*: assessing the effectiveness of Species Distribution Models temporal projection through herbarium data”
- **Niccolò Forin**, Sebastiano Nigris, Samuele Voyron, Mariangela Girlanda, Alfredo Vizzini, Giorgio Casadoro, Barbara Baldan
  “Importance of molecular studies involving nomenclatural types: the case of the Saccardo mycological collection”
- Isabella Bettarini, **Ilaria Colzi**, Cristina Gonnelli, Federico Selvi
  “Assessing metal hyperaccumulation using herbarium specimens: the case of *Odontarrhena sibirica* (Brassicaceae)”

17.10-18.00 Poster session

18.00-19.30 General Meeting of the Italian Botanical Society (members only)
During the General Meeting an agreement with Azerbaijan Botanists Society and the “Code of Conduct - Life ASAP” will be signed

20.30-23.30 Congress social dinner (SBI+SIBV)

Friday 6 September 2019

Symposium 4 SBI

“PLANT-ANIMAL INTERACTIONS: FROM MOLECULES TO LANDSCAPE”

(Chairpersons: M. Galloni and G. Aronne)

Key words: pollination, seed dispersal, plant defensive signals, plant-animal communication, plant-herbivore interaction, VOCs and chemical signalling

9.00-9.35 **Lilach Hadany**, University of Tel Aviv
“Can plants hear their pollinators?”

9.35-11.05 **Communications**

- **Massimo Nepi**, Gherardo Bogo, Massimo Guarnieri, Simona Sagona, Antonio Felicioli, Laura Bortolotti, Marta Galloni
  "Effects of non-protein amino acids of floral nectar on survival and locomotion of pollinators"
- **Luca Pegoraro**, Ellen C Baker, Manica Balant, Sarah Barlow, Lin Fu, Oriane Hidalgo, Andrew R Leitch, Ilia J. Leitch, Luis Palazzesi, Daniele Sommaggio, Jaume Pellicer
  "Pollinator monitoring in a mixed-ploidy population of *Senecio doronicum* in Alpes maritimes"
- **Angela Etcheverry**, Trinidad Figueroa, Antonella Ducci, María Alemán, Carlos Gómez, Carolina Yáñez, Diego López, Andrea, González Reyes
  "Asymmetrical flowers of Papilionoideae: functional morphology, morphometrics, and interactions with its pollinators"
- **Edy Fantinato**, Gabriella Buffa
  "Using pollination networks to detect tourism sustainability"
- Roberto Silvestro, Luigi Gennaro Izzo, **Giovanna Aronne**
  "Aerial seed bank benefits *Primula palinuri* Petagna in avoiding seed predation and run-off"
- **Stefano Vitti**, Francesco Boscutti, Valentino Casolo, Stefano Sponza
  "Seagrass - waterbirds interactions in a lagoon ecosystem of the northern Adriatic Sea"

11.05-11.25 Coffee Break

Symposium 5 SBI

"PLANT SPECIALIZED METABOLISM: CHEMISTRY AND BIOLOGICAL FUNCTIONS"

(Chairpersons: R. Caniato and L. Pistelli)

Key words: specialized metabolites, biotic and abiotic stress, plant-organism interactions

11.25-12.00 **Oliver Kayser**, TU Dortmund University, Germany
"Understanding cannabinoid biosynthesis by learning lessons from *Cannabis sativa*, *Helichrysum umbraculigerum* and *Radula marginata*"

12.00-13.30 **Communications**

- **Mandrone Manuela**
  "NMR-based metabolomics approach to detect frauds and adulterations of oregano samples"
- **Laura De Martino**, Luigi Aliberti, Lucia Caputo, Vincenzo De Feo, Filomena Nazzaro, Lucéia Fátima Souza
  "Chemical composition and biological activities of the essential oils of two different cultivars of *Citrus medica* L."
- **Daniela De Vita**, Chiara Toniolo, Claudio Frezza, Anastasia Orekhova, Giovanna Simonetti, Luigi Scipione, Roberto Di Santo, Roberta Costi
  "Antifungal activity of amaranth seed extract in combination with known antifungal drugs"
- **Giuseppe Malfa**, Barbara Tomasello, Alfonsina La Mantia, Francesco Pappalardo, Claudia Di Giacomo, Marcella Renis, Salvatore Ragusa, Rosaria Acquaviva
  "Anti-adipogenic and anti-oxidant effects of a standardized extract of *Citrus sinensis* (L.) Osbeck 'Moro' (Rutaceae) during adipocyte differentiation of 3T3-L1 pre-adipocytes"
- **Giuseppina Chianese**, Annalisa Lopatriello, Silvia Parapini, Annette Habluetzel, Donatella Taramelli, Harouna Sore, Alain R. Tenoh, Orazio Taglialatela-Scafati
  "Discovery of a selective and potent gametocytocidal antimalarial agent from *Lophira lanceolata*"

Roberta Ascrizzi, Laura Pistelli, Enrica Attuoni, Arianna Pelletti, Matteo Mancino, Guido Flamini, Luisa Pistelli

"Phytochemical and physiological evaluations of four *Citrus* species from the *horti simplicium* of the Charterhouse of Pisa over one year of analyses"

13.30-14.00 Congress Closure

Simposio Post Congresso SBI
(Orto botanico di Padova)

“GLI ORTI BOTANICI UNIVERSITARI: NUOVE OPPORTUNITÀ DI COORDINAMENTO E INDIRIZZO”
(a cura del Gruppo di Lavoro per gli Orti Botanici e i Giardini Storici)

"THE UNIVERSITY BOTANICAL GARDENS: NEW OPPORTUNITIES FOR COORDINATION AND COMMON OBJECTIVES”
(Symposium organized by The Working Group on Botanical and Historical Gardens)
(Moderatore: F. Pedrotti)

15.00 Apertura dei lavori e indirizzi di saluto del presidente della Società Botanica Italiana, del coordinatore del Gruppo di Lavoro per gli Orti Botanici e i Giardini Storici, del prefetto dell’Orto Botanico di Padova

15.05 **A. Cogoni**, Università di Cagliari
“Le piante aliene invasive e il riscatto degli Orti Botanici: da siti di introduzione e acclimatazione a promotori di buone pratiche. Gli Orti Botanici Italiani aderiscono al Codice di Condotta - Life ASAP”

Comunicazioni, parte prima: Gli Orti botanici in una prospettiva internazionale

15.15 **G. van Uffelen**, Hortus botanicus Leiden NL
“Hortus botanicus Leiden: 429 years of stability and change”

15.45 **B. Lainoff**, Botanic Gardens Conservation International, Kew UK
“The Largest Global Network of Botanic Gardens and Setting International Standards for Botanic Gardens through Accreditation”

Comunicazioni, parte seconda: Esperienze italiane

16.15 **B. Baldan**, Università di Padova
“L'Orto Botanico di Padova: dal 1545 al future”

16.25 **G. Bacchetta**, A. Caddeo, G. Iiriti, F. Meloni, M. Porceddu, Università di Cagliari
“Hortus Botanicus Karalitanus: un Orto Botanico universitario in cammino verso la sostenibilità sociale e ambientale”

16.35 **A. Chiarucci**, U. Mossetti, Università di Bologna
“Il rinnovamento dell’Orto Botanico di Bologna, dalla gestazione dell’idea alla gestione”

16.45 **L. Peruzzi**, Università di Pisa
“La sottile linea tra divulgazione e snaturamento, ovvero dell'importanza che degli Orti Botanici si occupino i Botanici. Evoluzione recente dell'Orto e Museo Botanico di Pisa”

16.55 **C. Siniscalco**, Università di Torino
“Gli Orti Botanici oggi tra priorità di comunicare la conoscenza delle piante e... mille altre richieste e necessità. Una Rete per condividere le scelte”

17.05 **M. Mariotti**, L. Minuto, Università di Genova
“I Giardini Botanici Hanbury: un orto botanico di frontiera con ruolo transdisciplinare nella formazione, nella ricerca scientifica e nella gestione del territorio”

17.15 Discussione e conclusioni

Saturday 7 September 2019

8.00-17.00 Escursione sociale al Bosco del Cansiglio e al Giardino Botanico Alpino G. Lorenzoni
(Social excursion to the Cansiglio Wood and Orto Botanico G. Lorenzoni)

Indice in ordine alfabetico in coda al volume degli Atti

INDEX

Keynote Lectures

SYMPOSIUM 1.
PLANTS IN AND FOR THE FUTURE

SYMPOSIUM 2.
FOREST BIODIVERSITY AND FUNCTIONING IN A CHANGING WORLD: CHALLENGES AND OPPORTUNITIES

SYMPOSIUM 3.
HERBARIA: STILL RELEVANT IN THE 21^{TH} CENTURY?

SYMPOSIUM 4.
PLANT-ANIMAL INTERACTIONS: FROM MOLECULES TO LANDSCAPE

SYMPOSIUM 5.
PLANT SPECIALIZED METABOLISM: CHEMISTRY AND BIOLOGICAL FUNCTIONS

Communications

Symposium 1.
Plants in and for the future

Symposium 2.
Forest biodiversity and functioning in a changing world: challenges and opportunities

Symposium 3.
Herbaria: still relevant in the 21TH Century?

SYMPOSIUM 4.
PLANT-ANIMAL INTERACTIONS: FROM MOLECULES TO LANDSCAPE

SYMPOSIUM 5.
PLANT SPECIALIZED METABOLISM: CHEMISTRY AND BIOLOGICAL FUNCTIONS

Posters

1. PLANTS IN AND FOR THE FUTURE

1.1 Cell biology

1.2 Signal transduction

1.3 Plant development

1.4 Stress responses

1.5 Biotechnology

2. FOREST BIODIVERSITY AND FUNCTIONING IN A CHANGING WORLD: CHALLENGES AND OPPORTUNITIES

2.1 Global change

2.3 Conservation

2.4 Functional responses

2.5 Vegetation dynamics

2.6 Vegetation classification

3. HERBARIA: STILL RELEVANT IN THE 21TH CENTURY?

3.2 Herbarium collections

3.3 Herbarium digitization

3.5 Systematics

4. PLANT-ANIMAL INTERACTIONS: FROM MOLECULES TO LANDSCAPE

4.1 Pollination

4.2 Seed dispersal

4.4 Plant-animal communication

4.5 Plant-herbivore interaction

5. Plant Specialized Metabolism: Chemistry and Biological functions

5.1 Specialized metabolites

5.2 Biotic and abiotic stress

5.3 Plant-organism interactions

FREE TOPIC

Post-congress “Orti botanici”

Biotecnologie e Differenziamento

Botaniche Applicate

Briologia

Conservazione della Natura

Ecologia

Fenologia e Strategie vitali

Lichenologia

Micologia

Palinologia e Paleobotanica

Piante Officinali

Specie Alloctone

Vegetazione

Post Congress

GLI ORTI BOTANICI UNIVERSITARI: NUOVE OPPORTUNITÀ DI COORDINAMENTO E INDIRIZZO

KEYNOTE LECTURES

Developing phloem – orchestrating plant organ formation

Christian S Hardtke
Department of Plant Molecular Biology, University of Lausanne, Switzerland

Angiosperms, the extant plants that dominate terrestrial ecosystems, are characterized by extensive vascular xylem and phloem networks, which permit nutrient distribution as well as systemic coordination of physiology and development. Molecular mechanisms of vascular differentiation and their relation to plant hormone pathways are the major research focus of my lab. In this context, the development of phloem sieve elements is particularly interesting, because during their differentiation, sieve elements reduce some organelles and lose others (notably the nucleus) as they interconnect via sieve plates to form continuous sieve tubes. Thus, sieve elements represent a unique cell type that retains complex functionality in the absence of a nucleus. A major question in our research is how the onset of the peculiar sieve element differentiation process is controlled at the molecular level? The Arabidopsis root is particularly well-suited to investigate this process, because vascular tissues are continuously formed from stem cells at its tip and phloem formation can be followed along the spatio-temporal gradient of single cell files. Over the last years we have built up an extensive molecular genetic network that governs the differentiation of sieve element precursors. This network comprises angiosperm-specific positive regulators, which drive sieve element differentiation through tipping a delicate quantitative balance, opposing negative regulators of the process. Both auxin transport regulation and brassinosteroid signaling are key aspects of the positive regulatory output, whereas CLE peptide signaling is central to the negative regulatory output. I will present data that illustrate how these pathways intersect to guide sieve element differentiation.

Forestal ecological history for the future

Jean-Luc Dupouey
National Institute for Agronomical Research, Silva research unit, F-54280 Champenoux, France, jean-luc.dupouey@inra.fr

Historical, archaeological and paleoecological data provide a better understanding of the current distribution of species, which cannot be inferred directly from the sole knowledge of their fundamental ecological niche, dispersal ability and interactions with other species. The long time is an additional component, which is the object of historical ecology, the branch of ecology that is interested in legacies of the past in the current functioning of ecosystems. We will present some examples of the significance of these long-term effects.

The first concerns the impact of past land-use changes on vegetation. Europe has known for the last two centuries a significant increase in forest area. We show the multiple impacts on the composition of plant communities of this forest expansion, in the Mediterranean area among others. This has led to the development of the important concept of ancient and recent forests, which is now classically included in assessments of the degree of naturalness of forests. We have tried to characterize the duration of these impacts: following the recolonization of a former agricultural soil, how long time does it take to erase the extinction debt of the species previously present but destined to disappear, and the colonization credit of the species that still have to arrive? The research that we have conducted on several Roman sites shows that these impacts have a multi-millennial span. We analyze the mechanisms of this strikingly long memory of ecosystems.

In a second example, we will search for anthropogenic traces in the current spatial range of Fagus sylvatica in France. The resampling of dendrometric inventories from the XVIIIth century, the distribution of toponyms of beech at the national scale and soil charcoal analyses show how this tree species has been disadvantaged by forest management, perhaps early in the Neolithic period. Knowledge of such large-scale manipulations is needed to properly characterize the beech climatic niche, in order to be able to project its future distribution range into different climate change scenarios.

The reaction times of plant communities to natural or anthropogenic disturbances can be very long, as shown by the examples presented. This has many consequences: climacic equilibria are rarely or never reached, the impacts of human actions should be appreciated over much longer time horizons than they are today, the understanding of our biotic environment cannot be done without an historical perspective. Time must be considered as a relevant attribute of the species niche.

The simple structure of forests: an allometric approach for assessing the degree of old-growthness

Anfodillo Tommaso[1], Maritan A.[2], Banavar J.R.[3], Sellan G.[4], Simini F.[5]
[1] Department Territorio e Sistemi Agro-Forestali, University of Padova, IT; [2] Department of Physics and Astronomy' G. Galilei', University of Padova, and INFN, IT; [3] Department of Physics and Institute for Theoretical Science, 1274 Unversity of Oregon, Eugene OR 97403, USA; [4] School of Science and the Environment, Manchester Metropolitan University, Chester Street, Manchester M1 5GD, UK; [5] Department of Engineering Mathematics, University of Bristol, Clifton BS8 1TR, UK

At global scale primary forests are dramatically disappearing. In Europe, primary forests represent 0.7% of the forested area and only about 50% of them are strictly protected, it is thus very urgent to promote actions for strengthening the protection schemes. It would also be important to increase the protected forest lands including forests that have been preserved by harvesting activity for a long time and that can be classified as old-growth forests (in Italian: foreste vetuste). However, for implementing protection initiatives and prioritizing the actions it is essential to find a quantitative method for estimating the degree of old-growthness.

We developed a theoretical framework (the so-called *H-model*), based on simple assumptions, allowing us to define a metric of the degree of disturbance in forest stands based on analysis of the community structure (i.e. tree-size distribution). The method is species-independent and can be applied in any forest around the globe.

The aim of this talk is to present the theoretical bases of the method and some applications in different forests (in temperate and tropical areas).

The method is based on two main assumptions: 1) forests, after a long period without major disturbances, can approach a condition of maximum use of available resources; 2) trees use the resources proportionally to their leaf area (or crown volume, Vcro).

When the condition of maximum resource use with trees of different sizes ($N_{\max,h}$) is met, it is possible to predict the tree-size distribution because the number of trees in each size class scales with tree height with an exponent that is proportional to – Vcro or, in other words $N_{\max,h} \propto h^{-Vcro}$ where h=tree height.

For implementing the method it is necessary to measure: 1) the scaling of the crown volume with tree height (by measuring crown traits of 60-100 trees in the whole range of sizes) and 2) the actual tree size distribution of the forest. Then comparing the theoretical distribution with the empirical one is possible to have a metric of the degree of old-growthness that is proportional to the difference of the slopes between the two tree-size distributions (empirical *vs.* theoretical).

Our approach appears theoretically sound and easy to apply, thus promising for assessing the actual status of forests around the globe.

Sellan G, Simini F, Maritan A, Banavar JR, de Haulleville T, Bauters M, Doucet JL, Beeckman H, Anfodillo T (2017) Testing a general approach to assess the degree of disturbance in tropical forests, J. Veg. Sci., 28(3), 659-668

Anfodillo T, Carrer M, Simini F, Popa I, Banavar JR, Maritan A (2013) An allometry-based approach for understanding forest structure, predicting tree-size distribution and assessing the degree of disturbance, Proc. R. Soc. B, 280, 20131751.

Simini F, Anfodillo T., Carrer M, Banavar JR, Maritan A (2010) Self-similarity and scaling in forest communities, *PNAS*, 107, 7658-7662

Moscow Digital Herbarium: a global approach through regional actions

Alexey P. Seregin
Herbarium, Lomonosov Moscow State University, Leninskie Gory 1, 119234 Moscow, Russia

Moscow Digital Herbarium is an initiative of the Moscow State University. In 2015, we started complete imaging of the Moscow University Herbarium (MW) at 300 dpi (1). This budget-consuming work was completely covered by the megagrant #14-50-00029 from the Russian Science Foundation (РНФ). Year by year, our commercial partner digitized the complete MW Herbarium.

In October 2016, we launched a web-portal of Moscow Digital Herbarium (available at http://plant.depo.msu.ru/), to deliver the images of our herbarium specimens to a wider audience, with some basic associated metadata like ID, species name and geographical area (2). From that time, we also began a complete databasing of our collections to include complete metadata: 1) transcriptions of labels as a result of manual text capturing, 2) OCR transcription of printed elements, and 3) geographical coordinates.

By the end of 2018, we finally published online 968,000 specimens (93%), captured 135,800 labels (13%), georeferenced 323,000 specimens (31%), and made all possible automatic OCR transcriptions using Tesseract 3 (it was successful for 330,100 specimens without text transcriptions).

Unfortunately, we failed to raise budget for the whole process to reach triple-100% for imaging, label capturing and georeferencing. Currently, Moscow University Herbarium is acting as an international institution with no more than 65% of holdings coming from Russia, but national funding agencies enforced us to focus our activity on the Russian projects. So, to finish the mission and to keep high standards of digitization efforts for new accessions, we proposed several regional projects which were later formally approved by funding agencies. We focused our proposals on a few regions which are especially well represented in our collection.

1. The City of Moscow and Moscow Oblast (project #19-34-70018 funded by RFBR and Moscow city Government). Within this project, we aimed to database and georeference all labels from Moscow and surrounding Moscow Oblast from two herbaria – Moscow University Herbarium (76,000 specimens) and Main Botanical Garden Herbarium (38,000 specimens). The project made Main Botanical Garden (MHA), which holds the second largest herbarium in Moscow, the second publisher of the Moscow Digital Herbarium.
2. European Russia (project #Russia2019_14 funded by FinBIF within Data mobilization in European Russia programme). The Moscow Digital Herbarium is the largest biodiversity database in Russia, and currently the largest Russian dataset published in GBIF (3), but only 31% of the records were georeferenced by the end of 2018. We expect that the project will allow georeferencing an additional 54,450 specimens from European Russia by the end of September 2019.
3. Western Caucasus (project #19-44-233012 funded by RFBR and Krasnodar Krai Government). The Caucasus is the only biodiversity hotspot of international importance in Russia, which makes it the top priority area for national floristic studies. The goal of the project is to create the most complete dataset on the floristic diversity of the Russia's richest regional flora. The portal will include at least 25,000 specimens from the Moscow University Herbarium, including new accessions.
4. Tula Oblast (project #19-44-710002 funded by RFBR and Tula Oblast Government). The goal of the project is to assess the actual diversity and spatial structure of the flora of the Tula Oblast, by combining various data sources on a single platform. To do this, it is supposed to combine the data of five local herbarium collections on our web-platform.

What lessons we learned after finishing of on-purpose financial support?

(1) Never give up! We applied for 16 grants, competition and prizes to keep our work moving. The most precious trophies were not conquered, but nonetheless we have five medium-sized grants to promote regional missions.

(2) Do not rely on volunteers in your work. You will spend dozens of hours to teach a volunteer with no guarantee that he will come tomorrow. Think twice, do you really need him?

(3) Virtual consolidation of lesser collections within a larger web-hub is a budget-efficient solution. Have no portal? Start publishing via GBIF.

1) A.P. Seregin (2016) Taxon, 65, 205-207
2) A.P. Seregin (2018) Taxon, 67, 463-467
3) A.P. Seregin (2019) Moscow University Herbarium (MW), occurrence dataset https://doi.org/10.15468/cpnhcc accessed via GBIF.org

Leveraging a global online herbarium to understand plant phenological response to climate

Charles C. Davis[1], Daniel S. Park[1], Ian Breckheimer[1], Goia Lyra[1], Jarvis Xie[2], Aaron M Ellison[3]
[1]Department of Organismic and Evolutionary Biology and Harvard University Herbaria, Harvard University, Cambridge, Massachusetts, USA; [2]David R. Cheriton School of Computer Science, University of Waterloo, Waterloo, Ontario, Canada; [3]Harvard Forest, Harvard University, Petersham, Massachusetts, USA

The digitization of natural history collections is one of the greatest advances in museum science during the past century (1). The online mobilization of herbaria in particular has been at the leading edge of these advancements: entire floristic collections once behind locked cabinets are now accessible at the touch of a button. One of the most timely and vibrant uses of this virtual herbarium are ongoing efforts to address species' phenology, i.e., the seasonal timing of an organism's life history. Phenological change is a critical baseline indicator for how plants will respond to climate in the Anthropocene. The tens of millions of herbarium records collected over time represent phenological events of species at specific places and times under varying climatic conditions (Fig. 1), thus offering spatial, temporal, and phylogenetic coverage far beyond existing field observational data. Here, I will discuss efforts to harness such phenological data from herbarium records, including i.) validating specimens for this purpose (2, 3); ii.) the development and application of massive online crowdsourcing experiments to harvest these data quickly and effectively (4); iii.) the important biological insights emerging from these efforts (5, 6, 7); and iv.) future directions to stimulate these efforts, including machine learning (8, 9, 10), and to characterize and help remedy the imperfections of these data for addressing phenological response to climate.

1) E.K. Meineke, T.J. Davies, B.H. Daru, C.C. Davis (2018) Phil. Trans. Roy. Soc. B, 374
2) C.C. Davis, C.G. Willis, B. Connolly, B. Kelly, A.M. Ellison (2015) Am. J. Bot., 102, 1599-1609
3) B.H. Daru, D.S. Park, R.B. Primack, C.G. Willis, D.S. Barrington, T.J. Whitfeld et al. (2018) New Phytol., 21, 939-955
4) C.G. Willis, E. Law, A.C. Williams, B.F. Franzone, R. Bernardos, L. Bruno et al. (2017) New Phytol., 215(1), 479-488
5) D.S. Park, I. Breckheimer, A.C. Williams, E. Law, A.M. Ellison, C.C. Davis (2018) Phil. Trans. Roy. Soc. B, 374, 20170394.
6) I.W. Park, S.J. Mazer. (2019) Global Ecol. Biogeogr., in press
7) E.K. Meineke, A.T. Classen, N.J. Sanders, J.T. Davies (2019) J. Ecol., 107, 105-117
8) I.W. Park, S.J. Mazer (2018) Global Change Biology, 24, 5972-5984
9) I.W. Park, A. Jones, S.J. Mazer (2019) Appl. Plant Sci., e01230
10) T. Lorieul, K.D. Pearson, E.R. Ellwood, H. Goëau, J.F. Molino, P.W. Sweeney et al. (2019) Appl. Plant Sci., 7, e01233

Can plants hear their pollinators?

Lilach Hadany[1], Marine Veits[1], Itzhak Khait[1], Uri Obolski[1], Eyal Zinger[1], Arjan Boonman[2], Aya Goldshtein[2], Kfir Saban[1], Udi Ben-Dor[1], Paz Estlein[1], Areej Kabat[1], Dor Peretz[1], Ittai Ratzersdorfer[1], Slava Krylov[3], Daniel Chamovitz[1], Yuval Sapir[1], Yossi Yovel[2]

[1] Department of Plant Sciences and Food Security, Tal Aviv University, Tel Aviv, Israel; [2] School of Zoology, Tel-Aviv University, Tel-Aviv, Israel; [3] School of Mechanical Engineering, Tel-Aviv University, Tel-Aviv, Israel

Can plants hear? That is, can they sense airborne sounds and respond to them? This question has intrigued humans since ancient times. The environment of plants includes many informative sounds, produced by biotic and abiotic sources. An ability to respond to these sounds could thus have a significant adaptive value for plants. I will discuss the ecological contexts where response to sound could be most advantageous to plants, focusing on one case of particular interest: the acoustic interaction between flowers and pollinators.

We found that evening primprose flowers (*Oenothera drummondii*), exposed to the playback sound of a flying bee or to synthetic sound-signals at similar frequencies, produced sweeter nectar within 3 minutes, potentially increasing the chances of cross pollination. We found that the flowers vibrated mechanically in response to these sounds, suggesting a plausible mechanism where the flower serves as the plant's auditory sensory organ. Both the vibration and the nectar response were frequency-specific: the flowers responded to pollinator sounds, but not to higher frequency sounds.

Pollination literature considers the way that pollinators respond to plant signals – including color, shape and smell. Our results document the other direction – that plants can also rapidly respond to signals emitted by the pollinators – the sound of their wingbeat. Plant sensitivity to pollinator sounds can affect plant-pollinator interactions in a wide range of ways: plants could identify the period of peak pollinator activity, which can differ daily, and allocate their resources more adequately; pollinators would then be better rewarded; flower shape may be selected for its functional effect on the hearing ability, and not only on signaling; pollinators may evolve to make sounds that the flowers can hear better; and plants may be affected by other sounds as well, including antropogenic ones.

Understanding cannabinoid biosynthesis by learning lessions from *Cannabis sativa*, *Helichrysum umbraculigerum* and *Radula marginata*

Oliver Kayser
Technical Biochemistry, TU Dortmund University, Dortmund, D-44227, oliver.kayser@tu-dortmund.de

Cannabinoids play a major role in pharmacology as new upcoming chemical scaffolds for the treatment of various diseases like chemotherapy, multiple sclerosis, trauma and ophthalmology. Besides their almost unique cannabinoids as terpenophenolics are found only in few plants like C. sativa, H. umbraculigerum und the liverworth R. marginata. Based on genome and transcriptome analysis followed by studies on the metabolic profile important differences are discussed. Cannabinoids are biosynthesized in plants in glandular trichomes as mostly less understood organ and trafficking and storage of THC and related precursors remains unclear. In this talk, we highlight the molecular basis for precursor delivery, localization of biosynthesis in trichomes by Imaging-MS and CARS microscopy, gene regulation by qPCR and metabolome analysis of THC and CBD over the standard cultivation time of 8 weeks. Briefly the heterologous expression and cannabinoid pathway assembly in yeast is explained. Here, the concept of bioengineering an artificial biosynthesis of cannabinoids following engineering principles (systems biotechnology, in silico prediction) is outlines. More recent cannabinoids have been detected in the liver moss Radula marginata. Latest findings will be introduced to the taxonomy, phylogenicity and anatomical structure of these unique cannabinoid producing mosses (1–3).

References
1) Schachtsiek J, Warzecha H, Kayser O, Stehle F. (2018) Current Perspectives on Biotechnological Cannabinoid Production in Plants, Planta Medica
2) Carvalho Â, Hansen EHEH, Kayser O, Carlsen S, Stehle F. (2017) Designing microorganisms for heterologous biosynthesis of cannabinoids. FEMS Yeast Res., 17(4)
3) Happyana N, Kayser O. (2016) 1H NMR-based metabolomics differentiation and real time PCR analysis of medicinal Cannabis organs, Acta Horticulturae, 1125

COMMUNICATIONS

Plant cell downstream control of conventional and unconventional membrane traffic contribution to the tonoplast.

Gian Pietro Di Sansebastiano[1], Fabrizio Barozzi[1],
[1]DISTEBA (Department of Biological and Environmental Sciences and Technologies), University of Salento, Via Lecce-Monteroni, Campus ECOTEKNE, 73100 Lecce, Italy

A new unexpected interaction was recently described (1) in *Arabidopsis thaliana* between the QcSNARE SYP51 normally localized on TGN and tonoplast and aquaporin NIP1;1 mainly localized on the endoplasmic reticulum (RE). This interaction has shown to be extremely specific and able to influence the vacuole's ability to accumulate some metals and metalloids (1). The interaction between these very different proteins seems to play a regulatory role in the membrane traffic towards the vacuole, determining its characteristics.

It is known that the tonoplast membrane originates mainly from the RE and it appears evident that direct Golgi-independent membrane traffic from the RE to the vacuole remains active in all cell types after vacuole biogenesis, providing examples of unconventional membrane traffic. It is equally well known that a large part of the vacuolar traffic is realized involving the Golgi apparatus and is considered more “conventional”. The most recent data on unpublished interactions between SNARE and aquaporin, are used here to describe a new system of regulation of the vacuolar traffic that would allow to maintain the characteristics of the tonoplast even in conditions of strong traffic alteration through the Golgi apparatus such as heterologous proteins overexpression. If some proteins transited from the Golgi exceed partner proteins that reach the tonoplast directly from the RE, the former would be concentrated in specific areas and invaginated within the vacuole for rapid degradation.

This new regulatory mechanism would make it possible coordinating direct traffic and Golgi-mediated vacuolar traffic while maintaining the identity of the tonoplast.

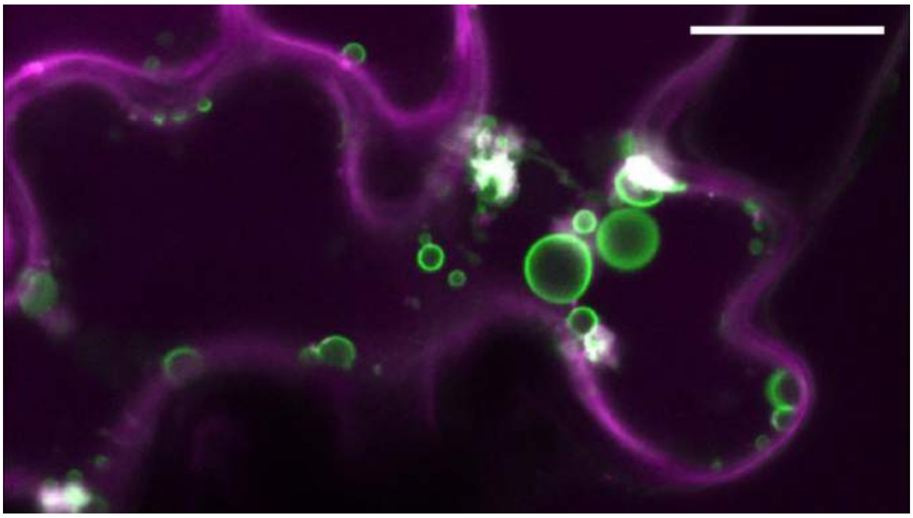


Fig. 1: Confocal image of an *Arabidopsis thaliana* epidermal cell transformed with RFP tagged NIP1;1 (in magenta) distributed in the ER and GFP tagged SYP51 (in green) distributed in the tonoplast. The two proteins have been shown to interact transiently (signal in white) on the tonoplast and induce the excess of GFP tagged SYP51 to invaginate in the vacuole.

1) F. Barozzi, P. Papadi, G. Stefano, L. Renna, F. Brandizzi, D. Migoni, F.P. Fanizzi, G. Piro, G.P. Di Sansebastiano (2019) Front Plant Sci, 9, 1949, doi: 10.3389/fpls.2018.01949

The heterologous expression of the *Rhizophagus irregularis RiPEIP1* gene in rice plants leads to enhanced growth and mycorrhizal colonization

Cristina Votta[1], Valentina Fiorilli[1], Jorge Gómez-Ariza[2], Fabio Fornara[3], Luisa Lanfranco[1]
[1]Department of Life Sciences and Systems Biology, University of Torino, Viale Mattioli 25, 10125 Torino, Italy; [2]Department of Molecular Genetics Centre for Research in Agricultural Genomics, Barcelona, Spain; [3]Department of Bioscience, University of Milano, Via Celoria 26, 20133 Milano, Italy

The majority of land plants establish a root symbiotic association with arbuscular mycorrhizal (AM) fungi that provide several benefits such as improved mineral nutrition and higher tolerance to biotic and abiotic stresses (1). The fungal molecular determinants controlling the formation of a functional symbiosis are still largely unknown. We recently characterize *RiPEIP1* (*Preferentially Expressed In Planta*), an orphan gene from the AM fungus *Rhizophagus irregularis,* which is strongly expressed in the intraradical phase, including arbuscules, in different host plants. When expressed as a GFP fusion in yeast cells, RiPEIP1 localizes in the endomembrane system, which is consistent with the *in silico* prediction of four transmembrane domains. In the absence of genetic transformation protocols for AM fungi, we exploited two different heterologous expression systems. Previous results showed that *RiPEIP1* expression in *Oidiodendron maius*, an ericoid endomycorrhizal fungus, led to enhanced colonization capacity compared to the *O. maius* wild type strain (2). To gain further insights on the biological role of *RiPEIP1* we generated rice *RiPEIP1*-expressing lines under the constitutive 35S promoter. A preliminary analysis showed that these transgenic lines have a higher number of crown roots, an increased shoot length, a higher root and shoot biomass, an early flowering phenotype, an increased number of panicles and seeds and a higher AM colonization level compared to wild type plants. Although the mechanism of action of *RiPEIP1* is still unknown, these findings suggest that *RiPEIP1* expression affects different host metabolic pathways which promote plant growth and mycorrhizal colonization.

1) L. Lanfranco, V. Fiorilli, C. Gutjahr (2018) New Phytologist, 220(4), 1031-1046
2) V. Fiorilli V., S. Belmondo, H.R. Khouja, S. Abbà, A. Faccio, S. Daghino, L. Lanfranco (2016) Mycorrhiza, 26(6), 609-621

Comparative transcriptomic profiling of peach and nectarine cultivars to elucidate chilling injury mechanisms

Antonella Muto[1], Ernesto Picardi[2], Leonardo Bruno[1], Hilary J. Rogers[3], Antonio Ferrante[4], Adriana Ada Ceverista Chiappetta[1], Maria Beatrice Bitonti[1], Natasha Damiana Spadafora[1],[5]
[1]Department of Biology, Ecology and Earth Sciences, University of Calabria, Via Ponte P. Bucci 6b, 87036 Arcavacata di Rende Cosenza, Italy; [2]Department of Biosciences, Biotechnology and Biopharmaceutics, University of Bari A. Moro, Bari, Italy; [3]School of Biosciences, Cardiff University, Sir Martin Evans Building, Museum Avenue, Cardiff, United Kingdom; [4]Department of Agricultural and Environmental Science – Production, Landscape, Agroenergy, Università degli Studi di Milano, Via Celoria 2, 20133, Milan, Italy; [5]Markes International, Gwaun Elai Medi-Science Campus, Llantrisant, Cardiff, United Kingdom.

Peach (*Prunus persica* (L.) Batsch) is a fruit tree belonging to *Rosaceae* family and is one of the most commercially relevant crop species. Namely, peach fruits are greatly appreciated for attractive uniqueness in flavour, texture, juiciness, and nutritional value and therefore widely consumed as a fresh and canned fruit, as well as in processed juices.

Italy is the second largest peach producer in the EU (1.362.000 MT in 2017/18- FAS) and peach fruit production plays a key role in the agricultural sector of several Italian regions, including Calabria. Currently, with the aim to extend peach commercialization producers mainly focus on the cultivation of peach local cultivars and varieties, particularly prized by consumers for the intense skin colour and high flavor of their fruits. Among these varieties, the nectarine *(P. persica* var. *nucipersica*), is one of the most relevant.

In this context, it must be mentioned that peach and nectarine fruits are characterized by a rapid deterioration at room temperature (1,2) and cold storage is widely used to delay fruit post-harvest ripening and extend fruit commercial life (3). However, several physiological disorders, collectively known as chilling injury (CI), can develop under low temperature storage and affect fruit quality. Therefore, it appears of considerable scientific and economic interest to improve the knowledge on the mechanisms by which fruit response to cold stress. Starting from this *scenario*, in the FRUITY (FRUIt qualiTY) project we planned to investigate the molecular basis of chilling injury fruit in peach, since in fruit biology research this area is lagging far behind. Currently, a very suitable support to fulfill this gap is provided by whole genomes sequencing data and concerning peach, a–fully sequenced and annotated genome is available (4). Moreover, peach fruit-specific cold responses have been recently investigated at the proteomic level (2). However, despite such information, the exact mechanism by which chilling injury affects a fruit, is not fully understood.

In the present work we performed a comparative transcriptomic analysis by Next Generation Sequencing (NGS) on one peach (cv Sagittaria) and one nectarine (cv Big Top) cultivars respectively. Fruits were sampled immediately after harvest (T0), and at 1 (T1), 5 (T2), 7 (T3) and 14 (T4) days during cold storage in an attempt to identify pre-symptomatic genetic changes of chilling injury. The following multiple pairwise comparisons have been performed: T1/T0, T2/T0, T3/T0, T4/T0 and differentially expressed genes (DEGs) were estimated. A comparable expression trend was observed in both cultivars, with a progressive increase of DEGs number along cold storage, together with a higher number of DEGs in the Big top (2261 DEGs) compared to Sagittaria (1484 DEGs) cultivar. Differences between cultivars were also detected by analyzing the fraction of DEGs up- and down-regulated. Additional analysis of the time course of transcriptomic changes allowed the detection of transient or monotone induced genes.

Globally, the results so far obtained evidenced common responses to cold treatments in nectarine and peach cultivar, such as a general downregulation of genetic pathway related to photosynthesis machinery, and an upregulation of genetic pathways related to amino sugar and nucleotide sugar metabolism as well to hormone signal transduction. However, genotype specific activation of some pathway was also observed, such as for sesquiterpenoid and triterpenois biosynthesis in Sagittaria cultivar, which could be relevant (play a role) in determining post-harvest fruit quality.

1) Lurie S., Crisosto C.H. (2005) Chilling injury in peach and nectarine. Postharvest Biol. Technol. 37, 195–208

2) Tanou G, Minas I. S., Scossa F., Belghazi M., Xanthopoulou A., Ganopoulos I., *et al.* (2017) Exploring priming responses involved in peach fruit acclimation to cold stress. Sci. Rep. 7, 11358

3) Aubert C., Bony P., Chalo G., Landry P., Lurol S. (2014) J. Agric. Food Chem., 62, 4707-4724

4) Verde I, Jenkins J, Dondini L, Micali S, Pagliarani G, Vendramin E, *et al.* (2017) The Peach v2.0 release: high-resolution linkage mapping and deep resequencing improve chromosome-scale assembly and contiguity. BMC Genomics, 18, 225

Evolution and functional differentiation of recently diverged phytochelatin synthase genes from *Arundo donax* L.

Claudio Varotto[1,#] , Mingai Li[1,*], Luca Stragliati[2,*], Erika Bellini[3], Ada Ricci[2], Luigi Sanità di Toppi[3,#]
[1]Department of Biodiversity and Molecular Ecology, Research and Innovation Centre, Fondazione Edmund Mach, via Mach 1, 38100, San Michele all'Adige (TN), Italy; [2]Dipartimento di Scienze Chimiche, della Vita e della Sostenibilità Ambientale, Università degli studi di Parma, Parco Area delle Scienze 11/A, 43125, Parma, Italy; [3]Dipartimento di Biologia, Università di Pisa, via Luca Ghini 13, 56126, Pisa, Italy

Phytochelatin synthases (PCS) play pivotal roles in the detoxification of heavy metals and metalloids in plants (1). Despite such relevance, little information on the evolution of recently duplicated *PCS* genes in plant species is available.

Here we characterize the evolution and functional differentiation of three *PCS* genes from the giant reed (*Arundo donax* L.), a biomass/bioenergy crop with remarkable resistance to cadmium (Cd) and other heavy metals (2). Phylogenetic reconstruction with *PCS* genes from fully sequenced monocotyledonous genomes indicates (3) that the three *A. donax* PCS, namely *AdPCS1-3*, form a monophyletic clade (Fig. 1). *AdPCS1-3* genes are expressed at low levels in many *A. donax* organs and, in particular, display different levels of Cd-responsive expression in roots (Fig. 2). Overexpression of *AdPCS1-3* in *Arabidopsis thaliana* and yeast reproduces the phenotype of functional *PCS* genes (4). Mass-spectrometry analyses confirm that AdPCS1-3 are all functional enzymes, but also evidence significant differences in the amount of the phytochelatins synthesized (Fig. 3). Moreover, heterogeneous evolutionary rates characterize the *AdPCS1-3* genes, indicative of relaxed natural selection (5).

These results highlight the elevated functional differentiation of *A. donax PCS* genes from both a transcriptional and an enzymatic point of view, providing evidence of the high evolvability of *PCS* genes and of plant responsiveness to heavy metal stress.

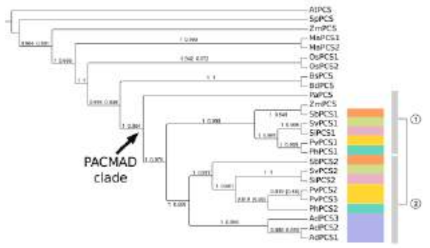


Fig. 1. *AdPCS1-3* phylogeny

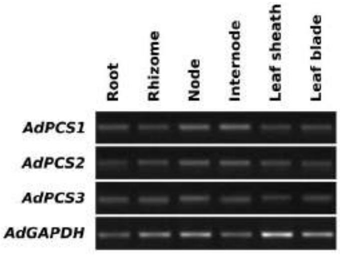


Fig. 2. *AdPCS1-3* expression

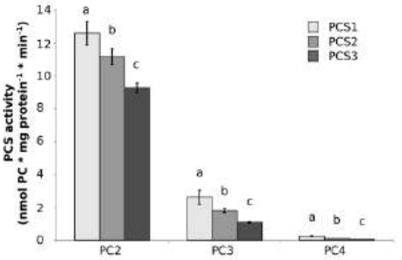


Fig. 3. *AdPCS1-3* specific activity

1) Clemens S (2006) Evolution and function of phytochelatin synthases, Journal of Plant Physiology, 163, 319–332

2) Papazoglou EG, Serelis KG, Bouranis DL (2007) Impact of high cadmium and nickel soil concentration on selected physiological parameters of *Arundo donax* L. European Journal of Soil Biology, 43, 207–215

3) Goodstein DM, Shu S, Howson R, et al. (2012) Phytozome : a comparative platform for green plant genomics. Nucleic Acids Research, 40, 1178–1186

4) Lee S, Moon JS, Ko TS, Petros D, Goldsbrough PB, Korban SS (2003) Overexpression of *Arabidopsis Phytochelatin Synthase* paradoxically leads to hypersensitivity to cadmium stress. Plant Physiology, 131, 656–663

5) Wertheim JO, Murrell B, Smith MD, Pond SLK, Scheffler K (2014) RELAX : Detecting relaxed selection in a phylogenetic framework. Molecular biology and evolution, 32, 820–832

Combining historical ecology, species distribution modelling and instrumental climate reconstruction to infer causes of change in forest distribution: the case of Monte Pisano

Gabriele Casazza[1], Michele Brunetti[2], Fabio Malfatti[3], Valentina Simonetti[4], Andrew S. Mathews[5]
[1]Aix Marseille Univ, Avignon Université, CNRS, IRD, IMBE, Marseille, France ; [2]CNR – ISAC, Consiglio Nazionale delle Ricerche – Istituto di Scienze dell'Atmosfera e del Clima, Bologna, Italy; [3]Centro Ricerche EtnoAntropologiche, CREA, Siena, Italy; [4]Archivio di Stato di Lucca, Lucca, Italy; [5]University of California, Santa Cruz, USA

Human-induced climate change is producing unprecedented changes on plant communities and is predicted to have still greater impacts over the next century. Furthermore, other human-induced changes may affect species distributions and species assemblage, including changing land use, and the spread of pests and pathogens. Climate change, species invasions and land use change combine to affect distributional range of species and in turn to promote change in biodiversity. In Europe, where human populations have altered the vegetation for thousands of years before the industrial era, socioeconomic changes are one of the main causes for land abandonment and related land-cover changes. In Southern European mountains where climate change has been greater than the global average, land use and climate change may have interacted favouring change in vegetation. However, there is a particular difficulty in separating the effects of climate change from changes in land use. Moreover, unlike climate change, for which possible scenarios can be hypothesized, future land use and ecological interactions are unpredictable. Nevertheless, interdisciplinary histories can provide a better knowledge, that can enrich our understanding of the range of biotic impacts we might see in the future. Here, we combine early-nineteenth century cadastral data (1850), present-day studies of vegetation (1999), secular climatic records and species distribution models to infer the effects of past land-use and climate change over two centuries on forest distribution in a key landscape of Central Italy, the Monti Pisani. Our systematic sample shows that while total forest area remained broadly stable (60% in 1850 vs 52% in 1999), chestnut decreased dramatically (from 27 to 12%), and pine increased (from 13% to 30%). Mean annual temperature increased gradually from 1820 until roughly the 1980's, then it increased sharply. On the other hand, precipitation showed a slightly negative trend. The predicted relative potential distribution of chestnut and pine during the past was similar to the present one. In line with projected distribution, chestnut now occurs in high altitude areas while in the past chestnut forests occurred at low altitudes in areas predicted more suitable for pine forests. The past distribution of chestnut forest in Monti Pisani does not correspond with what we might expect from its bioclimatic requirements, because peasant farmers cultivated chestnut in climatically marginal areas, affecting the past distributions of both chestnut and pine forests. Historical accounts of the Monti Pisani in about 1800 suggest that chestnut was probably cultivated at its climatic limit because these low altitude areas were not suitable for more profitable cultivations and were easily reachable from the villages at the foothill, favouring their management, harvest and processing. Moreover, the distribution of chestnut forests in Monti Pisani have deeply changed since 1850 even if the potential distributional range of chestnut and pine has shifted weakly. These results suggest that albeit in the past chestnut was widely cultivated in areas more suitable for pine, changing species distribution relocated each species to more suitable sites. The shift from chestnut to pine forest at low altitude may be explained both by the idea that climatically marginal populations are more prone to extinction than populations growing at the optimal climatic conditions, and by changes in land use (i.e., agricultural abandonment, the increase in firewood cutting to supply emerging industries and the arrival of ink disease and chestnut canker). Furthermore, the change in climatic conditions mainly recorded in the last forty years seems not to be the main cause of the change in distribution of pine and chestnut forests, probably because climate change acts on longer temporal scales than land-use change. Taken together our results are in line with the idea that land abandonment is the main driver of short-term landscape change in Europe, and that land-use changes and climate change will strongly interact to affect species distribution in the future.

Tree species diversity versus identity effects on understorey phylogenetic diversity in thermophilous deciduous forests

Andrea Coppi[1], Lorenzo Lazzaro[1], Evy Ampoorter[2], Lander Baeten[2], Kris Verheyen[2], Federico Selvi[3]
[1]Department of Biology, University of Florence, Via Micheli 1, 50121 Firenze, Italy; [2]Department of Environment, Ghent University, Geraardsbergsesteenweg 267, 9090 Gontrode, Belgium; [3]Department of Agriculture, Food, Environment and Forestry (DAGRI), University Firenze, P.le delle Cascine 28, 50144 Firenze, Italy

Understorey vegetation is a key biodiversity component of forest ecosystems, representing up to 80% of the total plant species richness of the community (1). Despite their negligible contribution to the total forest biomass, understorey plants are known to influence a number of ecosystem functions and services (2, 3). Previous studies examined the relationships between understorey and overstorey vegetation mainly in terms of taxonomic diversity, composition or productivity. So far, none focused on the phylogenetic aspect, which represents the evolutionary component of diversity in a community. Here, we explored the relationships between overstorey species richness and identity and the phylogenetic structure of the understorey vegetation. As a model system we used a network of 36 plots in thermophilous deciduous forests of Central Italy established for the FunDivEurope project on forest biodiversity and functions (4). The phylogenetic diversity of each plot was quantified using the Faith's phylogenetic diversity (PD) (5), which represents the simplest measure of the cumulative evolutionary age in a community and has the advantage of being a phylogenetic diversity metric used in conservation research (5, 6). The Net Relatedness index (NRI) and the Nearest Taxon Index (NTI) were also used to quantify the degree of the community phylogenetic clustering/overdispersion among species within each plot (7). These indices were inferred from a phylogenetic tree obtained from the ITS1, 5.8S and ITS2 regions of the nuclear ribosomal DNA. We assembled a sequence dataset retrieving 103 out of 109 accessions from GenBank (http://www.ncbi.nlm.nih.gov/). Hence, isolation and amplification of genomic DNA was performed ex-novo for six understorey species. Multiple alignment of the ITS-5.8S dataset was performed with MAFFT. Considering the high phylogenetic divergence of taxa in the analyzed communities, the following step by step multi-alignment procedure was performed: (i) each taxon was grouped at the order level and aligned using the Q-INS-i strategy; (ii) the multi-alignments were merged at higher ranks using the merge option in MAFFT, obtaining separate multiple alignments for Eudicots, Monocots, Gymnosperms; (iii) the three alignments were finally merged again. As in a recent study (8) such an approach allowed to obtain a fully resolved phylogeny and reliable indices of understorey phylogenetic diversity and structure. The resulting understorey phylogenetic tree allowed reliable estimation of PD, NRTI and NTI. Phylogenetic diversity was dependent on species richness and the strength of this relationship did not change along the gradient of tree species richness. NRI and NTI were both mostly positive but non-significant, indicating random phylogenetic structure of the understorey. The presence of *Quercus petraea*, an oak species usually found in more favorable site conditions, was associated with a significant phylogenetic clustering at the species level. In conclusion, overstorey species richness was not related to understorey phylogenetic structure, while species identity (*Q. petraea*) was associated with an increase in evolutionary relatedness in this forest layer, possibly due to competitive exclusion in favorable site conditions. Further studies should examine whether and how understory phylogenetic structure is affected by variables such as small scale variation in site conditions and presence of a shrub layer, both present but not accounted for in our model system. A better understanding of the role of these variables and their interaction may contribute to fill the current gap between the theoretical field of phylogenetic ecology and forest management

1) F.S. Gilliam (2007) BioScience, 57, 845-858
2) F.S. Gilliam (2014) Oxford University Press, New York
3) R.N. Muller (2014) Oxford University Press, New York, 622, 13-34
4) L. Baeten, K. Verheyen, C. Wirth, et al. (2013) Perspect Plant Ecol Evol Syst, 15, 529, 281-291
5) D.P. Faith (1992) Biol. Conserv, 61, 1-10
6) F. Forest, R. Grenyer, M. Rouget, T.J. Davies, R.M. Cowling, D.P. Faith (2007) Nature, 445, 757-760
7) C.O. Webb (2000) Am Nat, 156, 145-155
8) F. Selvi, E. Carrari, A. Coppi (2016) For Ecol Manage, 367, 1-11

Signs from the past: long-term changes in Italian mountain forests under changing management

Chiara Lelli[1], Juri Nascimbene[1], Davide Alberti[2], Nevio Agostini[2], Alessandro Chiarucci[1]
[1]Department of Biological, Geological and Environmental Sciences, Alma Mater Studiorum University of Bologna, Via Irnerio 42, 40126 Bologna, Italy; [2]Parco Nazionale Foreste Casentinesi, Monte Falterona e Campigna, via Guido Brocchi 7, 52015 Pratovecchio, Arezzo, Italy

Mountain forests represent an ideal study system to investigate the effects of human footprint on forest biodiversity. Indeed, human intervention is one of the main drivers of forest biodiversity, shaping the structure and composition of almost the totality of the European across centuries. However, since the mid-twentieth century, mountain forests are experiencing abandonment, thus providing a unique chance for investigating how forest systems are responding to the lack of human intervention and how strong is the human legacy. To investigate this topic, we carried out two studies using a i) diachronic and a ii) synchronic approach, respectively.

i) In 2018, we resampled 22 historical vegetation plots recorded by Pietro Zangheri between 1934 and 1961 in three main mountain forest types in Italy (i.e., European beech forests, chestnut forests, and oak forests). These data are among the oldest sources of information to quantify long term changes in forest structure and composition. Our final data set consisted of 88 plots (22 original and 66 resampled plots) and 366 plant species. All the forests were managed at the time of original sampling, while currently all of them are not actively managed. Species richness significantly decreased in the herb and shrub layers from the original to the resurveyed plots, with light-demanding species that were replaced by more shade-tolerant species, reflecting higher and denser forest canopy. In particular, chestnut and oak forests reached a more mixed composition as compared to the original plots, including several tree species typical of mixed-broadleaved forests, with a gradual loss of cultural habitats, but also with a trend toward a spontaneous rewilding.

ii) In the second study we focused on the beech forest in the Foreste Casentinesi National Park (Northern Apennines, Italy) using a multi-taxon approach. We compared species richness and composition of vascular plants, epiphytic lichens and bryophytes in actively managed and abandoned stands that shared similar past management history. We recorded 113 species of vascular plants, 60 species of lichens and 17 species of bryophytes. Plant and epiphyte species richness did not differ between managed and abandoned stands. Only plant species composition significantly differed between the two treatments, with more habitat generalist species in the managed stands. Narrow-range species (in terms of habitat and substratum affiliation) of vascular plants and lichens resulted strongly related to changes in forest structure.

Overall, despite a human legacy that is still strongly detectable in forest ecosystems, we are observing the first signals of long term dynamic processes. Our findings suggest that landscape level planning could help to maximize biodiversity accounting both for the contribution of traditional forest practices to structural and compositional diversity, and for the crucial role of set-asides for the recovery of natural dynamics and biodiversity in a long-term perspective.

Are the effects of the overstory on herb and shrub layers important in forest habitat monitoring? The case of *Quercus suber* woodlands

Claudia Angiolini[1], Bruno Foggi[2], Antonio Gabellini[1], Matilde Gennai[2], Paolo Castagnini[1], Michele Mugnai[2], Simona Sarmati[1], Daniele Viciani[2], Simona Maccherini[1]
[1]Department of Life Sciences, University of Siena, Via P.A. Mattioli, 4, 53100 Siena, Italy; [2]Department of Biology, University of Florence, Via G. La Pira 4, 50121 Firenze, Italy

Habitat conservation has been identified as relevant for the preservation of biodiversity in the European Union (92/43 EEC Habitats Directive, hereafter: HD). According to the Art. 11 and Art. 17 of this Directive (1), each European country is obliged to monitor the Annex I Habitats every six years to periodically evaluate if the conservation targets are achieved. In this context, the Tuscan Region promoted a project (Monito-Rare) aiming to develop a new monitoring system that allows the recovery of a better level of information. This approach adopts for each habitat type an "ad hoc methodology" taking as a starting point the directives proposed by ISPRA and MATTM (2). Our study has tested the effects of overstory data (structural data such as cover, average height and basal area of tree species) on the shrub and herb layers to check the importance of tree structure and composition for forest habitat monitoring. The research was carried out in the EU Habitat "*Quercus suber* forests" (code 9330), that includes woods characterized by dominance of the cork oak (*Quercus suber* L.), differentiated respect to the *Q. ilex* forests by a lesser tree coverage that leaves ample space for herbaceous and shrubby species (3). Tuscan cork oak woodlands represent a high proportion of this habitat on mainland Italy. In order to capture its habitat variability they have been investigated within two different Special Areas of Conservation (SACs) representative of habitat in Tuscany (Monte Leoni and Val di Farma), through a stratified sampling that takes into account the geological substrate (siliceous crystalline formation of quartzitic-anagenetic type named "Verrucano" versus other substrates), a variable considered discriminating for this type of habitat . Within each SAC we randomly selected a number of plots proportional to the surface covered by the habitat. We sampled a total of 49 circular plots of 200 m^2, 25 in Monte Leoni (17 patches) and 24 in Val di Farma (9 patches). To investigate the fixed effects of SAC (type of substrate and patch) on community composition (abundance data) of the understory, we used permutational multivariate analysis of variance (PERMANOVA; 4). We used as covariates the basal area of cork oak, other oaks and other trees. The results underline the key role of substrate in driving understory composition in the *Q. suber* woods, showing a statistically significant difference in species composition between the forests growing in the two types of substrate. While Verrucano substrate is characterised by an higher abundance of *Arbutus unedo* L., *Erica arborea* L., *E. scoparia* L. and *Myrtus communis* L., the other substrates are dominated by *Phillyrea latifolia* L. and *Brachypodium rupestre* (Host) Roem. & Schult. There is also great floristic variability between the patches within the SACs. The covariables basal area both of other oaks and of other trees have a significant effect on understory composition. Moreover, our results indicate that in the habitat type 9330 the understory varies significantly in function of structure and composition of the tree layer, highlighting the importance of considering the overstory data when monitoring forest communities included in HD.

1) European Commission (1992) Official Journal L 206, 22/07/1992, P. 0007-0050
2) P. Angelini, L. Casella, A. Grignetti, P. Genovesi (Eds.) (2016) ISPRA, Serie Manuali e Linee Guida, 142/2016
3) E. Biondi, C. Blasi, S. Burrascano, S. Casavecchia, R. Copiz, E. Del Vico,. R. Venanzoni (2009) Ministero dell'Ambiente e della Tutela del Territorio e del Mare, DPN http://vnr. unipg. it/habitat
4) M.J. Anderson (2001) Austral Ecology, 26, 32–46

An overview of the Italian forest biodiversity and its conservation level, based on the first outcomes of the 4th Habitat Report ex-Art. 17

Gigante Daniela[1,2], Selvaggi Alberto[2], Acosta Alicia T.R.[2], Adorni Michele[2], Allegrezza Marina[2], Angiolini Claudia[2], Armiraglio Stefano[2], **Assini Silvia**[2], Attorre Fabio[2], Bagella Simonetta[2], Barcella Matteo[2], Bazan Giuseppe[2], Bertacchi Andrea[2], Bolpagni Rossano[2], Bonari Gianmaria[2], Buffa Gabriella[2], Caccianiga Marco Stefano[2], Cacciatori Cecilia[2], Caria Maria.Carmela[2], Casavecchia Simona[2], Casella Laura[3], Cerabolini Bruno E.L.[2], Ciaschetti Giampiero[2], Ciccarelli Daniela[2], Cogoni Annalena[2], Cutini Maurizio[2], De Sanctis Michele[2], De Simone Walter[2], Del Vecchio Silvia[2], Di Cecco Valter[2], Di Martino Luciano[2], Di Musciano Michele[2], Fantinato Edy[2], Filesi Leonardo[2], Foggi Bruno[2], Forte Luigi[2], Frattaroli Anna Rita[2], Galdenzi Diana[2], Gangale Carmen[2], Gianguzzi Lorenzo[2], Giusso Del Galdo Gianpietro[2], Grignetti Alessandra[3], Guarino Riccardo[2], Lasen Cesare[2], Maneli Fabio[2], Marcenò Corrado[2], Mariotti Mauro Giorgio[2], Oriolo Giuseppe[2], Paura Bruno[2], Perrino Enrico[2], Pesaresi Simone[2], Pezzi Giovanna[2], Pisanu Stefania[2], Poponessi Silvia[2], Prisco Irene[2], Puglisi Marta[2], Rivieccio Giovanni[2], Sciandrello Saverio[2], Spampinato Giovanni[2], Stinca Adriano[2], Strumia Sandro[2], Taffetani Fabio[2], Tesei Giulio[2], Tomaselli Valeria[2], Venanzoni Roberto[2], Viciani Daniele[2], Villani Mariacristina[2], Wagensommer Robert Philipp[2], Zanatta Katia[2], Angelini Paola[3]

[1]Department of Agricultural, Food and Environmental Sciences, University of Perugia, Borgo XX giugno 74, I-06121 Perugia, Italy, daniela.gigante@unipg.it; [2]SBI-4RH: Working Group of the Italian Botanical Society for the 4th Report ex-Art. 17 of the 92/43/EEC Directive's Annex I Habitats in Italy; [3]ISPRA, Italian Institute for Environmental Protection and Research, Via Brancati 48, I-00144 Roma, Italy

In 2019 the 4th Report ex-Art. 17 on the conservation status (CS) of Annex I Habitats of the 92/43/EEC Directive was expected by every EU/28 country, with reference to the period 2013-18. In Italy, the process was in charge to the Italian Institute for Environmental Protection and Research (ISPRA), on behalf of the Ministry for Environment, Land and Sea Protection (MATTM), with the scientific support of the Italian Botanical Society (SBI). A large group of thematic and territorial experts elaborated the available data concerning the 124 types of terrestrial and inland water Habitats present in Italy, 39 of which are represented by Forest Habitats (Group 9),. The main aim of the work was the evaluation of the overall CS of each Habitat by Biogeographic Region (Mediterranean, Continental and Alpine), for a total amount of 294 assessments. A high proportion of these (92, corresponding to 31% of the total) referred to Forest Habitats, including 20 marginal types for which the CS was not requested.

The analysis was carried out at different scales: a) administrative territory, through the data contained in the ISPRA database, whose compilation was in charge to the Regions and Autonomous Provinces; b) Natura 2000 site, with the latest updates available (Standard Data Forms updated to 2018); c) national scale, implementing the distribution maps for each Habitat based on the European grid ETRS89-LAEA5210 (10x10 km^2 mesh); d) Biogeographic Region, scale of the final assessment. Cartographic outcomes, associated databases and additional data used for the assessments will be available online on the ISPRA Portal as soon as the validation process by the European Commission will be completed. A dedicated archive named "HAB_IT" has been created in the national database "VegItaly" (1), managed by the Italian Society of Vegetation Science, where the phytosociological relevés representative of the various Annex I Habitats in Italy will be archived and freely accessible. An overview of the results regarding the Forest habitats is here provided, including a comparison with the outcomes of the former reporting cycle, the 3rd Report ex-Art. 17 (2). In several cases (e.g. 9120, 91L0), the distribution maps have been remarkably improved due to better knowledge and more fitful interpretation. The conservation status resulted as Favourable (FV) for 6,7%, Inadequate (U1) for 58,7% and Bad (U1) for 32,0% of the 72 assessed forest Habitat types. In no case there was an improvement of the conservation status, while in 6 cases a worsening of the conditions resulted from the data analysis, pointing out the Habitats types with a higher need of action.

Similarly to other projects carried out as a team by the network of Annex I Habitat experts of the Italian Botanical Society and the Italian Society for Vegetation Science (e.g. 3, 4), this is another step in the direction of supporting the implementation of the 92/43/EEC "Habitat" Directive in Italy and Europe. On this ground, the high biodiversity of the Italian forest Habitats could be emphasized, however results pointed out that some rare or endemic types (e.g. *Alnus cordata* or *Betula aetnensis*-dominated forests) are still scarcely acknowledged by the most prominent EU conservation tools such as the Annex I to the "Habitat" Directive.

1) F. Landucci et al. (2012) Plant Biosyst., 146(4), 756-763
2) P. Genovesi et al. (2014) ISPRA, Serie Rapporti, 194/2014
3) E. Biondi et al. (2009) Società Botanica Italiana, MATTM, D.P.N., http://vnr.unipg.it/habitat/
4) D. Gigante et al. (2016) Plant Sociology, 53(2), 77-87

NDVI temporal pattern as functional responses of Mediterranean forest plant associations

Simone Pesaresi[1], Adriano Mancini[2]
[1]Department of Agricultural, Food and Environmental Sciences, Università Politecnica delle Marche, Via Brecce Bianche 10, 60131 Ancona, Italy; [2]Department of Information Engineering; Università Politecnica delle Marche, Via Brecce Bianche 10, 60131 Ancona, Italy

In the classification of vegetation with the method of Braun-Blanquet, plant association is the level of greater detail that can be defined. The plant association is defined by a typical combination of species and a quantifiable ecological value (1). In the forest landscape, this typical floristic composition is the result of the combination of the overstory canopy (tree species composition) with the understory canopy (shrub and herb species composition).

Since plant associations allow the diagnosis of habitats listed in the annex I of the habitat directive, their mapping is fundamental for monitoring, management and conservation of biodiversity and habitats of a territory. However, the mapping of plant associations is mostly based on methods (here defined as 'traditional') that integrate field surveys with the interpretation of orthophotos. These traditional methods produce high resolution maps, but they are hardly updatable and repeatable (2).

Here, a *Functional Data Analysis* (FDA) to the pixel-based NDVI Landast 8 time series was used to analyse the forested area of the Conero Regional Park (Marche, Italy). The main NDVI space-time variations identified by the FDA (predictors) and the data detected in the field (phytosociological surveys - training data) were processed by the Random Forest classifier in order to map the plant associations of the Monte Conero forests.

The field surveys made it possible to recognize four different forest plant associations. These showed different average annual NDVI curves (Figure 1), defined as ‘functional signature’ in (3). The Random Forest has mapped plant associations with a high global accuracy (85%).

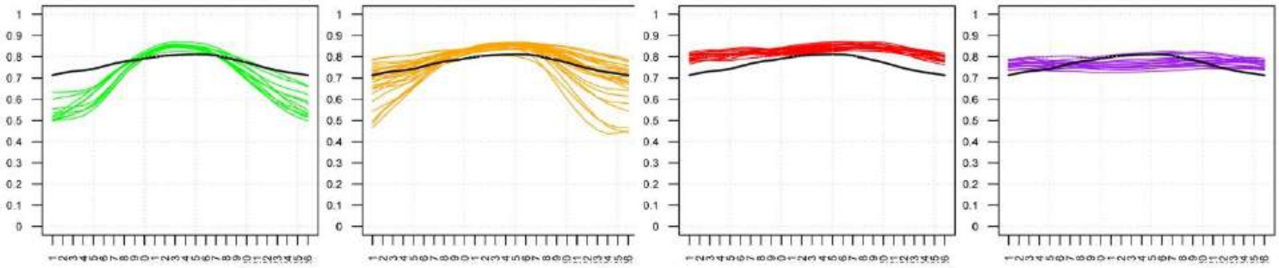


Figure 1. Annual NDVI curves of the Monte Conero (Marche, Italy) forest plant associations. Y-axis: NDVI. X-axis: bi-weeks (1-26) of the year. *Asparago acutifolii-Ostryetum caprinifoliae* (green), *Cephalenthero longifoliae-Quercetum ilicis* (orange), *Cyclamino hederifolii-Quercetum ilicis* (red), *Pinus halepensis* community (purple). Black line is average NDVI curve of the study area.

The mapping of the forest plant associations, based on NDVI curves (functional response of forests), was effective and accurate. Furthermore, as functionally linked to remote sensing data with high temporal resolution, compared to the traditional mappings, it allows to update and repeat the vegetation maps (in accord to the article 17 of Habitats Directive) and then monitor the changes that forest habitats could suffer from climate change and/or management.

1) Biondi, E. (2011) Plant Biosyst. 145, 19–29
2) Ichter, J., Savio, L., Evans, D., Poncet, L., (2017). Doc. phytosociologiques, 2017, 1 vol., 6, 334-352
3) Alcaraz-Segura, D., Cabello, J., Paruelo, J. (2009) Plant Ecol., 202, 13–29

Digitization of the *Lichenotheca Veneta*

Maria Zardini[1], Raffaella Trabucco[2], Juri Nascimbene[3], Stefano Martellos[4]
[1]Department of Biology, University of Padova, Via U. Bassi, 58/B, I-35121 Padova, Italy ; [2]Natural History Museum of Venice, Santa Croce 1730, I-30135 Venice, Italy; [3]Department of Biological, Geological and Environmental Sciences, University of Bologna, via Irnerio 42, I-40126 Bologna, Italy; [4]Department of Life Sciences, University of Trieste, via L. Giorgieri 10, I-34127 Trieste, Italy

The *Lichenotheca Veneta* is among the most important collections of *exsiccata* of the nineteenth century. It was published in a limited number of copies by the famous lichenologist Vittore Trevisan (1818-1897) (1). To our best knowledge, only two complete copies do exist, one preserved at the Natural History Museum of Venice, and one at the Herbarium of the University of Modena. The collection has a relevant scientific and historic value, and its digitization is a necessary step towards its preservation, and for increasing its visibility and accessibility. The *Lichenotheca Veneta* is made of eight issues, grouped in 4 volumes, each with a hardcover. Almost all specimens are glued to the herbarium sheet. The collection includes 268 specimens, all - but five - collected in NE Italy, and belonging to 74 genera and 197 species (1).

The digitization process was carried out by following a parallel pipelines scheme, which foresees on one side the digital imaging of all specimens, and on the other the digitization of the labels, captured from Lazzarin (1994) with an OCR software. The digital imaging process was carried on using a Canon digital camera (EOS 600D, with a 23 mm and 105 mm macro lenses). For each specimen, an image of the full sheet, with the label and the specimen, plus several images detailing the specimen alone, and its more relevant details were produced. After a first digital imaging run, all images where checked, in order to re-imaging the specimens which features were not properly captured. The digital capture of the text was carried on using the Tesseract Open Source OCR engine (version 3.04). The text was then reviewed for errors, and divided into several blocks of information: identification number, taxon name, correct name, three blocks for images numbers, and then literature references, notes to the name, synonyms, locality, and observations. A further information which was added to the database is the assessment of the conservation status of the specimens, according to the scale by McGinley (1993) (2).

The two pipelines converged into a relational database, in which the relation hosting the data was coupled with a relation hosting image metadata. A searchable online database will be soon available.

1) Lazzarin G. (1994) L'opera lichenologica di Vittore Trevisan. Museo Civico di Storia Naturale di Verona, Opera Naturalistica Classica, 2, 394 pp.
2) McGinley RJ (1993) Where's the management in collections management? Planning for improved care, greater use, and growth of collections. International Symposium and First World Congress on the Preservation and Conservation of Natural History Collections, Vol. 3, 309–338

Plant de-extinction: the importance of herbaria for recovering genetic diversity back to the wild

Simone Orsenigo[1], Graziano Rossi[1], Thomas Abeli[2]
[1]Department of Earth and Environmental Sciences, University of Pavia, Via S. Epifanio 14, 27100 Pavia, Italy; [2]Department of Sciences, University of Roma Tre, Viale Guglielmo Marconi 446, 00146, Roma, Italy

The alarming current and predicted species extinction rates urge conservationists to increase their efforts to avoid biodiversity losses but for species or populations already extirpated from the wild, what can we do? Sometimes, nothing can be done, but in other cases not all is lost. Every taxon validly described should correspond to at least one voucher specimen. Herbaria are not normally seen as a source of material for conservation actions, but spores or seeds preserved with dried herbarium voucher specimens can in some cases remain viable for decades, thus providing potential diaspores for the recovery of lost genetic diversity and translocation [1; 2]. So far, there have been few attempts to use herbarium specimens in translocation and only one attempt has progressed towards the establishment of viable plant populations from propagules gained from herbarium specimens (*Crepis foetida* L. subsp. *foetida)* [3]. However, several authors have obtained viable spores and seeds from herbarium vouchers up to 237 years old [4 and references therein], which indicates that propagules may remain viable in an herbarium for a long time. However, some critical issues could limit the use of herbarium spores or seeds. For instance, herbarium-sourced translocation material is generally scarce in terms of the number of specimens for rare species and number of propagules preserved within each specimen [5]. Moreover, spores and seeds typically show low viability and in old specimens the DNA is often degraded [6]. This low viability of herbarium propagules may also be linked to pest control treatments, including the application of chemicals and heat treatments [5]. Probably the most important caveat associated with using herbarium specimens is that delivering conservation benefit might undermine the primary use of herbaria collections; sampling spores or seeds from herbaria may irreparably damage the specimens limiting their use for taxonomic scopes [2; 7]. The assessment as to whether that risk is worth taking is something that needs consideration at species level and taking into account the availability of specimens for both systematics and conservation. Considering the sheer number of preserved specimens (more than 387 million specimens) [8], herbaria might play a more valuable conservation role by ensuring the availability of flowering and fruiting plants among their vouchers from which pollen and seeds may be collected. Such recommendations are of importance considering the recent debate on synthetic biology applied to conservation (including DNA synthesis; 9) as advancements in molecular engineering will make herbaria possible sources of genetic material.

1) Bowles ML, Betz RF, De Mauro MM (1993) Propagation of rare plants from historic seed collections: Implications for species restoration and herbarium management. Restoration Ecology, 1, 101-106
2) Shiga T (2013) A relationship among curator, collector, and user surrounding natural history specimens: toward a better specimen preservation, collection and usage. Japanese J of Ecology, 63, 375–383
3) Sears J (2011) Re-introduction of stinking hawk’s-beard into South-East England, UK. In: Soorae PS (ed.) 2011, Global re-introduction perspectives: additional case-studies from around the globe, pp 234-238. IUCN/SSC Re-introduction Specialist Group, Abu Dhabi, UAE
4) Molnár VA, Sonkoly J, Lovas-Kiss Á, Fekete R, Takács A, Somlyay L, Toeroek P (2015) Seed of the threatened annual legume, *Astragalus contortuplicatus*, can survive over 130 years of dry storage. Preslia, 87, 319–328
5) Godefroid S, Van de Vyver A, Stoffelen P, Robbrecht E, Vanderborght T (2011) Testing the viability of seeds from old herbarium specimens for conservation purposes. Taxon 60:565-569
6) Leino MW, Edqvist J (2010) Germination of 151-year old Acacia spp. seeds. Genetic Res Crop Evol 57:741-746.
7) Graves GR, Braun MJ (1992) Museums: storehouses of DNA? Science 255:1335–1336
8) Thiers B. 2018. Index Herbariorum: A global directory of public herbaria and associated staff New York Botanical Garden's Virtual Herbarium. Available from: http://sweetgumnybgorg/science/ih/
9) Piaggio AJ, Segelbacher G, Seddon PJ, Alphey L, Bennett EL, et al. (2017) Is it time for synthetic biodiversity conservation? Trends Ecol Evol 32:97–107

A cross-disciplinary study of the work and collections by Roberto de Visiani (1800–1878)

Moreno Clementi, Antonella Miola
Department of Biology, University of Padova, via Ugo Bassi 58 B, 35131 Padova, Italy

With over 600 newly described taxa and almost 1,000 newly established names, Roberto de Visiani (Šibenik 1800 – Padova 1878) was one of the most important scholars to direct the Botanical Garden of Padova, a position he held from 1837 to his death. He was the author of numerous works of taxonomy, mostly focussed on the vascular flora of his homeland, Dalmatia. After his death, he left in Padova not only a significant collection of about 12,000 specimens, but also thousands of letters, manuscripts, and other unpublished material, which remained almost entirely unstudied. Only thirteen of his names had been typified. In our work (1), we adopted a widely cross-disciplinary, author- and collection-centric approach with the main goal to correctly identify and formally designate type material in Visiani's collections. Visiani's herbarium in Padova was catalogued in its entirety. All of Visiani's published works on floristics were collected and discussed in detail, with special attention dedicated to *Flora Dalmatica* (2) and his works with Serbian colleague Josif Pančić. All of Visiani's unpublished material available at the Library of the Botanical Garden was digitised; over a third of his letters were transcribed, including all those received from and sent to Pančić and palaeobotanist Abramo Massalongo. Our research has led to the publication of eight scientifc papers on nomenclature and taxonomy (3) (4) (5) (6) (7) (8) (9) (10), with fifty-one type designations in total, plus detailed notes for twenty-three other names. A full treatment of all of the 275 names newly published in *Flora Dalmatica* is in preparation. The data from the herbarium and from Visiani's published works have all been integrated in a single database, with most data geolocated. We could greatly clarify Visiani's network of relations and exchanges and understand his scientific approach in detail. A rich personal biography of the botanist could be produced, dealing with topics such as his origins, studies, professional life, travels, character, religion, political ideas, and material legacy. His scientific standing at the national and international level was analysed. Unpublished material by Visiani is being made freely available online on the PHAIDRA platform by the University's Library Centre (11). Publishing of the correspondence between Visiani and Pančić, and Visiani and Massalongo is being discussed. All of the collected data should eventually be made available to the public with the development of a web application.

1) Clementi, M. (2017) A Cross-disciplinary Study of the Work and Collections by Roberto de Visiani (1800–1878). Doctoral thesis, University of Padova, 377 pp.
2) Visiani R. de (1851–1852) Flora Dalmatica, Leipzig, Friedrich Hofmeister, 3 vol.
3) Clementi M., D'Antraccoli M., Miola A. (2019) Nomenclatural and taxonomical notes on some taxa described by Roberto de Visiani for Egypt and Sudan. Phytotaxa, 339, 49–64
4) Clementi M., Niketić M., Miola A., Vukojičić S. (2016) Typification and nomenclature of the names published in Plantae Serbicae Rariores aut Novae – Decas III. Phytotaxa, 252, 85–98
5) Iamonico D., Clementi M. (2016) Nomenclatural notes about the names in Amarathaceae published by Roberto de Visiani. Hacquetia, 15, 101–106
6) Clementi M., Anačkov G., Miola A., Vukojičić S. (2015) Typification and taxonomical notes on the names published by Roberto de Visiani and Josif Pančić in Plantae Serbicae Rariores aut Novae – Decas II. Phytotaxa, 224, 29–44
7) Clementi M., Vukojičić S., Lakušić D. & Kuzmanović N. (2015) Typification of the names published by Roberto de Visiani and Josif Pančić in Plantae Serbicae Rariores aut Novae – Decas I. Phytotaxa, 202, 121–134
8) Bogdanović S., Ljubičić I., Clementi M. (2015) *Aegilops uniaristata* Vis. (Poaceae): typification and occurrence in Croatia. Acta Botanica Croata, 74, 165–72
9) Clementi M., Kuzmanović N., Barina Z., Lakušić D., Vukojičić S. (2014) Typification of five names published by Roberto de Visiani in Plantarum Serbicarum Pemptas. Phytotaxa, 170, 57–7
10) Kuzmanović N., Clementi M., Kabaš E., Vukojičić S. (2013) Retypificcation of the name *Eryngium palmatum* (Apiaceae). Phytotax, 105, 58–60
11) Phaidra – Digital collections | University of Padova, available: https://phaidra.cab.unipd.it/

'*Back to the future*': assessing the effectiveness of Species Distribution Models temporal projection through herbarium data

Marco D'Antraccoli[1], **David Dolci**[1], Francesco Roma-Marzio[2], Lorenzo Peruzzi[1]
[1]Department of Biology, University of Pisa, Via Derna 1, 56126 Pisa, Italy; [2]Orto e Museo Botanico dell'Università di Pisa, Sistema Museale di Ateneo, Via L. Ghini 13, 56126 Pisa, Italy

Species distribution models (SDMs) are useful tools to explain or to predict species range from various environmental factors (1). Accordingly, SDMs are widely used in conservation biology, especially to predict future distribution scenarios in the light of climate change. Even if these temporal extrapolations are a common practice in plant ecology and systematics, their effectiveness are not yet experimentally tested due to the absence of empirical data at the time when model are projected. To contribute to fill this gap, we planned an analytical exploration of advantages and critical points in projecting SDMs over time. We focused on two different chronological windows: past (around 1900) and present. Change ratios of climatic predictors from past to present were inferred from rasters provided by EcoClimate Database (2) at a cell resolution of ca. 50×50 km. These ratios were then applied in a spatially-explicit way to current climatic rasters at 1×1 km of resolution, as provided by WordClim database (3), allowing thus to obtain coupled (past/present) climatic predictors. Firstly, we modelled past species distributions using both past occurrence data and past climate data, then we projected the models to present-days climatic conditions. In parallel, using occurrence records and climatic conditions available for the present-days, we obtained a second independent model of current potential distribution. This made possible a congruency check between predictions returned by the model projected over time and the model carried out in the present-days, to verify whether trends of over- or under-estimation do exist when a temporal extrapolation is performed.

The majority of data available for modelling past plant distributions derive from herbaria, and they are typically presence-only data. In a general perspective, herbarium documentation represents the only reliable source for this kind of information. Recent advances offered by digital technologies, coupled with the increasing interest in the creation and dissemination of virtual herbaria, allow broad-scale research by botanists (4). On the other hand, it has been found that herbarium records provide limited accuracy in predicting distribution and do not meet current standards for sampling in ecological studies, due to inadequate geographic coverage or various collection biases (4, 5). Hence, we performed several modeling techniques, from parametric statistical to non-parametric rule-based (e.g., 'Generalised Linear Model', 'Regression Tree Analysis', 'Maximum Entropy, 'Artificial Neural Network'). Then, we applied an ensemble approach averaging single results in a single final model, which was assessed by True Skill Statistic (TSS) (6), setting the threshold at 0.7. Thanks to an ongoing digitization project, we obtained historical species occurrence data by surveying the herbarium of Michele Guadagno (1878–1930), currently stored at *Herbarium Horti Botanici Pisani* (official acronym: PI-GUAD). It represents an ideal study case, since the collections are focused on central-southern Italy and show a well-defined chronological window (1900–1925).

As case studies we selected 10 taxa endemic to Italy, represented by a minimum number of specimens collected in different localities (> 5), namely: *Adenostyles australis* (Ten.) Iamonico & Pignatti, *Aubrieta columnae* Guss. subsp. *columnae*, *Crocus biflorus* Mill., *C. imperati* Ten., *Galium magellense* Ten., *Gypsophila arrostii* Guss. subsp. *arrostii*, *Helleborus viridis* L. subsp. *bocconei* (Ten.) Peruzzi, *Saxifraga porophylla* Bertol. subsp. *porophylla*, and *Sedum magellense* Ten. subsp. *magellense*. Current species occurrence data were obtained from an ongoing project promoted by the Group for Floristics, Systematics, and Evolution of the Italian Botanical Society, aimed to map a selection of Italian endemics (7).

1) V.H. Gomes, S.D. IJff., N. Raes et al. (2018) Sci. Rep., 8, 1003
2) M.S. Lima-Ribeiro, S. Varela, J. González-Hernández et al. (2015) Biodiv. Inform., 10, 1-21
3) S.E. Fick, R.J. Hijmans (2017) Int. J. Climatol., 37, 4302-4315
4) B.A. Loiselle, P.M. Jørgensen, T. Consiglio et al. (2008) J. Biogeogr., 35, 105-116
5) S. Ungricht, J.Y. Rasplus, F. Kjellberg (2005) Biodiv. Cons., 14, 205-232
6) O. Allouche, A. Tsoar, R. Kadmon (2006) J. Appl. Ecol., 43, 1223-1232
7) L. Peruzzi (2018) Approfondimenti floristici e sistematici sulla flora italiana. Notiziario della Società Botanica Italiana, 2, 33-34 [provisional pages]

Importance of molecular studies involving nomenclatural types: the case of the Saccardo mycological collection

Niccolò Forin[1,2], Sebastiano Nigris[1,2], Samuele Voyron[3], Mariangela Girlanda[3], Alfredo Vizzini[3], Giorgio Casadoro[1], 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 U. Bassi 58/B, 35131 Padova, Italy; [3]Department of Life Sciences and System Biology, University of Torino, Viale P.A. Mattioli 25, 10125 Torino, Italy

The mycological collections, also known as fungaria, represent a source of molecular information that may be exploited to obtain important DNA sequences. Indeed, it has been demonstrated that DNA barcoding projects of fungarium material have the potential to enlarge the coverage of species-level DNA sequence information deposited in public databases (1). However, these collections, in particular the oldest ones, are an underused resource for building up voucher-based reference datasets, due to the difficulty to obtain DNA data from ancient biological material.

Pier Andrea Saccardo's mycological collection, started around 1874 and preserved in the herbarium of the Botanical Garden of Padova, is composed by almost 70,000 fungal specimens comprising over 18,500 different species. This collection has a huge scientific importance due to the presence of over 4,000 type specimens, mainly belonging to the phylum Ascomycota. These specimens have been borrowed by mycologists from all over the world for morphological revisions and consequent taxonomic reclassifications, but they have never been involved in sequencing projects.

Nomenclatural types are the only certain link to a specific Latin binomial name and the existence of molecular information from these important samples might help to solve taxonomic confusions because they represent the pillar for a stable classification (2).

In this study, an Illumina sequencing was apply to obtain ITS1/ITS2 sequences, that together with the 5.8S coding gene form the consensus barcode for the identification of fungal species (internal transcribed spacer region, ITS; 3), from types belonging to different fungal genera in the collection, overcoming the problems of the DNA fragmentation of Saccardo's fungarium samples and the presence of contaminations by exogenous DNA (4). Several of these types were morphologically revised in the past by expert mycologists and placed in synonymy with other species or reclassified as members of new genera.

The molecular analysis and the morphological observations have suggested that there is a need to reclassify some types previously revised only on a morphological basis or never considered for taxonomic revisions, demonstrating in this way that in fungi the morphology alone does not always lead to a correct fungal systematic evaluation.

These results provide material for a taxonomic revision of invaluable types and the new ITS sequences obtained will be a significant resource for future phylogenetic studies and species identification. In addition, this study suggests that the application of a next generation sequencing method to obtain genetic information from the type specimens stored in the Saccardo collection could result in a taxonomic reassessment of many fungal species.

1) T.W. Osmundson, V.A. Robert, C.L. Schoch, L.J. Baker, A. Smith, G. Robich, L. Mizzan, M.M. Garbelotto (2013) PLOS ONE 8, e62419–8

2) K. Liimatainen, T. Niskanen, B. Dima, I. Kyt. vuori, J.F. Ammirati, T.G. Frøslev (2014) Persoonia, 33, 98–140

3) C.L. Schoch, K.A. Seifert, S. Huhndorf, V. Robert, J.L. Spouge, C.A. Levesque, W. Chen, Fungal Barcoding Consortium (2012) PNAS, 109, 6241–6246

4) N. Forin, S. Nigris, S. Voyron, M. Girlanda, A. Vizzini, G. Casadoro, B. Baldan (2018) Frontiers in ecology and evolution, 6, 129

Assessing metal hyperaccumulation using herbarium specimens: the case of *Odontarrhena sibirica* (Brassicaceae)

Isabella Bettarini[1], **Ilaria Colzi**[2], Cristina Gonnelli[2], Federico Selvi[3]
[1]Department of Biomedical Experimental and Clinical Sciences, University of Florence, viale Morgagni 50, 50134, Florence, Italy; [2]Department of Biology, University of Florence, Via P.A. Micheli 1, 50129, Florence, Italy; [3]Department of Agriculture, Food, Environment and Forestry, University of Florence, P.le Cascine 28, Florence, Italy

Metal hyperaccumulators are unusual plants that possess the rare ability to accumulate particular metals or metalloids in their living tissues to levels that may be hundreds or thousands of times greater than is normal for most species (1, 2). These plants are of substantial interest for research as they represent models to investigate the mechanisms of metal uptake, transport and sequestration, as well as adaptation and evolution in extreme environments. An active field of enquiry is also the ecology of hyperaccumulators, especially anti-herbivore defense, allelopathy and biotic interactions. These plants are also of remarkable practical interests for biotechnological applications such as phytoremediation and agromining (3). At present ca. 700 species of Angiosperms are known to be hyperaccumulators for metals such as Cadmium, Cobalt, Lead, Zinc, and Nickel. Most of them are restricted to outcrops of ultramafic rocks that are naturally rich in some of these heavy metals.

Timely identification of hyperaccumulator species is essential to promote research and applications, as well as to preserve their diversity in a rapidly changing world (4). Herbarium specimens provide a unique resource to discover hyperaccumulator species by means of several techniques, such as Atomic Absorption Spectrophotometry (AAS), inductively-coupled plasma atomic emission spectroscopy (ICP-AES), and inductively-coupled plasma mass spectrometry (ICP-MS).More recently, new analytical technology such as X-ray fluorescence (XRF) has enabled mass analysis of herbarium collections at a rate of *c.* 300 specimens per day, in a non-destructive way. Hence, new substantial discoveries are expected in the next decades by systematic screening of existing collections in herbaria around the world.

In this contribution we investigated Nickel accumulation in a facultative serpentine species from the E Mediterranean and W Asia, *Odontarrhena sibirica* (Willd.) Španiel et al. (≡ *Alyssum sibiricum* Willd.). *Odontarrhena* is the most diverse genus of Ni accumulators in W Eurasia, with some 80 species most of which grow obligatedly or facultatively on ultramafic soils and accumulate well above 1000 mg/kg of Ni in their shoots. At present, *O. sibirica* is the only species in the genus that is not clearly able of accumulating Ni even when growing on Ni-rich ultramafic soils. Previous reports for this species gave contrasting results, some of them indicating shoot Ni levels < 1000 mg/kg even in plants from ultramafic soils (Global Hyperaccumulator Database; http://hyperaccumulators.smi.uq.edu.au/collection/). We applied AAS to assess Ni content in shoots collected from multiple herbarium samples from Greece, Turkey and other countries, conserved in major European herbaria such as B, FI, G, K, and W. Based on collections details reported on the label we could assess whether the specimens originated from serpentine or non-serpentine soils, and compare Ni-levels in the two groups of edaphic accessions. Results support that *O. sibirica* may actually be the only species in its genus that is unable to accumulate Ni even when growing on serpentine soils. This might suggest an evolutionary loss of accumulation capacity, which would make of this species an ideal model to investigate the genetic bases, physiological mechanisms and molecular pathways of this unique ability.

1) R.D. Reeves, A.J.M. Baker, T. Jaffré, P.D. Erskine, G. Echevarria, A. van der Ent (2018) New Phytol., 218, 407-411
2) M. Wójcik, C. Gonnelli, F. Selvi, S. Dresler, A. Rostański, J. Vangronsveld (2017) Adv. Bot. Res., 83, 1-42
3) P.S. Kidd et al. (2018) Front. Environ. Sci., 6, 44. doi: 10.3389/fenvs.2018.00044
4) S.N. Whiting et al. (2004) Restor. Ecol., 12, 106-116

Effects of non-protein amino acids of floral nectar on survival and locomotion of pollinators

Massimo Nepi[1], Gherardo Bogo[2,3], Massimo Guarnieri[1], Simona Sagona[4], Antonio Felicioli[4], Laura Bortolotti[3], Marta Galloni[2]

[1] Department of Life Sciences, University of Siena, Via P.A. Mattioli 4, 53100 Siena, Italy; [2]Department of Biological, Geological and Environmental Sciences, University of Bologna, Via Irnerio 42, 40126 Bologna, Italy; [3]Council for Agricultural Research and Economics, Research Centre for Agriculture and Environment (CREA-AA), Via di Saliceto 80, 40128 Bologna, Italy; [4]Department of Veterinary Sciences, University of Pisa, Viale delle Piagge 2, 56124 Pisa, Italy

A wide array of animals, from arthropods to birds and mammals, use floral nectar as a source of carbon and energy (1) and are engaged for pollination in a mutualistic relationship. The main solutes of floral nectar are simple sugars and its alimentary importance is complemented by the presence of all the 20 proteinogenic amino acids (2). Recently several secondary metabolites have been found in nectar and some affect the foraging behaviour of animals and potentially increase the benefits to the plant (3, 4). One class of such substances is the non-protein amino acids, i.e. amino acids that are not used for protein synthesis. GABA (γ-aminobutyric acid) and β-alanine are among the more abundant and frequently found in floral nectar (5) and they are important neurotransmitters in the insect nervous system. In this study we analyzed the effect of these substances on mason bees (*Osmia bicornis*), bumble bees (*Bombus terrestris*), and honey bees (*Apis mellifera*). Insects fed artificial diet enriched with the two non-protein amino acids at low (naturally occurring in nectar) and high concentration (twenty-fold more concentrated) and their survival and behavioral parameters (flying, walking, feeding, staying) have been assessed by scan sampling. GABA had a positive (bumble bees) or neutral effect on insect's survival (mason bees and honey bees) whereas β-alanine had a negative (bumble bees and mason bees) or neutral effect (honey bees). The enriched diets affects the behavioral parameters according to species and concentration. Bumble bees increased their walking activity when fed the β-alanine diet at high concentration, while they increased their flying activity with the same solution at low concentration. Mason bees were sensitive to high-concentration GABA diet that increased their walking activity. Behavioural parameters of honey bees were less significantly affected by both diets.

The ability of affecting survival and locomotion of pollinators through nectar composition may have important ecological consequences for the plants since their reproductive output is largely dependent on pollination that is in its turn affected by insects movements among flowers.

1) S.W. Nicolson (2007) Nectar consumers. In: Nectaries and nectar, Springer, 289–342
2) S.W. Nicolson, R.W. Thornburg (2007) Nectar chemistry. In: Nectaries and nectar, Springer, 215–264
3) M. Nepi (2017) Acta Agrobot., 70, 1704
4) M. Nepi, D.A. Grasso, S. Mancuso (2018) Front. Plant. Sci., 9, 1063
5) M. Nepi (2014) J. Ecol., 102, 108-115

Pollinator monitoring in a mixed-ploidy population of *Senecio doronicum* in Alpes maritimes

Luca Pegoraro[1,2], Ellen C Baker[1,2], Manica Balant[3], Sarah Barlow[4], Lin Fu[1,5], Oriane Hidalgo[1,6], Andrew R Leitch[2], Ilia J Leitch[1], Luis Palazzesi[7], Daniele Sommaggio[8], Jaume Pellicer[1]

[1]Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey, TW9 3AB, UK; [2]School of Biological and Chemical Sciences, Queen Mary University of London, London, E1 4NS, UK; [3]University of Primorska, Faculty of Mathematics, Natural Sciences and Information Technologies, Glagoljaška 8, SI-6000 Koper, Slovenia; [4]Red Butte Garden and Arboretum, University of Utah, Salt Lake City, UT 84108, USA; [5]South China Botanical Garden, Chinese Academy of Sciences, No.723, Xingke Road, Tianhe District Guangzhou 510650, P.R. China; [6]Facultat de Farmàcia i Ciències de l'Alimentació, Universitat de Barcelona, Campus Diagonal, Av. de Joan XXIII, 27-31, 08028 Barcelona, Spain; [7]Museo Argentino de Ciencias Naturales, CONICET, División Paleobotánica, Buenos Aires, C1405DJR, Argentina; [8]Dipartimento di Scienze Agrarie, Università di Bologna, viale Fanin 42, 40127 Bologna, Italy; l.pegoraro@kew.org

Pollination is a crucial aspect of a plant’s reproductive success and isolation, and has long fascinated biologists. However, its study has proven difficult due to the complexity of interactions and the challenges in recording insect visitations.

We used a novel automated pollinator monitoring system (Rana) to investigate pollinator visits in a mixed-ploidy population of *Senecio doronicum* in the South-Western Alps. To our knowledge, this is the first use of an automated monitoring system to record insect visitors to flowers in high altitude environments.

Polyploidy is widely regarded as a major driver of angiosperm diversification, and genome duplication is one of the few accepted mechanisms of sympatric speciation. Here we study a tetraploid and a recently formed octoploid cytotype of *Senecio doronicum*. These variants differ in size, number and size of capitula, floret number and phenology, although a large degree of overlap in all characters exists. They also occupy different spatial micro-niches.

Pollinators monitoring results reveal a partial overlap of pollinator visitations. However visitors exhibit different visitation rates and visit duration, which relates to variability in morphological characteristics of the inflorescence, number of capitula and other traits.

The pollinators monitoring data is being combined with inflorescence morphospace analysis to capture the most relevant traits in attracting visitors, and to provide further insight into the role of inflorescence architecture for pollination, especially in the context of generalist pollination, as is the case for most species in Asteraceae.

These data are being integrated into a larger endeavour investigating the role of genome size, polyploidy and chromosome number on the adaptation of Asteraceae in high altitude environments. Our hope is to increase understanding of how genomic processes, such as polyploidy, and ecological processes, such as pollination, shape plant diversity.

Asymmetrical flowers of Papilionoideae: functional morphology, morphometrics, and interactions with its pollinators

Angela Etcheverry, Trinidad Figueroa, Antonella Ducci, María Alemán, Carlos Gómez, Carolina Yáñez, Diego López, Andrea González Reyes
Laboratorio de Biología Reproductiva de Plantas, Facultad de Ciencias Naturales, Universidad Nacional de Salta, Avenida Bolivia 5150, 4400 Salta, Argentina

Flowers can be considered as combinations of organs that are functionally coordinated to attract pollinators and to mechanically interact with pollinators' bodies. Within Fabaceae Papilionoideae, some taxa show a complex floral morphology which involves asymmetry of the corolla, *i.e.*, vexillum, wings and keel petals (*e.g. Phaseolus, Macroptilium* and *Cochliasanthus*) (1, 2), or a part of it, as the keel petals in *Crotalaria* (3). In the first case, pollinators land on the left wing triggering the brush pollination mechanism, with secondary pollen presentation. In *Crotalaria* flowers, with a pump mechanism, the landing platform is the wings-keel complex. Selection might favour the evolution of asymmetrical flowers as a mechanism of character displacement to avoid heterospecific pollen-transfer (4).We analyze floral morphology and morphometrics and plant-pollinator interactions in five species pertaining to Tr. Phaseoleae, and one species of Tr. Crotalarieae, pollinated by native social bumblebees (*Bombus* spp., Fig. 1A), solitary bees (*Centris tarsata; Epanthidium* spp.; *Eufriesea mariana; Megachile* spp.-Fig. 1D-; *Mellissodes tintinnans; Psaenythia* sp.; *Thygater* sp.; *Xylocopa* spp.-Fig. 1B) and *Apis mellifera* (Fig. 1C). Given that the pollination mechanism operates on the left side of the flower (1), it was hypothesized that floral parts on that side would present qualitative and/or quantitative differences. The morphological variation was analyzed in nine floral traits; six of them were measured on right and left sides of the flower with digital calipers (width and length of wings, wing's sculptured surfaces, width and length of the keel petals and the length of the wings claw). In addition, the depth of the nectary and the height of the pollination chamber were analyzed. All plant species had transversal sculpturings in the outer surfaces of both wings. *Phaseolus vulgaris* var. aborigineus, presented significant differences in all the variables analyzed, resulting larger those dimensions corresponding to the left side of the flower, except for the length of the right wing. *Macroptilium gibbosifolium* presented a greater sculptured surface in the left wing, a wider left keel, and the right wing's claw significantly longer. In *Macroptilium erythroloma* both, the wing and the wing's claw were longer on the right side, while the sculptured surface was larger on the left side of the flower. *Cochliasanthus caracalla* presented differences in the sculptured surface of the left wing and claw's length, also larger on the left side. Finally, in *Crotalaria stipularia* only the length of the sculptured surface and the width of the keel were greater on the left side. Significant differences were observed between the species both in the depth of the nectary and in the height of access to the pollination chamber. We discuss the relationship between functional floral morphology and pollination groups.

Fig 1. A. *Bombus morio* in *Cochliasanthus caracalla*, B. Xylocopa sp. in *Macroptillum erythroloma*, C. *Apis mellifera* in *Macroptillum gibbosifolium* and D. *Megachile* sp. in *Crotalaria stipularia*.

(1) A.V. Etcheverry, S. Vogel (2018) Flora, 239, 141-150
(2) M.M. Le Roux, B.E. Van Wyk (2012) Flora, 207, 414-426
(3) A. Delgado-Salinas, M. Thulin, R. Pasquet, N. Weeden, M. Lavin (2011) Am. J. Bot., 98, 1694-1715
(4) W.S. Armbruster, N. Muchhala (2009) Evol. Ecol., 23, 159-179

Using pollination networks to detect tourism sustainability

Edy Fantinato*, Gabriella Buffa
Department of Environmental Science, Informatics and Statistics, University Ca' Foscari of Venice, Via Torino 155, 30172 Venice, Italy; *corresponding author: edy.fantinato@unive.it

Intense concentration of human activities and disturbance are increasingly leading to coastal dune loss and fragmentation, considerably affecting remnant natural areas. To prevent and possibly reverse degradation of remnant natural areas, it is mandatory to understand whether, and under what conditions, tourism can be allowed.

In coastal dune ecosystems, animal-mediated pollination has a marked influence on the dynamics and diversity of plant communities (1). Moreover, coastal dunes are a hotspot for several highly habitat-specialized pollinator species (especially Hymenoptera). Thus, the pollination networks approach may be useful to evaluate ecological community structure and depict interactions among species, providing the opportunity for a holistic assessment of ecosystem structure and functioning.

To address the issue, we evaluated the impact of human disturbance on the structure and resilience of pollination networks in coastal dune ecosystems in the North Adriatic coast. Selected study sites were freely accessible to people but subjected to different levels of human disturbance. We recorded pollination interactions together with descriptors of human disturbance along sea-inland belt transects during three surveys. The impact of human disturbance was summarised in three variables: path density, gamma connectivity of land cover types intercepted by each transect, based on a fine-scale habitat map (γ; 2), and the relative abundance of alien floral displays.

For each transect, we calculated the richness of animal-pollinated plant and pollinator species. Furthermore, we quantified four among the most used descriptors of the structure and resilience of pollination interactions: network connectance (C; 3), network selectiveness (H_2'; 4), weighted nestedness (wNODF; 5) and quantitative modularity (Q; 6).

An intermediate level of path density was positively related to the richness of animal-pollinated plant and pollinator species. Besides species richness, the resilience of pollination networks was also highest at intermediate levels of path density. Specifically, network connectance (C) and selectiveness (H_2'), reached their minimum at intermediate values of path density; while weighted nestedness (wNODF) reached its maximum at intermediate values of path density. No effects on networks resilience could be detected for gamma connectivity and the relative abundance of alien floral displays.

The use of the pollination network approach allowed to evidence that a moderate disturbance can co-exist with the long-term conservation of pollination interactions on coastal dunes and to identify quantitative thresholds for path density. Thus, to achieve sustainable tourism, recreational activities should be regulated, and visitor access to coastal sites managed, so as to prevent intense human disturbance from compromising both the structure and function of coastal dune ecosystems.

1) Fantinato, E., Del Vecchio, S., Silan, G., Buffa, G. (2018) Pollination networks along the sea-inland gradient reveal landscape patterns of keystone plant species. Sci. Rep., 8, 1–9
2) Forman, R.T., Godron, M., (1986) Landscape ecology. Wiley, New York, NY
3) Dunne, J.A., Williams, R.J., Martinez, N.D. (2002) Food-web structure and network theory: the role of connectance and size. Proc. Natl. Acad. Sci. U.S.A. 99, 12917–12922
4) Blüthgen, N., Menzel, F., Blüthgen, N. (2006) Measuring specialization in species interaction networks. BMC Ecology. 6, 9
5) Galeano, J., Pastor, J.M., Iriondo, J.M. (2009) Weighted-interaction nestedness estimator (WINE): a new estimator to calculate over frequency matrices. Env. Model. Soft., 24, 1342–1346
6) Dormann, C.F., Strauss, R. (2014) A method for detecting modules in quantitative bipartite networks. Methods in Ecology and Evolution, 5, 90–98

Aerial seed bank benefits *Primula palinuri* Petagna in avoiding seed predation and run-off

Roberto Silvestro[1], Luigi Gennaro Izzo[1], **Giovanna Aronne**[1]
[1]Department of Agricultural Sciences, University of Naples Federico II, Via Università 100, 80055, Portici (NA), Italy

Aerial seed bank and serotiny are considered effective strategies to cope with extreme environments (1) and to reduce the risk of offspring mortality allowing seed release only with the occurrence of a specific environmental trigger (2) and limiting seed predation (3). *Primula palinuri* Petagna is an endemic species that deals with extreme condition of vertical cliffs (4). Although serotiny and aerial seed bank are not reported for *P. palinuri* and in general for cliff plants, this species could benefit from these dispersal strategies.

The study was conducted to investigate the dispersal strategy adopted by *P. palinuri* Petagna, hypothesizing this species is strongly serotinous and develops a long-lasting aerial seed bank to minimize post-dispersal seed losses on vertical cliffs.

Seed predation was observed in the field and proved performing seed sowing and cafeteria trial experiments in sites along the coastal cliff where the few remnant populations of the species survive. Data showed that seed predation is intense and that granivorous ants are much attracted by seeds of *P. palinuri.* The aerial seed-holding on plants lasts up to three years. This period fits with seed longevity: seed germinability was proved to be high and similar within seeds up to three-years-old and strongly decreased from the fourth year of age.

Serotiny occurrence was verified performing a laboratory test aimed at identifying the main environmental trigger involved in capsules dehiscence focusing on mechanical damages, cycles of wet and dry condition and exposure to light. To assess the occurrence of a long-lasting aerial seed bank, a seed germination test was performed to investigate seed longevity and loss of viability within a range of six years after maturation. In addition, by observing capsules semi-thin cross sections, an anatomical analysis was performed to evaluate possible structural characteristic involved in capsules dehiscence. Capsules treated with wet/dry cycles dehisced within 20 and 75 days. The timing of capsules dehiscence varied according to the treatment combination of light exposure and mechanical damage. On the contrary, none of the capsules subjected to any combination involving dry condition dehisced, confirming the essential role of wet and dry cycles in capsules dehiscence.

Specific anatomical analyses of the capsule tissues revealed the mechanism that allow capsules, once dehisced, to promptly close-up when wet and to slowly open again upon drying. Indeed, in *P. palinuri* capsules we identified two chemical and anatomical different and overlapping tissues (i.e. resistance tissue and swelling tissue), which differently react with water inducing an open-close mechanism.

Our results proved the occurrence of serotiny in *P. palinuri* in which seed release takes place only in response to cycles of dry conditions followed by wet periods. After dehiscence, seeds are not released all in one time and capsules open when dry and close again when moistened gradually distributing seed dispersal over time. This mechanism combined to the longevity of seeds enable *P. palinuri* to develop an effective and long-lasting aerial seed bank. Moreover, storing seed on mother plant and gradually distributing seed release over time, the duration of seed persistence on ground is reduced, thus minimizing the risk of seed losses due to run-off and predation.

1) L. Van der Pijl (1969) Springer Berlin Heidelberg, Berlin, Heidelberg
2) B.B. Lamont, N.J. Enright (2000) Plant Species Biol., 15, 157–166
3) A. Günster (1994) J Arid Environ., 28, 195–205
4) G. Aronne, C. Arena, V. De Micco, M. Giovanetti, M. Buonanno (2018) Plant Biosyst., 152, 863–872

Seagrass - waterbirds interactions in a lagoon ecosystem of the northern Adriatic Sea

Stefano Vitti[1,2], Francesco Boscutti[2], Valentino Casolo[2], Stefano Sponza[3]
[1]Department of Life Science, University of Trieste, Via Weiss 2, 34128 Trieste, Italy; [2]Department of Agricultural and Environmental Sciences, Plant Biology Unit, University of Udine, Via delle Scienze 91, 33100 Udine, Italy; [3]Department of Mathematics and Geoscience, University of Trieste, Trieste, Italy

The main goal of this work is to understand the interactions between primary producers (plants) and consumers (herbivore birds) for the conservation of ecosystems such as lagoons, in the view of plant-herbivore interactions. We studied the relationships between the abundance of the herbivore bird Eurasian Wigeon (*Anas penelope*) and the distribution of three seagrass species (*Cymodocea nodosa*, *Zoostera marina* and *Nanozostera noltei*) occurring in the Marano and Grado lagoon. This is a large waterbody located in the north part of the Adriatic Sea, in Friuli Venezia Giulia region, Italy (1) and it is morphologically classified as a leaky lagoon (2). Twelve bird monitoring areas were monthly surveyed during three years and seagrass distribution data were collected. The overall number of individuals of *A. penelope* was related to seagrass meadow extension and species cover by using a multiscale approach in four circle buffers (with radius of 500 m, 750 m, 1,000 m and 1,250 m). Among the considered scales, the 500 m radius and 1,250 m radius showed similar statistical scores, having lower performances for all the considered statistical parameters. The 750 m radius scale had the best performances. The total number of Eurasian wigeon individuals was related with area occupied by seagrass meadows and the mean percentage cover of *C. nodosa* and *N. noltei*. In particular, the number of observed individuals of wigeon increased where there was a larger area occupied by seagrasses meadows. Results showed also that when *C. nodosa* mean percentage cover increased the number of wigeon decreased, while if *N. noltei* mean percentage cover increased wigeon number increased. Finally, *Z. marina* showed a not statistically relevant influence in all the tested scales. Our findings confirmed that, in lagoon ecosystems, wigeon wintering populations shows co-occurrence on the abundance of seagrass meadows.

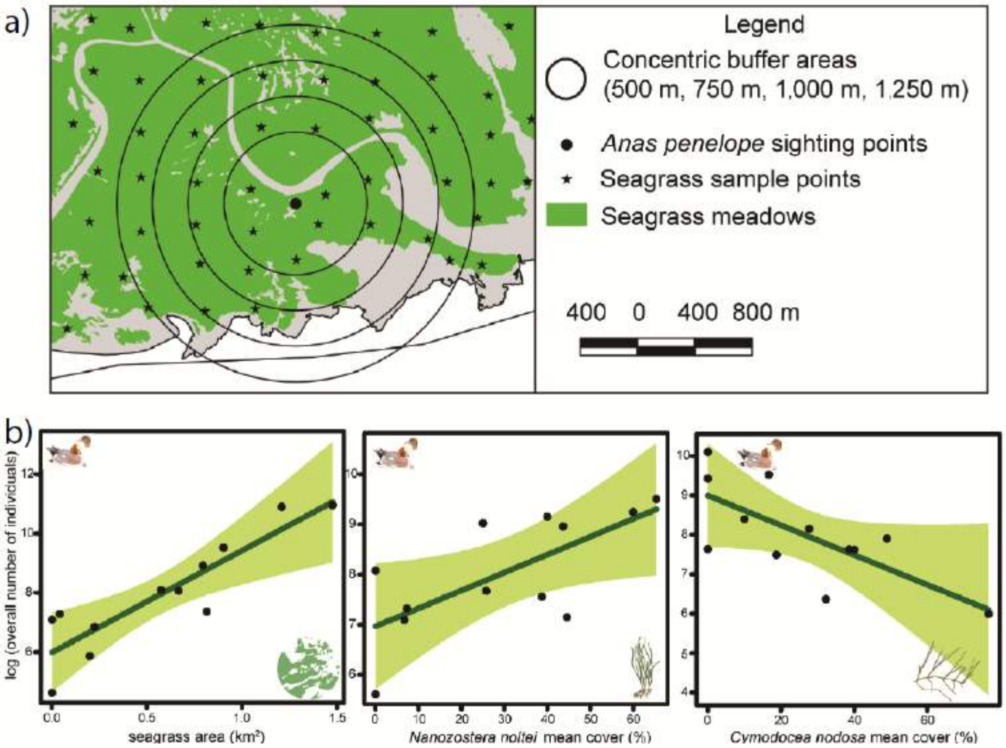


Fig. 1a. Seagrass sample points and concentric buffer areas (site 5), as an example of the multiscale analysis approach. It has been used for each representative point. Fig 1b. Relationship between *Mareca penelope* individuals (with log transformation) and cover of seagrass, *Cymodocea nodosa* and *Nanozostera noltei* in a 750 m buffer area.

1) F. Boscutti, I. Marcorin, M. Sigura, et al. (2015) Estuarine, Coastal and Shelf Science, 164, 183-193
2) B. Kjerfe (1994) Coastal Lagoon Processes. In: B. Kjerfve (ed.) Elsevier Oceanography Series. Elsevier, New York, NY, pp. 1-8

NMR-based metabolomics approach to detect frauds and adulterations of oregano samples

Manuela Mandrone
Department of Pharmacy and Biotechnology (FaBit), University of Bologna, Via Irnerio 42, 40126, Bologna, Italy

Metabolomics coupled with chemometrics resulted a valuable approach in several scientific disciplines, including plant and food science. Untargeted analysis and comparison of a wide number of samples allowed to deal with different aspects of plant science, such as geographical origin of plants, metabolic modifications upon stress, species-specific metabolites endowed with biological properties, as well as quality assessment and authenticity of spices and foods (i.e. balsamic vinegars, cereals, fruits, legumes, oils, wine, beer) (1).
In this work, NMR-based metabolomics was employed, for the first time, to perform quality control of oregano samples, which is one of the most adulterated spice worldwide (2). In particular, twenty-four oregano samples were provided by two different Companies to assess the degree of purity and/or fords. Samples were subjected to both morphological and metabolomics analysis and the results were compared. For metabolomics, ^{1}H NMR profiles were measured and spectra, after proper data reduction, were compared by PCA. The developed model proved able to detect differences among oregano species. In order to build even more predictive model, on the basis of PCA results, it was developed an OPLS-DA model validated by permutation test (R2y (cum)= 0.99 and Q2 (Cum)= 0.97) and CV-ANOVA ($p=1.71x10^{-8}$; F=57.49). *Origanum vulgaris* and *O. onnites* (the two marketable species of oregano) were clearly distanced on the base of their ^{1}H NMR profiles and the model was able to recognize samples differing from these species, thus to be considered frauds, such as *O. majorana*. The obtained results were generally in agreement with morphological analysis, and in case of not marketable species yielded even more satisfactory results, in fact, same samples rating high quality (99% pure oregano) by morphological analysis, resulted, non-marketable as oregano by metabolomics. Regarding the presence of impurities, different O2PLS models were developed for the two marketable oregano species, using as y variables the diagnostic signals of the main biomarkers of quality previously detected by PCA (mainly thymol and rosmarinic acid). The developed models were able to discriminate pure oregano by samples contaminated with other herbs, in agreement with the results of morphological analysis. The latter allowed to identify *Cistus incanus* as the most common adulterant. On this basis, samples spiked with different percentage of *C. incanus* (from 60 to 1%) were prepared in laboratory, leading to obtain a multivariate data model (OPLS) able to predict the degree of cistus presence in oregano samples on the basis of ^{1}H NMR profile. This model was developed using as y variable signal at δ 3.46, which resulted diagnostic NMR signal of cistus impurity by PCA. This analysis allowed to detect the presence of *C. incanus* at a concentration lower than 2% w/w, which is the maximum degree of impurity allowed (3). In conclusion, the results here reported testify the capability and the power of this approach, endorsing NMR spectroscopy and metabolomics as a valid, accurate and fast alternative or complement to the analysis routinely applied for the quality assessment of oregano.

1) Cagliani L.R., Scano P., Consonni R. (2017) NMR-based Metabolomics: Quality and Authenticity of plant-based foods. Modern Magnetic Resonance, 1-20
2) Galvin-King P., Haughey S.A., Elliott, C.T. (2017) Herb and spice fraud; the drivers, challenges and detection. Food Control, 88, 85-97
3) Lohumi S., Lee S., Lee H., Cho B. (2015) A review of vibrational spectroscopic techniques for the detection of food authenticity and adulteration. Trends in Food Science & Technology, 46(1), 85-98

Chemical composition and biological activities of the essential oils of two different cultivars of *Citrus medica* L.

Laura De Martino[1], Luigi Aliberti[1], Lucia Caputo[1], Vincenzo De Feo[1], Filomena Nazzaro[2], Lucéia Fátima Souza[1]

[1]Department of Pharmacy, University of Salerno, Via Giovanni Paolo II, 132, I-84084 Fisciano (Salerno), Italy; [2]Institute of Food Sciences, (ISA-CNR), Via Roma, 64, 83100 Avellino, Italy

Citron (*Citrus medica* L.), native to Southeast Asia, was imported to the Mediterranean area around 300 B.C. Probably, it arrived in Italy through the Hebrews who introduced the cultivation of the plant on the Calabrian coasts, Amalfi Coast, and Garda Lake (1).

Citrus medica cv. *'liscia'* and *C. medica* cv. '*rugosa'* are two taxa belonging to the biodiversity of South Italy, in particular of Amalfi Coast, in the Campania region (2).

Because of different studies reported evidence that *C. medica* consumption is associated with a reduced cancer incidence (3) and some essential oils and their components are known for their activity on the Central Nervous System (4), the aims of this paper were to study the chemical composition of the essential oils obtained from the peel of the fruits of these two cultivars grown in Amalfi Coast, to evaluate their potential antimicrobial activity, their cytotoxicity and the possible effects on CNS.

The chemical composition of the essential oils was studied by gas chromatography (GC) and gas chromatography-mass spectrometry (GC-MS) analyses and 100 compounds were identified, 82 for *C. medica* cv. '*liscia'* and 88 for *C. medica* cv. '*rugosa'*. Monoterpene hydrocarbons are the main constituents in both oils of *C. medica* cv. 'liscia' (79.1%) and *C. medica* cv. 'rugosa' (80.2%). In both oils, limonene (67.2%–62.8%) and camphene (8.5%–10.9%) are the main constituents.

The antimicrobial activity of the oils was assayed against some bacterial strains: *Bacillus cereus* (DSM 4313), *Bacillus cereus* (DSM 4384), *Staphylococcus aureus* (DSM 25693), *Pseudomonas aeruginosa* (ATCC 50071), and *Escherichia coli* (DSM 8579). Low concentrations of *C. medica* cv. 'rugosa' essential oil showed an inhibitory effect on *P. aeruginosa* and higher concentrations inhibited more *B. cereus* (4384) and *E. coli* than *S. aureus*. The antimicrobial activity registered confirm their traditional uses as food preserving agents and led us to hypothesize the possible use of these oils as antimicrobials. The cytotoxicity of the essential oils was evaluated against SH-SY5Y cell line: in particular, treatment of SH-SY5Y neuroblastoma cells with 800 µg/mL of limonene for 24 h resulted in a low cytotoxic activity. On the other hand, the treatment with 800 µg/mL of *C. medica* cv. '*liscia'* essential oil resulted in a stronger cytotoxicity than *C. medica* cv. '*rugosa'* essential oil with 38% cell death (Figure 1). The influence of the EO on the expression of adenylate cyclase 1 (ADCY1) was also studied. The alterations in ADCY1 expression suggested a role for limonene in the effects on the Central Nervous System.

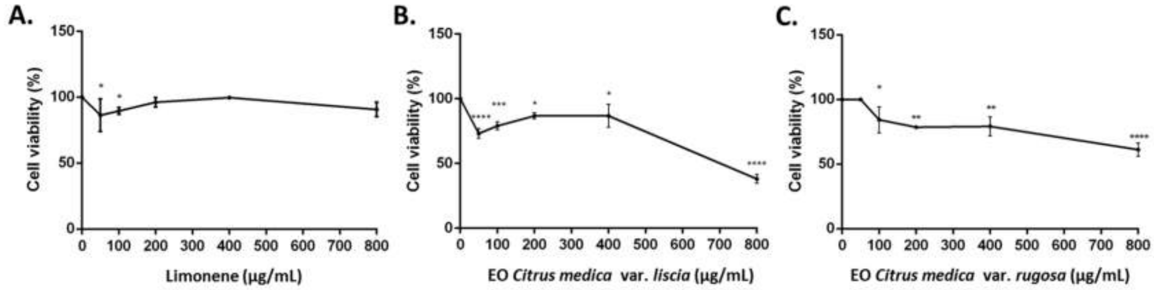


Fig. 1. Percentage of cell viability after 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide TT) assay. Cells were treated with different concentrations (50–800 µg/mL) of limonene (**A**); *C. medica* cv. '*liscia'* (**B**) and *C. medica* cv. '*rugosa'* essential oils, for 24 h and solvent (DMSO, 0.1%) alone. Data are the mean ± SD of three experiments * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$ vs. DMSO

1) B. Gabriele, A. Fazio, P. Dugo, R. Costa, L. Mondello (2009) J. Sep. Sci., 32, 99–108
2) A. Uzun, T. Yesiloglu, (2012) Caliskan M., Ed., InTech: Rijeka, Croatia, in Genetic Diversity in Plants, 213–230
3) W.Q. Li, S. Kuriyama, Q. Li, M. Nagai, A. Hozawa, Y. Nishino, I. Tsuji (2010) J. Cancer, 127, 1913-1922
4) F.M. Souto-Maior, F. Lélis de Carvalho, L.S. Soares Lima de Morais, S. Mendonça Netto, D. Pergentino de Sousa, R. Nóbrega de Almeida (2011) Pharmacol. Biochem. Behav., 100, 259–263

Antifungal activity of amaranth seed extract in combination with known antifungal drugs

Daniela De Vita[1], Chiara Toniolo[1], Claudio Frezza[1], Anastasia Orekhova[2], Giovanna Simonetti[2], Luigi Scipione[3], Roberto Di Santo[3], Roberta Costi[3]

[1]Department of Environmental Biology, Sapienza University of Rome, p.le Aldo Moro, 5, 00185, Rome, Italy; [2]Department of Public Health and Infectious Diseases, Sapienza University of Rome, p.le Aldo Moro, 5, 00185, Rome, Italy; [3]Istituto Pasteur-Fondazione Cenci Bolognetti, Department of Chemistry and Technology of Drug, Sapienza University of Rome, p.le Aldo Moro, 5, 00185, Rome, Italy

Amaranthus spp. (Amaranthaceae family) is a botanical genus known with the common name of amaranth. It is a group of tropical plants primarily cultivated in Central America and nowadays produced also by other countries, for example China and United States (1). Its seeds have high contents of lipids, proteins, carbohydrates, dietary fiber. Furthermore, other important components are presents, such as squalene (2), a triterpenoid compound extremely interesting for its use in food and cosmetics fields, but also in the pharmaceutical one. Indeed, it interestingly seems to potentiate the activity of an antifungal drug (3). Here, we report the evaluation of an amaranth seed extract activity. Not only its effects are presented, but also its effectiveness in combination with well-known antifungal drugs, against pathogens in charge of superficial mycoses, including dermatophytes and *Candida* spp. (typical of skin, hair, and nails infection in 20-25% of the world's population) (4). Fungicidal agents, such as terbinafine, are often preferred over fungistatic azoles since they may shorten treatment time by topical application; however, they are not very effective in infections caused by *Candida albicans* (5). Amaranth oil was obtained by supercritical carbon dioxide extraction of seeds and it was characterized by HPLC and NMR analyses, showing as main components squalene and triglycerides (TGs). This oil and its combination with conventional drugs such as Fluconazole and Terbinafine, were investigated in order to assess the *in vitro* susceptibility of fungi including *Candida* spp. strains relevant for topic infections. A broth-dilution method was performed and the obtained antifungal activity, expressed as the mean of minimal inhibitory concentration (MIC), showed that the natural extract alone did not exert antifungal activity against the tested strains. Interestingly, it increased the activity of a fungistatic agent that, when associated to the oil, showed fungicidal activity. In order to identify the extract fraction responsible for the effect, the main components of the oil were purified through a chromatographic approach. Therefore, squalene and TGs were tested alone and in combination with the antifungal drug. The obtained data suggest that the activity can be due to a minor component of the oil or, more interestingly, the biological activity is attributable to the phytocomplex, suggesting a possible topical application of the oil in combination with drugs.

1) L.M. Coelho, P.M. Silva, J.T. Martins, A.C. Pinheiro, A.A. Vicente (2018) Food Funct., 9, 5499-5512
2) O. Popa, N.E. Babeanu, I. Popa, S. Nița, C.E. Dinu-Parvu (2015) Biomed. Res. Int., 2015:367202
3) K. Masuda, S. I Akiyama, M. Kuwano, N. Ikekawa (1982) J. Antibiot., 35, 230-234
4) M. Ameen (2010) Clin. Dermatol., 28, 197-201
5) A.A. Kyle, M.V. Dahl (2004) Am. J. Clin. Dermatol., 5, 443-451

Anti-adipogenic and anti-oxidant effects of a standardized extract of *Citrus sinensis* (L.) Osbeck 'Moro' (Rutaceae) during adipocyte differentiation of 3T3-L1 pre-adipocytes

Giuseppe Malfa[1], Barbara Tomasello[1], Alfonsina La Mantia[1], Francesco Pappalardo[1], Claudia Di Giacomo Claudia[1], Marcella Renis Marcella[1], Ragusa Salvatore[2] Ragusa[2], Rosaria Acquaviva[1].
[1]Department of Drug Science - Biochemistry Section, University of Catania, Viale A. Doria, 6, 95125, Catania, Italy; [2]Department of Health Sciences, University *Magna Graecia* of Catanzaro, Viale Europa, Località Germaneto, 88100, Catanzaro, Italy

Blood orange (*Citrus sinensis* (L.) Osbeck), belonging to Rutaceae family, is a pigmented sweet orange variety, typical of the eastern Sicily (Italy). It is an evergreen tree with large spines on branches, dark green smooth leaves with a characteristic citrus smell while the flowers are small, white and fragrant.

Particular features in the pulp and in the peel of blood oranges are marked levels of anthocyanins that give the typical red coloration. The three most common varieties of red oranges are Tarocco, Moro (both native to Italy), and Sanguinello (native to Spain).

All blood oranges require strong day-night thermal excursion for intense colour formation and cultivar such as Moro, with the potential for high pigmentation, are strongly dependent on the prevailing climatic conditions during fruit ripening for full colour development.

Moro is the most colourful, with deep red flesh ranges from orange-veined with ruby coloration, to vivid crimson and nearly to black (1). Moreover, blood oranges contain others polyphenolic compounds such as hydroxycinnnamic acids and flavanones, well known to exert numerous health-promoting actions on human wellbeing (2).

Literature data reported that anthocyanins reduce lipid accumulation, negatively modulate the expression of some obesity-related transcription factors exhibiting scavenging and ant-inflammatory activities, concomitantly inducing decrease in lipogenesis and proinflammatory adipokine secretion during adipocyte differentiation (3). Therefore, the interest has grown in consuming blood oranges due to their high content in anthocyanins as well as in phenolic compounds.

In this scenario we innovatively utilized a standardized extract of Moro blood oranges (*Citrus sinensis* (L.) Osbeck), in order to investigate, *in vitro,* some effects on 3T3-Ll cell line, one of the best-validated models to study differentiation of pre-adipocytes into adipocytes (4).

Quantitative HPLC analysis of the extract to confirm its phenolic composition and the anthocyanin amount was performed; afterwards we evaluated the cytotoxicity by MTT assay and the antioxidant activity by DPPH test.

Subsequently, during adipocyte differentiation, 3T3-L1 cells were treated with concentrations of the extract containing 2.5, 5, 10, 25 µM of anthocyanins. After seven days of treatment and differentiation, we analyzed redox profile (ROS, GSH) and adipokine levels together with some key transcription factors related to the regulation of energy metabolism, lipogenesis and lipid accumulation.

Results showed that the extract exerts antioxidant and anti-adipogenic activities during adipocyte differentiation of 3T3-L1 pre-adipocytes.

1) G. Grosso, F. Galvano, A. Mistretta, S. Marventano, F. Nolfo, G. Calabrese, S. Buscemi, F. Drago, U. Veronesi, A. Scuderi (2013) Oxid. Med. Cell Longev., 2013:157240
2) B. Fallico, G. Ballistreri, E. Arena, S. Brighina, P. Rapisarda (2017) Food Chem., 215, 67-75
3) R. Naseri, F. Farzaei, P. Haratipour, S.F. Nabavi, S. Habtemariam, M.H. Farzaei, R. Khodarahmi, D. Tewari, S. Momtaz (2018) Front. Pharmacol., 9, 1310-1315
4) H. Green, O. Kehinde (1975) Cell, 5, 19-27

Discovery of a selective and potent gametocytocidal antimalarial agent from *Lophira lanceolata*

Giuseppina Chianese[1], Annalisa Lopatriello[1], Silvia Parapini[2], Annette Habluetzel[3], Donatella Taramelli[2], Harouna Sore[3], Alain R. Tenoh[3], Orazio Taglialatela-Scafati[1]

[1]Department of Pharmacy, University of Naples 'Federico II', via Domenico Montesano 49, Napoli, Italy; [2]Department of Biomedical, Surgical and Dental Science, University of Milan, via Commenda 10, Milano, Italy; [3]School of Pharmacy, University of Camerino, Via Gentile III da Varano, Camerino (MC), Italy

Malaria is an infective disease, causing, according to WHO estimates, about 219 million new clinical cases and 429,000 deaths have occurred worldwide in 2017. Among these, more than 90% were recorded in sub-Saharan Africa, the remaining occurring in South-East Asia and South America (1). The development of resistance engages researchers to identify new lead compounds as antimalarial agents or new phytomedicines. At same time, there is a strong need for the identification of new transmission blocking agents able to interrupt the malaria transmission. In this field, targeting the transmissible stages from mature gametocytes to elongated and motile ookinetes of *Plasmodium* is a crucial strategy for malaria elimination and eradication (1). Medicinal plants remain a large source for new antimalarial drugs discovering. Our approach to find antimalarial transmission-bocking agents is based on the investigation of African plants in order to isolate new lead compounds. Malaria parasite's specific life cycle assays conducted on the extracts of these plants indicated a promising potential for *Lophira lanceolata* Tiegh. ex Keay. In particular, a methanol extract of stem bark of *L. lanceolata* showed a relevant inhibitory activity on different stages: on gametocytes 3D7 strain, the percentage of viability at dose of 100 μg/ml was 7%; besides ODA (Ookinete development assay) results indicated a percentage of inhibition of around 95% at a dose of 50 μg/ml. Bioassay-guided fractionation through different chromatographic purification steps (e.g. MPLC, HPLC) of an organic extract led to the identification of the single secondary metabolite responsible for this stage-specific gametocytocidal activity with an IC$_{50}$ value in the nanomolar range. The phytochemical analysis of these fractions permitted the isolation of structural analogues allowing preliminary structure-activity relationships to be drawn.

Fig. 1a-b *Lophira lanceolata*

1) World Health Organization (2018) World Malaria Report 2018, WHO, Geneva
2) L.P. Ouattara et al. (2014) Parasitology Research, 113, 405–416
3) S. Sanon, et al. (2013) African Journal of Laboratory Medicine, 2, 81

Phytochemical and physiological evaluations of four *Citrus* species from the *horti simplicium* of the Charterhouse of Pisa over one year of analyses

Roberta Ascrizzi[1], Laura Pistelli[2,3], Enrica Attuoni[1], Arianna Pelletti[1], Matteo Mancino[2], Guido Flamini[1,3], Luisa Pistelli[1,3]

[1]Department of Pharmacy, University of Pisa, Via Bonanno 6, 56126 Pisa, Italy; [2]Department of Agriculture, Food and Environment, University of Pisa, Via del Borghetto 80, 56126 Pisa, Italy; [3]Interdepartmental Research Center "Nutraceuticals and Food for Health", University of Pisa, Via del Borghetto 80, 56126 Pisa, Italy

The present study is the result of one full year (from November 2017 to November 2018) of phytochemical and physiological analyses carried out on four *Citrus* spp. (*Citrus limon* (L.) Osbeck, *Citrus sinensis* (L.) Osbeck, *Citrus* × *aurantium* L., and *Citrus reticulata* Blanco) growing in the *horti simplicium* of the monks of the Charterhouse of Pisa, where they are not subjected to any agronomic treatment, as they are wild specimens.

The phytochemical analyses investigated the composition of the essential oils (EOs) extracted from the peel, the juice and leaves of each sample by means of gas chromatography coupled with mass spectrometry (GC-MS). The physiological analyses evaluated the photosynthetic efficiency, the relative water content (RWC), chlorophylls, carotenoids and polyphenols content.

The peel essential oils of all the samples were mainly rich in limonene, as expected for *Citrus* spp. Other relevant peel EO constituents were myrcene, linalool, nerol and geraniol and their related aldehydes. The leaf EOs, instead, showed more varied compositions, with monoterpenes as the most abundant chemical class of compounds, of which sabinene and linalool showed the highest amount. The sesquiterpenes were more represented in the leaf EOs, while in the juice EO valencene was particularly relevant, together with β-caryophyllene and β-sinensal as the most abundant.

The physiological analyses showed a correlation of responses with the climatic conditions: plants contain lower efficient photosynthetic activity in winter than during summer. Moreover, the high temperature and lack of agronomic treatment influenced the metabolites content and the RWC. *Citrus limon* appeared to be the better adapted species to the environmental conditions. The phytochemical data was also analysed by multivariate statistical analysis, using the principal component and the hierarchical cluster analyses to evidence evolution patterns in the EO compositions.

The complete analysis of the volatile profiles and of the main physiological parameters allowed to assess the plant evolution over one entire year, evidencing the variability induced by the climate and how the species influence the metabolite production in these four *Citrus* spp. growing in the same environment.

Funding information: This research was funded by INTERREG Italia Francia Marittimo 2014-2020 MARE DI AGRUMI "MARchio turistico e biotEcnologie verDi per lo sviluppo di Imprese su una risorsa comune: gli AGRUMI", grant number CUP C26D16007240007.

POSTERS

1.1 = Long-term photoacclimation of photosynthetic limitations and related morpho-anatomical determinants in the microphylls of *Selaginella martensii* Spring

Lorenzo Ferroni[1], Marian Brestič[2], Riccardo Cantelli[1], Alex Zeri[1], Tommaso Carassiti[1], Simonetta Pancaldi[1]
[1]Department of Life Sciences and Biotechnology, University of Ferrara, C.so Ercole I d'Este 32, 44121, Ferrara, Italy;
[2]Department of Plant Physiology, Slovak University of Agriculture in Nitra, Tr. A. Hlinku 2, 94901 Nitra, Slovak Republic

Ferns and fern allies (so-called "pteridophytes") share a generally much lower photosynthetic capacity as compared to seed plants (1). This physiological feature is probably related to their evolutionary history. In fact, the appearance of the most ancient pteridophytes is documented in the Silurian and Devonian periods (more than 400 Myr ago), when the atmospheric CO_2 concentration was 10-fold higher than present. They included the two diverging groups of lycophytes and euphyllophytes. Among the properties characterizing the former, which represent only 1% of all extant vascular plant species, microphyll morphology was possibly favoured under the selection pressure of a high CO_2 concentration. In general, many recent comparative studies point indeed to some evolutionary trends from the most ancient pteridophytes to angiosperms, influencing especially the diffusional limitations of photosynthesis (1-4). Stomatal conductance (g_s) relates to stomatal density and size (4), while mesophyll conductance (g_m) seems to be mainly related to mesophyll cell wall thickness and chloroplast surface exposed to intercellular spaces (3). The older the species, the lower the conductance values, and the few species of lycophytes included in studies laid at the scale bottom (3,4). In a previous research, the lycophyte *Selaginella martensii* Spring (Selaginellaceae) was reported to be capable of sun/shade acclimation with respect to chloroplast morphology and composition, resulting in an increased photochemical activity under sun conditions (5). However, the maintenance of a high capacity of thermal dissipation suggests that the plant could be inefficient in the long-term regulation of diffusional CO_2 limitations.

S. martensii was long-term acclimated to deep shade (L), shade (M), and full sunlight (H) in a humid greenhouse at the Botanical Garden of Ferrara. Microphylls were analysed with respect to CO_2 assimilation and chlorophyll fluorescence emission as a function of increasing irradiance (light curves) or CO_2 concentration (A/C_i curves); morphological aspects relevant to CO_2 diffusion were analysed by scanning and transmission electron microscopy. Stomata were typically distributed along the vein at the underside of the microphyll; their size and density markedly increased from L to H plants, with a consequent comparable increase in g_s, which yet remained in the range of the low values usually found in ferns (1). A recently published method was used to estimate g_m from A/C_i curves measured in parallel with photosystem II fluorescence (6). The lowest g_m was found in L plants and increased in M and H plants, possibly related to a change in the chloroplast surface exposed to mesophyll airspaces. Long-term acclimation to H conditions positively influenced also the maximum carboxylation rate, which however remained quite low. Therefore, we also checked the relative importance of electron sinks alternative to carboxylation (7).

Results suggest that in a lycophyte the modulations of microphyll morphological traits are coordinated with the biochemical acclimation of the photosynthetic apparatus; however, the gain in carboxylation capacity under sun conditions is limited. This explains the strong need for thermal dissipation of excess absorbed energy and the enhancement of alternative electron sinks to preserve the chloroplast integrity.

1) H. Carriquí, H.M. Cabrera, M.A. Conesa, R.E. Coopman, C. Douthe, J. Gago, A. Gallé, J. Galmes, M. Ribas-Carbo, M. Tomas, J. Flexas (2015) Plant Cell Environ., 38, 448-460
2) T. Tosens, K. Nishida, J. Gago, R.E. Coopman, H.M. Cabrera, M. Cariquì, L. Laanisto, L. Morales, M. Nadal, R. Rojas, E. Talts, M. Tomas, Y. Hanba, U. Niinemets, J. Flexas (2016) New Phytol., 209, 1576-1590
3) L-L. Veromann-Jurgenson, T. Tosens, L. Laanisto, U. Niinemets (2017) J. Exp. Bot., 68, 1639-1653
4) D. Xiong, C. Douthe, J. Flexas (2018) Plant Cell. Environ., 41, 436-450
5) L. Ferroni, M. Suorsa, E-M. Aro, C. Baldisserotto, S. Pancaldi (2016) New Phytol., 211, 554-568
6) D.P. Moualeu-Ngangue, T-W. Chen, H. Stueztel (2017) New Phytol., 213, 1543-1554
7) M. Živčak, M. Brestič, Z. Balatova, P. Drevenakova, K. Olsovska, H.M. Kalaji, X. Yang, S.I. Allakhverdiev (2013) Photosynth. Res., 117, 529-546

Research supported with the contribution of the Slovak Academic Information Agency.

1.1 = *In vitro* microcosms as a tool for the study of ecological interactions in microbial biofillms

Mariagioia Petraretti[1], Angelo Del Mondo[1], Antonino De Natale[1], Gabriele Pinto[1], Antonino Pollio[1]
[1]Department of Biology University of Naples “Federico II”, Via Cupa nuova Cinthia 21, 80126 Napoli, Italy

Microbial biofilms grow on many type of surfaces, including rocks material that affects the structure and behavior of the biofilms. Organisms involved in lithic biofilm formation represent a huge variety of microalgae, cyanobacteria, soil fungi and bacteria (1). The ecological success of biofilms growing on lithic surfaces is the result of a balance between cooperation and competition of different microbial species (2). However, few studies have focused on the social interactions and evolutionary dynamics within this kind of biofilm communities. In order to reproduce and investigate the social interactions among microorganisms and to examine how they evolve over time and space, an evaluation of the microbial community on the basis of its main actors has been needed. Thus in vitro colonization experiments were carried out for the assessment of growth on a lithic substratum by several groups of phylogenetically distinct microrganisms such as fungi, microalgae and cyanobacteria isolated from archaeological site.
In particular three differential experiental set were carried out:
in the first experimental condition the growth of single microorganisms was evaluated, in the second experimental condition the qrowth of microrganisms in pairs was evaluated, finally the growth of the three microrganisms together was evaluated.
During experimental simulations, growth in the three experimental conditions has been monitored with CLSM microscopy to investigate relationships among microrganisms and between microrganisms and substratum in order to understand how spatial structure of these communities influence the ecology and community diversity of biofilms growing on lithic surfaces. Our tests have shown that the heterotrafa component can be considered as a pioneer of subaerial biofilms; infact, in all the combinations tested the fungus colonizes the deepest part of the substrata and its hyphae give rise to a reticular structure, which allows a better rooting of the phototrophic species, providing a biological substrate to them and probably cooperating through the transfer of nutrients. Understanding the extent of cooperation and coordinationd and the evolution of biofilm communities, is essential for any predictive model of biofilm formation and for the design of strategies to remove biofilm infections.

1) Gorbushina, A. A. (2007). Life on the rocks. Environmental Microbiology, 9(7), 1613–1631
2) Nadell, C. D., Xavier, J. B., & Foster, K. R. (2009). The sociobiology of biofilms. FEMS Microbiology Reviews, 33(1), 206–224

1.2 = Establishment and evaluation of novel fluorescent reporters for the analysis of calcium-mediated communication in the arbuscular mycorrhizal symbiosis

Enrico Cortese[1], Francesca Pettiti[1], Alice Giletta[2], Andrea Genre[2], Diego De Stefani[3], Alessandro Negro[3], Lorella Navazio[1]

[1]Department of Biology, University of Padova, Via U. Bassi 58/B, 35131 Padova, Italy; [2]Department of Life Sciences and Systems Biology, University of Torino, Viale Mattioli 25, 10125 Torino, Italy; [3]Department of Biomedical Sciences, University of Padova, Via U. Bassi 58/B, 35131 Padova, Italy

Arbuscular mycorrhizal (AM) symbiosis is one of the most important and widespread beneficial plant-microbe interactions. This symbiotic association, which involves most land plants and fungi belonging to the Glomeromycotina subphylum, improves plant mineral nutrition and decreases the environmental impact of agriculture through a drastic reduction of the need for chemical fertilizers.

A complex molecular dialogue, based on the exchange of diffusible signals between the symbiotic partners, underlies the establishment of the AM symbiosis. Whereas the calcium-mediated signalling pathway activated in the plant host has been extensively studied (1), information about the perception and transduction of symbiotic signals in the fungal partner is still scarce. In this work we have established new tools to investigate the Ca^{2+}-based signalling mechanisms activated by plant symbiotic signals in AM fungi. In particular, we took advantage of the transduction properties of the TAT peptide, derived from the human immunodeficiency virus type 1 (HIV-1) transactivator of transcription (TAT) protein. This cell-penetrating peptide is known to function as an efficient nanocarrier for the intracellular delivery of cargoes of interest in both animal and plant cells (2), and it has also been successfully applied for the internalization of proteins in AM fungi (3). Novel genetically encoded fluorescent Ca^{2+} reporters fused to the TAT peptide were engineered, targeted to either the cytosol or the nucleus. They are based on GCaMPs, GFP-based Ca^{2+} biosensors that exhibit a higher fluorescent yield and signal change compared with cameleon (4). Constructs encoding cytosolic GCaMP6 and nuclear GCaMP7 (containing a nuclear localization signal) fused to the TAT peptide were expressed in *E. coli* and the recombinant proteins were isolated in bulk quantities from the bacterial lysates by affinity chromatography on Ni-NTA agarose column. Experiments are now ongoing to evaluate the internalization and correct targeting of these probes in animal and plant cell cultures. Once a suitable protocol of protein transduction is successfully set up, it will be applied to germinating spores of the AM fungus *Gigaspora margarita*. These novel fluorescent reporters will be used in Ca^{2+} imaging assays to monitor cytosolic and nuclear calcium dynamics in the fungal partner in response to plant symbiotic signals, *i.e.* strigolactones. Analyses of intracellular Ca^{2+} signals activated during the pre-symbiotic phase will provide novel insights into the signal transduction mechanisms underlying AM symbiosis.

1) P. Bonfante, A. Genre (2015) Trends Plant Sci, 20,150-154
2) C. Bechara, S. Sagan (2013) FEBS Lett, 587, 1693-1702
3) R. Moscatiello, S. Sello, M. Novero, A. Negro, P. Bonfante, L. Navazio (2014) New Phytol, 203, 1012-1020
4) A. Costa, L. Navazio, I. Szabo (2018) J Exp Bot, 69, 4175-4193

1.3 = Intra-seasonal variations in the genesis of phloem in Mediterranean pines

Angela Balzano[1]*, Katarina Čufar[1], Jožica Gričar[2], Martin de Luis[3], Veronica De Micco[4]
[1]Department of Wood Science and Technology, University of Ljubljana, Biotechnical Faculty, Ljubljana, Slovenia; [2]Department of Forest Yield and Silviculture, Slovenian Forestry Institute, Večna pot 2, 1000 Ljubljana, Slovenia; [3]Department of Geography and Regional Planning, University of Zaragoza, 50009 Zaragoza, Spain; [4]Department of Agricultural Sciences, University of Naples Federico II, via Università 100, 80055, Portici (Naples), Italy
*Angela.balzano@bf.uni-lj.si

In Mediterranean species, annual periodicity of cambial production is dectectable in xylem, by the alternation of earlywood and latewood during the seasons. The phloem in Mediterranean species, instead, has no marked boundaries and the difference between early-phloem and late-phloem is not clearly defined.
We studied the phloem formation focusing on seasonal response in *Pinus halepensis* and *Pinus pinea* growing at drought-prone sites in southern Italy and southern Spain. Microcores, containing phloem and xylem tissue, were collected at bi-weekly intervals throughout the calendar year, afterwards cut in thin cross section, stained and analysed under a light microscope. The variation of anatomical traits of phloem increments were quantified applying digital image analysis method.
Although it was not possible to detect clear annual boundaries within the phloem increments, we could differentiate between the collapsed and the non-collapsed phloem and measure the traits of the latter. Radial dimensions of sieve cells fluctuated throughout the year, although not clearly identifiable as early or late-phloem. The variation in the size of phloem cells was likely due to the slowing down of cambial production during the drought period and its incresing during the wet-warm months of the year. Moreover, also the frequence of axial parenchyma varied during the year, following seasonal trends.
The variations in phloem traits found in the analysed trees suggest the ongoing production of new phloem cells throughout the entire year. The quantification of phloem anatomical traits resulted to be helpful to follow the dynamics of phloem formation in Mediterranean trees. The analysis of phloem genesis, combined with xylogenesis, could provide more precise information on secondary-growth responses to the variations in climatic conditions.

1.3 = Polarity-driven cell divisions: the case study of stomata development

Camilla Betti[1,2,3], Anaxi Houbert[1,2], Eugenia Russinova[1,2]
[1]Department of Plant Biotechnology and Bioinformatics, Ghent University, 9052, Ghent, Belgium; [2]VIB Center for Plant Systems Biology, VIB, 9052, Ghent, Belgium; [3]Department of Medicine, University of Perugia, Perugia, Italy

Plant development requires exact positioning of cells and tissue organization to produce functional organs and viable organisms. To this aim, precisely oriented cell divisions, typically classified as asymmetric or symmetric, are crucial. Asymmetric cell divisions (ACD), also defined as formative, give rise to daughter cells with two distinct cell fates which often have different sizes and/or shapes. Conversely, symmetric cell divisions (SCD) generate two identical daughter cells and are denominated as proliferative. The orientation of the division plane in both SCD and ASD is tightly controlled by a combination of signals both intrinsic, occurring inside cells, and extrinsic, originating outside them.

Stomatal cell lineage is an archetypal example of ACD, which is necessary for plant survival. In Arabidopsis, the GLYCOGEN SYNTHASE KINASE3 (GSK3)/SHAGGY-like kinase BRASSINOSTEROID INSENSITIVE 2 (BIN2) phosphorylates both the mitogen-activated protein kinase (MAPK) signalling module (1, 2) and its downstream target, the transcription factor SPEECHLESS (SPCH) (3), to respectively promote and restrict ACDs in the same stomatal lineage cell. However, the mechanisms that balance these mutually exclusive activities was still not completely clarified. Recently, we have identified the plant-specific protein POLAR as a stomatal lineage scaffold for a subset of GSK3-like kinases that confines them to the cytosol and subsequently transiently polarizes them within the cell, together with BREAKING OF ASYMMETRY IN THE STOMATAL LINEAGE (BASL), before ACD. As a result, MAPK signalling is attenuated, enabling SPCH to drive ACD in the nucleus. Furthermore, POLAR turnover requires phosphorylation on specific residues, mediated by GSK3. This study reveals a mechanism by which the scaffolding protein POLAR ensures GSK3 substrate specificity, and could serve as a paradigm for understanding regulation of GSK3 in plants (4).

1) Kim TW, Michniewicz M, Bergmann DC, Wang ZY (2012) Brassinosteroid regulates stomatal development by GSK3-mediated inhibition of a MAPK pathway. Nature 482, 419-422
2) Khan M, Rozhon W, Bigeard J, Pflieger D, Husar S, Pitzschke A, Teige M, Jonak C, Hirt H, Poppenberger B (2013) Brassinosteroid-regulated GSK3/Shaggy-like kinases phosphorylate mitogen-activated protein (MAP) kinase kinases, which control stomata development in Arabidopsis thaliana. J. Biol. Chem. 288, 7519-7527
3) Gudesblat GE, Betti C, Schneider-Pizoń J, Mayerhofer J, Vanhoutte I, van Dongen W, Boeren S, Zhiponova M, de Vries S, Jonak C, Russinova E. (2012) SPEECHLESS integrates brassinosteroid and stomata signalling pathways. Nature Cell Biology 14, 548-554
4) Houbaert A, Zhang C, Tiwari M, Wang K, de Marcos Serrano A, Savatin DV, Urs MJ, Zhiponova MK, Gudesblat GE, Vanhoutte I, Eeckhout D, Boeren S, Karimi M, Betti C, Jacobs T, Fenoll C, Mena M, de Vries S, De Jaeger G, Russinova E. (2018) POLAR-guided signalling complex assembly and localization drive asymmetric cell division. Nature 563, 574-578

1.3 = From ovaries to fruit in plum species

Martina Cerri[1*], Marika Bocchini[1], Adolfo Rosati[2], Emidio Albertini[1], Franco Famiani[1], Lara Reale[1]
[1]Department of Agricultural, Food, and Environmental Sciences, Borgo XX Giugno 74, University of Perugia, Italy; [2]CREA, Research Centre for Olive, Citrus and Tree Fruit, Spoleto, PG, Italy
*Correspondence: martina.cerri@unipg.it

The mechanisms driving drupe differentiation have been investigated in several plant species. Often, bigger fruits derive from bigger ovaries but in some species, ovary size is similar and differences in fruit size are determined by differences in post-bloom tissue development. The aim of this work was to investigate whether, in three *Prunus* species (*P. salicina, P. domestica* and *P. cerasifera*), fruit size is related to ovary size or whether it depends on post-bloom developmental processes. This study represents a starting point to understand the mechanisms of cell division and expansion in *Prunus* species and their implication for fruit size management. We used microscope image analyses to evaluate ovary wall thickness at anthesis and fruit mesocarp thickness at final fruit size. In the same tissues (i.e. ovary wall and fruit mesocarp), we also measured cell area and number of cellular layers. Ovary wall thickness did not differ among the genotypes tested (except for Mirabolano, the smallest-fruited genotype, having slightly bigger ovaries) and was not correlated to either cell size or to the numbers of cell layers. Fruit mesocarp thickness varied three fold among genotypes and was strongly and positively correlated to the number of cell layers, and weakly and negatively correlated to cell size. No correlation was found between fruit weight (or mesocarp thickness or number of cell layers) and the respective ovary characteristic: bigger fruits did not derive from bigger ovaries. Fruit weight was instead related to the fruit-to-ovary weight and cell layer number ratios, indicating that fruit size in these species is related not to ovary characteristics, but to processes that occur to a different extent after bloom, particularly to a greater cell division. In particular, *P. salicina* cv. TC Sun, which had the biggest fruit, had a longer post bloom cell division activity and/or a higher post bloom cell division rate. Indeed, the number of TC Sun cell layers increased four times, while in the other species the mesocarp had twice the ovary layers. TC Sun also had the lowest increase in mesocarp cell area.
Moreover, previous work about ovary abortion in olive demonstrated a correlation between starch storage and ovary development. So, gene expression analyses are in progress through real time PCR and *in situ* hybridization for the genes related to sucrose and sorbitol metabolism, including two sucrose synthases, a vacuolar and a cytosolic invertase and sorbitol dehydrogenase, transporter and oxidase.

1.3 = Identification of novel genes involved in phosphate accumulation in *Lotus japonicus* through Genome Wide Association mapping of root system architecture and anion content

Marco Giovannetti[1], Christian Göschl[1], Stig U. Andersen[2], Stanislav Kopriva[3], Wolfgang Busch[1,4]
[1]Gregor Mendel Institute (GMI), Austrian Academy of Sciences, Vienna Biocenter (VBC), Vienna, 1030 Austria; [2]Department of Molecular Biology and Genetics, Aarhus University, 8000 Aarhus C, Denmark; [3]University of Cologne, Botanical Institute and Cluster of Excellence on Plant Sciences (CEPLAS), D-50674 Cologne, Germany; [4]Salk Institute for Biological Studies, Plant Molecular and Cellular Biology Laboratory, and Integrative Biology Laboratory, La Jolla, CA, 92037 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 architecture and metabolic profile to the evolution of widespread mutualistic interactions, such as arbuscular mycorrhizal (AM) symbiosis. Here we elucidated plant responses and their genetic basis to different phosphate levels in a plant species that is widely used as a model for AM symbiosis: *Lotus japonicus*. Rather than focusing on a single model strain, we measured root growth and anion content in response to different levels of phosphate in 130 Lotus natural accessions. This allowed us not only to uncover common as well as divergent responses within this species, but also enabled Genome Wide Association Studies by which we identified new genes regulating phosphate homeostasis in Lotus. Under low phosphate conditions, we uncovered a correlation between plant biomass and the decrease of plant phosphate concentration in plant tissues, suggesting a dilution effect. Altogether our data of the genetic and phenotypic variation within a species capable of AM complements studies that have been conducted in Arabidopsis, and advances our understanding of the continuum of genotype by phosphate level interaction existing throughout dicot plants.

1.3 = Leaf structural responses help explain single-leaf gas exchange, but not overall growth responses to narrow-band lighting

Luigi Gennaro Izzo[1], Matthew Mickens[2], Giovanna Aronne[1], Celina Gómez[3]
[1]Department of Agricultural Sciences, University of Naples Federico II, Via Università 100, 80055, Portici (NA), Italy; [2]NASA Utilization and Life Sciences Office, Mail Code: UB-A, Kennedy Space Center, FL 32899, USA; [3]Environmental Horticulture Department, University of Florida, 1549 Fifield Hall, Gainesville, FL 32611-0670, USA

Understanding plant responses to light quality is critical to advance plant production in controlled environments. Recent research with light-emitting diodes (LEDs) has shown that narrow-band lighting triggers a variety of functional responses in plants (1). In general, increasing blue light (400 to 500 nm) has been found to produce compact plants with low capacity for growth and productivity (2). In addition, some blue photons are absorbed by inactive and accessory pigments, and as a result, their energy is not efficiently used for photosynthesis (3). However, while some studies have found that blue light has a relatively small effect on photosynthesis, others have shown that the photosynthetic capacity of leaves increases up to a point in response to blue light (4). Therefore, although it is widely accepted that plants with smaller leaves will have limited photosynthetic capacity due to a reduction in radiation capture, the mechanisms that drive the increase in leaf photosynthesis in response to blue light are poorly understood. Our objective was to quantify the effects of blue light on leaf anatomy to correlate leaf structural responses with plant growth and single-leaf gas exchange. We grew 'Waldmann's Green' and 'Outredgeous' lettuce, which are green and red leaf cultivars, respectively, under five different red-to-blue-light ratios: 100% red (0B); 7% blue + 93% red (7B); 26% blue + 74% red (26B); 66% blue + 34% red (66B); 100% blue (100B). All treatments provided an average daily light integral of 11.5 $mol \cdot m^{-2} \cdot d^{-1}$ (200 ± 2 $\mu mol \cdot m^{-2} \cdot s^{-1}$ over a 16-h photoperiod). Growth, gas exchange, and leaf structural traits were measured. Overall, both cultivars showed similar responses to the different red:blue ratios and plants grown under 100B had a different trend relative to the other treatments. Gas exchange increased in response to blue light up to 66B. Conversely, at 100B all parameters decreased and the values were in general, similar to those produced by plants grown under red and blue light. Similarly, we found that chloroplast abundance (Chl_A) in leaves significantly increases in response to blue light, which is most likely responsible for the increase in photosynthetic rate per unit leaf area that is typically reported for plants grown under higher percentages of blue light. Our data confirms that although growth generally decreases with the addition of blue light, single-leaf gas-exchange responses increase in response to blue light. Based on our data, increasing blue light may increase the physiological capacity of plant leaves by increasing Chl_A thus, possibly regulating the CO_2 diffusive resistance within plant leaves. However, the photomorphogenic responses governed by blue light limit the capacity of plant to photosynthesize and thus, counteract the increase in gas exchange from increasing blue light.

1) C. Gómez, L.G. Izzo (2018) AIMS Agriculture and Food, 2018, 3(2), 135-153
2) B. Bugbee (2016) VIII International Symposium on Light in Horticulture, 1134
3) C. Barnes, T. Tibbitts, J. Sager, G Deitzer, D. Bubenheim, G. Koerner, B. Bugbee (1993) HortScience, 28(12), 1197-1200
4) M. Olle, A. Viršile, (2013) Agricultural and Food Science, 22(2), 223-234

1.3 = MADS-box genes expressed in floral buds of *Trithuria submersa*

Silvia Moschin[1,2], Sebastiano Nigris[1,2], Leonardo Bruno[3], Adriana Chiappetta[3], Maria Beatrice Bitonti[3], Giorgio Casadoro[1], 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 58/B, 35121 Padova, Italy; [3]Department of Biology, Ecology and Earth Sciences (DiBEST), University of Calabria, Via Pietro Bucci, 87036 Arcacavata di Rende (CS), Italy

Current botanical research shows growing interest in extant plant species that trace back their ancestry to the early angiosperms. Among the early-diverging extant angiosperm lineages, *Trithuria*, the only genus of the Hydatellaceae family, is particularly interesting as its phylogenetic position has been quite recently reconsidered. Indeed, this family has been transferred from their former placement in the monocots, close to the grasses, to the water-lily order Nymphaeales (1, 2).

The genus *Trithuria* currently comprises 12 species: 10 from Australia, one from India, and one from New Zealand. They are small and inconspicuous plants that received little attention from botanists prior to their taxonomic reassignment.

Trithuria submersa is a species found in seasonal, rain-fed wetlands of south-western Western Australia, as well as parts of southern New South Wales, South Australia, Victoria and Tasmania (3). *In vitro* cultivation of *T. submersa* has been recently developed, thus allowing to set up a deeper investigation of its biology (4). To date the studies of *Trithuria submersa* have mostly concentrated on characterizing vegetative and reproductive morphologies, but also some ecological and genomic aspects (5). Instead, molecular data are still very scarce, as they mostly regard sequences of few molecular markers.

Giving this lack of molecular data and its considerable phylogenetic position among the early-diverging angiosperms, we cultivated *Trithuria submersa in vitro* and set up an RNA-sequencing of its floral buds, with the first main purpose to identify MADS-box players involved in the formation of its reproductive units. Among the isolated transcripts we identified many different MADS-box sequences belonging to the MIKC-type group. We are currently studying and characterizing them, performing also some phylogenetic analyses. One interesting result regards the isolation of only one expressed *SEP*-like, belonging to the *AGL2* clade, differently from what has been shown in *Amborella trichopoda*, but consistently with data concerning other water-lilies.

A careful morphological observation of its tiny reproductive structures is flanking the molecular study. Moreover, we are setting up in situ hybridization on the tiny floral buds, with the purpose to do expression analyses of the MADS-box isolated.

Trithuria may offer important clues to the evolution of reproductive function among early angiosperms and Nymphaeales *sensu lato* in particular, and this study could be helpful to better define which type of key regulatory genes are necessary to develop these interesting flowers.

1) J.M. Saarela, H.S. Rai, J.A. Doyle, P.K. Endress, S. Mathews, A.D. Marchant, B.G. Briggs, S.W. Graham (2007) Nature, 446, 312–315
2) T. Borsch, C. Löhne, J. Wiersema (2008) TAXON, 57, 1052-1081
3) D.D. Sokoloff, M.V. Remizowa, T.D. MacFarlane, P.J. Rudall (2008) TAXON. 57, 179-200
4) R.G. Kynast, J.A. Joseph, J. Pellicer, M.M. Ramsay, P.J. Rudall (2014) Am. J. Bot. 101, 1447–1455
5) M.L. Taylor, T.D. MacFarlane, J.H. Williams (2010) Ann. Bot. 106, 909–920

1.3 = Genetic and molecular pathways regulating the development of fleshy structures involved in the reproduction of *Taxus baccata* and *Ginkgo biloba*

Sebastiano Nigris[1,2], Silvia Moschin[1,2], Chiara Tessari[1,2], Cheyenne Dell'Aquila[1,2], Giorgio Casadoro[1], 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 58/B, 35121 Padova, Italy

Seed plants evolved a wide range of mechanisms for seeds dispersal, a critical step for their reproductive success. Development of fleshy fruits is a distinctive trait of many angiosperms, from early diverging angiosperms to eu-dicots. The production of characteristic aromatic molecules, attractive colours and nourishing tissues attracts frugivore animals and allows an efficient seeds dispersal. Fleshy fruits are extensively studied as model of fruit development, in particular for their social and economic relevance in human nutrition. However, the appearance of fleshy structures related to the seed is not an angiosperm prerogative. In fact, the development of different forms of fleshy tissues has been observed in some gymnosperms. In yaw (*Taxus baccata*) the mature seed is enveloped by a fleshy, soft, non-poisoning red aril, that attracts birds. Similarly, also *Ginkgo biloba* seeds, at their full maturation, present the embryo surrounded by an external fleshy and smelly tissue.

The mechanisms regulating the development of these fleshy structures have been poorly investigated so far; however, the few data already existing indicate that these regulative pathways might somehow resemble the molecular pathway involved in fleshy fruit development ad maturation. In this context, different RNA-seq experiments were performed to identify the most differentially expressed genes during the formation of the fleshy structures in *Taxus baccata* and *Ginkgo biloba*. In particular, we focused on transcription factor coding genes and hormone biosynthesis-perception related genes, isolating a dataset of putatively involved genes. These genes encompass MADS-box genes, ethylene and auxin related genes, and a group of cell growth genes linked to the ovule and aril thickening. These preliminary data will allow an extensive study of the ovule developing and aril formation in the gymnosperm *Ginkgo biloba* and *Taxus baccata*.

1.3 = Influence of nitrogen supply to wheat caryopsis development

Lara Reale[1], Martina Cerri[1], Adolfo Rosati[2], Paolo Benincasa[1]
[1]Department of Agricultural, Food, and Environmental Sciences, Borgo XX Giugno 74, University of Perugia, Italy; [2]CREA-OLI, Spoleto (PG), Italy; lara.reale@unipg.it

Wheat is an important cereal crop with a high nitrogen (N) fertilization requirement to enable the grain protein accumulation necessary for high quality processing. On the other hand, high N fertilization rates cause potential negative environmental impact via leaching and/or gaseous N losses, so crop production and N management present a sustainability dilemma. Previous researches carried out, by our group, in two cultivar of common wheat (BORA, with large sized grains of about 45-50 mg, and BOLOGNA, with small sized grains of about 30-35 mg) indicated that ovary size varies significantly with agronomical/environmental conditions (i.e., nitrogen and density treatments, and season), while grain size is more conservative within cultivars [1]. The cultivar's typical grain size can be achieved, if resources are not limiting, independent of large variations in ovary size. In new studies, a protocol based on N supply at different rates and times was applied to the same cultivars (BORA and BOLOGNA) to verify the effects on caryopsis development also in condition of limiting resources. Results obtained may contribute to optimize N fertilization for obtaining high yield and quality together with reduced environmental impact. The fresh and dry weight and the size of caryopsis were measured, as well as the grain protein and N contents. Results highlighted the importance of the timing of N supply in the caryopsis development and in its N and protein content and evidenced some differences between the investigated cultivars.

1) P. Benincasa, L. Reale, E. Tedeschini, V. Ferri, M. Cerri, S. Ghitarrini, B. Falcinelli, G. Frenguelli, F. Ferranti, B.E. Ayano, O. Porfiri, A. Rosati (2017) The relationship between grain and ovary size in wheat: An analysis of contrasting grain weight cultivars under different growing conditions. Field Crop Research, 210, 175–182

1.3 = Effect of Temporary Immersion System on *in vitro* development of *Himantoglossum robertianum* (Loisel.) P. Delforge (Orchidaceae)

Elisabetta Sgarbi[1], Raffaella Ranieri[1], Giulia Santunione[1]
[1]Department of Life Sciences, University of Modena and Reggio Emilia, Via Amendola, 2, 42122 Reggio Emilia, Italy

Orchids reveal to be amazing plants when their life cycle, reproductive strategies and ecological relationships are investigated. They go through different and peculiar steps, from seed germination to adult plant, in which they change their trophic status. In the first phase of germination they can establish a specific relationship with symbiotic fungi, which supply nutrient to embryos – seeds have not storage tissues - and protocorms. In these early developmental stages, orchids are myco-heterotrophic, *i.e.* totally dependent by fungi for sustenance (1, 2). This condition is particularly binding for terrestrial orchids, which also in the adulthood maintain a symbiotic relationship with fungi at root level (3). To grow orchids out of their natural habitat is possible by applying *in vitro* technics, providing them inorganic macro- and microelements, source of carbon and nitrogen, often in organic form (4). Despite appropriate media and favourable culture conditions are now available, the seeds of some orchid species require a long time to germinate and to develop protocorms, and a lot of months to grow plantlets. The main aim of this work has been to evaluate if the application of a Temporary Immersion System (TIS), in which plants are periodically immersed in liquid medium following optimized cycles, is effective to improve orchids growth in comparison with traditional *in vitro* culture systems. In this study, we selected three accessions of seeds of *Himantoglossum robertianum*, maintained in conservation in the Unimore Seed Bank. The seeds, after scarification treatment with NaOCl solution, were sown on BM_2 medium (5) with some modification: 1g/L activated charcoal, 1ml/l PPM®, 6g/l Plant Agar were added before autoclaving. Only one accession of seeds, namely SBOA0205, gave high percentage of germination and a sufficient number of protocorms to set up all the following tests. In a first test, 50 little protocorms (diameter 2-3 mm) were taken from agarized medium and transferred both on the same medium without activated charcoal in Microbox vessels, and in Plantform Bioreactors (TIS) (6), in this last using BM_2 medium without activated charcoal and Plant Agar. Air inside Plantform was renewed for 10 min every 4 hours, whereas the condition of temporary immersion were 10 min every 8 hours. This first test has been repeated twice, within two months of each other. A second test has been carried out with the same experimental design using larger protocorms (about 5 mm diameter), which already presented a shoot apex. All cultures were maintained in a growth chamber at 23 °C, 12-h photoperiod for 33-36 days. The growth of protocorms were evaluated as Relative Growth Rate (RGR) index, based on the initial and final fresh weights of the plant material and the time of culture. The comparison of protocorm growth on agarized medium and in TIS in first test did not show any difference, resulting an RGR of 2.05 and 2.23, respectively. Interestingly, when this first test was considered by maintaining separate each of two repetitions, a significant difference emerged. In the first one, RGR was 2.78 whereas in the second was 1.49. This result could be related to the characteristics of a natural population of seeds, as protocorms firstly used are those that firstly developed in culture, likely individuals with higher growth potential. During the second test an evident and definite protocorm development was observed, as many plantlets with green leaves and roots occurred both in Microbox and in Plantform within one month. However, a noticeable increase of growth was obtained in TIS (RGR 2.06) in comparison with that observed agarized system (1.36 RGR).

TIS is known to be a useful device to obtain a rapid and effective propagation *in vitro*, until now widely applied in micropropagation. A lot of advantages, *i.e.* better nutrients supply and more effective dispersal of toxic metabolites can be obtained with bioreactors based on TIS (7, 8). In the light of these first results, Plantform system may be considered a good biotechnological device to grow terrestrial orchids in comparison with traditional systems, mainly to increase plant biomass.

1) H.N. Rasmussen (1995) Terrestrial orchids, from seed to mycotrophic plant. Cambridge Univ. Press.
2) J. Leake (1994) New Phytol, 127, 171-216
3) M. Girlanda, R. Segreto, D. Cafasso, H.T. Liebel, M. Rodda, E. Ercole, *et al.* (2011) Am J Bot, 98, 1148-1163
4) E. Sgarbi, M. Grimaudo, C. Del Prete (2007) J. Eur. Orch, 39, 611-624
5) J.M Van Waes, P. C. Debergh (1986) Physiol Plant, 67, 253–261
6) M. Welander, J. Persson, H. Asp, L.H. Zhu (2014) Sci Horticult, 179, 227–232
7) M. Escalona, G. Samson, C. Borroto, Y. Desjardins (2003) InVitro Cell Dev Biol Plant, 39, 651–656.
8) E. Gatti, E. Sgarbi, E. A. Ozudogru, M. Lambardi (2017) Plant Biosystems, 151, 1129-1136

1.4 = Anatomical and eco-physiological responses of Salanova Green and red lettuce (*Lactuca sativa* L.) Plants under different VPD conditions

Chiara Amitrano[1], Antonio Pannico[1], Youssef Rouphael[1], Stefania De Pascale[1], Carmen Arena[2], Veronica De Micco[1]
[1]Department of Agricultural Sciences, University of Naples Federico II, Via Università 100,80055 Portici (NA), Italy;
[2]Department of Biology, University of Naples Federico II, Via Cinthia 21, 80125 Napoli, Italy

Vapour Pressure Deficit (VPD) is one of the main drivers for transpiration in plants and it mainly changes with Temperature (T) and relative humidity (RH). Through VPD control in indoor cultivations, it is possible to ameliorate plant productivity and photosynthetic performance in terms of Evapo-transpiration (ET), Water Use Efficiency (WUE) and stomatal conductance (g_s). Since VPD increases when air T rises, climate warming conditions are expected to upraise its level (1) with negative consequences especially under water shortage (2,3). The optimization of the water use for crop production is a challenge for assuring an eco-friendly and sustainable agriculture, especially in arid and semi-arid areas. VPD regulation in controlled environment represents a strategy to achieve a valuable crop production (yield and product quality) in a sustainable way.

The aim of this study was to investigate the relationships between structural and functional leaf traits, in plants growth in controlled chambers (Fig.1) under a VPD changing environment. Green and red cultivars of *Lactuca sativa* L. 'Salanova' were grown under two different VPD conditions, namely VPD 0.59 kPa (Low VPD) and 1.76 (High VPD). More specifically, 1-week old plants were transplanted into pots on peat and perlite substrate and incubated into a growth chamber where the two different levels of VPD were achieved by keeping temperature at 24 °C and varying relative humidity. Plants were irrigated to field-capacity and weighted every-day in order to record daily ET. Evaporation losses from soil were minimized by covering the substrate with a plastic film. Furthermore, every 3 days, plant growth was assessed measuring plant total area and number of leaves, while infra-red measurements were carried out in order to record leaf temperature. After 30 days, on fully developed leaves, both anatomical and eco-physiological analyses were performed. The anatomical analyses were aimed to characterize the conductive resistances and plant-related specific traits such as stomata quantification, vein and overall functional anatomical traits in the mesophyll. Gas exchange and chlorophyll "a" fluorescence were utilised to assess the plant physiological behaviour in response to different VPD condition (Fig.2). Results showed that changes in VPD induced a fine tuning of anatomical and eco-physiological traits in lettuce plants and were responsible for their different development. Plants grown under the low VPD environment, reduced daily ET, also decreasing the daily water requirement, and enhanced WUE. Such a phenomenon should be taken into account when crop species are grown in protected cultivation.

Fig. 1. Green and red lettuce plant in growth chamber

Fig. 2. Leaf gas-exchange measurements in lettuce plants in chamber

1) Will, R. E., Wilson, S.M., Zou, C.B., Hennessey, T.C. (2013) Increased vapor pressure deficit due to higher temperature leads to greater transpiration and faster mortality during drought for tree seedlings common to the forest-grassland ecotone.*NewPhytologyst,* 200, 366-374.

2) McDowell, N.G., Allen, C.D. (2015) Darcy's law predicts widespread forest mortality under climate warming. Nature ClimateChange, 5, 669-672.

3) Anderegg, W.R.L., Flint, A., Huang, C.-y., Flint, L., Berry, J.A., Davis, Frank W., et al. (2015) Tree mortality predicted from drought-induced vascular damage. Nature Geoscience, 8, 367-371

1.4 = Metal detoxification and homeostasis: the key role of phytochelatin synthase from cyanobacteria to land plants

Erika Bellini[1,2], Lorenza Rugnini[2], Andrea Andreucci[1], Monica Ruffini Castiglione[1], Alessandro Saba[3], Laura Bruno[2], Luigi Sanità di Toppi[1]
[1]Dipartimento di Biologia, Università di Pisa, via Luca Ghini 13, 56126, Pisa, Italia; [2]Dipartimento di Biologia, Università di Roma "Tor Vergata", Via della Ricerca Scientifica 1, 00133, Roma, Italia; [3]Dipartimento di Patologia Chirurgica, Medica, Molecolare e dell'Area Critica, Università di Pisa, via Paolo Savi 10, 56126, Pisa, Italia

The enzyme phytochelatin synthase (PCS) is a ubiquitous enzyme in land plants, and it is involved in the synthesis of some peculiar thiol-peptide compounds, the so-called phytochelatins (PCn) (1). PCn production represents indeed a widespread strategy addressed to detoxification of several harmful metal(loid)s, e.g. cadmium, mercury, lead, silver, arsenic (2). However, previous sporadic studies carried out in angiosperms (especially in the model-plant *Arabidopsis thaliana*) suggest that PCS and PCn might also play a role in regulating the intracellular homeostatic needs of metal micronutrients, mainly zinc and copper. The possible involvement of the PCS in a primeval physiological phenomenon such as the homeostatic control of metal micronutrient needs might be also supported by the widespread and constitutive presence of PCS not only in angiosperms, but also in early plants, including some bryophytes, charophytes, marine and freshwater algae, in some fungi, lichens, and even in certain animal species (3,4). Moreover, PCS-like proteins, which share significant sequence homologies with plant PCSs, were identified in some photoautotrophic prokaryotes, such as the cyanobacterium *Nostoc* sp. PCC 7120 (5). Proteins encoded by the cyanobacterial genes seem to be progenitor or primitive forms of PCS and to represent an early stage in the evolution of the enzyme in photoautotrophic organisms. However, only a few handful investigations have been carried out in order to functionally characterize early PCSs from bryophytes and cyanobacteria, despite the importance of these autotrophic organisms as fundamental landmarks in plant evolution. Thus, in the study here presented we focused our efforts on the evolutionary relevance of PCS, by investigating its activity and function in phylogenetically-relevant photoautotrophic organisms. First of all, we investigated functional differences in eukaryotic and prokaryotic PCS, studying the enzyme activity in the model liverwort *Marchantia polymorpha* and in three cyanobacterial strains (*Nostoc* sp. PCC 7120, *Geitlerinema* sp. PCC 7407 and *Gloeobacter violaceous* PCC 7421). Accordingly, we set up and validated an efficient and versatile HPLC-ESI-MS-MS method to simultaneously assay GSH and $PC_{2\text{-}4}$ in these organisms and in *A. thaliana*. In all species investigated, the results demonstrated that PCSs – even the prokaryotic ones – were able to catalyze a full transpeptidation reaction leading to the production of PCn in the presence of heavy metals such as cadmium, thus evidencing a remarkable conservation of the PCS function among different photoautotrophic organisms. The second mechanistic point we investigated is based on the following hypothesis: is the PCS necessary for the homeostatic control of physiological needs of some metal micronutrients? Consistently, we are studying in all the above-mentioned species the expression and activity of the PCS enzyme in conditions of total starvation and in the presence of physiological concentrations of iron(II)/(III), zinc and copper.

1) Grill, E., Winnacker, E.-L., Zenk, M.H., 1985. Phytochelatins: The Principal Heavy-Metal Complexing Peptides of Higher Plants. Science 230, 674–676. https://doi.org/10.1126/science.230.4726.674

2) Gupta, D.K., Vandenhove, H., Inouhe, M., 2013. Role of Phytochelatins in Heavy Metal Stress and Detoxification Mechanisms in Plants, in: Gupta, Dharmendra K., Corpas, F.J., Palma, J.M. (Eds.), Heavy Metal Stress in Plants. Springer Berlin Heidelberg, Berlin, Heidelberg, pp. 73–94. https://doi.org/10.1007/978-3-642-38469-1_4

3) Fontanini, D., Andreucci, A., Ruffini Castiglione, M., Basile, A., Sorbo, S., Petraglia, A., Degola, F., Bellini, E., Bruno, L., Varotto, C., Sanità di Toppi, L., 2018. The phytochelatin synthase from Nitella mucronata (Charophyta) plays a role in the homeostatic control of iron(II)/(III). Plant Physiology and Biochemistry 127, 88–96. https://doi.org/10.1016/j.plaphy.2018.03.014

4) Degola, F., De Benedictis, M., Petraglia, A., Massimi, A., Fattorini, L., Sorbo, S., Basile, A., Sanità di Toppi, L., 2014. A Cd/Fe/Zn-Responsive Phytochelatin Synthase is Constitutively Present in the Ancient Liverwort Lunularia cruciata (L.) Dumort. Plant and Cell Physiology 55, 1884–1891. https://doi.org/10.1093/pcp/pcu117

5) Tsuji, N., Nishikori, S., Iwabe, O., Shiraki, K., Miyasaka, H., Takagi, M., Hirata, K., Miyamoto, K., 2004. Characterization of phytochelatin synthase-like protein encoded by alr0975 from a prokaryote, *Nostoc* sp. PCC 7120. Biochemical and Biophysical Research Communications 315, 751–755. doi:10.1016/j.bbrc.2004.01.122

1.4 = Growth and stress-related biochemical parameters respond to biostimulant activity of spelt husk extracts in maize plants subjected to salinity

Chiara Ceccarini[1], Fabiana Antognoni[2], Stefania Biondi[3], Alessandra Fraternale[1], Giancarlo Verardo[4], Andrea Gorassini[5], Valeria Scoccianti[1]

[1]Dipartimento di Scienze Biomolecolari, Università di Urbino Carlo Bo, 61029 Urbino, Italy; [2]Dipartimento di Scienze per la Qualità della Vita, Università di Bologna, 47921 Rimini, Italy; [3]Dipartimento di Scienze Biologiche, Geologiche e Ambientali, Università di Bologna, 40126 Bologna, Italy; [4]Dipartimenti di Scienze Agroalimentari, Ambientali e Animali, Università di Udine, 33100 Udine, Italy; [5]Dipartimento di Studi Umanistici e del Patrimonio Culturale, Università di Udine, 33100 Udine, Italy

In the natural environment, plants are exposed to a complex set of biotic and abiotic stresses that can negatively influence their survival and growth. Owing to limited rainfall and high evapotranspiration, coupled with poor soil and water management practices, salinity represents a major abiotic stress that affects plants growth and biomass production as well as yield of staple food crops in arid and semi-arid regions of the world[1]. Although the general perception is that salinization only occurs in the regions mentioned above, no climate zone is free from this problem[2]. Biostimulants are proposed as an innovative solution to address the challenge of a sustainable agriculture. Due to their heterogeneous composition, they are able to improve the nutrient use efficiency of plants and enhance tolerance to biotic and abiotic stresses[3]. In this work, we evaluated the possible biostimulant effect of two extracts obtained from spelt (*Triticum dicoccum* L.) husks against salt stress (100-200 mM NaCl) in maize plants. The phytochemical composition of the two extracts, containing respectively the soluble-conjugated (SC) and the insoluble-bound (IB) fraction of phenolic compounds, was investigated through GC-MS and quantification was carried out through HPLC-DAD. In both extracts, five phenolic acids were detected, two simple phenolic and three hydroxycinnamic acids, with a predominance of ferulic and *p*-cumaric acids, that are 25-fold higher in IB compared to SC. In addition, GC-MS analysis showed the presence of fatty acids in SC that are absent in IB extract. In order to estimate the efficacy of the extracts against salinity, we investigated their effects on several stress-associated parameters, such as proline, malondialdehyde, H_2O_2, GSH, photosynthetic pigments, and ion contents. The responses of salt-stressed maize plants appear to be differentially modulated by the two extracts, and their composition may account for these differences. IB showed a stronger protective effect and, even at the lower concentration tested, was able to recover the performance of stressed plants in terms of growth and levels of the stress markers. Results also indicate that the biostimulant activity of IB strictly depended on timing of treatment. In particular, both pre-treatment (i.e., during the two days preceding exposure to salt) and post-treatment (i.e., starting two days after and throughout exposure to salt) of maize plants with IB, allowed for a recovery of shoot growth at control levels and a reduction of stress-induced proline accumulation. The positive effect on photosynthetic pigments only occurred when IB was applied to plants before salt stress, and only the pre-treatment with the higher dose was effective in mitigating oxidative stress. Although in some cases SC and even methanol alone exerted some positive effects, they could also be deleterious, whereas IB never was. Overall, results indicate that a polyphenol-containing extract obtained from spelt by-products can behave as biostimulant in maize plants and can mitigate their response to salt stress, by acting on different biochemical targets. Experiments are in progress to evaluate changes in phenolic profile, and in the antioxidant response of seedlings treated with both extracts, supplied either as pre- or as post-treatment.

1) Munns R. (2002) Comparative physiology of salt and water stress. Plant, Cell and Environ. 25, 239-250

2) Rengasami P. (2006) World salinization with emphasis on Australia. J. Exp. Bot. 57, 1017-1023

3) Povero G., Mejia J., Di Tommaso D., Piaggesi A., Warrior P. (2016) A systematic approach to discover and characterize natural plant biostimulants. Front. Plant Sci. 7, 1-9

1.4 = Bryophytes in mining areas: the case of study of “Barraxiutta”, South-West Sardinia (Italy)

De Agostini Antonio[1], Hernández Antonio[3], Esteban Raquel[4], Cortis Pierluigi[1], Vacca Andrea[2], Cogoni Annalena[1]

[1]Department of Life and Environmental Sciences, University of Cagliari, Botany Section, Viale S. Ignazio 1, 09123, Cagliari (CA), Italy; [2]Department of Chemical and Geological Sciences, University of Cagliari, Cittadella Universitaria (Blocco D) – S.S. 554, 09042, Monserrato (Ca), Italy; [3]Departamento de Biología Vegetal y Ecología, Universidad del País Vasco (UPV/EHU), Bilbao, Spain; [4]BC3-Basque Centre for Climate Change, Scientific Campus of the University of the Basque Country, Leioa, Spain

The characterization of bryophyte diversity in abandoned mining areas, strongly compromised by heavy metals and metalloids pollution (both water and soil), could help identify those *taxa* able to survive extreme ecological conditions and heavy metal stress. These species could be suitable for phytoremediation and phytostabilization of environmental polluted sites (1). Sardinia (Italy), hosted an intense mining activity and nowadays many abandoned mining areas are present in the island. In this study, we report the species that are present on a tailing dump of about 2000 m^3, located in the abandoned mining area of “Barraxiutta” (South West of Sardinia). This heap consists of the residues from the flotation process of the extracted material (2) and its granulometry consist of mainly sand and coarse silt. The dump is characterized by elevated concentrations of many heavy metals and metalloids (among the others Fe 55,98 mg/g; Pb 5,21 mg/g; Cd 0,15 mg/g; Zn 13 mg/g etc.) and as it concerns the extractable fraction in acetic acid in respect to the total content, it consist in the 60% of Cd, 49% of Pb, and in the 37% of Zn (unpublished data). A sampling of the bryophytic component was carried out during the late winter/early spring of 2018 on the tailing dump, leading to the identification of several species, mainly belonging to the *Bryophyta* division, forming dense populations all over the tailings, preferentially in the moist and shady erosion channels. To follow, the species list: *Dicranella howei*, *Tortella humilis*, *Rhyncostegium murale*, *Didymodon luridus*, *Fossombronia caespitiformis* and *Fissidens gracifolius*.

Among these, *D. howei* and *R. murale* were the wider distributed, reaching high ground coverage. On these two species, different physiological parameters (photochemical efficiency and antioxidant capacity) were measured and results compared with individuals from control area close to the mining area. We aim to determine if soil pollution could have led to the occurrence of physiological stress/adaptation in the individuals growing on the tailing dump.

1) Barrutia, O., Artetxe, U., Hernández, A., Olano, J. M., García-Plazaola, J. I., Garbisu, C., & Becerril, J. M. (2011) Native plant communities in an abandoned Pb-Zn mining area of Northern Spain: Implications for phytoremediation and germplasm preservation. International Journal of Phytoremediation, 13(3), 256-270. https://doi.org/10.1080/15226511003753946

2) De Waele, J., Pisano, M. (1998) Interazione fra attività mineraria ed un acquifero carsico: l’esempio di Barraxiutta (Sardegna sud-occidentale). In Convegno Nazionale sull’Inquinamento delle Grotte e degli Acquiferi carsici e possibili ricadute sulla collettività (pp. 195-209)

1.4 = Redox-dependent molecular responses activated in rice under salt stress exposure: a focus on roots and leaves

Valentina Giacinti[1], Sara Cimini[1], Vittoria Locato[1], Laura De Gara[1]
[1]Unit of Food Science and Human Nutrition, Campus Bio-Medico University of Rome, via Alvaro del Portillo 21, 00128 Rome, Italy

Rice is an interesting species for its agronomic relevance and for being one of the most sensitive cereals to abiotic stresses, salinity first[1]. The generation of specific ROS signature is pivotal for the activation of homeostatic responses against stress and for ensuring plant fitness in resistant species[2].

In order to improve our knowledge on signalling pathways triggering defence responses against salinity, rice varieties showing different salt sensitivity have been investigated, with particular attention to the alteration of redox metabolism of leaves and roots. Analysis of key pathways regulating ROS intracellular content and cellular viability/development have been performed at level of metabolic profiles, gene expressions and enzyme activities. Moreover, redox-dependent regulatory mechanisms, such as post-translational modifications have been evaluated.

Data here presented indicate that plants with different capability to counteract salt stress are characterized by different basal metabolic profiles as well as by different capability to modulate various players of redox metabolism. Our results contribute to describe ROS and different antioxidative pathways as a part of a complex redox network for optimizing plant responses against salinity.

A better knowledge of the mechanisms acting in tolerant varieties will allow the identification of effective strategies aimed at increasing rice resilience toward salt stress.

1) Maiti, R., Satya, P., Research advances in major cereal crops for adaptation to abiotic stresses. GM Crops & Food, 5(4), 259-279

2) Formentin, E., Sudiro, C., Ronci, M.B., Locato, V., Barizza, E., Stevanato, P., Ijaz, B., Zottini, M., De Gara, L., Lo Schiavo F., H2O2 signature and innate antioxidative profile make the difference between sensitivity and tolerance to salt in rice cells. Frontiers in Plant Science, 871, 1549

1.4 = Effects on nutrient uptake and rhizo-microbiome in quinoa grown on compost amended soil irrigated with saline water

Francesco Guarino[1], Angela Cicatelli[1], Guido Lingua[2], Karina B. Ruiz[3], Giovanni Vigliotta[1], Gianmaria Oliva[1], Fatima Maietta[1], Stefano Castiglione[1]
[1]Department of Chemistry and Biology "A. Zambelli", University of Salerno, Fisciano (SA), Italy; [2]Department of Science and Innovation Technology, University of Piemonte Orientale, Alessandria (AL), Italy; [3]Department of Desert Agriculture and Biotechnology, University Arturo Prat (UNAP), Iquique, Chile

Salinization represents a constant and heavy legacy of modern agriculture, due to the use of saline water for irrigation, seawater intrusion in cultivated coastal areas, etc.. Salinization causes land degradation, chemical, physical and biological alterations of soils, resulting in loss of fertility and, as consequence, it affects agriculture productions. Among the halophyte plant species, quinoa (*Chenopodium quinoa* Willd.) has recently drawn the attention of researchers for its nutrient content, and its capability to withstand several abiotic stresses, making it a precious resource for food security in a climate change scenario. All these features make quinoa a good plant species candidate for environmental reclamation, in particular of salt affected soils.

The purpose of our research was to study the phyto-desalisation capability of quinoa in the presence of compost (CMP), and the effects of salt on macro- and micro-nutrient uptake and rhizo-microbiome. The CMP soil amendment introduces new organic matter and microorganisms, providing environmental and nutritional conditions useful to select bacteria or fungi suitable for saline soil reclamation. Therefore, the aims of the present study were to: i) evaluate the effects of NaCl on growth and biomass production of quinoa on soils amended, or not, with CMP; ii) investigate the phyto-desalination capability of quinoa; iii) isolate and characterize genetically and biochemically salt tolerant rhizo-bacterial strains; iv) study the microbiome associated to the quinoa rhizosphere, through both culture dependent and independent approaches. To these purposes, quinoa plants, cultivated with or without CMP, were exposed to 150 or 300 mM of NaCl for two months. At the end of the experimentation, plants were harvested, separated into roots, stems and leaves, and their dry weight determined. Sodium, macro- and micro-nutrients (K, Mg, Ca, P, Fe) were measured both in soil (at beginning and at end of the experimentation) and in the different plant organs. Moreover, fine roots were harvested and shaked in sterile physiological solution (9 g L^{-1} NaCl) in order to investigate the quinoa rhizo-bacterial community. The rhizo-microorganisms were, in part, plated on agar (PCA) with or without NaCl. Bacteria were initially selected using a NaCl concentration of 500 mM. The bacteria colonies, grown on the control plates (no NaCl addition) and those selected for NaCl resistance (NaCl 500 mM), were rescued and used for Next Generation Sequencing (NGS) analysis. The remaining physiological solution was processed for the total rhizo-microbiome analysis (NGS culture independent approach).

Our study highlighted that quinoa tolerates and easily grows on highly saline soils (conductivity > 16 dS m^1); in fact, its biomass was unaffected by the high salt soil content but, increased consistently with CMP amendment. The chemical analyses of the macro- and micro-nutrients revealed that NaCl did not affect K and Mg concentrations in the different plant organs. The Ca concentration, in the experimental theses amended with CMP, increased when we considered 150 mM NaCl, whilst it decreased in the other NaCl experimental thesis (300mM). Furthermore, the addition of salt (for both NaCl experimental theses) reduced the P concentration in stems and leaves respect to the CMP thesis. The presence of salt induced an increase of Na uptake and translocation in quinoa stems and leaves in agreement with the increasing NaCl concentrations used for irrigation. This was the opposite respect to the trend of Fe uptake, in fact, it decreased considering the two NaCl experimental theses. Salt addition heavily affected the quinoa rhizo-microbiome, even in the presence of CMP, though, in this case, the microbiome resulted more resilient respect to the NaCl thesis without CMP amendment, both in terms of unit forming colonies (UFC), and rhizo-bacteria biodiversity. Moreover, from the quinoa rhizosphere, exposed to increasing NaCl concentration, we isolated and genetically characterized, 15 bacterial strains resistant up to 3.0 M NaCl. Among these, three strains showed plant promoting features.

In conclusion, our study confirmed the capability of quinoa to grow in presence of saline water, accumulating Na in its epigeous organs without any effects on its health and biomass. Furthermore, we found an interesting relation among the concentrations of saline water added to soils, the Na and Fe contents in the different plant organs. In the perspective of interaction between rhizo-microbiota and plant, new interesting information were produced on the effects of salt on rhizo-microbiome. In fact, we were able to isolate high salt tolerant rhizo-bacteria showing plant growth promoting features.

1.4 = High temperatures affect pollen viability of micro-tom: a model crop for seed-to-seed cycle in space

Maurizio Iovane[1], Luigi Gennaro Izzo[1], Giovanna Aronne[1]
[1]Department of Agricultural Sciences, University of Naples Federico II, via Università 100, 80055 Portici, Italy

In recent years research activities in agricultural science are mainly focused on maximizing crop productivity in high-tech controlled environments. This approach is particularly relevant to implement bioregenerative life support systems (BLSS) for long-duration spaceflight manned missions.

In the BLSSs plants have the key role to recycle water and air and produce fresh food. The choice of candidate plant species for cultivation in BLSS is a critical issue that goes beyond plant survival, and mainly point on criteria of nutritional value, growth rate, productivity and resources use efficiency. Although many of the plant species currently studied for cultivation in space are grown to produce leafy crops starting from seeds produced on ground, the efficient realization of a crop cultivation in self-sufficient closed habitats requires the full completion of the reproductive cycle. In addition, compared to leaf and other vegetative parts, seeds and fruits have higher nutritional value. In this framework, *Solanum lycopersicum* L. represents one of the best candidate crop able to achieve a full seed-to-seed cycle for space missions.

Among tomato cultivars, Micro-Tom has been considered as a model crop for cultivation in space because of its dwarf habitus and short life cycle. Micro-tom has already been reported to achieve a full seed-to-seed cycle under simulated space environmental factors, such as simulated microgravity and high-LET ionising radiation (1, 2). As for most space biology researches, these experiments were performed focusing mainly on environmental constrains occurring in space and setting the other environmental factors at optimal level. The interactions between space factors and common Earth environmental factors, such as temperature, have been generally overlooked.

General aim of our research was to investigate interactions of simulated gravity and temperature on microsporogenesis and microgametogenesis of Micro-tom. Taken individually, both factors are reported to affect pollen biology of other species (3, 4), but they were not investigated on tomato. More specifically, in this work we hypothesized that temperatures higher than optimal could endanger the microsporogenesis and/or microgametogenesis of Micro-tom.

In order to test the hypothesis, we grew Micro-tom plants in a growth chamber with an air temperature of 22°C ± 1°C, from sowing to the development of the first inflorescences. Thereafter we moved half plants in a growth chamber with an air temperature of 30°C ± 1°C in order to compare the effect of different temperatures on pollen viability during different flowering stages. Pollen viability was assessed by Sigma® DAB Test (for peroxidases) and pollen abortion during microsporogenesis by lactophenol cotton blue stain (LCB). Pollen germinability was tested in a proper medium optimized for tomato pollen. Plants were grown in plastic pots on a 1:1 (v/v) soil and perlite medium. A photosynthetic photon flux (PPF) of 200 μmol m^{-2} s^{-1} with a 16 h d^{-1} photoperiod was provided by white LEDs and relative humidity kept at 70% ± 5% throughout the whole experiment.

Plants grown under the two temperature conditions developed flowers with no visual difference and data showed that viability and germinability of pollen coming from 22°C treatment remained high throughout all flowering stages. Conversely, viability and germinability of pollen coming from 30°C treatment resulted significantly lower than the control treatment. The 30°C pollen showed less than 10% viability and germinability at anther dehiscence when pollination naturally occurs. Furthermore, LCB stain highlighted that microsporogenesis occurs regularly even at high temperature, but pollen loses viability at very early stage of microgametogenesis.

Our results revealed that high temperatures could seriously endanger the seed-to-seed-cycle in Micro-tom. In order to overcome possible constrains in plant life cycle, future studies should focus on the effects of different factors on microsporogenesis and microgametogenesis as possible bottlenecks in seed-to-seed cycle of space model crops.

1) V. De Micco, S. De Pascale, R. Paradiso, G. Aronne (2014) Plant Biology, 16, 31–38
2) C. Arena, E. Vitale, B. Hay Mele, P. R. Cataletto, M. Turano, P. Simoniello, V. De Micco (in press) Plant Biology
3) V. De Micco, M. Scala, G. Aronne (2006) 228, 121-126
4) G. Aronne (1999) Grana, 38:6, 364-367

1.4 = A split root investigation of the physiological response to heterogenous elevated Zn exposure in poplar and willow

Emily Palm[1], Werther Guidi Nissim[1], Elisa Azzarello[1], Stefano Mancuso[1]
[1]Department of Agriculture, Food, Environment, and Forestry (DAGRI), University of Florence, viale delle idee 30, 50019 Sesto Fiorentino Italy

Essential and toxic ions are often heterogeneously distributed within a soil landscape (1,2). This fact complicates phytoremediation efforts to remove toxic ions such as heavy metals from the soil. Plants that are generally tolerant to heavy metal stress with moderate uptake could demonstrate a flexibility in root architecture that leads to changes in physiology and growth, and allow them to select more favorable microenvironments to avoid heavy metal accumulation (3,4). This avoidance strategy is especially relevant to plants selected for their phytoremediation potential based on their rapid biomass accumulation, such as poplar and willow (5). In this study a split-root hydroponic experiment was used to test whether root level exposure to zinc (Zn) stress to all, half or none of the root systems of *Populus deltoides x nigra* (poplar) and *Salix matsudana* (willow) plants alters root and shoot physiology (Fig. 1). Cuttings of both species were split to create two root system halves that were exposed to either control (-Zn) or stress (+ 1mM Zn) solutions for 42 days (Fig. 1). Weekly measurements of gas exchange and photosynthetic pigments showed that poplar was more sensitive to the split stress (-Zn/+Zn) treatment than willow, with lower gas exchange rates, and total chlorophyll and carotenoid values relative to their respective controls (-Zn/-Zn). Split-stress willow plants had increased root growth in the half without Zn, demonstrating a reallocation of carbon with no negative impact to shoot physiology as compared to poplar (Fig. 2). Water consumption was also reduced in split-stress willow but not in poplar, indicating both an increase in water use efficiency in response to heterogeneous Zn exposure, and a potential mechanism to avoid Zn accumulation (Fig. 3) Electrophysiological measurements of membrane electrical activity using the MEA system (6,7) showed an increased number of action potentials generated in response to 1 mM Zn in willow roots (both control and split stress), but not in the non-treated poplar roots in split stress. This difference in membrane electrical activity could play a role in signalling toxic Zn concentrations between root portions and between the root and shoot systems, altering the function and growth of both below and aboveground tissues. On a larger scale, these signficant differences among species in response to patchy distribution of toxic Zn concentration may impact the efficiency of phytoremediation efforts.

Fig. 1 Example of hydroponic set up

Fig. 3 Visual observation of water consumption A = -Zn, B = +Zn

Fig. 2 Harvested roots after 42 days in treatment solutions

1) Jackson RB, Caldwell MM (1993) Ecology 74, 612-614
2) Hodge A (2006) J of Exp Botany 57, 401-411
3) Hodge A (2009) Plant, Cell and Environment 32, 628-640
4) Remans T, Thijs S, Truyens S, Weyens N, Schellingen K, Keunen E, Gielen H, Cuypers A, Vangronsveld J (2012) Annals of Botany 110, 239-252
5) Sheoran V, Sheoran AS, Poonia P (2016) Pedosphere 26, 148-166
6) Masi E, Ciszak M, Stefano G, Renna L, Azzarello E, Pandolfi C, Mugnai S, Baluška F, Arecchi FT, Mancuso S (2009) PNAS 106, 4048-4053
7) Redwan M, Spinelli F, Marti L, Bazihizina N, Azzarello E, Mancuso S, Masi E (2017) Env Exp Botany 143, 20-28

1.4 = Rhizosphere response to Ni stress in the facultative hyperaccumulator *Alyssoides utriculata* (L.) Medik.

Enrica Roccotiello[1*], Stefano Rosatto[1], Grazia Cecchi[2], Simone Di Piazza[2], Luigi Vezzulli[3], Mirca Zotti[2], Mauro Mariotti[1]
[1]Lab. of Plant Biology; [2]Lab. of Micology; [3] Lab. of Microbiology - Università degli Studi di Genova, Corso Europa 26, 16136 Genova, Italy
*corresponding author: enrica.roccotiello@unige.it

The contamination of metals like Nickel (Ni) represents a serious threat worldwide in the soil. To counteract this phenomenon, hyperaccumulator plant species, able to remove metal from soil and store it at high concentration in shoots, are employed for metal phytoremediation purposes. Native microbial communities occurring in the rhizosphere of hyperaccumulators often promote plant growth and metal uptake.

So far, each abiotic and biotic rhizospheric components (soil, root system and microbiota) have been used without considering the reciprocal interactions within the responses to Ni stress. The present study aims to develop for the first time an innovative and multidisciplinary approach to examine the rhizosphere of Ni-hyperaccumulators as a holistic model, promoting the plant development and the Ni uptake.

Among metalliferous soils, specific attention was given to serpentine which display extremely hostile conditions (nutrient shortage and highly toxic concentration of metals - e.g., Ni, Cr, Co, Mg) for most plants except for some hyperaccumulator species.

Early response to Ni in plant development was assessed with micro- and mesocosm germination tests under Ni stress in the Ni-hyperaccumulator species *Alyssoides utriculata* (L.) Medik., *Noccaea caerulescens* (J. Presl & C. Presl) F. K. Mey., *Odontarrhena bertolonii* (Desv.) L. Cecchi & Selvi, and non-accumulator species *Alyssum montanum* L., and *Thlaspi arvense* L. used for comparison. Afterwards, the response to increasing Ni concentrations in terms of root surface area, root and shoot biomass and photosynthetic efficiency was evaluated.

Subsequently, *A. utriculata* was selected as a good candidate to study rhizospheric components because of its Ni-facultative hyperaccumulation traits and its ability to thrive in harsh metalliferous soils. Related rhizosphere and bare soil samples were collected from serpentine and non-serpentine sites.

Plant and soil samples were processed and analysed with specific attention to isolation and identification of culturable microbiota, then selected for their Ni-tolerance and Plant Growth Promoting (PGP) traits.

Results demonstrate that increasing Ni concentrations can induce marked inhibition of germination in hyperaccumulator species, despite their accumulation ability. However, hyperaccumulator species exhibit a positive response in terms of root surface area, biomass and photosynthetic efficiency, compared to non-hyperaccumulator species in which there is a dose-response effect by Ni, except for *T. arvense* in pot test.

Specifically, *A utriculata* reveals an increased aboveground biomass and sample vitality in pot test, suggesting an adaptation to harsh environmental conditions.

Microbiota isolates (bacteria and fungi) are more abundant in non-serpentinitic and rhizospheric soil, without selectivity between microorganisms and Ni.

Some bacterial and fungal strains (*Pseudomonas* sp. SERP1, *Streptomyces* sp. SERP4 and *Penicillium ochrochloron* Biourge Serp03S, *Trichoderma harzianum* Rifai Serp05S respectively) reveal high Ni tolerance (up to 20 nM) and PGP traits. The selected strains could be promising candidates as natural chelators in the rhizosphere of *A. utriculata,* to enhance plant development and Ni uptake.

This research represents the first step of integrated plant-bacteria-fungi tool, in the perspective to improve Ni uptake from polluted soil, using native Ni-hyperaccumulator species and associated rhizobiota, although further investigations are required to ascertain the efficiency of the field application.

1.4 = Polyphenol, antioxidant, flavonoid and chlorophyll content as a tool for heavy metal stress assessment

Mirko Salinitro[1], Alessandro Zappi[2], Dora Melucci[2], Annalisa Tassoni[1]
[1]Department of Biological Geological and Environmental Sciences, University of Bologna, Via Irnerio 42, 40126, Bologna, Italy; [2]Department of Chemistry "Giacomo Ciamician", University of Bologna, Via Selmi 2, 40126, Bologna, Italy

The aim of the present work was to assess the correlation between secondary metabolites produced by plants and the heavy metal content in aerial parts. We aimed at the use of these metabolites as tools for the evaluation of metal stress and tolerance in non- accumulator plants. We use as a case study the species *Polygonum aviculare* L., an annual plant that grows in disturbed environments. *P. aviculare* seeds were collected in 5 different environments to verify possible differences among populations: 2 urban localities (Milan and Bologna city centres), 2 woodlands localities (Milan and Bologna countryside), 1 serpentine locality (mount Prinzera, Parma). We tested six heavy metals singularly (Cu, Pb, Ni, Cr, Cd, Zn) at four increasing concentrations to evaluate possible differences in the response to each metal. The concentrations used were calculated on the basis of the total concentration of each metal in urban soil. Plants were cultivated in hydroponics for 4 weeks (1 in Hoagland's half strength solution and 3 in Hoagland's half strength solution + heavy metal), before harvesting the shoots. After collection, plants were grinded in liquid nitrogen and used fresh for spectrophotometric analyses or dried for ICP-OES analyses.

Overall, all plants treated with high dosage of metals (independently from the metal) showed toxicity symptoms like stunted growth, chlorotic leaves and in some cases necrosis. Metal concentration in the nutrient solution, was in fact negatively correlated with biomass production. The most severe effect on growth were caused by high copper and lead treatments while chromium resulted to be the less toxic for plants. Interestingly the lowest dosage of cadmium enhanced plant growth if compared to the control treatment, a phenomenon called "hormesis" (1). No differences were found among the five populations when considering metal uptake and the metabolic response to oxidative stress.

The concentrations of metals found in shoots linearly reflected the one in the nutrient solution, therefore *P. aviculare* could be considered as an indicator plant. This linear increase in metal content in shoots, caused a consequent increase in the production of polyphenols, flavonoids and antioxidants in stressed plants (fig. 1a, 1b, 1d). This secondary metabolites in fact, play an important role in plant detoxification systems by acting as metal chelators or directly scavenging reactive oxygen species (2). All the six tested metal appeared to induce similar responses in terms of plant metabolites production, therefore we assumed that plant reaction against oxidative stress was not specific to each metal but only dose-controlled.

Instead the determination of chlorophyll content, showed a decrease of photosynthetic pigments proportional to the increase of metal concentrations in shoots (fig. 1c). This trend was similar for all metals, but interestingly Ni, Cr, Cd and Zn at low dosage stimulated the production of chlorophylls that resulted slightly higher compered to control treatment. Our results, showed a strong positive correlation between metal uptake and polyphenols, flavonoids and antioxidants production, making these molecules good candidates as indicator of plant metal stress.

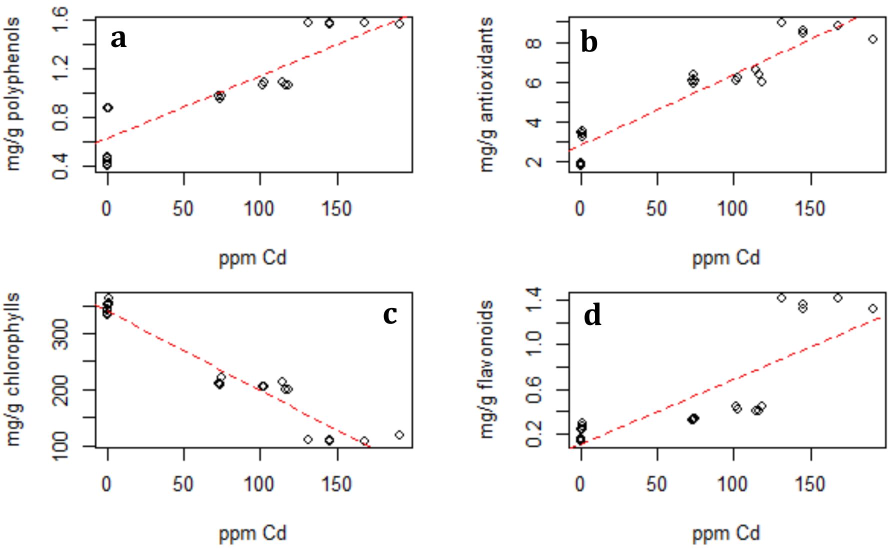


Fig. 1. Effect of cadmium on polyphenols (**a**), antioxidants (**b**) chlorophylls (**c**) and flavonoids (d) content in *P. aviculare*, after 3 weeks of metal stress.

1) E.J. Calabrese, R.B. Blain (2009) Hormesis and plant biology. Environmental Pollution, 157, 42–48
2) Michalak, A. (2006) Phenolic compounds and their antioxidant activity in plants growing under heavy metal stress. Polish Journal of Environmental Studies, 15(4)

1.4 = Effects of abiotic stress induced by a standard mix of atmospheric pollutants in *Chlorella mirabilis* (Chlorophyceae)

Giulia Santunione[1], Giulio Boni[1], Alberto Muscio[1], Elisabetta Sgarbi[1]
[1]Department of Life Science, University of Modena and Reggio Emilia, Via Amendola, 2, 42124 Reggio Emilia, Italy

This study evaluates the level of oxidative stress induced by a mix of standard atmospheric pollutants in the green microalga *Chlorella mirabilis*. Genus *Chlorella* has been found as one of the most widespread among the phototrophic organisms occurring also in urban context, where it forms biofilms on monumental and building surfaces (1, 2). Aerial and sub-aerial algae are considered suitable bio-indicators to study the effects of pollutants because their species-specific sensitivity may be greater than in higher plants along with faster physiological responses. Furthermore, they are easy to handle and to maintain in controlled conditions (3). For these reasons, *Chlorella mirabilis* was used in the present study as testing organism to evaluate the response to atmospheric pollutants. With the aim to apply a lot of pollutants without increase the variability of the system, we chose a standard mix of atmospheric pollutants, *i.e.* ASTM D-7897-2015 (American Society for Testing and Materials International). As result of studies set up on urban surfaces depositions (4,5), used to stress building materials and evaluate their "accelerate ageing" (6), it consists in four kinds of atmospheric particles: soot (carbon), dust, salts, and particulate organic matter, which together represent a dark solution called "soiling mix" (7).

Chlorella mirabilis was firstly grown in a specific culture medium (Bold's Basal Medium, BBM) in aseptic conditions at 23 °C, under 14 h photoperiod. To evaluate the effects of pollutants, algal cultures were carried out at the same time in a new medium (BBM-SM) obtained by mixing "soiling mix" with BBM (1:5 v/v).

Stress level in algae growing inside BBM-SM was assessed through the quantitative determination of photosynthetic pigments (Chlorophyll *a, b* and carotenoids) and malondialdehyde (MDA), this last considered as marker of lipid peroxidation. The extraction of photosynthetic pigments and oxidative metabolites was performed according with the methods of Lichenthaler (8) and Heath and Packer (9), respectively. Cell density was monitored during the experimental tests and the algal growth curve was described in both cultures conditions, after 15 day of algal growth.

Results of repeated tests on algae cultures stressed by pollutants underline an increase over 60% of MDA compared with control cultures, highlighting the occurrence of oxidative stress. At the same time the concentration of chlorophyll *a* and *b* and carotenoids decreases over 60%, 20% and 70%, respectively. Observation under light microscope shows shape and colour changes in algal cells during their growth in BBM-SM. Nevertheless, *C. mirabilis* proved to be able to survive in critical environmental conditions characterized by high concentration of pollutants and dark particulate. These results may represent an important first step to use this species in studies on cell response to different stress factors and moreover may have interesting effects on many fields of applied plant biology.

1) L. Tomaselli, G. Lamentia, M. Boscob, P. Tianoc (2000), International Biodeterioration & Biodegradation, 46, 251 - 258
2) M.F.Macedo, A.Z. Miller, A. Dionisio, C. Saiz-Jimenez (2009) Microbiology, 155, 3476–3490
3) S.M. Geelani, S.J.A. Bhat, H. Geelani, S. Haq S. (2012) Journal of Plant Science and Research, 28, 55-59
4) P. Berdahl, H. Akbari, L.S. Rose (2002) Applied Optics, 41, 12
5) S.E. Bretz, H. Akbari, Hashem Akbari (1997) Energy and Buildings, 25, 159-167
6) G. Santunione, C. Ferrari, C. Siligardi, A. Muscio, E.Sgarbi (2018) Advances in Building Energy Research, 1-18
7) ASTM D7897-18 (2018) American Society for Testing and Materials International
8) H.K. Lichenthaler (1987), Methods in Enzymology, 148
9) R.L. Heath, L. Packer (1968) Archives of Biochemistry and Biophysics, 126, 189-198

1.4 = Cambial zone-specific hormonal profiles of woody poplar root under bending stress

Gabriella S. Scippa[1], Elena De Zio[1], Antonio Montagnoli[2], Mattia Terzaghi[2], Donato Chiatante[2], Karin Ljung[3], Dalila Trupiano[1]

[1]Dipartimento di Bioscienze e Territorio, University of Molise, 86090 Pesche (IS), Italy; [2]Dipartimento di Biotecnologie e Scienze della Vita, University of Insubria, 21100 Varese (VA), Italy; [3]Department of Forest Genetics and Plant Physiology, Swedish University of Agricultural Sciences, 90183 Umeå, Sweden

Root responses to mechanical stress have been extensively investigated on model plant of herbaceous species *Arabidopsis thaliana*, where root bending induces a recruitment of new lateral roots on the convex side (1, 2).

By using a simple experimental system to mimic bending stress condition in poplar woody taproots (*Populus nigra* L.), we observed that the application of mechanical forces in root in secondary structure, as showed in the primary root, induces new lateral roots production on the convex side of bent taproot. Furthermore, on the opposite concave side, reaction wood formation and lignin deposition were also observed. These asymmetrical responses, triggered by different tension and compression forces distribution along the taproot, resulted to be modulated by a complex signaling involving phytohormones and several molecular factors (3, 4, 5, 6, 7, 8). Additionally, we observed between the convex and concave side of the bent taproot a different cambium cells activity and differentiation. Indeed, the number of cambium cells in the concave side was significantly higher than in the convex side (9).

Starting from these observations, in the present work, we investigated if the type (tension and compression) and the intensity of mechanical forces activate specific phytohormone signaling pathways across the two sides of the cambial zone (CZ) to modulate the different responses.

For this purpose, a tissue-specific hormonal profile was carried out, combining:

i) cryosectioning procedures, to isolate from the poplar root CZ of each side the cambial cells, the developing phloem (Ph dev) and xylem (Xy dev);

ii) an innovative and sensitive method based on liquid chromatography coupled to tandem mass spectrometry analysis (LC-MS/MS), to quantify auxins, abscisic acid and cytokinins metabolites.

Results showed a strong modulation of auxin gradient across the CZ of the concave side, where stress-related compression wood (CW) was developed. The auxin metabolites (oxIAA, IAAsp and IAGlu) also followed the same distribution trend of auxin in the CZ of the two bent sides, suggesting a key role of auxin and correlated metabolites in CW formation. The asymmetric distribution of abscisic acid and cytokinins between concave and convex sides, with their higher level in concave side, lead us to hypothesize a role of these hormones in the CW formation (10).

1) Laskowski M, Grieneisen VA, Hofhuis H, et al. (2008) Root system architecture from coupling cell shape to auxin transport. PLoS Biology 6: e307. doi: 10.1371/journal.pbio.0060307
2) Richter GL, Monshausen GB, Krol A, Gilroy S. (2009) Mechanical stimuli modulate lateral root organogenesis. Plant Physiology, 151, 1855-1866
3) Scippa GS, Trupiano D, Rocco M, et al. (2008) Unravelling the response of poplar (*Populus nigra*) roots to mechanical stress imposed by bending. Plant Biosystems, 142, 401-413
4) Trupiano D, Renzoni G, Rocco M, et al. (2012a) The proteome of *Populus nigra* woody root: response to bending. Annals of Botany, 110, 415-432
5) Trupiano D, Di Iorio A, Montagnoli A, et al. (2012b) Involvement of lignin and hormones in the response of woody poplar taproots to mechanical stress. Physiologia Plantarum, 146, 39-52
6) Trupiano D, Rocco M, Renzone G, et al. (2013b) Poplar woody root proteome during the transition dormancy–active growth. Plant Biosystems, 147, 1-6
7) Trupiano D, Rocco M, Renzone G, et al. (2014) Temporal analysis of poplar woody root response to bending stress. Physiologia Plantarum, 150, 174-193
8) Rossi M, Trupiano D, Tamburro M, et a. (2015) MicroRNAs expression patterns in the response of poplar woody root to bending stress. Planta, 242, 339-351
9) De Zio E, Trupiano D, Montagnoli A, et al. (2016) Poplar woody taproot under bending stress: the asymmetric response of the convex and concave sides. Annals of Botany, 118, 865-883
10) De Zio E, Trupiano D, Karady M, et al. (2019) Tissue-specific hormone profiles from woody poplar roots under bending stress. Physiologia Plantarum, 165(1), 101-113

1.4 = The aquatic fern *Azolla filiculoides* can partially overcome cadmium injuries in co-treatment experiments with TiO_2 nanoparticles

Carmelina Spanò, Stefania Bottega, Carlo Sorce, Giacomo Bartoli, Monica Ruffini Castiglione
Department of Biology, University of Pisa, Via Ghini 13, 56126 Pisa, Italy

Water ecosystems polluted by heavy metals, such as cadmium, may also be affected by the increasing presence of TiO_2 nanoparticles. While the toxicological profile of cadmium is well known (1), the effects of TiO_2 nanoparticles on ecosystems and biota are indeed not yet fully clear and unambiguously interpretable, especially in a scenario of a co-presence with other contaminants (2).

Previous studies reported an induction of antioxidant response in *Vicia* spp. treated with TiO_2 nanoparticles, suggesting a possible protective action of NPs in plants exposed to this type of contaminant (3, 4). On this basis we aimed to assess if TiO_2 nanoparticles might alleviate Cd injuries and improve the ability of plants to cope with this metal, focusing on the hydrophyte *Azolla filiculoides* Lam. as a model system.

With this mechanistic hypothesis, after a pre-treatment with TiO_2 nanoparticles, *A. filiculoides* plants were transferred to cadmium contaminated water, with or without TiO_2 nanoparticles. Commercial powder of TiO_2 anatase + rutile nanoparticles (1:1 ratio, nominal size of 30 nm, 99.9% of purity) were employed.

After five days of treatment, cadmium uptake, morpho-anatomical and physiological aspects were studied in plants.

The continuous presence of TiO_2 nanoparticles, though not increasing the uptake of cadmium in comparison with a priming treatment, were able to preserve root hair patterning (Fig. 1) and induced a higher translocation of this heavy metal to the aerial portion. Despite the translocation factor was always well below 1, cadmium contents in the fronds, generally greater than 100 ppm, ranked *A. filiculoides* as a good cadmium accumulator.

Higher cadmium contents in leaves did not induce damages to the photosynthetic machinery probably thanks to a compartmentalization strategy aimed at confining most of this pollutant to less metabolically active peripheral cells (Fig. 2). The permanence of NPs in growth medium ensured a better efficiency of the antioxidant apparatus (proline and glutathione peroxidase and catalase activities), induced a decrease in H_2O_2 content, however was not able to lower the oxidative damage in terms of TBARS.

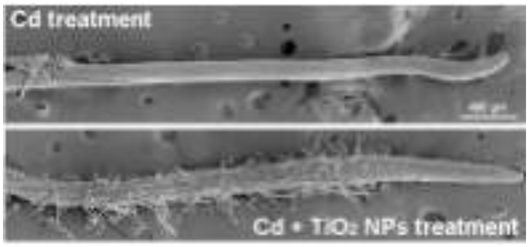


Fig. 1. *Azolla filiculoides* roots as observed under scanning electron microscope

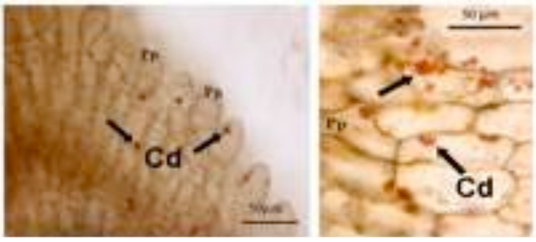


Fig. 2. *In situ* Cd localization in mature leaves of plants treated with Cd in co-presence of TiO_2 NPs. Pp = papillae (one-celled trichomes) of leaf epidermis. Dithizone staining

1) ATSDR (2012) Toxicological profile for Cadmium. https://www.atsdr.cdc.gov/toxprofiles/tp5.pdf

2) R. Deng, D. Lin, L. Zhu, S. Majumdar, J.C. White, J.L. Gardea-Torresdey, B. Xing (2017) Nanotoxicology, 11, 591-612

3) M. Ruffini Castiglione, L. Giorgetti, R. Cremonini, S. Bottega, C. Spanò (2014) Protoplasma, 251, 1471-1479

4) M. Ruffini Castiglione, L. Giorgetti, L. Bellani, S. Muccifora, S. Bottega, C. Spanò (2016) Environmental and Experimental Botany, 130, 11–21

1.4 = Air pollution modifies IgE binding of the main allergens of *Hesperocyparis (Cupressus) arizonica* (GREEN) Bartel pollen

Emma Tedeschini, Giuseppe Frenguelli
Department of Agricultural Food and Environmental Science, University of Perugia, Via Borgo XX Giugno 74, 06121 Perugia, Italy

Beckground. Air pollution is considered the main responsible of the increased incidence of respiratory diseases including allergies, in industrialized countries. The pollen of Cupressaceae have been frequently studied in relationship with air pollutants. The first report on this issue was at the end of the '80s in Japan (1), where that the people living in urban areas, or near intensive vehicular traffic locations, tended to be more allergic *to Cryptomeria japonica* pollen than people living in rural areas. Even today, international research is talking about this because the cypress pollen is the main cause of winter pollinosis due to large amount of pollen released and to wide distribution of the species ones (2). In the Mediterranean Regions peoples are exposed to their pollen from autumn to spring but the main pollination period is during winter when is higher the presence of air pollutants coming from vehicular traffic, heating of buildings and industrial activity. the concentration of environmental pollutants, and their interaction with the airborne pollen. Could explain the growing diffusion of pollinosis and, always more often, in individuals who have never had symptoms previously (3). The present work, attended to analyse the effect of air pollutants on the allergenic power of the cypress pollen, evaluating the protein profile of the pollen and the IgE reactivity in sensitized patients. The study was conducted in four areas in Umbria (Central Italy), characterized by different levels of air pollution.

Methods. The pollen of *C.arizonica* was collected in four sites, subjected to constant environmental monitoring. The pollen proteins were extracted and quantified according to the Bradford protocol. The specific IgE were quantified by ImmunoCAP test, on 100 Umbrians allergic to cypress pollen The Western blotting assay was performed using protein extracts of pollens from polluted and unpolluted sites. Proteins identification: Immune-reactive bands were carefully excised from Coomassie-stained 1D gels and digested to in-gel trypsin. The tryptic fragments were analysed by LTQ Orbitrap XLhybrid mass spectrometer. Peptide sequences identity were compared by *Uniprot database.*

Results. The analysis by mass spectrometer showed that the signals amplified both in the SDS-PAGE and in the immunoblot correspond to Cup a 1 and Cup a 2 the main allergens of *C.arizonica* and the appearance of new bands on polluted sites. The present study shows that air pollution is able: i.to interact with the main allergens of the cypress, increasing their IgE-binding ability; ii.to induce structural modifications of other surface proteins that become new allergens.

Conclusions. Air pollution increase the allergenic power of cypress pollen.

1) Ishizaki T, Koizumi K, Ikemori R, Ishiyama Y, Kushibiki E (1987) Studies of prevalence of Japanese cedar pollinosis among the residents in a densely cultivated area. Ann Allergy, 58(4), 265–270

2) Sénéchal H, Visez N, Charpin D, Shahali Y, Peltre G, Biolley J-P, Lhuissier F, Couderc R, et al. (2015) A review of the effects of major atmospheric pollutants on pollen grains, pollen content, and allergenicity. The Scientific World J, 2015, 1–29

3) D'Amato G., Tedeschini E., Frenguelli G., D'Amato M. (2019) Allergens as trigger factors for allergic respiratory diseases and severe asthma during thunderstorms in pollen season.Aerobiologia, 35, 379–382

1.5 = Nitrogen and phosphorous removal by microalgae isolated from sludge surnatants of a urban wastewater treatment plant

Costanza Baldisserotto[1], Ornella Accoto[1], Linda Benetti[2], Roberta Marchesini[1], Michele Maglie[1], Simonetta Pancaldi[1]
[1]Department of Life Sciences and Biotechnology, University of Ferrara, C.so Ercole I d'Este, 32, 44121 Ferrara, Italy;
[2]HERA SpA – Direzione Acqua, Via C. Diana, 40, 44044 Cassana, Ferrara, Italy

Microalgae are photosynthetic microorganisms and are considered very good candidates for a wide range of biotechnological applications, including phytoremediation processes. They have been suggested as an option for wastewater treatments since several decades, however this technology has not been expanded on the industrial scale and, nowadays, only a few small-scale facilities exist (1). The reason is that microalgal-based processes need technical improvements and microalgal strain selection represents a crucial step. Conventional urban wastewater treatments are based on aerobic/anaerobic processes able to convert contaminants (nutrients and other molecules) into inert compounds suitable for safe water reuse or disposal. The conventional steps for water depuration involve the production of sludge, still rich in contaminants, so needing further water-consuming treatments. Since nitrogen and phosphorus are the main contaminants that must be removed from waters and sludge before they can be inlet in natural environment or dispose (91/676/CEE and 91/271/CEE), research interest is usually focussed on the potential of microalgae in the removal of these nutrients as a tertiary treatment (2,3). Moreover, the employment of autochthonous microalgae isolated from wastewaters is proved to improve their survival, growth and nutrient removal capability (4).

In this context, a screening program is aimed for the selection of autochthonous algal strains suitable for nutrients removal from surnatants of the sludge treatment. This research, recently granted from Emilia Romagna Region (5), is currently conducted using wastewaters from the depuration plant located in HERA-Ferrara (Holding Energia Risorse Ambiente). The isolation program of microalgae involves surnatants from three crucial phases during sludge depuration: thickening, digestion and desiccation steps. The first microalgal isolates derive from the thickening step and were characterised in terms of growth capability, morphology, photosynthetic pigment content, PSII maximum quantum yield. Ammonia and phosphate ions removal by selected microalgae was also tested.

The most frequent isolates were *Chlorella*- and *Scenedesmus*-like strains, even if other organisms were observed (i.e. *Botrydiopsis* sp.). The first isolate, suitable for the characterization tests, was a *Chlorella*-like strain. Microalgae cultures were prepared both using the thickener surnatant wastewater (treated samples) and using a standard mineral medium supplemented with the same nitrogen and phosphorous concentrations (controls). This strain showed its better growth capability, both in terms of cell density and biomass yield, when cultivated in the surnatant wastewater. However, its photosynthetic response, evaluated as F_V/F_M of PSII, was similar in both cultures. In parallel, chlorophylls and carotenoids increased inside treated cells, while maintaining values of chlorophyll *a/b* and total chlorophyll/carotenoids molar ratios comparable to that of controls. Nutrients removal in waters treated with the microalgae was about 70 and 96% for ammonia and phosphates, respectively.

These biological findings open to the possibility of designing a success pilot system for the treatment of larger volumes of surnatants from urban wastewater treatment systems.

1) F.G. Acién, C. Gómez-Serrano, M.M. Morales-Amaral, J.M. Fernàndez-Sevilla, E. Molina-Grima (2016) Appl. Microbiol. Biotechnol., 100, 9013-9022
2) N. Abdel Raouf, A.A. Al-Homaidan, I.B.M. Ibraheem (2012) Saudi J. Biol. Sci., 19, 257-275
3) L. Delgadillo-Mirqueza, F. Lopesc, B. Taidic, D. Pareau (2016) Biotechnol. Reports, 11, 18-26
4) S. Pancaldi, L. Ferroni, C. Baldisserotto, M. Giovanardi, L. Mai (2012) Patent n. 1412445, dep.15.05.2012
5) POR-FESR 2014-20 - Progetto VALUE CE-IN "VALorizzazione di acque reflUE e fanghi in ottica di economia CircolarE e simbiosi Industriale

1.5 = Fungi and arsenic: tolerance and bioaccumulation by soil saprotrophic strains

Andrea Ceci[1], Veronica Spinelli[1], Lorenzo Massimi[2], Samuele Guadagnino[1], Silvia Canepari[2], Anna Maria Persiani[1]
[1]Department of Environmental Biology Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy; [2]Department of Chemistry Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy

Arsenic, a common metalloid, is worldwide recognised as important toxic element for human beings and living organisms (1,2). Natural processes as well as anthropogenic activities contribute to its diffusion and occurrence in the environment (1,2). Fungi, as geoactive agents, can play very important geological roles in several processes, including decomposition, biogeochemical cycling, element biotransformations, metal and mineral transformations, bioweathering and soil formation (3,4). Fungi can tolerate and accumulate high concentration of arsenic and for some species, biovolatilization via methylation was reported (5).

In this research, relationships between some soil saprotrophic microfungi and arsenic in relation to growth responses and bioaccumulation were investigated. In particular, *Absidia spinosa* Lendn., *Purpureocillium lilacinum* (Thom) Luangsa-ard, Houbraken, Hywel-Jones & Samson (formerly *Paecilomyces lilacinus*), *Metarhizium marquandii* (Massee) Kepler, S.A. Rehner & Humber (formerly *Paecilomyces marquandii*) and *Cephalotrichum nanum* (Ehrenb.) S. Hughes (formerly *Doratomyces nanus*), previously isolated from areas with high natural As concentrations, were studied in two different cultural media, namely Malt Extract Agar and Czapek-Dox Agar, and three different concentrations (10, 20 and 50 mg/L) of sodium arsenite ($NaAsO_2$) were tested. Metabolic responses and fungal tolerance to As have been investigated by tolerance indices, namely Rt:Rc (%) and T.I. (%), based on growth data, diametric extension and dry weights, respectively. Most of fungi resulted tolerant to all tested As concentrations, and values of tolerance indices varied according to cultural media and As concentrations. pH medium after fungal growth was measured to study pH variation and metabolic responses. As bioaccumulation in all fungi was observed with chemical analyses by hydride generation atomic fluorescence spectrometry. As tolerance and bioaccumulation by fungi and their metabolic responses shed further light in fungal geoactive roles in the environmental fate of As and provide potential applications in bioremediation.

1) R. Singh, S. Singh, P. Parihar, V. P. Singh, S. M. Prasad (2015) Ecotox. Environ. Safe., 112, 247-270
2) A. Sarkar, B. Paul (2016) Chemosphere, 158, 37-49
3) A. Ceci, M. Kierans, S. Hillier, A. M. Persiani, G. M. Gadd (2015) Appl. Environ. Microbiol., 81, 4955-4964
4) A. Ceci, F. Pinzari, F. Russo, A. M. Persiani, G. M. Gadd (2019) Appl. Microb. Biotechnol., 103, 53-68
5) M. Singh, P.K. Srivastava, P.C. Verma, R.N. Kharwar, N. Singh, R.D. Tripathi (2015) J. Appl. Microb., 119, 1278-1290

1.5 = In vitro culture of plants for the new chain of edible flowers

Andrea Copetta[1], Ilaria Marchioni[2], Laura Pistelli[2], Carlo Mascarello[1], Barbara Ruffoni[1]
[1]CREA – Research Centre for Vegetable and Ornamental Crops, Corso Inglesi 508, 18038 Sanremo, Italy; [2]Department of Agriculture, Food and Environment (DAFE), University of Pisa, Via del Borghetto 80, 56124 Pisa, Italy

The crisis that the floriculture sector has had in recent years has led to a re-orientation of many small and medium farms on species suitable for alternative markets while maintaining the same type of production. This process has recently successfully involved aromatic plants and is starting to involve those edible species that are positioned on the food market both in the tradition and in the cuisine of innovation. Edible flowers, in addition to their intrinsic decorative value, have important nutritional characteristics that must be well defined and valued. The Interreg Alcotra ANTEA project was created with the aim of reinforcing the emerging chain of edible flowers through the application of innovation in production and analysis methods and in the assessment of the safety of use, conservation and distribution strategies. The edible flower chain brings with it the added value of being a productive activity that is born linked to organic and sustainable cultivation and is therefore an economic activity that does not affect environmental costs. Furthermore, technological and communication innovations have been applied which guarantee immediacy and visibility between producer and consumer. Dissemination of the results to consumers and restaurateurs are essential for the same purpose too. Indeed, the ensuring food safety will help to expand the range of restaurateurs who will begin to have a constant supply of many flowers with high quality.

Several edible species for food purposes have been propagated *in vivo*; while plants with low multiplication rate (bulbous) or difficult to propagate by seed or cutting were multiplied *in vitro*. Among these last plants two varieties of *Agastache* (*A. aurantiaca* "Sunset Yellow" with lemon/mint taste - Fig. 1 - and *A. mexicana* "Sangria" with anise/mint taste); *Mertensia maritima* renowned because flowers and leaves have an oyster taste (Fig. 2); fifteen different clones of *Polianthes tuberosa* (fourteen from seed and one from bulb) and five types of *Tulbaghia*: *T. cominsii* with garlic butter taste (Fig. 3), *T. simmleri* (two varieties) with garlic asparagus taste, *T. violacea* (two varieties) with garlic taste. Microcuttings (for *Agastache* spp and *M. maritima*) with at least two axillary buds, and seeds (for *P. tuberosa* and *Tulbaghia* varieties) were disinfected with a solution sodium hypochlorite 1.5% or 2%, respectively, a few drops of Tween 20 per 20'and rinsed twice with distilled sterile water per 10'. The seeds were pre-germinated on moist sterile filter paper at 23°C in the dark. Microcuttings and germinated seeds were transferred in flasks containing semi solid medium which was composed of MS (1) salt and vitamins, 3% sucrose and 0.8% agar (MS0). For each variety, different propagation media added with plant hormones were tested.

The best multiplication medium is: a) basal MS added with BA 0.3 for *Agastache* varieties; b) basal MS added with BA 0.2 and active carbon 0.5% for *M. maritima*; c) basal MS added with BA 1.5 and IAA 0.5 for *Polianthes* clones (Fig. 4); d) basal MS added with BA 3 and NAA 0.1 for *Tulbaghia* varieties.


Fig. 1. *A. aurantiaca* "Sunset Yellow" inflorescence


Fig. 2. Detail of *M. maritima* flower


Fig. 3. *T. cominsii* plant cultured in greenhouse


Fig. 4. *In vitro* culture of a *P. tuberosa* clone

1) Murashige, Skoog (1962) Physiologia Plantarum, 15, 473-497

1.5 = Draft genome sequence and annotation of *Rhizopus arrhizus* FBL 578 (Mucoromycota): insights into the mycoremediation of DDT-contaminated soils

Domenico Davolos[1], Fabiana Russo[2], Andrea Ceci[2], Eligio Malusà[3], Małgorzata Tartanus[3], Oriana Maggi[2], Anna Maria Persiani[2]
[1]Department of Technological Innovations and Safety of Plants, Products and Anthropic Settlements (DIT), INAIL, Research Area, Via R. Ferruzzi 38/40, 00143 Rome, Italy; [2]Department of Environmental Biology Sapienza University of Rome, Piazzale Aldo Moro 5, 00185 Rome, Italy; [3]Research Institute of Horticulture, Konstytucji 3 Maja 1/3, 96-100 Skierniewice, Poland

Fungi can tolerate and transform anthropogenic contaminants such as persistent organic pollutants (POPs), thanks to their metabolic and enzymatic versatility (1, 2, 3). Indeed, fungal biodegradation of POPs, e.g. DDT, has been recognized as an environmentally-friendly, feasible, integrated, cost-effective remediation biotechnology (1, 2, 3). However, the mechanisms underlying detoxification metabolism of organochlorine compounds are still unclear and further investigations could support applications in pesticide-contaminated soils.

Strains of the monophyletic genus *Rhizopus* (Mucoromycota) are widely known for their importance in industrial, medical and environmental sectors (4, 5). *Rhizopus* sp. FBL 578 (deposited in the fungal collection of the Fungal Biodiversity Laboratory, Sapienza University of Rome), was isolated from Polish DDT-contaminated soil. The tolerance of this strain to 1 mg/L DDT has been investigated by tolerance indices (Rt:Rc (%); T.I. (%)). Metabolic responses to DDT were also evaluated through growth data (diametric extension and dry weight) and medium pH after fungal growth (6). Our results pointed out the potential of FBL 578 in DDT bioremediation.

An analysis of DNA sequences from the RPB2, EF-1α, and RNA helicase genes indicated that *Rhizopus* sp. FBL 578 is closely related to *Rhizopus arrhizus* A. Fisch (formerly *Rhizopus oryzae*) of the *R. arrhizus* complex, which are well-known producers of proteolytic enzymes, but certain strains can act as opportunistic human pathogens (mucormycosis) (7). In this study, FBL 578 was the target for whole-genome sequencing (WGS), assembly and annotation to better assess the safety of biotechnological applications in mycoremediation. Mitochondrial sequences were also identified. The assembly for *Rhizopus* sp. FBL 578 genome consisted of 6,027 contigs totaling a size of 39.3 Mbp with a 35.08% GC content. A total of 14,399 protein-coding genes were predicted, and then annotated with the GO and the CAZy databases. Bioinformatic analyses were conducted on putative genes involved in the metabolism of DDT. Prediction of biosynthesis gene cluster was also carried out to shed light on the genes potentially involved in the production of compounds and secondary metabolites of biotech value. The WGS project of *R. arrhizus* FBL 578 has been submitted to GenBank (NCBI).

The Next Generation Sequencing (NGS) work for the WGS project was financially supported by INAIL-DIT (Rome, Italy).

1) J. Foght, T. April, K. Biggar, J. Aislabie (2001) Bioremediation J., 5, 225-246
2) A. Mansouri, M. Cregut, C. M.-J. AbbesDurand, A. Landoulsiand, G. Thouand (2017) Appl. Biochem. Biotechnol., 181, 309-339
3) H. Harms, D. Schlosser, L.Y. Wick (2011) Nat. Rev. Microbiol., 9, 177-192
4) L. Londoño-Hernández, C. Ramírez-Toro, H. A. Ruiz, J. A. Ascacio-Valdés, M. A. Aguilar-Gonzalez, R. Rodríguez-Herrera, C. N. Aguilar (2017) Int. J. Food Microbiol., 257, 110-112
5) G. Barnita, R. Rinarani (2011) J. Appl. Sci., 11, 2470-2486
6) F. Russo, A. Ceci, F. Pinzari, A. Siciliano, M. Guida, E. Malusà, M. Tartanus, A. Miszczak, O. Maggi, A. M. Persiani (submitted) Appl. Environ. Microb.
7) G. Walther, S. Dolatabadi, G. S. de Hoog (2014) Mycoses, 57, 36-36

1.5 = Biochemical profile of *Pleurotus ostreatus* cultivated on unconventional substrate added with agricultural waste of lavender

Di Piazza Simone[1], Benvenuti Mirko[2], Damonte Gianluca[2], Salis Annalisa[2], Zotti Mirca[1]
[1]Laboratory of Mycology, Department of Life, Earth and Environmental Science (DISTAV), University of Genoa, Corso Europa 26-16132 Genoa, Italy; [2]Department of Experimental Medicine (DIMES), Center of Excellence for Biomedical Research (CEBR), University of Genoa, Via Leon Battista Alberti 2-16132 Genoa, Italy

In recent decades, the increase in population, especially in some areas of the planet, and the increasingly competition among industries, including those of agricultural sector, push them to optimize and exploit resources in a circular economy perspective aimed at reducing the production of waste. In the context of the agricultural circular economy, mushrooms, thanks to their natural ecological role as degraders, can undoubtedly find employments in the field of recycling and valorisation of waste.

Our work framed in the European ALCOTRA project FINNOVER aims at: i) exploiting *Pleurotus ostreatus* to reuse the lavender solid waste ii) finding new products to revamp agriculture in the western Liguria coast.

Following a pilot test carried out during 2018 (1), we selected the best growing substrates and to set up an *ad hoc* biochemical analysis protocol for the extraction of fungal matrices.

In the second pilot test, carried out in 2019, we used 4 substrates with a growing concentration (0%, 25%, 50%, 75%) of lavender (*Lavandula angustifolia* Mill. and a hybrid called *Boscomare*) inoculated with 2 strains of *P. ostreatus* (one commercial and one native).

After a total cultivation period of 4 months we observed that the native strain produces a greater biomass than the commercial one. This fact is particularly evident in the spawns with a concentration of 25% and 50% lavender. This suggests that the native strain, being more adapted to the local environmental conditions, can better withstand the stress of growing on a substrate enriched with lavender residues which obviously is not optimal for the development of this fungus.

The qualitative biochemical profile was characterized in HPLC-MS, in particular the presence of numerous families of compounds of interest: polyphenols and other phenolic compounds (chlorogenic acid, caffeic acid, dihydrocaffeic acid, cinnamic acid, vanillic acid, resveratrol, various flavonones), fatty acids and their derivates (linoleic acid, linolenic acid, stearic acid, sphingosine, phosphatidylethanoamines, leukotoxines) vitamins (riboflavin, pantothenic acid) sesquiterpens (koningic acid) statins (lovastatin) (2, 3). It should be noted the presence of molecules with known antioxidative and potential farmacological proprerties but also the production of molecules with potential negative effects, especially leukotoxin the 9, 10 epoxide of linoleic acid (a fatty acid greatly present in fungi) produced as a consequence of oxidative burst (4).

The present results encourage further experiments aimed at investigating how different growth substrates can affect the biochemical profiles of different *P ostreatus* strains.

1) S. Di Piazza, F. Turrini, R. Boggia, G. Damonte, M. Benvenuti, M. Zotti (2018) V INTERNATIONAL PLANT SCIENCE CONFERENCE (IPSC). Poster n 90. Fisciano (SA), 12 - 15 September 2018
2) M.Gąsecka, M. Mleczek, M. Siwulski, et al. (2016) Eur Food Res Technol, 242(5), 723-732
3) L. M. Papaspyridi, N. Aligiannis, P. Christakopoulos, A. L. Skaltsounis, N. Fokialakis, (2011) Procedia Food Science, 1, 1746-1752
4) J. D. Miller, & D. R. (2014) McMullin Appl Microbiol Biotechnol, 98, 9953-9966

1.5 = Optimization of the co-cultivation of *Tisochrysis lutea* and *Nannochloropsis oculata*: preliminary tests

Michele Maglie[1], Costanza Baldisserotto[1], Alessandra Guerrini[2], Simonetta Pancaldi[1]
[1]Department of Life Sciences and Biotechnology, University of Ferrara, C.so E. I d'Este, 32, 44121 Ferrara, Italy;
[2]Department of Life Sciences and Biotechnology, University of Ferrara, P.le L. Chiappini, 3, 44121 Ferrara, Italy

Nowadays, the biotechnological potential of microalgae is crucial for biomass production, human food, feed in aquaculture, cosmeceutical and pharmaceutical products, energy and phytoremediation(1). Moreover, the great biodiversity of microalgae and their different biochemical composition make them attractive as commercial sources of a wide range of biomolecules (vitamins, aminoacids, PUFA, pigments, etc.)(2;3). Many studies aim at lowering production costs, optimizing culture systems and modifying culture conditions, in order to improve the yield and at the same time reduce water use and nutrient supply. In this context, some studies have focused on co-cultivations of different organisms, a strategy based on community ecology approaches. Mixed cultures may involve different species of microalgae, or microalgae and fungi, or microalgae and bacteria, or fungi and bacteria(4). Many other studies have focused on the influence that irradiance and spectral characteristics of cultivation light may have on growth rates and biochemical composition of microalgae(5). In this regard, the aim of our research is to test the co-cultivation of *Tisochrysis lutea* (Haptophyta) and *Nannochloropsis oculata* (Ochrophyta) to obtain important compounds, such as pigments and lipids. Moreover, the co-cultivation of these two species can allow the obtainment of a lipid profile overall similar to that of fishmeal, for which some issues arise especially when used as a feed for herbivorous animals. In addition to the responses of the two organisms to co-cultivation, we also aim at evaluating the impact that different lighting conditions could have on cells in either single or mixed cultures. In the preliminary phase of experimentation, we used white light and observed that both microalgae did not benefit from co-cultivation, especially *T. lutea*. However, an increase in the concentration of chlorophylls and carotenoids was noted in both strains. At the end of experiment, the non-polar lipid fraction was analysed by gas chromatography-mass spectrometry. The results showed that there was no increase in the amount of neutral lipids in co-cultivated cells as compared to the mono-cultivated ones. Analyses showed that, in mono-cultivation, the two microalgal strains accumulated fatty acids different in nature and proportion. The lipid profile obtained from the co-cultivated cells was qualitatively suitable to produce ω-3 fatty acid similar to fishmeal. An improvement of algal co-cultivation still needs further research in order to also increase the proportion of *T. lutea*. To this purpose, we tested red (626 nm peak) and blue (470 nm peak) LEDs, in comparison with the white LEDs that simulate daily light. Red and blue lights, in fact, are known to stimulate the growth of microalgae because of their selective excitation of chlorophylls(5). LEDs were frequently used for microalgae cultivation because of their advantages, such as low energy consumption, small size, high operation reliability and long irradiation lifetime(6). At present, we have tested the effect of white light enriched in red or blue wavelengths on monocultures of *T. lutea*. Red light enrichment improved the growth of *T. lutea* and, at the 10th day of cultivation, these cultures were significantly different in comparison to cells exposed to blue-enriched and white light. Some slight differences were also recorded in the production of photosynthetic pigments and lipid globules. The cultures exposed to white and red-enriched light showed higher levels of chlorophylls and carotenoids than those exposed to blue light. On the contrary, an early appearance of lipid globules was observed in the cultures exposed to blue-enriched light. Further studies are being performed to evaluate the response of *N. oculata* under the same experimental light conditions. The results obtained from this research will permit to set up optimal lighting conditions for the co-cultivation of the two microalgal strains.

1) Rizwana M. et al (2018) Renewable and Sustainable Energy Reviews, 92, 394-404
2) Matos A.P. (2017) J Am Oil Chem Soc, 94, 1333-1350
3) Borowitzka M.A. (2013) J Appl Phycol, 25, 743-756
4) Zhu L. (2015) Biofuels, Bioprod. Bioref., 9, 801-814
5) del Pilar Sánchez-Saavedra et al. (2016) J Appl Phycol, 28, 839-847
6) Lin C.Y. and Tseng Y.M. (2018) J Renewable Sustainable Energy, 10, 02310

1.5 = Reuse of reclaimed wastewater from hexavalent chromium through iron based nanoparticles on tomato plant growth

Valerio Petruccelli[1+], Elisa Brasili[1+], Irene Bavasso[2+], Alessio Valletta[2], Giorgio Vilardi[2], Luca Di Palma[2*], Gabriella Pasqua[1*]

[1]Sapienza University of Rome, Department of Environmental Biology, Rome, 000185, Italy; [2]Sapienza University of Rome, Department of Chemical Engineering Materials Environment, Rome, 00185, Italy
[*]corresponding authors: gabriella.pasqua@uniroma1.it; luca.dipalma@uniroma1.it
[+]these authors contributed equally to this work

Water pollution by heavy metals is a very common and serious environmental problem. Anthropogenic Cr(VI) contamination is generally related to industrial applications such as metallurgical, refractories and chemicals (1). In the pH range 5-8, Cr(VI) shows a high mobility and solubility in aqueous media, whilst Cr(III) is almost insoluble (2). Although Cr(III) in traces is an essential element for the human body, Cr(VI) has been shown as possible carcinogenic compound. Several technologies for Cr(VI) removal from industrial wastewater have been successfully proposed. In recent years, zero valent iron nanoparticles (nZVI) have attracted a great attention due to their efficiency in removal of different types of contaminants from aqueous solutions, including Cr(VI) (3). The present research deals with a comprehensive study of Cr(VI) removal from wastewater by nZVI. NZVI particles were continuously synthetized in a Spinning Disk Reactor (SDR) by borohydride reduction method, without the addition of any stabilizer. The reuse of the reclaimed wastewater on tomato plant growth, in terms of seed germination, length of hypocotyl and roots, levels of carotenoids and chlorophyll A, B and total, was investigated, in a wide range of Cr(VI) and nZVI concentration. Results show that seed germination percentage was significantly reduced after treatment with Cr (VI) in the range between 1,5 ppm and 1000 ppm, compared to control. The combined effect of chromium and nanoparticles resulted in a general reduction of the seeds germination rate, though, at a Cr(IV) concentration lower than 100 ppm, a substantial recovery was observed. The treatment with 5 ppm Cr (VI) showed a significant decrease of hypocotyl length but not of the root, compared to the control. The combined treatment with 5 ppm Cr (VI) and nZVI particles showed a significant increase of both hypocotyl and root length compared to 5ppm Cr (VI) treatment. Plants grown in pots treated only with nZVI (5ppm) showed a significant increase of chlorophyll B and carotenoid content at 35 days. At 65 days of growth, a significant increase of chlorophyll A and B as well as total chlorophyll and carotenoid level was observed in nZVI (5ppm) treated plants compared to the control.

1) M. Nourbakhsh, Y. Sag, D. Ozer, Z. Aksu, T. Kutsal & A. Caglar, (1992) Process Biochemistry, 29(1994), l-5
2) John C. Seaman, Paul M. Bertsch, L. Schwallie, (1999) Environmental Science & Technology. 33, 938-944
3) Vilardi, G., Di Palma, L., Verdone, N., (2019) Chemosphere, 220, 590-599. DOI: 10.1016/j.chemosphere.2018.12.175

1.5 = Plant products as a green solution in the fight against biodeterioration of stone monuments

Lorenza Rugnini[1], Neil William Thomas Ellwood[1,2], Anna Rosa Sprocati[3], Giada Migliore[3], Flavia Tasso[3], Chiara Alisi[3], Laura Bruno[1]
[1]LBA-Laboratory of Biology of Algae, Dept. of Biology, University of Rome "Tor Vergata", via Cracovia 1, 00133 Rome, Italy; [2] Department of Science, University of Roma Tre, Viale G. Marconi 446, 00146 Rome, Italy; [3]ENEA, RC Casaccia, Via Anguillarese 301, 00123, Rome, Laboratory of Environmental Biogeochemistry, Italy

Stone materials in archaeological sites and confined environments are exposed to *biodeterioration* due to the presence of living organisms that endanger their durability. Weathering and natural or artificial light favour the growth of phototrophic biofilms that are generally composed of cyanobacteria, bacteria, microalgae, mosses and fungi (1). These microbial communities can cause aesthetic damage to valuable surfaces by causing discoloration and, in some cases, structural problems due to the ability of microorganisms to attack stone, by penetrating the material also through the painted layers. Nowadays, the restoration practices in use include the mechanical removal by brushes combined with spraying chemical biocides that are frequently hazardous towards human health and environment and the effect on the treated surface is not always well understood. Moreover, the employment of chemicals seems to be selective for resistant microorganisms (2). As a result of these negative factors, more sustainable and safer practices are being investigated for their biocidal action on typical biofilms of lithic surfaces (3-5).
The site of interest for this study is the Domus Aurea (Rome, Italy), built after the fire of 64 AD as the new residence of the Emperor Nero, the Domus's walls were covered with precious marbles and vaults decorated with gold and precious stones, then Nero's successors buried the residence, in-filling it entirely with soil. To date, among the restoration efforts to recover the monument, the disinfection of biodeteriogens growing on the walls and ceilings has become a highly important aspect. Based on prior knowledge it was decided to carry out tests on biofilms using the essential oil from *Lavandula angustifolia* and alcohol extracts from leaves of *Glycyrrhizza glabra (*Trifolio-M GmbH*)* and *Capsicum* spp. These natural compounds were tested at different concentrations, as alternative biocides of phototrophic biofilms. Biofilms samples were collected in 'room 93' from an undecorated wall and were then inoculated in agarized BG11 growth medium, to reconstruct homogeneous biofilms to be tested in laboratory. Observations of the biofilms showed that *Scytonema julianum* was the dominant species. This cyanobacterium has often described from hypogea environments, such as the Roman Catacombs, and it is well known for its biodeteriogenic activity due to the precipitation of calcium carbonate in its sheaths after the dissolution of minerals from the substrate. The tested products were applied to the biofilms twice (day 1 and 5), and the photosynthetic response on the biofilm was followed for five days with a mini-PAM portable fluorometer. As photosynthesis is highly susceptible to this kind of treatment, measurements of rates were used as a proxy for cell health. Changes in photosynthetic activity of the samples treated with the extracts were compared to control biofilms receiving no treatment. Results show that the essential oil of *L. angustifolia* and *G. glabra* extract 30% had a higher inhibition potential, followed by *G. glabra* extract 10%. *Capsicum* extract was the least efficient. These first results are encouraging enough to continue beyond the laboratory trials in the hope to realise an eco-friendly, non-toxic and sustainable strategy for the conservation of lithic cultural heritage.

1) Albertano P (2012) Cyanobacterial biofilms in monuments and caves. In: Whitton (ed.) Ecology of Cyanobacteria II: Their diversity in Space and Time, Springer, Netherlands, 317-343
2) Urzi C, De Leo F, Krakova L, Pangallo D, Bruno L (2016) Effects of biocide treatments on the biofilm community in Domitilla's catacombs in Rome. Science of Total Environment, 572: 252–262
3) Ruffolo SA, De Leo F, Ricca M, Arcudi A, Silvestri C, Bruno L, Urzì C, La Russa MF (2017) Medium-term in situ experiment by using organic biocides and titanium dioxide for the mitigation of microbial colonization on stone surfaces. International Biodeterioration and Biodegradation, 123, 17-26
4) Bruno L, Valle V (2017) Effect of white and monochromatic lights on cyanobacteria and biofilms from Roman Catacombs. International Biodeterioration & Biodegradation, 123, 286-295
5) Bruno L, Rugnini L, Spizzichino V, Caneve L, Canini A, Ellwood NTW (2019). Biodeterioration of Roman hypogea: the case study of the Catacombs of SS. Marcellino and Pietro (Rome, Italy) Annals of Microbiology https://doi.org/10.1007/s13213-019-01460-z

1.5 = Phytochemical profile of olive oil mill vegetative waters

Fabio Sciubba[1], Alberta Tomassini[1], Daniele Pizzichini[2], Daniela Bellincampi[3,4], Vincenzo Lionetti[3,4], Alfredo Miccheli[4,5,6]

[1]Department of Chemistry, Sapienza University of Rome, Italy; [2] Laboratory Bio-products and Bio-processes BIOAG, ENEA Centro ricerche Casaccia, Italy; [3]Department of Biology and Biotechnology "Charles Darwin", Sapienza University of Rome, Italy; [4] Research Center for Applied Sciences for the Protection of the Environment and Cultural Heritage, Sapienza University of Rome, Italy; [5]Department of Environmental Biology, Sapienza University of Rome, Italy; [6]NMR-based Metabolomics Laboratory, Sapienza University of Rome, Italy

Olive (*Olea europaea L.*) belongs to the *Oleaceae* family and it has been cultivated for olive oil, fine wood, olive leaf and its fruits from 2000 years ago and is one of the most diffused crops in the Mediterranean basin. Its fruits can be consumed as it is, but traditionally they are employed to produce olive oil, which is one of the cornerstones of the Mediterranean diet. It is a commercial good whose market is projected to reach a revenue of 1 billion of US dollars in 2022 worldwide and is a major contribution to Italian economy (1). The production of olive oil consists in several steps that produce, along the final product, several wastes among which pomace is a wet solid waste composed by peels, pulp residues and seed fragments. In modern implants (decanter multiphase) waste can include a dehydrated pomace containing peels and hazelnuts suitable for production of pellet for stoves and a second by-product, called "paté", consisting in hydrated pulp with a high humidity (75-80%) usually used to produce biogas. In the process for the latter, vegetative water is also produced, which is an aqueous toxic waste usually discarded (2). Olive mill wastes are toxic for both natural beneficial and pathogenic microrganisms. Nowadays, in the optic of a better employment of the natural resources, these approaches are no longer sustainable, and as such several national and international projects have been founded in order to use these waste materials for other applications.

The aim of this project, which is the first step of the larger ABASA (Agricultural By-products into valuable Assets for Sustainable Agriculture) project founded by LazioInnova- Regione Lazio, is to characterize the phytochemical composition of patè and vegetative waters fractionated by various stages of membrane filtration in order to identify and quantify molecules with biological activity which could be employed in agriculture as phytopesticides, agents in crop protection, soil biofertilizers or as a source of bioactive compounds.

In the present study, both pomace and vegetative waters fractions were analyzed by high-resolution NMR spectroscopy. The matrices have been treated with a multi-solvent extraction protocol, and both the hydro soluble and lyposoluble fractions have been analyzed. Mono and polyunsaturated fatty acid, triglycerides, triterpenes derived from ursolic acid, oleuropein, lingstroside and their aglycones, tyrosol and hydroxytirosol were observed in both matrices, with the major difference between them being the relative amount of triglycerides. Of great interest is the presence of oleuropein and lingstroside in waste waters since they are both seco-iridoids with a known antifungal and antimicrobical activity (3) Carbohydrate profile has been obtained for the same fractions. The presence of pectin and oligogalacturonides (OGs) were identified by anion exchange HPLC-Dionex. OGs are cell wall derived pectic fragments elicitors of plant immune responses (4). This finding highlights the high potential of these by-products as biostimulant of plant defense responses. The evaluation of the fractionation efficiency and the biological activity of the different fractions are in progress.

1) International Olive Council (2019) http://www.internationaloliveoil.org

2) M. Niaounakis, C.P. Halvadakis Olive Processing Waste Management Literature Review and Patent Survey (2nd ed.), Elsevier Ltd., Kidlington, Oxford (2006)

3) Y. Liu, L.C. McKeever, N.S.A. Malik; 2017 Assessment of the Antimicrobial Activity of Olive Leaf Extract Against Foodborne Bacterial Pathogens Frontiers Microbiology, 8, 113

4) D.Bellincampi et al (2014) Plant cell wall dynamics and wall-related susceptibility in plant-pathogen interactions. Front Plant Sci., 5,228

1. 5 = Are mosses effective in the interception on microplastics in water environment?

Maria Cristina Sorrentino[1], Fiore Capozzi, Rosa Carotenuto, Simonetta Giordano, Valeria Spagnuolo
[1]Department of Biology, University Federico II of Naples, Via Cintia 4, 80126 Napoli, Italy

The present work highlighted the potential use of mosses for the monitoring of plastic particles in water environment. Nowadays, plastic pollution has become a critical issue worldwide (1,2) and, although mosses are well known as biomonitors of metal pollutants in fresh water (3), no studies were carried out to evaluate their capacity in the interception of microplastics. This study represents a first laboratory experiment aimed at detecting the interaction between the moss *Spagnum palustre* L. cultured in vitro and polystyrene nanoparticles (NPs). The interception of NPs onto moss surface, the effect of moss vitality on NP interception, and the effect of post-exposure water washing on NP retention, were evaluated by fluorescence microscope. The results showed an increase of particles number directly proportional to the exposure time and the retention of polystyrene NPs as small and large aggregates on moss leaves (Figg. 1 and 2). The accumulation of NPs was also favored by the devitalization of the moss probably caused by damages in dead moss material; the post exposure washing induced a loss of large NP aggregates. These results encourage to test mosses for monitoring microplastics contamination in field experiments.

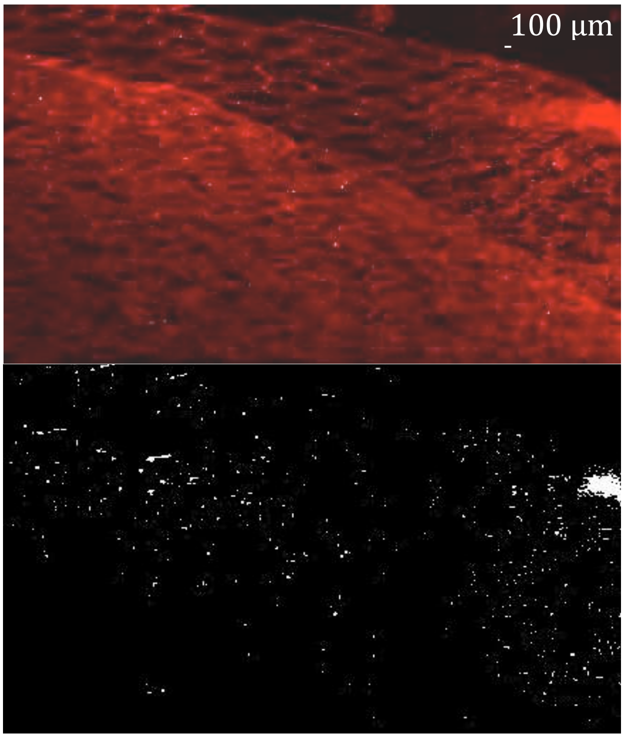
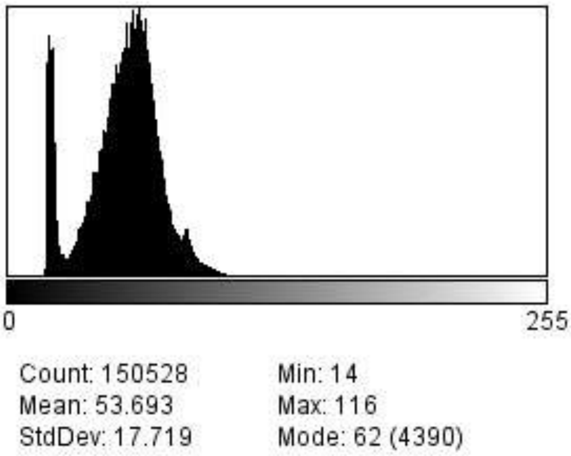


Fig. 1. Moss leaf treated with polystyrene NPs (a) and relative image for particle counting (b)



```
Count: 150528      Min: 14
Mean: 53.693       Max: 116
StdDev: 17.719     Mode: 62 (4390)
```

Fig. 2. Particles counted on a moss leaf area and their size distribution

1) S. Yoshida, K. Hiraga1, T. Takehana, I. Taniguchi, H. Yamaji, Y. Maeda, K. Toyohara, K. Miyamoto, Y. Kimura, K. Oda, (2016) Science, 351, 1196-1199
2) Plastic Europe, (2015) http://www.plasticseurope.org/it/resources/publications/93-plastics-facts-2015
3) S. Debén, J.A. Fernandez, A. Carballeira, J.R. Aboal, (2016). Environ. Pollut. 210, 315-322

1.5 = Contaminant assessment and bioremediation of urban soils: a case study

Federica Spina[1], Matteo Florio Furno[1], Giorgio Bordiglia[2], Marco Mucciarelli[1], Perugini Iolanda[1], Valeria Tigini[1], Debora Fabbri[2], Elisa Gaggero[2], Mery Malandrino[2], Anna Fusconi[1], Giovanna Cristina Varese[1], Paola Calza[2]
[1]Department of Life Sciences and Systems Biology, University of Torino, viale P. Mattioli 25, 10125, Torino, Italy; [2]Department of Chemistry, University of Torino, via P. Giuria 7, 10125, Torino, Italy

Urban agriculture has grown rapidly in the last years, thanks to the possibility of producing fresh foodstuffs at low cost and to reduce socio-economic and environmental issues. However, urban gardens are exposed to continuous pollution caused by emission from road traffic, domestic heating, previous industrial use of the sites, atmospheric deposition from industrial activities, with the main pollutants being represented by heavy metals and organic compounds, i.e polycyclic aromatic hydrocarbons (PAH). Plants may absorb and accumulate toxic elements, depending on species and environmental conditions. Hence, the evaluation of the content and bioavailability of the heavy metals in the soil, of the presence of PAHs and of the pollutants uptake and accumulation capacity of plants are fundamental to estimate the risks resulting from the consumption of plants and to properly manage the areas. Several technologies for remediation of contaminated soils have been developed. Bioremediation, in particular phytoextraction for metals and biodegradation for organic pollutants, is a passive, low cost, in situ approach, which uses plants and/or soil microorganisms, bacteria and fungi, to reduce concentration of pollutants.

The results here presented have been obtained within the project “Re-Horti”- Strategies to reuse urban areas for horticulture, aimed (1) to identify an experimental site between different areas in the city of Turin, chosen to be reused as urban gardens; (2) to purify the identified area by using phytoexctraction and biodegradation through soil fungi; (3) to test the effect of an organic soil conditioner on plant and fungus growth and soil restoration. Plants for phytoremediation were chosen on the basis of the literature, sunflower (*Helianthus annuus*) and maize (*Zea mais*) for their great biomass and high rate of growth, *Brasssica juncea* for the fast reproductive cycle that allows more crops per year, the fern *Pteris vittata* for its capacity to accumulate arsenic and different metals. Biodegradation technology included: (i) the characterisation of cultivable autochthonus mycoflora from different stratigraphic layers; (ii) the isolation and selection of fungal strains with a biodegradation potential through a degradation-focused screening process; (iii) the analysis of the suitable technique for the inoculum in urban horticultural soil. The soil was characterized and metals, both in the soil and the plants, were chemically analyzed through ICP-OES, GF-AAS, ICP-MS. Among the organic pollutants the sixteen most frequent and/or dangerous PAHs selected by US-EPA were studied using HPLC-UV/VIS and GC-MS techniques.

Results showed that the soil chosen for the experiments was strongly polluted by heavy metals (in particular by lead and, to a lesser extent, by Zn, Cr, Ni, As) and by organic pollutants, especially pyrene, perylene and anthracene.

Preliminary results on phytoextraction showed the highest leaf concentrations of lead in *B. juncea* and, in the same species, the highest translocation factor (TF) from roots to shoots for all metals. The only exception was represented by the TF for the As in *P. vittata*. The addition of the soil improver had a contrasting effect on leaf accumulation, depending on both plant species and metal.

Since the choice of the correct consortium to be applied in a fungal bioaugmentation process is a critical step, particular effort was given to the isolation and the characterization of the expressed metabolic pathway of the isolated fungi. Strains belonging to 29 species were isolated. Among them, 19 strains were capable of use as sole carbon source at least one pollutant (pyrene, phenanthrene, paraffin oil) more than the glucose, showing impressive adaptation skills. The tested strains showed a peculiar growth behaviour; the growth skills on each pollutant were strain-dependent. *Thermoascus crustaceus* and *Trichoderma asperellum* were the only ones capable of growing both on pyrene and phenanthrene with a growth comparable to the control. Very good growth skills were observed for *Fusarium proliferatum, F. oxysporum, Aspergillus terreus* and *Coniochaeta canina* in the presence of alkanes. The choice of the microbial consortium to be applied was not limited only to the degradation capabilities, but it was designed accordingly also to the production of biosurfactants and the capability to grow on ligninocellulose substrates that were used as carriers to vehiculate the microorganisms into the soil. The best microbial consortium has been inoculated *on site* and trials are in due course.

1.5 = Characteristics of Monacolin K production by *Pleurotus* (Fr.) P. Kumm. strains from Umbria (Italy), in liquid and solid culture

Roberto Venanzoni[1], Bruno Tirillini[2], Giancarlo Bistocchi[1], Andrea Arcangeli[1], Giancarlo Angeles[1], Federica Bastioli[1], Lorenzo Monarca[1], Bernard Fioretti[1], Paola Angelini[1]
[1]Department of Chemistry, Biology and Biotechnology, University of Perugia, Via del Giochetto, 06123 Perugia, Italy;
[2]Department Department of Biomolecular Sciences, University of Urbino, Via Saffi, 2, 61029 Urbino, Italy

The genus *Pleurotus* (Fr.) P. Kumm. (*Pleurotaceae*, *Basidiomycota*) is the most exploitable of xylotrophic mushrooms, with valuable biotechnological, medical, and nutritional properties. It is represented in Umbria by six species [*Pleurotus columbinus* Quél., *P. cornucopiae* (Paulet) Rolland, *P. dryinus* (Pers.) P. Kumm., *P. eryngii* (DC.) Quél., *P. ostreatus* (Jacq.) P. Kumm., *P. pulmonarius* (Fr.) Quél.] found growing attached to the cortex of *Salix* spp., *Populus* spp., *Quercus* spp. and *Fagus sylvatica* (1).The aims of this work were to evaluate the biotechnological feasibility of *Pleurotus* strains from Umbria and to produce Monacolin K in high quantity. Monacolin K is a fungal polyketide-based secondary metabolite widely used as a prominent drug in hypercholesterolemia (2).

Fruiting bodies of *Pleurotus* spp. were collected in different woods from Umbria in the autumn and spring of 2017 and 2018. Pure cultures of 25 strains were obtained by excising pieces of trama from inner parts of fruiting bodies and transferring them onto malt extract agar medium which were then incubated for 14 days at 25 °C. A voucher specimen of the mushrooms was deposited in the PeruMyc herbarium (Department of Chemistry, Biology and Biotechnology, University of Perugia). Fermentation was carried out in modified Melin-Norkrans liquid medium for 15 days. Mycelium for inoculation of cultivation substrates was prepared on wheat grain using the traditional method (3). The experimental substrate comprised of oak sawdust and different mixtures of beech, willow and poplar sawdust or wheat straw. The prepared substrates were placed in polypropylene bags with a microporus filter. Experimental substrates were sterilized for 4 h at 104-108 °C, then cooled down to 21 °C, and inoculated with 20 g of grain spawn per bag. The bags were subsequently kept in a spawn running room at 23 ± 1 °C. Incubation was continued until the entire surface of the substrate was colonized by the mycelium. For the induction of *Pleurotus* strains fruiting bodies, the bags were first incubated at 2-4 °C for 24h and then were kept at 23 ± 1 °C and 65-68 % relative humidity with a 10 h photoperiod. In the cultivation experiment, five replications were carried out for each combination of strain, substrate mixture and its moisture content as well as the level of organic supplementation.The mycelial mass from liquid culture and the fruiting bodies (collected after 2-3 months from solid substrate) were processed for extraction using methanol until exhaustion. The identification and quantification of Monacolin K (lactone and acid forms) were carried out on the culture filtrate and extracts by HPLC-TOF/MS.

The current study allowed for a comparison between the content of Monacolin K present in the mycelia liquid cultures and fruiting bodies with that of *Pleurotus* spp. cultivated in solid substrate. The mycelial development in liquid cultures and fruiting bodies in conservatories, present a Monacolin K level that fluctuates between 0.04 to 2.00% measured in dry fungus, depending on strain and substrate culture.

1) H. Caballero Ortega, R. Pereda-Miranda, L. Riverón-Negrete, J.M. Hernández, M. Medécigo-Ríos, A. Castillo-Villanueva, F.I. Abdullaev (2004) Acta Hort., 650, 321-326
2) S. H. Mousavi, N. Z. Tayarani, H. Parsaee (2010) Cell. Mol. Neurobiology, 30, 185-191
3) S. Samarghandian, M. H. Boskabady, S. Davoodi (2010) Pharmacognosy Magazine, 6, 309-314

2.1 = Snow cover influence on phenology and primary growth of *Juniperus communis* L.

Elisabetta Candeago[1], Martina Pollastrini[1], Marco Carrer[2]
[1]elisabetta.candeago@gmail.com martina.pollastrini@unifi.it Department of Agriculture, Food, Environment and Forestry, University of Firenze, Piazzale delle Cascine 18, Firenze, Italy; [2]marco.carrer@unipd.it Department of Land, Environment, Agriculture and Forestry, University of Padua, Viale dell'Università 16, Legnaro (PD), Italy

Global warming and climate change are the most discussed topics in the last decades. It is well known that plants communities and forests ecosystems are affected by the increasing of average temperatures and by extreme precipitations and drought, events that are always more frequent and intense (1). The response of plants to climate changes is an important aspect to keep in mind in order to predict the effects of temperature on plant communities. Even a small rise of average temperature (+1,5/2,0°C) can influence plant phenology with an anticipation of buds burst and flowering or cause a variation of growth rhythm. Temperature increase also causes the reduction of snow cover and humidity of the soil. Plant photosynthetic efficiency is affected by these environmental changes, with consequences on CO_2 uptake. Furthermore, a migration of woody species from lower to higher altitudes has been observed in the Alps due to the increase of temperatures (2). These environmental changes threat the species living in colder areas (3). The purpose of this study is to evaluate the responses of common juniper (*Juniperus communis* L.) to the changes in the period and duration of snow cover in terms of phenology and primary growth of the shoots. A snow manipulation experiment was carried out in a treeline area, in the eastern Alps near to Passo Giau (San Vito di Cadore, Belluno, Italy, Fig.1), in order to study the effects of the alterations of environmental conditions (duration of snow cover, air, soil and bark temperatures and soil water availability). The experiment setup included 15 plants of common juniper, grown in 3 plots with 3 different treatments (5 plants in each plot): S (covered by snow), U (uncovered by snow) and C (control) plots (Figs. 2, 3). Starting from March 2018, the snow has been removed from the U plots and, at the same time, some snow has been collected from the area around the site and placed on the plants in S. Indeed, from May 2018 the snow was melted on the C and U plots. From this study we observed that the manipulation of the snow cover affects (i) the shoot length in junipers grown covered by snow, with an anticipated beginning of growth and a more elongation than junipers grown in U and C plots; (ii) the plant phenology, with a delay in maturation of the reproductive structures in S plant respect to U and C junipers. This research is the first step of a long-term experiment in alpine treeline environmental conditions. This kind of studies are essential to know the phenology and growth of woody species grown in extreme environments, such as the treeline, to evaluate changes in plant communities, both in their spatial structure and species composition driven by the current and future environmental changes.

Fig. 1 *Study site*

Fig. 2 *Covered plot (S)*

Fig. 3 *Uncovered plot (U)*

1) Walther GR (2004). Plants in a wormer world. Perspectives in plant Ecology, Evolution and Systematics 6: 169-185
2) Cannone, N., Sgorbati, S., Guglielmin, M. (2007). Unexpected impacts of climate change on alpine vegetation. Frontiers in Ecology and the Environment
3) Körner C (1998). Worldwide positions of the alpine treelines and their causes. The Impacts of Climate Variability on Forests (Baniston M, Innes JL eds). Lecture Notes in Earth Science, 74, 221-229. Springer, Berlin

2.1 = Warmer temperatures determine the germination window of *Platanus orientalis* L.

Francesca Carruggio[1], Maria Castrogiovanni[1], Carmen Impelluso[1], Antonia Cristaudo[1]
[1]Department of Biological, Geological and Environmental Sciences, University of Catania, Via Empedocle 58, 95128 Catania, Italy

Recent anthropogenic climate change is disrupting natural environmental patterns, which is affecting species' distributions and life history events, putting natural populations at risk and creating challenges for biodiversity conservation (1). The most threatened species are those with restricted ecology. This might be the case of wetland species in the Mediterranean bioregion.

Platanus orientalis L. is a long-lived tree distributed from the central Mediterranean to the Himalaya (2) that occupies lowland riparian forests, a geographically restricted ecological niche within its distribution (Habitat Natura 2000 code 92C0). *P. orientalis* is currently in decline in Europe, where it is considered vulnerable due to habitat loss, fungal infection by *Ceratocystis platani* and ongoing climate change (3).

The aim of our work was to better understand seed germination behaviour of Sicilian populations from Iblei and Peloritani Mountains at the western range edge of *P. orientalis* in Europe. We recently observed that the number of individuals of *P. orientalis* in natural populations has significantly declined in the last few decades, and seedling recruitment is very low in almost all monitored locations. Here, we conducted a series of experiments to explore the effects of temperature and light on seed germination of *P. orientalis*. Experiments started two weeks after seed collection and tested germination performance at constant temperature (5°C increments from 5-35°C), either with 12/12h light/dark (L/D) or full darkness (D) photoperiod.

Our results allowed us to exclude the presence of primary dormancy. Seeds are dispersed between late winter and early spring and are immediately germinable, but require temperatures above 10°C, with almost complete thermo-inhibition of germination below 15°C, either in light and dark conditions. In the light, final germination percentage reached 90% in the optimal temperature range (20 to 30°C) and remained high at temperatures either side of the optimal range (around 80% at both 35 and 15°C). At temperature above 20°C, mean germination time was very short (3 days), and increased with decreasing temperature (up to 10 days at 15°C). The presence of light improved germination at 15 and 20°C (near zero germination in full darkness), but had no effect at higher temperatures.

Our laboratory experiments supported the likelihood of a late-spring germination window (April to May), when the temperature increases and water availability in the soil is assured. In agreement with this, we observed seedlings of *P. orientalis* in the field in early May. Seed germination characteristics in *P. orientalis* were quite similar to those reported for many temperate wetland species that require high temperature, temperature fluctuation and light for ideal germination conditions. For example, seeds of *Salix* spp. are also non-dormant at the time of dispersal and can germinate over a wide range of temperatures (4). This combination of characteristics is known as the 'typical wetland germination requirement (TWGR)' (5,6). Considering the thermal germination requirements observed in our experiments, long-term population survival of *P. orientalis*, particularly at the western edge of its distribution, could be further threatened by reduced spring precipitation, increased temperatures and more frequent extreme weather events that are predicted to occur with ongoing climate change (7). There may be a second window of suitable germination conditions between late summer and early autumn, and our future research will test this to better understand the natural germination seasons of *P. orientalis*, and how they may shift between spring and autumn as climate change continues to alter natural environmental regimes. Indeed, understanding how germination strategies will respond to ongoing climate change is important for implementing active conservation measures.

This research was supported by the programme INTERREG V-A Italia-Malta 2014-2020, Axis III - SO 3.1, 'SiMaSeed' project - Code C1-3.1-16.

1) T.P. Dawson, S.T. Jackson, J.I. House, I.C. Prentice, G.M. Mace (2011) Science 332, 53–58
2) N. Wazen, B. Fady (2016) FAO and Plan Bleu, Rome, Italy
3) M. Barstow, M.C. Rivers (2017) http://dx.doi.org/10.2305/IUCN.UK.2017- 3.RLTS.T33951A68135880.en
4) R. Densmore, J.C. Zasada (1983) Canadian Journal of Botany, 61, 3207-3216
5) M.A. Albrecht, Q.G. Long (2014) Seed Science Research, 24, 239-245
6) C.C. Baskin, J.M Baskin. (2014) Elsevier/Academic Press, San Diego, CA, USA
7) S. Planton, F. Driouech, K. EL Rhaz, P. Lionello (2016) In J.P. Moatti, S. Thiébault. IRD Éditions, Marseille

2.1 = Oak decline: the study case of Lucanian Apennine (southern Italy) in summer 2017

Antonio Luca Conte[1], Donato Lucia[2], Giuseppe Cillis[3], Piera Di Marzio[1], Duilio Iamonico[4], Romeo Di Pietro[4], Paola Fortini[1]

[1]Department of Bioscience and Territory, University of Molise, Pesche (IS) Italy; [2]MSC in forestry and environmental science, Potenza (Pz) Italy; [3]SAFE School of Agriculture, Forestry, Food and Environmental Sciences - University of Basilicata, Potenza (Pz) Italy; [4]Department PDTA, University of Rome Sapienza, Via Flaminia 70, 00196 Rome, Italy

The recent forest oaks decline observed in the Mediterranean basin has been triggered by an increase of climatic anomalies such as heat waves, droughts, and extreme cold (1).

This study intends to deepen the relationships between climate anomalies and oak decline based on the observations made in the Lucanian Apennine (southern Italy) in summer 2017.

The affected areas by oak decline have been identified through the interpretations of the remote sensing data (visible image interpretation).

Five stands of these areas were chosen for supplementary investigation on oak-decline damage.

In addition, we analysed the thermometric and pluviometric data recorded in 2015 and 2017, these years being the hottest and the driest, respectively, since 1800 (2).

Remote sensing data [Normalised Difference Water Index (NDWI), Temperature Condition Index (TCI)], and the field collected data [(Decline Severity (DES), Deficit/Surplus (D/S), Rainfall Anomaly Index (RAI), Standardised Precipitation Index (SPI)] were examined using GIS techniques to evaluate the spatio-temporal distribution of the damage by oak decline.

Spring and winter rainfall deficit were found to be strictly related to the oak decline during summer 2017. With this work, we experimented a method that combined remote sensing techniques and field surveys. This approach turned out as a useful tool to follow the spatio-temporal distribution of oak decline and study better this worrying phenomenon.

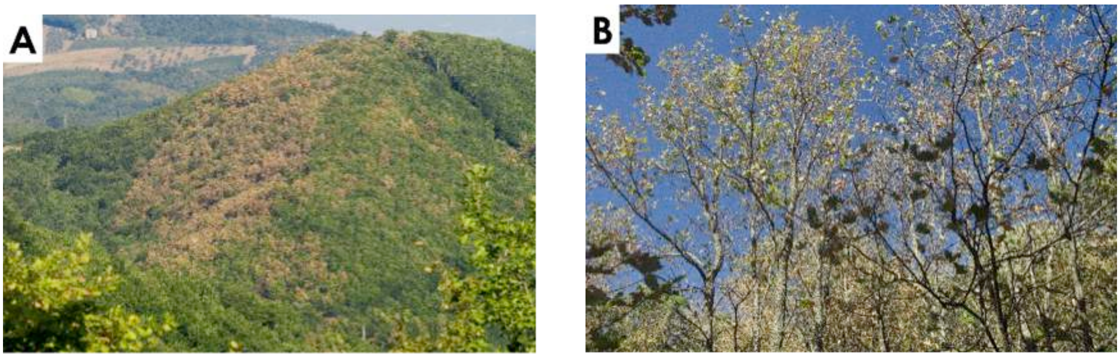


Fig. 1A-1b. (A) oak decline at the 2017-08-27, San Martino d'Agri. (B) defoliation of trees at the 2017-09-17, Montemurro

1) M. Lindner, M. Maroschek, S. Netherer, A. Kremer, A. Barbati, J. Garcia-Gonzalo, R. Seidl, S. Delzon, P. Corona, M. Kolström, M.J. Lexer, M. Marchetti (2010) Forest Ecology and Management, 259, 698–709
2) F. Desiato, G. Fioravanti, P. Fraschetti, W. Perconti, E. Piervitali, V. Pavan (2018) Ed. ISPRA

2.1 = The role of Urban Green Infrastructure in regulating climate at the local level: the case of the city of Rome

Federica Marando[1], Elisabetta Salvatori[1], Alessandro Sebastiani[1], Lina Fusaro[1], Fausto Manes[1]
[1]Dipartimento di Biologia Ambientale, Sapienza Università di Roma, P.le Aldo Moro 5, 00185, Roma

Cities are the kind of human settlement that characterize the most the current time, and can be framed both as focal centers of human activities and of environmental imbalances. Urban areas contribute greatly to climate change through greenhouse gas emission, but at the local level, the most prominent climatic phenomenon is Urban Heat Island effect (UHI), which is caused by the alteration of energy fluxes mainly through land cover change (1). In this context, Green Infrastructure can greatly contribute to the improvement of the urban microclimate, providing regulating Ecosystem Services (ES). In the present work, the ES of climate regulation at the local level provided by GI was assessed in the Municipality of Rome, through an integrated approach, for the period 2013-2017. UHI was analyzed through both an air temperature measurements and a spatially explicit approach, by mean of modeling Land Surface Temperature (LST) on a seasonal basis, retrieving Landsat 8 OLI/TIRS data and an emissivity map derived by the integration of different high spatial resolution datasets (2). The role of different structural and functional parameters of the GI in regulating the city's temperature was investigated using a multiple regression, and the cooling capacity of different elements of the GI, namely an urban, a peri-urban forest and street trees, was then evaluated through a buffer analysis. It emerges that GI significantly mitigate summer UHI, and that this effect strongly depends by structural vegetation characteristics such as NDVI and spatial extent. Therefore, GI might contribute greatly to an ecosystem-based climate adaptation strategy. Nevertheless, it is underlined the importance of environmental constraints in reducing GI capacity to provide ES, therefore the need of knowledge based criteria and indicators is essential for an effective decision-making aimed to maximize the provision of ES in a Mediterranean cities.

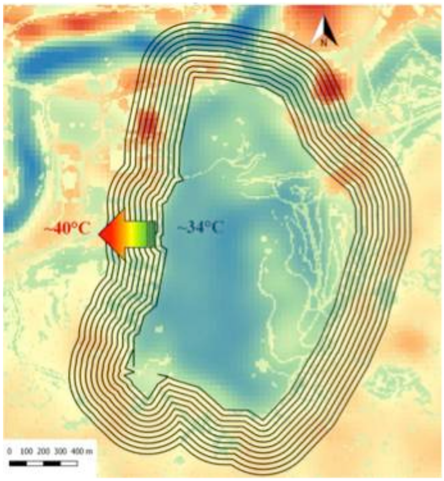


Fig. 1. Schematic representation of the buffer analysis and cooling capacity of an urban forest (Villa Ada Savoia) of the Urban Heat Island effect (average summer values)

1) T. R Oke (1982) Q. J. R. Meteorol. Soc., 108, 1-24
2) F. Marando, E. Salvatori, A. Sebastiani, L. Fusaro, F. Manes (2019) Ecological Modelling, 392, 92-102

2.1 = Modelling Invasive Alien Species distribution using data from citizen science

Stefano Martellos[1], Silvia Ongaro[1], Marco Massimi[2], Fabio Attorre[2]
[1]Department of Life Science, University of Trieste, Via Licio Giorgieri 10, 34127 Trieste, Italy; [2]Department of Environmental Biology, University of Roma "La Sapienza", Piazzale Aldo Moro 5, 00185 Roma, Italy

Invasive alien species (IAS) are a major component of global change, affect ecosystem services and human societies (1) and directly threat the local biodiversity. Species Distribution Models (SDMs) can be used for depicting potential invasion areas. Thus, they are useful for prioritizing alien species surveys, supporting conservation efforts (2). However, solid SDMs do require a (possibly) high number of up-to-date and verifiable occurrence data. Given the limited number of researchers, the number of species to monitor, the wide extent of areas which need to be explored and the necessity of acquiring a number of observations in a relatively short amount of time (in order to capture the "current" situation), the support provided by citizens in monitoring activities can be a viable and valid opportunity. Citizens' participation to research activities is far from being new (3). Citizen science programs can have many purposes: long-term monitoring, environmental education, preservation of traditional ecological knowledge (4), etc. In this study, we used occurrence data collected by volunteers in the framework of CSMON-LIFE project (LIFE13 ENV/IT/842), to build distribution models for 9 IAS: *Ailanthus altissima* (Mill.) Swingle, *Ambrosia artemisiifolia* L., *Carpobrotus edulis* (L.) N.E.Br., *Datura stramonium* L., *Euphorbia prostrata* Aiton, *Opuntia ficus-indica* (L.) Mill., *Oxalis pes-caprae* (L.), *Phytolacca americana* L., *Robinia pseudacacia* L., *Senecio inaequidens* DC. Occurrence data, verified and validated by a team of experts, were cleaned and georeferenced in Q-GIS software (version 2.18.4). Rasters with a spatial resolution of 1 km were processed for bioclimatic, soil and land cover predictors: 19 bioclimatic variables were obtained from Chelsa database (http://chelsa-climate.org/), 6 soil variables from SoilGrid portal (https://soilgrids.org) and 3 land use categorical variables from Corine Land Cover grids. To verify whether predictors were correlated, a Spearman test was performed. Principal Component Analysis were carried out to depict species requirements and on the whole study area for depicting environmental space and how species occurrences relate to it. Since different algorithms can provide similar predictions, with slight differences, models built using 3 well-established algorithms, were combined in a final ensemble, which averages their results on the basis of individual model performance, for obtaining more reliable predictions (5).

Fig.1. *Ailanthus altissima*

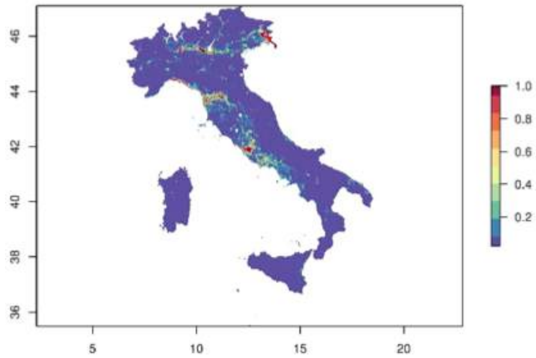


Fig. 2. Suitability map for *A. altissima*

1) L. Pejchar, H. Mooney (2009) Trend in Ecology and Evolution, 24, 497-504
2) J. Franklin (2013) Diversity and Distributions, 19, 1217–1223
3) A. Miller-Rushing, R. Primack, R. Bonney (2012) Frontiers in Ecology and Environment, 10, 285-290
4) J. L Dickinson, R. Bonney (2012) Citizen science: Public participation in environmental research, Cornell University Press, 1-14
5) M. B. Araújo, M. New (2007) Trends in Ecology and Evolution, 22(1), 42-47

2.1 = Hazelnut phenological phases and environmental effects in two central Italy areas

Fabio Orlandi[1], Aldo Ranfa[1], Luigia Ruga[1], Chiara Proietti[1], Marco Fornaciari da Passano[1]
[1]Department of Civil and Environmental Engineering, University of Perugia, Borgo XX Giugno 74, 06121 Perugia, Italy

Plants of Corylus avellana L. from two phenological gardens located near Perugia and in the plain of Rieti, were observed to analyze the phenology of their growth stages in relation to different climate characteristics. The following phenological phases were carried out during a 13-year period (2005-2017): BBCH 02, beginning of bud swelling; BBCH 11, first true leaf; and BBCH 95, leaves have mostly fallen (50% of leaves have fallen). Also, BBCH 65 (full flowering) was reported to highlight reproductive developments during the study period.

Temperature and solar radiation proved to be the factors which affected the different development phases the most in both of the study areas, while other variables (Precipitation and RH) presented heterogeneous behaviors also when considering the particular water table levels of one of the two observation areas (Rieti).

In addition, two periods: "first leaf development" FLD (BBCH 11-02) and "leaf presence period" LPP (BBCH 95-11) were calculated on the basis of previous observations.

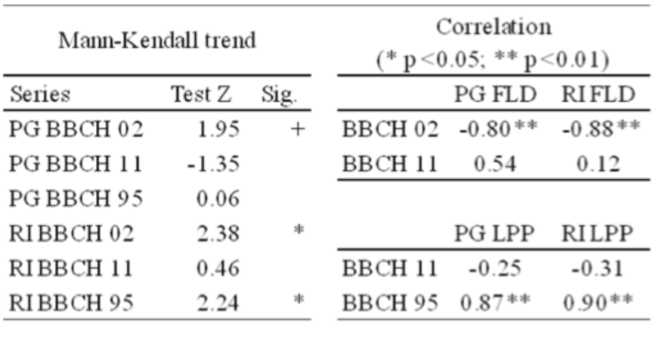
The flowering of the observed Hazelnut trees marked a progressive, but not significant, advance during the years when the study was carried out. The only meteorological variable with a significant trend in both areas was Solar radiation which tended to progressively decrease but apparently not enough to influence the major reproductive phenomenon (probably mostly linked to photoperiod).

The statistical results revealed a significant decreasing trend in solar radiation during the study period linked to the similarly significant progressive delay of bud swelling and leaves' fall (in Rieti) which determined a FLD shortening and LPP lengthening. The FLD shortening in both of the study areas is likely to be a consequence of the progressive delay in the beginning of bud swelling (BBCH 02) and the simultaneous lack of change in the first true leaf dates (BBCH 11) across the years. The influence of temperature but also of Rain (in Perugia Garden) and Solar radiation practically allowed the young leaves to develop over shorter periods and reach complete development (BBCH 11) in the same weeks during the study period. In contrast, the trends of the LPP period in both of the areas, were influenced by the variation in BBCH 95 starting dates. The lengthening of the LPP period, particularly significant in Rieti, may be related to the progressive delay in the senescence phase across the years, as the BBCH 95 moved from an average of the 43rd week to an average of the 45th week during the last observation years.

By a physiological point of view the combined statistical analyses may suggest that the closed buds, protected and insulated by the perulae, were more influenced by trends in Solar radiation than by trends in temperature.

After the bud break phase, the growing young leaves were probably more influenced by temperature and Rain that permitted the progressive recovery of the leaf vegetative development delay.

The effect that spectral composition has on the timing of bud burst and leaf out in tree species has been studied much less than that of photoperiod, even though light composition changes depending on various local parameters and due to cloud cover that may alter the ratio between far-red near-infrared light received by phytochrome photoreceptors influencing also plant bud swelling. Recent studies have found a negative correlation between heat requirement and insolation sum during dormancy, showing that years with more insolation tend to have earlier leaf flushing. This evidence could suggest that the amount of insolation during dormancy influence on heat requirement for leaf flushing, although the impact varies between species (1).



Mann-Kendall trend

Series	Test Z	Sig.
PG BBCH 02	1.95	+
PG BBCH 11	-1.35	
PG BBCH 95	0.06	
RI BBCH 02	2.38	*
RI BBCH 11	0.46	
RI BBCH 95	2.24	*

Correlation
(* $p<0.05$; ** $p<0.01$)

	PG FLD	RI FLD
BBCH 02	-0.80**	-0.88**
BBCH 11	0.54	0.12

	PG LPP	RI LPP
BBCH 11	-0.25	-0.31
BBCH 95	0.87**	0.90**

Table 1. Trend analyses of all the vegetative phenological phases (BBCH 02; 11; 95) and correlation between them and the elaborated periods (FLD, LPP), in both gardens (PG=Perugia; RI=Rieti).

1) Y.H. Fu, S. Piao, Y. Vitasse, H. Zhao, et al. (2015) Global change biology, 21(7), 2687-2697

2.1 = Ecosystem services of urban forest, quantification, and evaluation. Study case in Real Bosco of Capodimonte, Naples

Antonello Prigioniero[1], Pierpaolo Scarano[1], Mario Marziano[1], Rosaria Sciarrillo[1], Carmine Guarino[1]
[1]Department of Science and Technology, University of Sannio, Via Port'Arsa 11, 82100 Benevento, Italy

In the Urban forestry study, emphasis is often placed on Ecosystem Services (ES) provided by urban forests. To estimate the benefits provided by an urban forest, multiple relationships between the structure and the ecological processes associated with it must be evaluated (1-2). ES are classified into four macro-categories: supporting, provisioning, regulating, cultural services (3). Different structures, deriving from management, lead to the maximization of one or more ES at the expense of others; it is necessary to identify the structure (and therefore the management) which leads to the maximum expression of the desired ES. In this work a study was conducted on the Real Bosco di Capodimonte of Naples. The area extends for 132 ha, a cartography has been created in a GIS environment (4); following a random sampling, 79 sample areas of 400 m^2 were materialized. To assess the absorption of pollutants from the atmosphere by the plants, surveys of the arboreal component were conducted and the data required for the i-Tree application were collected. The results obtained from the model show the structure existing in the forest, the quantities removed of each pollutant (kg / year), the percentage contributions of the species detected in absorption, the monetary value of air purification. Subsequently three sub-areas were highlighted in the park, based on the different structures and management. In these areas, a floristic survey was carried out on 30 test areas, extrapolated from the previous ones, and the b-biodiversity was calculated. The relationship between the park structure and biodiversity variation was highlighted. In the final analysis, the recreational forest service was evaluated through questionnaires proposed to park users. Also in the questionnaire, the three sub-areas were highlighted, based on the different structure and management. The results obtained show the differences relating to the quantity and quality of the ecosystem services provided, in relation to the type of urban forest management.

1) R. A Rowntree (ed) (1984) Urban Ecol., 8, 1–178
2) D. J. Nowak (1994) J. Forestry, 92, 36–41
3) Millennium Ecosystem Assessment 2005
4) ESRI 2011. ArcGIS Desktop: Release 10. Redlands, CA: Environmental Systems Research Institute
5) i-Tree applications (2018) Retrieved April 3, 2018, from http://www.itreetools.org/applications.php

2.3 = Conservation status of *Boletales* in Umbria (Italy): an evaluation at regional level

Paola Angelini[1], Robert P. Wagensommer[1], Claudia Perini[2], Giancarlo Bistocchi[1], Andrea Arcangeli[1], Giancarlo Angeles[1], Roberto Venanzoni[1]
[1]Department of Chemistry, Biology and Biotechnology, University of Perugia, Via del Giochetto 6, 06123 Perugia, Italy; [2]Department of Life Sciences, University of Siena, Via P.A. Mattioli 4, 53100, Siena, Italy

Recently, the intensive use of molecular tools applied to the study of the systematics of boletoid mushrooms and related groups (order *Boletales* E.-J. Gilbert) has upset the traditional classifications based on morphological characters (1). At present, the *Boletales* (Basidiomycota) has been divided into six suborders, about 19 families and 70 genera, grouping taxa with different morphology of fruiting bodies including poroid, gilled, resupinate, hypogeous and epigeous gasteroid forms (1).

In this work, a Red List of all 84 *Boletales* species (belonging to 35 genera and 10 families) recorded in Umbria region is provided (2). The conservation status has been assessed according to the IUCN categories and criteria, adapted for sub-global level assessments (3, 4, 5, 6).

Data on species distribution were collected during field surveys,and published and unpublished data since 1990 have been considered. In the last ten years, some localities in the province of Perugia, such as Collestrada, Castel Rigone, Mt. Subasio and Polvese Island, were monitored continuously, and all collected data were georeferentiated. All records were identified according to the most recent bibliography.

According to the IUCN categories and criteria, most of the assessed species results to be threatened. As a consequence of the applied downlisting, the majority of the assessed taxa is classified as Vulnerable. One of the most threatened species in Umbria is *Suillellus dupainii* (Boud.) Blanco-Dios, classified as EN (Endangered). This species, listed among the 33 fungal species threatened at European level and proposed for Annex 1 of the Bern Convention (7), has been categorized as 'VU' (Vulnerable) in the Red List of the Italian flora (8, 9), according to IUCN Criteria and Categories.

1) M. Gelardi, C. Angelini, F. Costanzo, F. Dovana, B. Ortiz-Santana, A. Vizzini (2019) MycoKey, 49, 73-97
2) P. Angelini, A. Arcangeli, G. Bistocchi, A. Rubini, R. Venanzoni, C. Perini (2017) Plant Biosyst., 151, 915-923
3) IUCN (2012) IUCN red list categories and criteria: version 3.1. 2nd ed
4) IUCN (2012) Unified classification of direct threats: version 3.2
5) IUCN (2012) Guidelines for application of IUCN red list criteria at regional and national levels: version 4.0
6) IUCN (2017) Guidelines for using the IUCN red list categories and criteria. Version 13
7) A. Dahlberg, H. Croneborg, eds. (2006) Nat. Environ., 136, 131
8) C. Perini C., G. Venturella (2008) Informatore Botanico Italiano, 40(1), 149-150
9) G. Rossi, C. Montagnani, D. Gargano, L. Peruzzi, T. Abeli, S. Ravera, et al. (2013) Italian Committee of IUCN and the ministry for the environment, land and sea. Roman Printing House, 58

2.3 = Species distribution models of the narrow endemic *Primula apennina* Widmer (Primulaceae): a valuable tool for conservation purposes

Giovanni Astuti[1], Francesca Olivieri[1], Marco D'Antraccoli[1], Gianni Bedini[1]
[1]Department of Biology, University of Pisa, Via Derna 1, 56126 Pisa, Italy

Primula apennina Widmer is a hypsophylous small herbaceous plant that grows exclusively on sandstone, preferentially on northern exposures. This species only occurs along the ridge of the Tuscan-Emilian Apennines, in a 45-km stretch between Monte Orsaro to the north and Monte Vecchio to the south (2).

It is heterostylous and reproduces either sexually or vegetatively (clonal propagation); rare pollinating insects seem to ensure a discrete gene flow among the various populations, thus maintaining a low inter-populational genetic distance and a high intra-populational genetic diversity (1). The peculiar ecology of the species, the specialisation to ridge environments, the restricted area, which configures it as a global and local micro-endemism, make *P. apennina* of particular conservation interest, especially in view of climate changes and consequent loss of habitat (3, 4), and justify its inclusion in Annex II of the Habitats Directive.

Because of the conservation importance of the species, we focused mainly on the reconstruction of its current distribution and find new potential areas of occurrence. Therefore, as a first step, we mapped the known sites of occurrence of *P. apennina* using the available bibliographic sources, and then we conducted the demographic surveys on the field. In each site, through direct inspections, we verified the presence of the species, estimated the number of ramets and georeferenced the most abundant nuclei by means of GPS. The occurrences were analysed using Species Distribution Modeling (SDM) techniques, running the model using as environmental predictors eight geographical, vegetation and climate factors (temperature factors), and as background those areas that met the ecopedological features found in the known area of occurrence of *P. apennina*. Two models were produced: one using only occurrences derived from bibliographic source and the other implemented with occurrences obtained from field surveys.

Bibliographic sources allowed identifying 17 sites, all located within the National Park of the Tuscan-Emilian Appennines. We confirmed the presence in all known sites, which are characterised by siliceous walls with chasmophilous vegetation. Our results showed that the two models performed better than random predictions (AUC > 0.5) and had a similar spatial distribution pattern. However, the field model was more accurate and informative in detecting the relative contribution of each variable and the complex non-linear interactions between the predictive factors. Notably, we found that elevation provided the main contribution to the model, followed by the annual temperature range. Both models allowed identifying new potential areas of occurrence both inside and outside the known range. Therefore, the best performance and predictive accuracy of the model constructed from field data highlighted the advantage of field surveys and justified the efforts made to conduct them. However, we did not find any significant correlation between the probability of finding the species and the abundance of individuals in a certain cell.

Since we found that elevation and temperature were effective in shaping the species' distribution, we expected a range shrinkage from the prediction analysis made under a CCSM4 model using RCP 8.5 scenario projections on the year 2070. Indeed, the distribution model under this climate conditions returned a severe decline of cell occurrence coupled with a low occurrence probability of *P. apennina* for all cells, hence predicting a "next-to-the-extinction scenario". This prediction may be due to an upward range shift in elevation, which has been documented in many mountain plants (5, 6, 7), combined to a "no place left to go" effect as northern Apennines reach the maximum elevation at little more than 2100 m.

1) S. Crema, G. Cristofolini, M. Rossi, L. Conte (2009) Plant Systematics and Evolution, 280, 29–36
2) A. Alessandrini, G. Branchetti (1997) Flora Reggiana. Cierre Edizioni, Verona
3) J.R. Malcolm, C.R. Liu, R.P. Nelson, L. Hansen, L. Hannah (2006) Conservation Biology, 20, 538–548
4) H. Pauli, M. Gottfried, S. Dullinger, O. Abdaladze, M. Akhalkatsi et al. (2012) Science, 336, 353–355
5) I.C. Chen, J.K. Hill, R. Ohlemüller, D.B. Roy, C.D. Thomas (2011) Science, 333, 1024–1026
6) J. Lenoir, J.C. Gégout, P.A. Marquet, P. de Ruffray, H. Brisse (2008) Science, 320, 1768–1771
7) C. Parmesan, G. Yohe (2003) Nature, 421, 37–42

2.3 = Institutional projects to monitor plant species targeted by the Habitats Directive in Tuscany

Gianni Bedini[1], Giovanni Astuti[1], Angelino Carta[1], Marco D'Antraccoli[1], Lorenzo Peruzzi[1]
[1]Department of Biology, University of Pisa, Via Derna 1, 56126 Pisa, Italy

The E.U. Habitats Directive requires each member state, *inter alia*, to monitor the plant species listed in the Directive Annexes II, IV, and V and to assess their conservation status (art. 11). The assessment of the conservation status of the species targeted by the Directive forms an essential part of the national report that each member state must send to the European Commission every six years, compliant with art. 17.

In Italy, administrative regions have the institutional responsibility to carry out species monitoring, to assess their conservation status, and to provide monitoring data to the Ministry of Environment following national guidelines (1). To this end, in 2018 Tuscany Region launched the project "Monito-Rare", aimed at collecting the required data for species and habitats targeted by the Habitats Directive, in collaboration with the Universities of Florence, Siena, and Pisa. This paper reports some results of the project "Monito-rare", discusses their usefulness and looks at the perspective opened by the project.

Tuscany hosts 25 plant taxa targeted by the Directive: 5 bryophytes, two pteridophytes, and 18 angiosperms. Three of them are listed in Annex II only, three in Annex IV only, twelve in Annexes II and IV, and seven in Annex V. Five species are endemic to Italy, two of which are exclusive of Tuscany. Only one species - the endemic *Primula apennina* Widmer - is a priority species. Three taxa are super-specific: *Cladonia* L. subgenus *Cladina* (Nyl.) Vain. (= *Cladonia* Browne), with five species occurring in Tuscany, *Sphagnum* L. sp. pl. (21 species), *Lycopodium* L. sp. pl. (7 species). Before performing the monitoring campaign, the nomenclatural or systematic changes intervened since the adoption of the Directive had to be taken into account. Namely, the populations of *Crocus etruscus* Parl. living on Elba Island have been transferred to *Crocus ilvensis* Peruzzi & Carta (2), while the taxon formerly known as *Galanthus nivalis* L. subsp. *reginae-olgae* (Orph.) Gottl.-Tann. has been transferred to specific rank as *Galanthus reginae-olgae* Orph. The populations of *Aquilegia alpina* L. living in the Tuscan-Emilian Apennine is now considered *Aquilegia lucensis* E.Nardi.

Based on literature data, an extensive field survey campaign was planned and executed in 2018 to monitor the status of 13 target species - 2 pteridophytes, one bryophyte, ten angiosperms - across Tuscany. Field surveys allowed to update the distribution records, to assess the population size, and to evaluate main pressures and threats that impact the target species. Unfortunately one species - *Marsilea quadrifolia* L. - was not confirmed in the last known location in Tuscany; however, for other species - namely *Crocus etruscus*, *C. ilvensis*, *Galanthus nivalis*, *G. reginae-olgae*, *Ionopsidium savianum* (Caruel) Arcang., *Primula apennina* - several new populations were found and censused. New distribution records were also used to test the effectiveness of monitoring protocols (3) and to develop spatially-optimised distribution models (4). The new findings resulting from field surveys confirm the importance of empirical field research in the management of biological systems. Field research has been neglected in the last decades, with the consequence that important tools to analyse biodiversity conservation data, like synthetic analysis and big-data approaches, can be flawed by a lack of sound observational data collected through fieldwork (5). In perspective, the monitoring campaign will continue in 2019, 2020, and 2021 thanks to a new project, named "NatNeT", led by Tuscany Region in collaboration with the three Tuscan universities. Under NatNet, all remaining angiosperms and many bryophytes will be assessed. Furthermore, in 2021, the second monitoring of some species will be carried out to evaluate a quantitative trend. Finally, we strongly recommend that the taxonomic framework adopted in the Directive Annexes be updated according to current views in European plant taxonomy, to serve more effectively the conservation goals set in the Directive.

1) S. Ercole, V. Giacanelli, G. Bacchetta, G. Fenu, P. Genovesi (ed.) (2016) Manuali per il monitoraggio di specie e habitat di interesse comunitario (Direttiva 92/43/CEE) in Italia: specie vegetali. ISPRA, Serie Manuali e linee guida, 140/2016
2) L. Peruzzi, A. Carta (2011) Nord. J. Bot., 29, 6-13
3) G. Astuti, M. Adorni, G. Bedini, A. Carta, F. Carli, M D'Antraccoli, L. Ghillani, S. Picollo, L. Peruzzi (2018).113mo Congresso della Società Botanica Italiana, Book of Abstracts, Poster N. 94
4) G. Astuti, F. Olivieri, M. D'Antraccoli, G. Bedini (2019) 114mo Congresso della Società Botanica Italiana, Book of Abstracts (in press)
5) C.A. Ríos-Saldaña, M. Delibes-Mateos, C.C. Ferreira (2018) Global Ecol. Cons., 14, e00389

2.3 = Challenges for lichen diversity conservation in forest ecosystems

Renato Benesperi[1], Paolo Giordani[2], Elisabetta Bianchi[1], Gabriele Casazza[3], Alessandro Chiarucci[4], Matteo Dainese[5], Lorenzo Lazzaro[1], Chiara Lelli[4], Paola Malaspina[2], Lorenzo Marini[6], Pier Luigi Nimis[7], Chiara Vallese[4], Juri Nascimbene[4]
[1]Department of Biology, University of Florence, 50121 Firenze, Italy; [2]Department DIFAR, University of Genova; 16148 Genova, Italy; [3]Aix Marseille Univ, Avignon Université, CNRS, IRD, IMBE, Marseille, France; [4]Department BiGeA, University of Bologna 40126, Bologna, Italy; [5]EURAC Research, 39100 Bolzano, Italy; [6]Department DAFNAE, University of Padova, 35020 Legnaro, Padova, Italy; [7]Department of Life Sciences, University of Trieste, 34127 Trieste, Italy

Despite the fact that lichens greatly contribute to forest ecosystem diversity and functioning, conservation policies are almost missing to protect them, resulting in an alarming extinction risk for several forest species. Forest management is probably among the major causes of epiphytic species loss at the local and landscape level since several environmental factors relevant to the dispersal, establishment, and maintenance of epiphytic lichens are affected by forest management.

In the last decade, we dedicated research effort to explore the effects of forest management on lichen diversity in mountain and Mediterranean forests, carrying out a number of case studies across Italy under contrasting management regimes.

In general, our results revealed that higher lichen diversity is related with lower management intensity, even if in some cases non-intensively managed forests may provide better conditions for epiphytic lichens than recently abandoned forest. The main negative effects of forestry are related to the lack of old trees, short rotation cycles, excessive canopy cover, or excessive exposure to direct light in the final part of the rotation cycle, lack of substrate, particularly for dead-wood dwelling species, decrease of structural diversity, lack of forest continuity and forest fragmentation. In addition, inappropriate forest management may expose forest habitat to the effect of climate change as well as to a higher degree of threat of invasion by alien species (such as *Robinia pseudoacacia*). Strict protection of old growth remnants hosting lichen hotspots, coupled with integrative approaches applied at multiple spatial scales in production forests may provide a win-win strategy to reconcile timber production and lichen conservation.

2.3 = Update to the Liguria (NW Italy) checklist of macrofungi: new reports of over 1000 species of Basidiomycota and Ascomycota

Fabrizio Boccardo, Dario Gisotti[1], Grazia Cecchi[1], Simone Di Piazza[1], Mirko Benvenuti[2], Mirca Zotti[1]
[1]Department of Environmental Earth and Life Sciences (DISTAV), University of Genoa, Corso Europa 26, 16132, Genoa, Italy; [2]Dipartimento di Medicina Sperimentale (DIMES), Università degli Studi di Genova, Via Leon Battista Alberti 2, 16132 Genova

Liguria is a region of NW Italy characterized by a great variety of closely intertwined habitats, ranging from Mediterranean scrubs to alpine forests, and by a large number of wild macrofungi species.
The checklist of Liguria macrofungi, last updated in 2008, counted 1693 *taxa* (1, 2).
Through the effort of several ligurian mycologists, the present work lists new 1068 *taxa* of macrofungi previously never recorded in Liguria: 1018 of them belong to *Basidiomycota* and the remainder to *Ascomycota*. The major part of fungi was identified by macro-micromorphological methods, whereas the species that presented difficulties during the identification were molecularly analysed.
The findings come from surveys carried out in a very wide range of habitats, from the maritime *Cistus* shrubs of Punta Baffe (Sestri Levante, Genoa) to the *Quercus* and *Castanea* woods of Sassello (Savona), the *Abies* forest of Testa d'Alpe (Imperia), and Passo delle Salse (Imperia). Each species report includes the following items: latin name, author, habitat, height, and GPS coordinates.
This work represents an important contribution to the knowledge of the Ligurian mycoflora and integrates the national checklist.

1) M. Zotti, F. Orsino (2001) Flora Mediterranea, 11, 115-294
2) M. Zotti, A. Vizzini, M. Traverso, F. Boccardo, M. Pavarino, M. G. Mariotti (2008) Mycotaxon, 105, 167-170

2.3 = A multidisciplinary approach for the conservation of *Orchis patens* Desf.

Jacopo Calevo[1], Roberta Gargiulo[2], Samuele Voyron[1], Francesco Saverio Robustelli della Cuna[3], Leif Bersweden[2], Annalisa Giovannini[4], Cinzia Boselli[3], Michael F. Fay[2], Mariangela Girlanda[1]
[1]Department of Life Sciences and Systems Biology, University of Torino, Viale Mattioli 25, 10125 Torino, IT; [2]Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, Surrey, UK; [3]DDS Department of Drug Sciences, University of Pavia, via Taramelli 12, 27100 Pavia, Italy; [4] CREA Research Centre for Vegetable and Ornamental Crops, Corso degli Inglesi, 508 - 18038 Sanremo (IM), Italy

Effective conservation science relies on species-relevant knowledge obtained from multidisciplinary research such as population genetics, mycorrhizal associations and pollinator interactions. The Mediterranean *Orchis patens* is the flagship species in the European LIFE project LIFEorchids (LIFE17NAT/IT/000586, a project on the conservation of orchids and their habitats) because of an alarming decreasing number of individuals in its natural populations. In this work we used genetic diversity and structure data from microsatellite markers, fungal metabarcoding from the orchid roots and the analysis of floral VOCs to better understand the causes of its rarity and its interaction with the other organisms of the ecosystem. Our results will improve the conservation assessment and management of this Mediterranean and Endangered orchid species.

2.3 = Phylogenetic signal as a predictor of extinction risk in spermatophytes: woody species are at greater risk

Angelino Carta[1], Simone Orsenigo[2]
[1]Department of Biology, University of Pisa, Via Derna 1, 56126 Pisa, Italy; [2]Department of Earth and Environmental Sciences, University of Pavia, Via S. Epifanio, 14, 27100 Pavia, Italy

Examining biological diversity in an evolutionary context has been the subject of research for several decades, but only recently, the increasing availability of species-level phylogenies stimulated a blossoming number of investigations (1, 2). One approach is to quantify phylogenetic signal to determine the statistical nonindependence among species trait values due to their phylogenetic relatedness (3). When the phylogenetic signal of phenotypic evolved traits is detected, related taxa exhibit similar traits while in the absence of phylogenetic signal, taxa are not more similar than expected by chance (4). Whilst extinction risk is not a hereditable trait, there is emerging evidences that species at risk are non-randomly distributed across the phylogeny likely because extinction-proneness is shared among closely related taxa (5). Nonetheless, the degree of phylogenetic clustering of threat status at global scale has not been evaluated yet.

Here, we first quantified phylogenetic signal in extinction risk using all the species assessed at a global level according to the IUCN categories and criteria (https://www.iucnredlist.org/) and a pruned version of a recently published mega-tree of seed plants (6) leading to a final dataset embracing 7840 species. We then tested for an asymmetry in the degree of extinction risk due to growth form. We hypothesized that “woody” species (i.e., trees/shrubs) exhibit either higher risk, because they take many years to reach reproductive maturity (7) or a lower risk compared to herbaceous species, because woody species are reported to have larger and stable population sizes (8).

Our comparative analysis of 7480 plant species (among which 2666 threatened) indicates that extinction risk is not spread randomly across the phylogeny. Instead, the phylogenetic signal detected implies that closely related species are more prone to have the same extinction risk compared to species selected randomly from the phylogeny. This pattern may be explained because usually the species share intrinsic aspects of their biology (i.e.: life history, reproductive physiology, geographical distribution, and ecology; see 9). In addition, we also found a higher phylogenetic signal in some clades (e.g. Monocots) but not in others (e.g. Fabids) suggesting distinct phylogenetically driven susceptibility to extinction. We also found that herbaceous and woody species appear to be significantly ($P<0.0001$) associated with opposite values of extinction risk. Apparently, this latter result may be biased by the higher number of threatened woody species (1952) compared to the herbaceous one (714) in our dataset. However, randomization and species resampling confirm the higher proneness of woody species to be threatened which suggests differential sensitivities to extinction drivers between life forms.

In the light of phylogenetic signal in extinction risk, the integration of evolutionary and ecological data would thus help in predicting conservation status of data-deficient and not evaluated species (10, 11). Hence, we conclude that a complete understanding of extinction risk also requires consideration of species' evolutionary histories.

1) H. Sauquet, S. Magallón (2018) New Phytol., 219, 1170-1187
2) A. Carta, D. Gargano, G. Rossi, G. Bacchetta, G. Fenu, C. Montagnani, T. Abeli, L. Peruzzi, S. Orsenigo (2019) Sci. Tot. Env., 665, 1046-1052
3) L. Revell, L. Harmon, D. Collar (2008) Syst. Biol., 57, 591-601
4) J. Felsenstein (1985) Am. Nat., 125, 1-15
5) A. Purvis (2008) Ann. Rev. Ecol. Evol. Syst. 39, 301-319
6) S. Smith, J. Brown (2018) Am. J. Bot., 105, 302-314
7) M. Verdú (2002) Evolution, 56, 1352-1361
8) R. Petit, A. Hampe (2006) Ann. Rev. Ecol. Evol. Syst., 37, 187-214
9) J. Vamosi, S. Magallón, I. Mayrose, S. Otto, H. Sauquet (2018) Ann. Rev. Plant Biol., 69, 685-706
10) L. M. Bland, B. E. N. Collen, C. D. L. Orme, J. O. N. Bielby (2015) Conservation Biology, 29, 250-259
11) W. Jetz, R. Freckleton (2015) Philos. Trans. R. Soc. Lond. B. Biol. Sci., 370, 20140016

2.3 = Morphometric, biochemical and genetic analyses revealed a high biodiversity of Italian ancient olive trees

Nicola Criscuolo[1], Francesco Guarino[1], Claudia Angelini[2], Stefano Castiglione[1], Tonino Caruso[1], Angela Cicatelli[1]

[1]Department of Chemistry and Biology 'A. Zambelli', University of Salerno, 84084, Fisciano, Salerno, Italy; [2]Institute for Applied Mathematics 'Mauro Picone', CNR, 80131, Naples, Italy

The present study is focused on *Olea europaea* L. of Campania, an area of Southern Italy highly suitable for the cultivation of olive trees, with a recognized socio-economic and environmental value, and for the production of extra virgin olive oil (EVOO). Morphometric, biochemical and genetic analyses were conducted on olive drupes and leaves in order to elucidate the distribution of phenotypic, biochemical and genetic diversity in this area of cultivation, and to contribute to the development of a practical tool of EVOO traceability. To this aim, drupes and leaves were collected from trees living in four different areas of the Campania Region and morphometric characteristics were evaluated. Biochemical and genetic diversity were estimated on the basis of the fatty acid composition of manually extracted EVOOs, and with microsatellite markers, respectively. Moreover, in order to perform the statistical analysis we provided an open source tool as a novel *R* package namely *OliveR.* This software tool is useful in performing multivariate data analysis using a point-click interactive approach. The analyses, we conducted on the data set, highlighted a clear correlation among the morphological, biochemical and genetic profiles of olive trees and their collection sites. Our observations confirmed once more that Southern Italy, and especially Campania Region, represent a wide and relevant reservoir of phenotypic and genetic variability for this cultivated tree.

2.3 = Marine litter and psammophytes: a case study in the Migliarino-San Rossore-Massaciuccoli Regional Park coastal sand dunes

Alessio Mo, Marco D’Antraccoli, Gianni Bedini, Daniela Ciccarelli
Department of Biology, University of Pisa, Via L. Ghini 13, 56126 Pisa, Italy

Coastal sand dunes are one of the most impacted ecosystems in the world (1). They host various habitats of Community interest under the Habitats Directive 92/43/EEC but are profoundly affected by pollution and waste management, even within protected areas. The factors that define the amount, type and distribution of beach litter are complex and relate more to human intervention and natural variables.

This work aims to analyse the composition, abundance and distribution of marine litter within a protected area in two different chronological windows, i.e. before and after the bathing season. We also attempted to verify whether the presence of waste could alter the coverage of the psammophilous vegetation.

The protected area examined in this project is the Migliarino-San Rossore-Massaciuccoli Regional Park (Tuscany, Italy), where we selected three dune sites stretching along the coastline with a North/South gradient: Lecciona (2 Km^2), Bufalina (1 Km^2) and Calambrone (1 Km^2), respectively. We adopted a stratified random sampling design, using permanent multiscale squared-plots of 16 m^2, with two nested plots placed at a fixed corner of 4 m^2 and 1 m^2, respectively. Each site was divided into same-area strata with a spatially optimised algorithm. Inside each layer two points were randomly selected, corresponding to the field plots; overall, a total of 22 plots were sampled. Sampling took place in two sessions, one in May and the other one in November.

The data collected were the total percentage coverage of litter and the individual coverage of each type of litter for each plot. Classification of marine litter followed the directives of the "Master List of Categories of Litter Items", a list drawn up by the Joint Research Centre (JRC) of the European Commission based on several classification protocols (2). At the same time, we censused the plant species in each plot and measured their total percentage coverage at each investigated spatial scale.

We compiled a litter × plot matrix with the percentage of coverage of each type of waste found in the plots at the three spatial scales and in the two periods. The PERMANOVA analysis of the matrix with 4 factors (month, site, layer and plot area) highlighted that the interaction term month × site explained significantly ($P < 0.001$) the variance in the composition of waste at the plot level. NMDS analysis (non-metric multidimensional scaling) showed that the categories most significantly related to the variability between plots were the following: "plastic fragments less than 2.5 cm (G78)" and "plastic fragments between 2.5 and 50 cm (G79)". These two types of litter showed contrasting patterns over time: G78 increased from June to November, G79 decreased in the same period. Multivariate analyses were carried out using the software PRIMER v.7 (3) and PERMANOVA+ (4).

The diachronic study of the relationship between area and the number of litter categories, carried out using the Arrhenius power law equation (5), showed that in Lecciona and Bufalina there was in November a greater growth in types of litter as the area increased in respect to the first sampling period, while in Calambrone there were no differences. According to Pearson correlation test, temporal turnover of litter categories, quantified partitioning β-diversity following (6), and the total percentage coverage of vegetation resulted inversely correlated ($cor = - 0.44$, $P = 0.038$).

In conclusion, artificial polymer materials were found to be the dominant waste type (85%) at the three study sites examined, two of which were characterised by an increase in waste after the bathing season. In the third site, however, probably due to regular manual cleaning actions, no differences were found. Where vegetation is more abundant, the total number of litter categories tends to be more stable across the seasons, suggesting that psammophytes are a relevant biotic component to be considered in the analysis of spatio-temporal dynamics of coastal litter.

1) Ciccarelli D., 2014. Environ. Manag. 54, 194–204
2) Galgani F., Hanke G., Werner S., et al. (2013) JRC Scientific and Policy reports
3) Clarke K., Warwick R. (2001). Change in marine communities: an approach to statistical analysis and interpretation Ed. 2. PRIMER-E, Plymouth
4) Anderson M.J., Gorley R.N., Clarke R.K. (2008). PERMANOVA+ for PRIMER: Guide to software and statistical methods. PRIMER-E, Plymouth
5) Arrhenius O. (1921) J Ecol, 9, 95-99
6) Baselga A. (2010). Glob Ecol Biogeogr, 19, 134-143

2.3 = Effects of increasing coverage values of the invasive *Robinia pseudoacacia* on the sub-Mediterranean *Quercus pubescens* forests

Silvia Montecchiari[1], Giulio Tesei[1], Marina Allegrezza[1]
[1]Department of Agricultural, Food and Environmental Sciences, Marche Polytechnic University, Via Brecce Bianche 10, 60131 Ancona, Italy

Robinia pseudoacacia (black locust), a leguminous tree species from north America, is one of the 100 worst alien species presents in Europe (1). Its ecological and physiological characteristics let *R. pseudoacacia* to determine substantial changes in target ecosystems in comparison with natural forest habitats. Has been proven that *R. pseudoacacia* cause impacts on soil characteristics, arthropods and lichen communities and understory plant composition and diversity, forming characteristic plant communities (2); this allows black locust to be strongly competitive in natural vegetation succession processes (3). To assess the level of ecosystem change in invasion processes is necessary to consider a gradient of alien plant abundance (4) in homogeneous environmental conditions. In Italy *R. pseudoacacia* is strongly present in the central-northern sectors, where is in contact with several natural habitats such as *Quercus pubescens* natural forest habitats, threatening the integrity of this ecosystem. We want to investigate the *R. pseudoacacia* hilly communities of central-northern Italy in sub-mediterranean bioclimate, along cover classes, in the potentiality of the *Q. pubescens* natural forests. Through this investigation we highlighted the relations between increasing levels of *R. pseudoacia* cover values and vegetation indexes related to disturbed conditions. The preliminary results shows a clear separation between black locust coverage groups and the natural *Q. pubescens* forests. The behavior of the groups along the set of ecological indices let us say that high values of *R. pseudoacacia* coverage shows an homogenization effect; moreover already low coverage levels of *R. pseudoacacia* are linked to an initial enrichment in nitrophilous and alien species, and changes in nutrient, moisture and reaction indicators.

1) P. W. Lambdon, P. Pyšek, C. Basnou, M. Hejda, M. Arianoutsou, F. Essl, V. Jarošík, et al. (2008) Preslia 80 (2), 101–149
2) M. Allegrezza, S. Montecchiari, C. Ottaviani, V. Pelliccia, G. Tesei (2019) Plant Biosystems, in press
3)V. Wagner, M. Chytrý, B. Jiménez-Alfaro, J. Pergl, S. Hennekens, I. Biurrun, I. Knollová, et al. (2017) Diversity and Distributions 23 (9), 969–981
4) F. D. Panetta, B. Gooden (2017) NeoBiota 34, 53–66

2.3 = Monitoring of coastal dunes habitats in Tuscany through the "MONITO-RARE" project

Michele Mugnai[1], Simona Sarmati[2], Matilde Gennai[1], Simona Maccherini[2], Claudia Angiolini[2], Daniele Viciani[1], Andrea Bertacchi[3], Daniela Ciccarelli[4], Bruno Foggi[1].

[1]Department of Biology, University of Florence, 50121 Firenze, Italy; [2]Department of Life Sciences, University of Siena, 53100 Siena, Italy; [3]Agriculture, Food and Environment, University of Pisa, 56124 Pisa, Italy; [4]Department of Biology, University of Pisa, 56126 Pisa, Italy

"MONITO-RARE" is a project involving Tuscany Region and Universities of Firenze, Siena and Pisa, aimed to improve the knowledge and develop monitoring methodologies for species and habitats included in Natura 2000 Network and considered of community interest, according to Art. 11 and Art. 17 of the Directive 92/43/EEC. Among the habitat of interest (Annex I), coastal sand dune is one of the most threatened by multiple human pressures (1, 2), such as pollution, coastal erosion, effects of global warming, farming practices, urban development, and pressure from tourism (3). As part of this project, was carried out a first year of monitoring of dunal habitats (Natura 200 code 2110, 2120, 2210, 2230, 2240, 2250* and 2260) in five SACs (Special Areas of Conservation) in order to cover different latitudes of Tuscany coast ("Dune litoranee di Torre del Lago/Selva Pisana", "Tombolo da Castiglion della Pescaia a Marina di Grosseto", "Dune costiere del Parco dell'Uccellina" and "Duna del Lago di Burano"). The coastal areas to the north and south of Arno basin are very different by climatic, geomorphologic (erosion) and anthropic factors. The northern coast is twice rainy as the southern one.

Nevertheless, sand dune habitats are distributed in a fine scale mosaic, not distinguishable through aerial photos and maps. For this reason, we opted for a stratified sampling in three EUNIS habitat types (B1.3, shifting coastal dunes; B1.4, coastal stable dune grassland; B1.6, coastal dune scrub) for psammophilous vegetation. According to Sperandii et al. (4), we recorded these communities by 262 random plots of 2 x 2 m surface in which were surveyed pressures and threats, floristic composition and an estimate of abundance using a percentage cover scale ranging from 1 to 10. Our sampling is congruent with RanVegDunes (GIVD ID EU-IT-020), the first Italian database gathering standardized, randomly-sampled vegetation data in coastal dune environments.

We detected significative differences in abundance and species composition between EUNIS habitat types, between SACs, and between habitat types within SACs. In particular, our data shows particular differences between communities of north and south of Tuscany: the northern SACs to Serchio river are heavily impacted by the presence of mass tourism, with bathing establishments, roads and human trampling. From these evidences can be deduced that trails installed within the DUNETOSCA Life Projects and aimed to reduction of tourism impact may be insufficient to reduce anthropic pressures in those contexts and adequate conservation strategies are required.

Fig. 1. Coastal dune vegetation (Parco dell'Uccellina)

Fig. 2. Plot of 2 x 2 m for floristic surveys

1) EEA (1999) State and pressures of the marine and coastal Mediterranean Environment. Environmental Issues Series No. 5, European Environment Agency, Copenhagen

2) Barcelona Convention, Athens (2012) UNEP/MAP 2012. State of the Mediterranean Marine and Coastal Environment https://wedocs.unep.org/bitstream/handle/20.500.11822/364/sommcer_eng.pdf?sequence=4&isAllowed=y

3) Ciccarelli D. (2014) Environmental Management 54, 194–204

4) Sperandii M.G., Prisco I., Stanisci A., Acosta A.T.R. (2017) Phytocoenologia 47, 231–232

2.3 = Fungal Species Conservation: *Alessioporus ichnusanus* (Alessio, Galli & Littini) Gelardi, Vizzini & Simonini and *Poronia punctata* (L.) Fr. two new entries IUCN red list in 2019 thanks to a national collaboration network

Anna Maria Persiani[1]*, Italian Botanical Society mycology working group
[1]Department of Environmental Biology, Sapienza University of Rome, Piazzale Aldo Moro, 00185 Roma, Italy; *Coordinator of the Italian Botanical Society micology working group

On the 30 November 2018 in Rome was held the Workshop "Matching fungal conservation in Italy: the current state and future challenges" sponsored by The Italian Botanical Society and organized by the mycology working group with the participation of Anders Dahlberg. Following the workshop, the mycology working group committed to work, within The Global Fungal Red List Initiative, on the assessment of the threat status of two fungal species, in order to get them included in the IUCN red list. To pursue the assessment in the best possible way, thanks to a first opportunity of collaboration during the workshop, was promoted the initiative of developing a national collaboration network including the Italian Botanical Society mycology working group, research institutes and amateur associations.

The two species that were chosen to be assessed within The Global Fungal Red List Initiative were *Alessioporus ichnusanus* (Alessio, Galli & Littini) Gelardi, Vizzini & Simonini (Basidiomycota) and *Poronia punctata* (L.) Fr. (Ascomycota).

Alessioporus ichnusanus is an ectomycorrhizal species that grows in thermophilous broadleaved forests, associated with pure or mixed evergreen sclerophyllous and deciduous oak communities (1). The analysed data showed that this species has a range restricted to European Mediterranean region and grows in small scattered localities.

Poronia punctata is a coprophilous fungal species mainly associated with horse dung. Therefore, major threats to this species include the reduction of natural grazing of equines and other domestic animals and the reduction or loss of typical semi-natural habitats (2). Despite this species has a worldwide distribution, a substantial decline and several local extinctions have been observed during the last century in its population.

Data provided by members of the Italian Botanical Society Mycology Working Group, ISPRA Special Fungi Project, Associazione Micologica Ecologica Romana (AMER) and Agaricwatching Mycology Association, together with data reported in literature and in online databases, were crucial to estimate the current distribution and geographic range of these species.

The assessments proposed by this working group to The Global Fungal Red List Initiative (http://iucn.ekoo.se/en) have been reviewed within the ECCF Workshop in Cambridge, 25–29 March 2019 during which the following assessments have been formalized.

Alessioporus ichnusanus qualifies for the IUCN red list as Endangered under criterion C2a(i) because of less than 250 mature individuals in largest subpopulation and population decline.

Poronia punctata is assessed as Least Concern, because, even though the magnitude of its past population decline and several local extinctions, the risk of global extinction is currently low. However, *Poronia punctata* is considered of conservation concern and in case the decline restart, then it may quickly move towards a threatened category.

The upcoming publication (2019) of these two fungal species in the IUCN red list represents a remarkable result in order to promote fungal species conservation. Moreover, this experience showed the great importance of a collaboration network in order to achieve relevant results in nature conservation both on a national and international level.

Fig. 1. *Alessioporus ichnusanus* (Photo by Giancarlo Bistocchi)

Fig. 2. *Poronia punctata* (Photo by Veronica Spinelli)

1) M. Gelardi, G. Simonini, E. Ercole, A. Vizzini (2014) Mycologia, 106, 1168–87
2) A. Szczepkowski, A. Obidziński (2016) Acta Mycologica, 51(2): 1084

2.4 = Functional variation in forest understory at different spatio-temporal scales

Roberto Canullo[1], Giandiego Campetella[1], Stefano Chelli[1]
[1]Plant Diversity and Ecosystems Management Unit, School of Biosciences & Veterinary Medicine, University of Camerino, Via Pontoni 5, 63032 Camerino, Italy

In a context of noticeable climate and land use changes a lot of studies tried to elucidate the mechanisms supporting observed patterns of biodiversity and their environmental drivers. The taxonomic approach for the study of the understory dynamics in forests has been recently linked to the functional trait approach. Plant functional traits demonstrated to be a very effective tool in understanding the crucial mechanisms shaping the species patterns within ecosystems.

In this contribution, we present a series of approaches conducted at different spatial scales (biogeographic-landscape-stand level) and along time (0-100 yrs chronosequence, long-term diachronic observations) in order to elucidate patterns and mechamisms of functional variation of the understory.

Several datasets have been used, including the ICP Forests Level I and II of the Italian CONECOFOR network and local datasets from Central Italy. Approaches were mainly performed at community level (by using community weighted mean values, CWM), but included also aspects related to specific level.

At biogeographic scales, climatic factors showed a pervasive effect in determining LHS traits, i.e., specific leaf area-SLA, plant height, seed mass (1), as well as clonal and bud bank trait patterns in Italian forest understories. Soil, forest structure and land use factors alone showed a marginal effect. However, soil features exerted a certain influence on trait patterns only when joined with climate. Land-use related parameters demonstrated to influence only SLA, probably because they determine the light conditions of the ground layer. This finding is confirmed at landscape scale, where the shifting mosaic of light and dark phases of coppice regeneration processes after logging leads to significant changes in understory functioning, in terms of SLA (2), but also clonal traits (3,4). In beech coppice forests, this functional shift seems to depend on two distinct processes: the turnover of generalist species (i.e., species replacement) and the intraspecific adjustement of beech forest specialist species. In particular, specialist species increased their abundance-weighted mean SLA and variability during the chronosequence (i.e., years since the last logging) thanks to their intraspecific trait plasticity.

By using a series of fine-scale diachronic observations on four old-growth beech forests across the Italian peninsula, we also explored which traits are linked to the process of species persistence. Leaf traits (leaf anatomy and SLA) demonstrated to be key in determining fine-scale persistence in these environments. However, we revealed that different attributes (i.e., helomorphic vs. scleromorphic leaves) were of importance in the northernmost and southernmost sites, respectively, probably depending on the intensity of water stress. Persistent species in sites experiencing mild drought demonstrated an acquisitive resource strategy, while in forests with stronger potential water stress, persistent species seem to adopt a conservative resource strategy, supported by clonal reproduction.

Our overview highlights the importance of some functional traits (i.e., related to photosynthetic functions and to clonality) for the challenges of species adaptation and persistence in forest ecosystems. In addition, our results open new insigths on conservation practices aimed at increasing the functional structure of plant diversity.

1) Westoby, M. (1998) Plant and Soil 199: 213-227

2) Campetella, G., Botta-Dukat, Z., Wellstein, C., Canullo, R., Gatto, S., Chelli, S., Mucina, L. Bartha, S. (2011). Agriculture Ecosystem Environment 145: 38-48

3) Canullo, R., Campetella, G., Mucina, L., Chelli, S., Wellstein, C., Bartha, S. (2011) Folia Geobotanica 46: 271-288

4) Canullo, R., Simonetti, E., Cervellini, M., Chelli, S., Bartha, S., Wellstein, C., & Campetella, G. (2017) Folia Geobotanica 52: 71-81

2.4 = Root functioning in forest ecosystems

Antonio Montagnoli[1], Mattia Terzaghi[1], R. Kasten Dumroese[2], Bruno Lasserre[3], Gabriella Stefania Scippa[3], Donato Chiatante[1]
[1]Department of Biotechnology and Life Science, University of Insubria, Via Dunant 2, 21100 Varese, Italy; [2]U.S. Department of Agriculture, Forest Service, Rocky Mountain Research Station, Moscow, Idaho; [3]Department of Biosciences and Territory, University of Molise Pesche (IS), Italy

The root system represents a significant component of the total biomass in forest ecosystems. The finest component (diameter [d] < 2 mm) rarely corresponds to more than 5% of total tree biomass, but its annual production accounts for 33-67% of the total NPP (Jackson et al., 1997). Fine roots absorbing water and nutrients are of crucial importance to mature tree survival potential, and in either seedling recruitment or outplanting establishment. Modulation of fine root production and turnover rate is also a product of plant response to changing environment, and therefore may be a good indicator of forest adaptation to climate change (Eissenstat et al. 2000). Furthermore, coarse root component (d > 2 mm) is responsible for plant anchorage. Root plasticity allows trees to shape their root system architecture in response to mechanical forces such as gravity (slope conditions) and/or dominant wind, as well as to the soil hydrological perspective (Ghestem et al. 2011). Plasticity also plays a key role in modulating fine root morphology and specific root length enhancing the cost vs. benefit ratio. Here we present a series of case studies concerning root functioning in response to water shortage and fire, and mechanical forces at the mature-tree stage. Our work for all tree species examined (*Fagus sylvatica*, *Quercus cerris*, *Quercus ilex*) demonstrates a complex fine root pattern related to season, with biomass and length peaking in summer and late autumn (Figure 1). The summer maximum was characterized by an increase of the thinner part of the root population (smallest diameter size and highest specific root length [SRL]), while autumn maximum was characterized by an increase in mean diameter size of the fine root population (largest mean diameter size and lowest SRL). Thus, our results suggest that our tree species growing at different latitudes in the Mediterranean environment adopted an intensive strategy modifying the root length per unit mass, channeling carbon preferentially into the production of very fine roots. This allowed trees to exploit transient periods of low soil water content by accessing a greater soil volume and thereby facilitating nutrient and water uptake. On the contrary, in fall once precipitation sufficiently recharged soil moisture, the percentage of very fine roots that did not die after the summer flush continued their growth in a radial pattern to function for starch storage. When data collected after a moderate fire occurred in late November 2017 in a beech forest was analysed, fine root length and biomass revealed that fire damaged roots at the soil surface while stimulating new fine root production at a deeper soil layer (20-30 cm) (Figure 2). In the case of coarse roots, we excavated the root systems of *Pinus ponderosa* trees growing on a steeply sloped, volcanic ash-influenced soil in the northern Rocky Mountains of the United States to assess their functional coarse-root traits and root system architecture. We found that the trees had produced more roots, in terms of length and volume, in the downslope and windward quadrants than in their upslope and leeward quadrants, likely a response to mechanical forces toward improving stability (Figure 3).

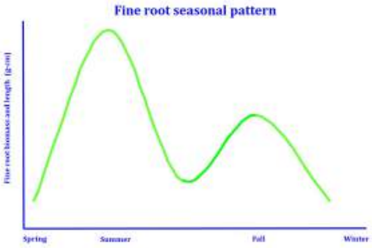


Fig. 1 Fine root seasonal variation in length and biomass

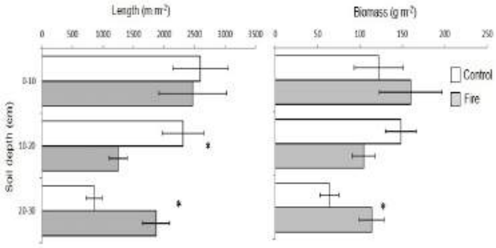


Fig. 2 Fine root biomass and length for beech trees experiencing moderate fire

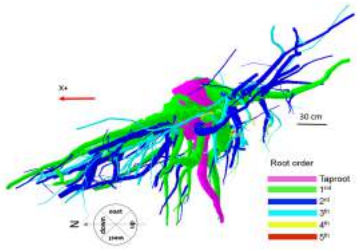


Fig. 3. Reconstruction of a 32-years old *Pinus ponderosa* root system

1) R. B. Jackson, H. A. Mooney, E. D. Schulze (1997) Proc. Natl. Acad. Sci. 94, 7362– 7366
2) D. M. Eissenstat, C. E. Wells, R.D. Yanai, J. L. Whitbeck (2000) New Phytol. 147, 33–42
3) M. Ghestem, R. C. Sidle, A. Stokes (2011) BioScience 61, 869–879

2.4 = What will the future forests look like? Comparison of performance between Mediterranean oak species in a common garden experiment

Martina Pollastrini[1], Elisabetta Salvatori[2], Fusaro Lina[2], Riccardo Marzuoli[3], Wolfgang Brüggemann[4], Filippo Bussotti[1]

[1]Department of Agriculture, Food, Environment and Forestry (DAGRI), University of Firenze, Piazzale delle Cascine 28, 50144 Firenze, Italy; [2]Department of Environmental Biology, University “La Sapienza”, Piazzale Aldo Moro, 5, 00185 Roma, Italy; [3]Department of Mathematics and Physics, University of Cattolica del Sacro Cuore, Via dei Musei 41, 25121 Brescia, Italy; [4]Institut für Ökologie, Evolution und Diversität, Goethe Universität Frankfurt am Main, Max-von-Laue-Str. 13, D-60438 Frankfurt, Germany

Forests are subjected to impacts of climate changes, including extreme drought phenomena, which can be very harmful for seedlings therefore compromising the regeneration. Phenotipic plasticity of tree species under environmental changes can be threatened and it is expected a decrease of tolerance to climate events (1). Modifications of forest structure and composition, including the substitution of mesophytic deciduous formations to xeric or evergreen forests and shrublands may occur. Such ecological changes will take place at landscape level, slowly and in uneven way, since the natural migration speed of trees, until reaching a new potential equilibrium with the modified conditions, may be too slow for an ordered successional process (2).

Before planning any management and conservative actions to address the adaptability of forests to the changing environment, it is essential to know the physiology and ecology of tree species under current climate. The project ‘European Oaks under Climate Change’ (FUTUREOAKS) addresses this issue, simulating the assisted migration (i.e. replacement of existing forest species/genotypes by neighbouring species/genotypes filling the same ecological function) of tree species that could move from Mediterranean area to Central Europe.

The performance (i.e. plant grow, photosynthesis, resource use efficiency, mortality) of *Quercus ilex* L., *Quercus pubescens* Willd., *Quercus frainetto* Ten., were tested, using two accessions (from Greece and Italy), grown in common gardens in Germany, Greece and Italy. The purpose was to assess the potential for the acclimation of species and provenances for planting as alternative forest species under the expected future drier and warm condition (3). This contribute refers to the field survey carried out in Italy, where a common garden has been established in Umbria (Santa Anatolia di Narco, Perugia). Results will be then compared with those obtained in the correspondent common gardens in Greece and Germany.

The common garden was established in April 2017, by using seedling produced from acorns collected in selected forests in Italy and Greece and ripened in the autumn 2016. Our analyses started in September 2017, when the plants were one year-old. In September 2018 measures of plant growth (stem height), Specific Leaf Area, gas exchange (net carbon assimilation, stomatal conductance to water vapour, water use efficiency) and chlorophyll fluorescence were carried out on each species and provenance.

Q. ilex and *Q. pubescens* resulted the best performing species, showing higher growth and photosynthetic efficiency respect to *Q. frainetto*. Seedlings of this last species exhibited greater mortality after one year than the other species, especially for the greek provenance. Differences between the provenances also resulted in the stem height, chlorophyll fluorescence and gas exchange parameters, with *Q. ilex* and Q. *pubescens* showing a better performance of the Italian seedlings compared to Greek ones. The xerophytic *Q. ilex* and *Q. pubescens* resulted the species more suitable for sustainable climate-adapted forests under the constrains of climate change. An increase of their distribution area in Central Europe is expected (4) and an *ad hoc* management strategy should be adopted to safe forest ecosystems and to adapt them to changing environment.

1) S. Noce, A. Collalti, M. Santini (2017) Ecology and Evolution doi 10.1002/ece3.3427
2) F. Bussotti, M. Pollastrini, V. Holland, W. Bruggemann (2015) Environmental and Experimental Botany 111, 91-113
(3) F. Bantis, J. Graap, E. Früchtenicht, F. Bussotti, K. Radoglou, W. Brüggemann (2019) Photosynthetica, *submitted*
(4) T. Hickler, K. Vohland, J. Feehan, P.A. Miller, B. Smith et al. (2012) Global Ecology and Biogeography 21, 50-63

2.5 = Transformation of floral composition and plant cover of coastal part of Caspian Sea in Azerbaijan

Valida Alizade, Kamalya Asadova, Rena Abdieva, Naiba Mehdiyeva, Shahla Mirzayeva
Institute of Botany, Azerbaijan National Academy of Sciences, Badamdar highway 40, Baku AZ1004, Azerbaijan

The vegetative cover of the coastal ecosystems of the Caspian Sea in the whole is distinguished by rich floristic diversity [5]. Coastal part of the Caspian Sea in Azerbaijan is 1205 km. Over the past 100 years, plant diversity has exposed to a serious anthropogenic transformation. Transgression leads to a periodic change in sea level, which inevitably affects the vegetative cover of this unique part of the coastal strip [3]. The aim of the research was to monitoring of early spring and summer plants, assessment of their composition and structure in the changing anthropogenic and environmental conditions by using classic and modern approaches. The observed vegetation transformation is confirmed by the processed aerospace images (AZERSKY), images were linked to the WGS-84 coordinate system. Also GPS coordinates was applied on the basis of which electronic maps of vegetation cover of coastal ecosystems have been created.

The study of current state of the coastal part of the Sea reveled that plant species such as *Convolvulus persica, Argusia sibirica, Artemisia arenaria, Cakile euxina, Melilotus caspica, Ammochloa palaestina*, *Xanthium strumarium* L. are widely distributed (Figure). At the same time, the specific landscape plant formations of the middle part of the coastal zone are represented with *Suaedeta, Salicornieta, Phragmiseta*, *Juncuseta, Calamagrostiseta, Elagnuiseta* and *Herbosa*. In addition, due to the transgression in recent years, wetland communities with the participation of *Phragmites australis*started become dominant.

At the present time, an increase in anthropogenic impact leads to the destruction of individual taxa, especially rare and endemic plant species (*Calligonum bakuense, Alcea kusariensis, Crocus speciosus, Tulipa* biebershteinii), the reduction of habitats, the spread of weeds, and conversely, the expansion of habitats and the emergence of new taxa in new specific biotopes. This relates to large-scale technogenic transformations of ecotopes in the development of oil fields, as well as to intensive construction and human activities [1]. Anthropogenic pressure observed against the background of global climate warming leads to disruption of the structure of vegetation cover, redistribution of the dominant composition of communities and the progressive development of synanthropic and invasive vegetation [2, 4]. The current ecological human impact requires urgent measures in the field of the modern assessment of biodiversity and human resources development [6]. In condition of climatic, antropogenic and technogenic environmental changes the prediction of development of flora and plant cover on the base of obtained results will be useful for elaborate scenarious and creation of protected areas in this region.

Some plant species in the coast of the Caspian Sea. From left to right *Convolvolus persica*L, *Calligonum bakuense* Litv, *Xanthium strumarium* L.

1) Antropogenic Transformation of the South Caucasus Natural Ambience.Tbilisi. 2008
2) Abdieva R.T. (2018) The list of invasive flora of Azerbaijan. Baku
3) Budagov B., Mamedov R., Ismatova H., Mikailov A. (2002) The Current State and Causes of Desertification of the Azerbaijan Coastal Area of the Caspian Sea. The issues of drought and desertification in the countries of South Caucasus, 31-38
4) Zernov A.S., Mirzayeva Sh.N. (2014) New Invasions and Expansions in Flora of the Absheron Peninsula. ANAS News(Division of Biological and Medical Sciences), 69, 22-30
5) Karyagin I.I. (1952) Flora of Absheron. Baku: Publishing House of the Academy of Sciences of the Az SSR
6) Caucasus. Plant diversity between the Black & Caspian Seas. Berlin 2014

2.5 = The effect of wind and land cover on the diffusion of *Quercus* pollen

Francesco Ciani[1], Giovanna Marchi[2], Lorella Dell'Olmo[1], Bruno Foggi[1], Marta Mariotti Lippi[1]
[1]Department of Biology, University of Florence, Via La Pira 4, 50121, Florence, Italy; [2]Regional agencies for environmental protection of Tuscany, Laboratorio Area Vasta Sud - UO Biologia, Viale Maginardo 1, 52100 Arezzo, Italy

The study on pollen transport and dispersion must take into account the weathers variables. Wind has been recognized as one of the major factors affecting pollen air circulation (Palacios et al., 2000). Wind vectors as direction, persistence, and speed cause a pollen dispersion over a long distance. The jointed analysis between these variables and the distribution of the source plants can allow forecasting the transport of airborne pollen grains from the areas of greatest pollen contribution.

The present study was conducted in Florence, Italy (Fig. 1), and focused on the effects of wind and land cover on *Quercus* pollen type. The pollen data were furnished by Regional agency for environmental protection of Tuscany (ARPAT) whose pollen sampling has been carried out through the use of a volumetric Hirst-type spore trap (Hirst, 1952). Meteorological data concerning wind were supplied by the weather station of the University of Florence. Wind variables as mean daily direction, maximum daily speed and the frequency of calm days were statistically correlated with *Quercus* pollen data. A buffer of 10 km in radius surrounding the volumetric sampler was created using GIS software in order to detect the presence of oak woods and thickets in the area surrounding Florence. The buffer was further divided into eight parts (octants) corresponding to the predominant wind directions (Fig. 1).

The statistical model shows a rather good correlation between pollen data, wind maximum intensity, and north-east wind mean direction. A negative correlation is instead showed between pollen data, wind maximum intensity and north-west mean direction and between pollen values, north wind mean direction and the frequency of wind calm days.

The land cover map (Fig. 2) shows that the northeastern area of the buffer, i.e. N-NE and NE-E octants, hosts the highest extension of broad-leaved forests (both pure broad-leaved forest and mixed coniferous/broad-leaved formations), about the 8% of all the buffer area. In the W-NW and NW-N octants these formations reached about 2% of the total area.

The jointed analysis between pollen values, the land cover and the wind variables suggests a significant pollen contribution from the northeastern reliefs that surrounding the urban area of Florence. The highest contribution is reached when the wind blows at high speed.

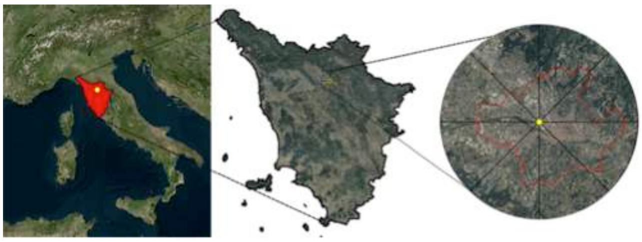


Fig. 1. Geographical location of Florence and the studied area.

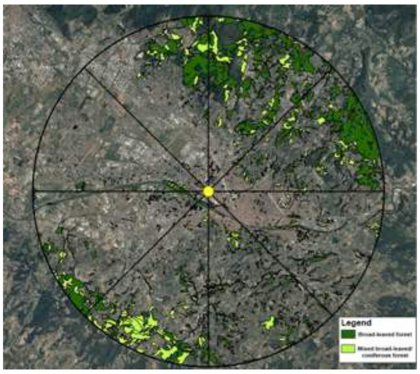


Fig. 2. Distribution of broad-leaved forest.

1) I. S. Palacios, R. Molina T., A.M. Rodríguez (2000 International Journal of Biometeorology, 44(3), 128-133
2) J. Hirst (1952) Annals of applied Biology, 39(2), 257-265

2.5 = Tree species richness modulates the effect of drought and heat stress on tree mortality and understory diversity in thermophilous deciduous forests

Giovanni Iacopetti[1], Martina Pollastrini[1], Filippo Bussotti[1], Federico Selvi[1]
[1]Department of Agriculture, Food, Environment and Forestry, University of Florence, Piazzale delle Cascine 18, 50144 Florence, Italy

Biodiversity is known to enhance ecosystem stability (1). Overstorey species composition, being the dominant tree species the major autogenic "engineers" of forest ecosystems (2), is expected to have a strong influence on forest responses to biotic and abiotic stress factors. Both tree diversity level and species identity play a role in modulating the effects of climatic extremes.

The Italian network of plots established in 2011 for the FunDivEurope project (3) includes 36 permanent quadrats of 30 x 30 m, located in Central Tuscany and belonging to the category "thermophilous deciduous forest" of the European Environment Agency system. The tree layer is composed by different mixtures of five species, *Castanea sativa*, *Ostrya carpinifolia*, *Quercus cerris*, *Q. ilex* and *Q. petraea,* representing four species richness levels (1, 2, 3, and 4+5 species). The stands share similar environmental and structural conditions, being old coppices with no management since 50-60 years, and are mostly included in the Alto Merse, Farma and Berignone-Tatti Natural Reserves. The experimental design of FunDivEurope was optimised to test the effect of tree layer species richness on a pool of forest functions and processes.

To this purpose, in 2012 we measured DBH, height, crown diameter, crown condition and width of tree rings for all trees in the overstorey (> 7 cm), in each of the 36 plots. In addition, we analyzed the understorey vegetation (< 1.3 m) by recording cover and maximum height of each species. In 2018, we repeated the same sampling to examine the changes in structure and composition of the forests after the repeated heat waves and consecutive peaks of the yearly average temperature, that occurred together with prolonged periods of drought (2014, 2015, 2016). Based on preliminary observational data, in fact, these events stressed the forest vegetation of the sites.

We found that that heat and/or drought, combined with the biotic attack of *Dryocosmus kuriphilus* on *C. sativa* affected the tree layer composition of the canopy and, consequently, its shade casting ability. This caused cascading effects on the understory by changing the ecological conditions at also the forest floor, similarly to other case studies (4).

Many herbaceous species present in the first survey were no longer found in 2018, and the change in composition reflected the expected shift towards a more arid-tolerant and light demanding community.

This contribution explores how tree diversity and composition may have modulated the climatic effect on ground conditions through tree mortality and crown health, by causing species loss in the herb layer and community compositional shifts, along with changes in Ellenberg's Indicator and C-S-R mean values.

1) S. Naeem, S. Li (1997) Nature 390, 507–509
2) J. H. Lawton, C. G. Jones (1995) Linking Species and Ecosystems 141–150
3) F. Bussotti, A. Coppi, M. Pollastrini, M. Feducci, L. Baeten, M. Scherer-Lorenzen, K. Verheyen, F. Selvi (2012) Rivista Di Selvicoltura Ed Ecol For. 9 251–259
4) T. Nagel, G. Iacopetti, J. Javornik, A. Rozman, P. De Frenne, F. Selvi, K. Verheyen (in press) Journal of Vegetation Science

2.5 = Distribution and ecological role of selected shrubby species in the Mediterranean basin

Emanuela Lombardo, Gianniantonio Domina, Giuseppe Venturella
Department of Agricultural, Food and Forest Sciences, University of Palermo, Viale delle Scienze, Bldg. 5, 90128, Palermo, Italy

In many natural and semi-natural habitats trees and shrubs represent two major growth forms (1). In particular shrublands, which can be considered a mixture of several sclerophyllous shrubs and tree species, represent a Mediterranean type of vegetation rarely found in other ecosystems. The distribution of shrubs in an ecosystem is connected to complex ecological processes. Indeed, shrubs provide substrate, food and shelter for organisms, play an important role in the nutrient cycle providing organic matter input to soils and they protect watersheds from erosion. Moreover shrubs are very important for many associated species such as mammals, birds, invertebrates, other plants (that favour thanks to their role of nurse plants), and their distribution also influences the variety and abundance of mycorrhizal fungi, fundamentals in ecological terms and nutritional relationships (2). This study represents a review on the ecological role of some selected Mediterranean shrubby species. In particular the attention is focused on the following species: *Amelanchier ovalis* Medik, *Astragalus nebrodensis* (Guss.) Strobl., *Crataegus laciniata* Ucria, *Lycium intricatum* Boiss., *Prunus spinosa* L., and *Viburnum tinus* L. These species have been selected because they are representative of different Mediterranean vegetation types, distributed in relation to the bioclimate type and the altitude. In fact, various habitats have been considered in relation to these species, for example coastal and sub-coastal environments for *Lycium intricatum*, sclerophyllous woodlands for *Viburnum tinus*, mantle vegetation and pre-forest structures for *Amelanchier ovalis*, *Prunus spinosa* and *Crataegus laciniata*, orophilous thorny-cushions vegetation for the Sicilian endemic *Astragalus nebrodensis*. For each species the geographical distribution in the Mediterranean Basin has been investigated, considering its borders according to the limits of Mediterranean climate (3), and the definition of terrestrial ecoregions of the world (4). The species distribution as well as their taxonomy (especially in relation to the presence of subspecies in the Italian territory) have been analyzed thanks to a number of scientific publications and web-based sources (e.g. Euro + Med PlantBase, The International Plant Names Index, ILDIS- International Legume Database & Information Service, PAN-European Species directories Infrastructure, Portale della Flora d'Italia, Flora of Greece Web, Anthos, African Plant Database, Silene-Flore database, etc.) at regional, national and international scale. Moreover for each species distribution maps have been prepared geo-referencing the species location data obtained from the labels of digital herbaria samples of the Muséum National d'Histoire Naturelle, MNHN – Paris, P; Naturhistorisches Museum Wien, W; Herbarium Mediterraneum Panormitanum, PAL; Herbarium Plant Biology Section, CAT; Botanischer Garten und Botanisches Museum Berlin-Dahlem, Zentraleinrichtung der Freien Universität Berlin, B, and from the data portal Global Biodiversity Information Facility- GBIF (always in relation to herbarium samples), also in order to obtain historical distribution data. The ecological role of species in every ecosystem, but also of their successional dynamics and their response to global changes and disturbances was investigated making an analysis of the literature data and also in relation to some botanical aspects and functional traits, available for some of the studied species, from TRY Plant Trait Database (https://www.try-db.org) and form BROT 2.0 database, for example: Growth form, Leaf phenology, Spinescence, Flower color, Dispersal mode, Fruit type, Nutritional relationships (the analysis of these data is in progress especially with regard to mycorrhizal symbioses). This has allowed to clarify why shrubs are successful in many habitats and to evaluate the critical role that they play in Mediterranean ecosystems.

1) Götmark Frank, Götmark E., Jensen A.M. (2016). Front. Plant Sci.7:1095
2) Kerns B.K., Ohmann J.L. (2004). Ecological Indicators 4, 83–98
3) Quézel & Médail (2003). Ecologie et biogéographie des forêts du basin méditerranéen. Elsevier, Paris
4) Olson *et al.* (2001). BioScience 51:933–938

2.5 = Monitoring of shrub and larch encroachment in a subalpine grassland after grazing exclusion: a new methodological approach

Ludovica Oddi[1], Edoardo Cremonese[2], Gianluca Filippa[2], Marta Galvagno[2], Umberto Morra Di Cella[2], Consolata Siniscalco[1]

[1]Department of Life Sciences and Systems Biology, University of Turin, Via Pier Andrea Mattioli 25, 10125 Turin, Italy; [2]Environmental Protection Agency of Aosta Valley (ARPA VdA), Località Grande Charrière 44, 11020 Saint-Christophe (AO), Italy

Land-use change is considered the factor with major effects on ecosystem functioning and biodiversity. Currently, the abandonment of meadows and pastures in mountain areas is the first cause of recolonization of open areas by forest through the encroachment of shrubs and trees. This process threatens habitat and species conservation of semi-natural grasslands. Moreover, land-use change may also alter the physical and chemical characteristics of soils together with vegetation, ultimately affecting ecosystem functioning.

This work aims at monitoring and analysing shrub and larch (*Larix decidua*) encroachment in a subalpine grassland that was abandoned in 2008, and at testing an alternative method of monitoring that might be faster and more efficient than filed surveys. The study area is located on the western Italian Alps at 2200 m a.s.l. and vegetation is dominated by *Nardus sricta* (Habitat Directive - 6230*). Two different approaches were followed to reach the aims, so that vegetation dynamics was investigated from both an ecological and methodological point of view. A vegetation survey of shrubs and larches was carried out at the end of the growing season 2015 and 2018 (October) along 45 transects 100 m long. Maximum trunk diameter, height and canopy dimension were measured for each larch in the grassland, whereas among shrubs only those occurring along the transect were considered and measured for maximum width and length. Furthermore, images acquired by drone in October 2015 and 2018 were photo-interpreted with ArcGis software (version 9.1). Afterwards, data collected in the field were analysed and compared with results of photo-interpretation.

Results showed that larch and shrub encroachment occurred at a very rapid rate, with the number of larches that almost doubled in just three years. Moreover, larches distribution followed a patchy pattern with areas of significant higher similarity in density and dimensions. The comparison between methods showed that photo-interpretation had a good performance for shrub cover estimation and the detection of larches higher than 10 cm.

The presence of a patchy distribution of larches can be explained referring to ecological variables, such as distance from forests, wind and water transport of larch seeds along preferential directions, and the presence of ‘islands’ formed by woody shrubs that might provide a favourable microenvironment for seed germination or a physical barrier to seed dispersion. Finally, the efficiency of phot-interpretation of drone images showed that this method might be a good alternative to vegetation survey for monitoring vegetation dynamics in wide areas, although an improvement of this method is still needed.

2.5 = Forest dynamics in Sacred Natural Sites – A long-term multi-scale analysis across Italy

Piero Zannini[1], Filippo De Vigili[1], Juri Nascimbene[1], Fabrizio Frascaroli[1], Marco Cervellini[1], Alessandro Chiarucci[1]
[1]Department of Biological, Geological and Environmental Sciences, University of Bologna, Via Irnerio 42, 40126 Bologna, Italy

According to IUCN-UNESCO, Sacred Natural Sites (SNS) are portions of land or sea having special spiritual significance human communities. Due to their spritual value SNS have been non-intensively managed for centuries, if not for millennia. Indeed, they are regarded as the oldest protected areas in human history and are now recognized as OECM (Other Effective Area-Based Measures) that can preserve biodiversity and provide multiple ecosystem services. On this basis, it is expected that they represent areas with lower disturbance and higher stability in forest dynamics. With this study we tested the hypotheses that SNS show lower changes in forest cover than reference sites (RS) and a greater number of patches have mantained the same landuse type in SNS than in RS. For testing this we use a time frame of ca. 80 years for whihc detailed forest cover data are available.
We used a georeferenced national-level inventory of about 1,600 SNS and an equal number of random sites. These RS have similar landuse features as compared to SNS and are distributed in the same elevational belts. As land use cartographies, we used the Italian Kingdom Forest Map, dated 1936 (IKFM), and Corine Land Cover Map 2012 (CLC). In order to reduce inconsistencies between the data we limited our analyses to forest-non forest land use types, irrespectively of forest type. Moreover, to account for different minimum mapping units we rasterized the data cartographies at 500m resolution. We then calculated forest change and patch persistency for each SNS and RS within nested circular windows of 1,000m, 1,500m, 2,000m and 2,500m. We are aware that a major limitation of this work is due to the several georeferencing inconsistencies of IKFM, nevertheless results can be considered reliable due to the large number of SNS and RP analyzed. Although our results are sensitive to different spatial scales they indicate that forest dynamics of SNS differ from those of RS, being more stable. Hence, according to our hypotheses, SNS seem to be more resistant to landuse modifications. Further studies should evaluate this processes in a finer way, by analysing the role of different forest types, in particular dominant species and degradation stages.

2.6 = Notes on syntaxonomy, chorology and dynamics of *Olea europaea* L. var. *sylvestris* (Mill.) Lehr. forests in the Mediterranean landscape

Lorenzo Gianguzzi[1], Giuseppe Bazan[2]
[1]Department of Agricultural, Food and Forest Sciences, University of Palermo, Via Archirafi 38, 90123 Palermo, Italy;
[2]Department of Biological, Chemical, and Pharmaceutical Sciences and Technologies, University of Palermo, Via Archirafi 38, 90123 Palermo, Italy

The wild olive or oleaster [*Olea europaea* L. var. *sylvestris* (Mill.) Lehr.] is a typical woody plant of the Mediterranean landscape, of which is an emblematic element of natural forest vegetation (1, 2). Forest community dominated by oleaster that has been described up until now in the Mediterranean basin, as more or less evolved aspects of woods, microwoods and high shrubland that principally tends to make up climax and edaphic climax "series heads". These formations are currently quite rare and fragmented in the wake of large-scale deforestations and the impoverishment of old-growth communities dominated by a millennial species; however, they maintain a significant large-scale distributive potential within the infra- and thermomediterranean bioclimate belt (also with small penetrations into the mesomediterranean) with a dry-subhumid (and sometimes humid) ombrotype. The study was conducted through the analysis of phytosociological data taken from the scientific literature and other unpublished data regarding North Africa (Morocco, Algeria), the Iberian Peninsula, the Balearic Islands as well as other islands from the Tyrrhenian area (Sardinia, Corsica, Sicily and its minor islands), the Italian Peninsula, the Balkan Peninsula, the Aegean region, Turkey and the southern Anatolian coast. A comparison between the different communities has shown a high floristic and physiognomic-structural homogeneity that justifies their categorization in the class *Quercetea ilicis* Br.-Bl. in Br.-Bl., Roussine & Nègre 1952.

The biogeographic and ecologic vicariance shown by the same formations within the large Mediterranean distribution range make it possible to subdivide them into the following three orders and respective alliances and sub-alliances:

1) *Pistacio lentisci-Rhamnetalia alaterni* Rivas-Martínez 1975 [a) all. *Tetraclini articulatae-Pistacion atlanticae* Rivas-Martínez, Costa & Izco 1986 (suball. *Pistacienion atlanticae* Barbero, Quézel & Rivas-Martinez 1981); b) all. *Asparago albi-Rhamnion oleoidis* Rivas Goday ex Rivas Martinez 1975; c) all. *Arbuto unedonis-Laurion nobilis* Rivas-Martínez, Fernández-González & Loidi 1999 (suball. *Arbuto-Laurenion nobilis* Rivas-Martinez & Sanchez-Mata 2001); d) all. *Oleo sylvestris-Ceratonion siliquae* Br.-Bl. ex Guinochet & Drouineau 1944];

2) *Quercetalia calliprin*i Zohary 1955 [e) all. *Ceratonio-Pistacion lentisci* Zohary et Orshan 1959];

3) *Quercetalia ilicis* Br.-Bl. ex Molinier 1934 [f) all. *Querco rotundifoliae-Oleion sylvestris*n Barbéro, Quézel & Rivas-Martínez in Rivas-Martínez, Costa & Izco 1986; g) all. *Fraxino orni-Quercion ilicis* Biondi, Casavecchia & Gigante ex Biondi, Casavecchia & Gigante in Biondi, Allegrezza, Casavecchia, Galdenzi, Gigante & Pesaresi 2013; h) all. *Erico arboreae-Quercion ilicis* Brullo, Di Martino & Marcenò 1977].

In the thermomediterranean belt most affected by farmland, the oleaster formations is generally made up of small fragmented nuclei; in these cases, they are located on isolated rocky outcrops or between hedgerows – for example along dry-stone walls – as a phytocoenological expression of regeneration. The most complete residual aspects are found on steep ridges which are not suited to agriculture, or in semi-natural environments in the upper thermomediterranean or mesomediterranean belts; this is the case, for example, of sites located near various kinds of rocky outcrops or on ledges, in areas where fire can't easily spread, and where forest vegetation can therefore evolve more easily.

Olea europaea L. var. *sylvestris* (Mill.) Lehr. forests are protected by European Habitats Directive (43/92 CEE; code "9320 - Olea and Ceratonia Forests") (3, 4). Considering the relictual nature of these formations in the Mediterranean landscape, it would be opportune extent the conservation measures to the other countries within the distribution range.

1) S. Rivas-Martìnez, F. Fernàndez-Gonzàlez, J. Loidi, M. Lousa, A. Penas (2001) Itiner Geobot., 14(2), 5-341
2) S. Rivas-Martínez, T.E. Díaz, F. Fernández-González, J. Izco, J. Loidi, M. Lousã, A. Penas (2002) Itiner Geobot., 15(1-2), 5-922
3) L. Gianguzzi, E.V. Perrino (2016). In: P. Angelini, L. Casella, A. Grignetti, P. Genovesi (editors). Roma. ISPRA, Serie Manuali e Linee Guida; p. 248-249
4) D. Gigante, F. Attorre, R. Venanzoni, A.T.R. Acosta, E. Agrillo, M. Aleffi, N. Alessi, M. Allegrezza, P. Angelini, C. Angiolini, et al. (2016) Plant Sociol., 53(2), 77-87

2.6 = Syntaxonomical and ecological comparison between termophylous *Quercus* woodlands of Calabria (S-Italy) and Southern Iberian Peninsula

Carmelo Maria Musarella[1,2], Ana Cano-Ortiz[2], Antonio Morabito[1], José Carlos Piñar-Fuentes[2], Ricardo Quinto-Canas[3], Catarina Meireles[4], Carlos Pinto-Gomes[4], Eusebio Cano[2], Giovanni Spampinato[1]

[1]Dept. of AGRARIA, "Mediterranea" University of Reggio Calabria, Località Feo di Vito, 89122 Reggio Calabria, Italy; [2]Dept. of Animal and Plant Biology and Ecology, Botany Sector, University of Jaén, Paraje las Lagunillas s/n, 23071 Jaén, Spain; [3]Faculdade de Ciências e Tecnologia, Universidade do Algarve,Campus de Gambelas, 8005-139 Faro, Portugal; [4]Dept. de Paisagem, Ambiente e Ordenamento/Instituto de Ciências Agrárias e Ambientais Mediterrânicas (ICAAM). Universidade de Évora, Portugal

The genus *Quercus* L. includes about 663 taxa, including species, subspecies and hybrids (1). It is distributed throughout in the whole northern hemisphere where they characterize a great variety of forests of economic, environmental and naturalistic importance (2). Altogether, the genus *Quercus* is one of the most disputed and problematical ones from the taxonomical point of view. In this genus, hybridization and introgression have determined the presence of plants with intermediate morphological characters compared to the parental species ones, which make the systematic of this group particularly controversial. The high morphological variability caused different taxonomic interpretations by the authors concerned with this genus. In the Mediterranean area, the oak woods that has a considerable ecological importance are certainly the sclerophyllous woods. In them, it is possible to find different plant communities where the dominant species are evergreen oaks (e.g. *Q. suber* and *Q. ilex*) and semi-deciduous species of *Q. pubescens* group (e.g. *Q. virgiliana* and *Q. amplifolia*).

The aim of this research is to update the knowledge on structure, floristic composition and ecology of evergreen oak forests of Calabria (Southern Italy) and the Southern of Iberian Peninsula (Spain and Portugal).

The field survey of forest vegetation was carried out in the period 2000-2013, through the phytosociological method of the Zurich-Montpellier school (3). The phytosociological relevés carried out were subsequently processed with a multivariate statistical analysis with the aim of defining coenological groups on an actual statistical basis.

Both Calabrian and Iberian woodlands belong to the class *Quercetea ilicis* Br.-Bl. ex A. Bolòs 1950, and to the order *Quercetalia ilicis* Br.-Bl. ex Molinier 1934, that include evergreen and semi-deciduous thermo- to supramediterranean oak forests of the Mediterranean territory. The two main groups distinguished in the cluster analysis correspond to the two compared territories. In Calabria, evergreen oak woods (with *Q. ilex* subsp. *ilex* and *Q. suber*) or semi-deciduous oak woods with several associations extend from the hilly to the sub-mountain belt and are included in the alliance *Fraxino orni-Quercion ilicis* Biondi, Casavecchia & Gigante in Biondi et al. 2013. In the Southern of Iberian Peninsula, the various plant communities highlighted are gathered in three clusters that correspond to a same number of alliances: *Quercion ilicis* Br.-Bl. ex Molinier 1934, in thermo-supramediterranean bioclimate on calcareous substrates; *Quercion broteroi* Br.-Bl., P. Silva & Rozeira, 1956 em. Rivas-Martinez, 1975 corr. Ladero, 1974, in meso-supramediterranean bioclimate belt; *Querco rotundifoliae-Oleion sylvestris* Barbéro, Quézel & Rivas-Martínez in Rivas-Martínez, Costa & Izco 1986 in the thermo-mesomediterranean bioclimate belt on deep soils.

1) The Plant List (2013). Version 1.1. Published on the Internet; http://www.theplantlist.org/ (accessed 30 april 2019)
2) C.S. Sargent (1965) Manual of the trees of North America (exclusive of Mexico). Dover Publ., Inc. New York., 934 p.
3) J. Braun-Blanquet (1964) Pflanzensoziologie.Grundzüge der Vegetationskunde. Springer, Wien

3.2 = Herbarium samples for the phenological characterization of Mediterranean shrubs in conditions of range marginality: the case of *Cistus albidus* L. near the Lago di Garda

Fabrizio Buldrini[1], Giovanna Puppi[1], Bruno Bertolasi[2], Luisa Zago[2], Lorenzo Gui[2], Tommaso Sitzia[3], Isabella Vanetti[4], Giorgio Binelli[4], Giovanna Pezzi[1]
[1]Dipartimento di Scienze Biologiche, Geologiche e Ambientali, Università di Bologna, Via Irnerio 42, 40126 Bologna, Italy; [2]Centro Nazionale Biodiversità Forestale, Via Ponte 256, 37020 Peri (Verona), Italy; [3]Dipartimento Territorio e Sistemi Agro-Forestali, Università degli Studi di Padova, Viale dell'Università 16, 35020 Legnaro (Padova), Italy; [4]Dipartimento di Biotecnologie e Scienze della Vita, Università degli Studi dell'Insubria, Via J.H. Dunant 3, 21100 Varese, Italy

The potential effects of climate change on the distribution of Mediterranean plants at their peripheral range make them extremely vulnerable to environmental fluctuations. Thus, long-term data are needed to correctly evaluate such effects. The northernmost Italian population of *Cistus albidus* (45° 37' N, 10° 42' E, < 500 individuals) provides an outstanding case-study, as it grows at the extreme northern edge of the species' range, and because both *exsiccata* (since the early XIX century) and current phenological monitoring data are available. *Exsiccata* for this population came from Italian university herbaria (1) and natural history museums of northern Italy. A total of 41 herbarium specimens were found, spanning almost 200 years (period 1824-2013). Among them, 22 had a complete date of collection and 11 were collected for the *Flora Italica Exsiccata* (2). A phenological analysis was performed on the specimens with a complete date of collection (day-month-year). The following flowering stages were detected: presence of floral buds, open flowers, withering, and fructification (3). The collection date of a specimen (written on the herbarium sheet label) was used as the day of the year (DOY) of occurrence of a certain flowering stage. The flowering cycle of *C. albidus*, studied in living individuals, lasts 28-37 days and goes from April to early June. Generally, the anthesis starts on DOY 113 (23 April), has a peak on DOY 130 (10 May), and ends on DOY 145 (25 May), but showing considerable inter-annual variations (4). The presence of flowers in June was recorded several times in the *exsiccata* dataset, in very cold years as 1824, 1846, 1984, and 1988.

To understand the relation between the DOY of the flowering stages and average temperatures and/or precipitation of the previous months, a multiple linear regression analysis was performed, processing the variable «date of flowering» as a dependent variable, whereas the flowering stages, average temperatures and rainfall (5) were used as predictors. Such analysis was performed considering 4 different periods (January-April, February-April, March-April, and April), significant for current monitoring data. Regression analyses on herbarium data confirmed the general findings obtained by current monitoring data (4): the temperatures of the previous months were in most cases significant, whereas rainfall coefficients were never significant. The anticipation of flowering as a phenological response to winter-spring warming is considerably high (more than 8 days $°C^{-1}$) and seems to be much higher (16-21 days) in the *exsiccata* dataset than in the case of current living populations (8 days). Even if the direct comparison of the results is biased by the small number of herbarium specimens and by the large time-span considered, such a clear convergence in the results of the two datasets is worth noting, and shows that the population is very sensitive and responsive to temperature. Accordingly, if the increasing temperature trend will continue in the near future, we will face a substantial shift forward of flowering time in *C. albidus*, whose consequences on the species fitness will have to be carefully evaluated. We take these results as an indication that the integrated use of historical herbarium and current monitoring data could be a cornerstone for studying population changes over long periods of time. In addition, herbarium data are important also because, thanks to their long continuity, they are the sole concrete proof of the constant presence of *C. albidus* in this site, being historical floras not always supported by *ad hoc* herbarium collections. The amount of the specimens available today is also considerable, especially thinking to the small size of the population (4). The 11 specimens belonging to the *Flora Italica Exsiccata* (2) underline the great importance of herbarium collections, not only from a systematic viewpoint, but also for investigations on the reproduction biology of a given species.

1) F. Taffetani (2012) Herbaria. Il grande libro degli erbari italiani. Nardini Editore, Firenze
2) A. Béguinot (1905) Nuovo Giorn. Bot. Ital., n.s., XII (II), 167
3) A. Marcello (1935) Nuovo Giorn. Bot. Ital., 42, 543-556
4) B. Bertolasi, L. Zago, L. Gui, I. Vanetti, G. Binelli, T. Sitzia, G. Puppi, F. Buldrini, G. Pezzi (2019) Flora, 252, 26-35
5) M. Brunetti, G. Lentini, M. Maugeri, T. Nanni, C. Simolo, J. Spinoni (2012) Phys. Chem. Earth, 40-41, 9-22

3.2 = A dry herbarium of a Tyrolese medical student is the key to identify the plant taxa cultivated in the Padua Botanical Garden in the 17th century

Elsa Mariella Cappelletti[1], Giancarlo Cassina[2]
[1]Past Prefect of the Botanical Garden, University of Padova, Via Andrea Meneghini 1, 35122 Padova, Italia; [2]Past Curator of the Botanical Garden, University of Padova, Via Ezzelino il Balbo 1, 35141 Padova, Italia

The dry herbarium kept in the Pharmacy Museum of Bressanone (Brixen, Südtirol, Italy), and dated 1653, is a bound volume (Fig. 1) containing 950 well preserved specimens collected by the gardener Giulio Pizzi in the Botanical Garden of Padua. The specimens are mounted in a roughly alphabetical sequence following their names. For each specimen polynomial in Latin is provided, often complemented by the German vernacular name, and, sometimes, by a concise note on the plant part which was used in pharmacology (Fig. 2).

The herbarium was bought by the medical student Johann Baptista Angermann from Innsbruck, paying 10 Venetian silver *scudi*. A printed *ex libris* indicates the name of another owner, Johann Georg Franckh, who was the holder of the *Bischöfliche apotheke* in Bressanone until 1724. In 1852 another holder, Anton Peer, sold the *Bischöfliche apotheke* transferring the herbarium to another pharmacy, the Stadtapotheke, where it was kept until 2002.

The high resolution digitized images allowed a careful interdisciplinary study of this plant collection, and the identification of all specimens (1). The plant names were written by Angermann making several mistakes, only a few of them being detected and fixed by Angermann himself.

The names of the plants cultivated in the Padua Botanical Garden in the 17th century are known from several lists, published by the Prefects Johann Wesling in 1642 and 1644, and Giorgio Dalla Torre in 1660. However, there are difficulties in correlating the polynomials used at that time with the modern scientific names, and frequently they may be misapplied or otherwise misleading. In our herbarium the species have been identified and therefore they can help to highlight misidentifications of earlier authors. For instance, the species indicated as “Granadilla seu flos passionis” is *Passiflora caerulea* L. according to Saccardo (2), while the well preserved specimen F21, “Flos passionis”, is *Passiflora incarnata* L. (Fig. 3).

The herbarium kept in the Pharmacy Museum of Bressanone documents that in the Padua Botanical Garden dry plant collections were prepared by the gardeners for didactic purposes and sold to students. This herbarium is particularly valuable in confirming the exact identity of the plants cultivated in the Garden in the 17th century. In the meantime, it offers empirical evidence for the date of introduction into cultivation of particular alien plants, namely the North American taxa *Helianthus strumosus* L., *Heliopsis helianthoides* (L.) Sweet, and *Verbesina alternifolia* (L.) Britton ex Kearney.

Fig. 1. The herbarium

Fig. 2. Specimens C1 - C5

Fig. 3. “Flos passionis” (F21)

1) E.M. Cappelletti, G. Cassina, E. Feherenbach Peer, O. Peer, N. Giovè (2017) Atti Mem. Accad. Galileiana Sci., Lett. ed Arti, CXXIX, 197-320
2) P.A. Saccardo (1909) Cronologia della Flora italiana. Padova, Tipografia del Seminario

3.2 = Are historic herbaria still important? A long-term databasing project at NAP

Paolo Caputo[1,2], Paola Cennamo[3], Olga De Castro[1], Emanuele Del Guacchio[2], Annalisa Santangelo[1], Roberta Vallariello[1]
[1]Department of Biology, University of Naples "Federico II", via Cupa Nuova Cintia, 21, 80126, Naples, Italy; [2]Botanical Garden of Naples, University of Naples "Federico II", via Foria 223, 80139, Naples, Italy; [3]University "Suor Orsola Benincasa", Corso Vittorio Emanuele, 292, 80132, Naples, Italy

The so-called "*Herbarium Neapolitanum*", i.e. the Herbarium kept at the Botanical Garden of the University of Naples "Federico II", mainly hosts historical collections, which especially include central and Southern Italian specimens, collected from the most important XIX century botanists operating in Naples (1). The collections have never been combined in a single one, and therefore preserve the original arrangement given by their authors.

The great majority of the specimens is indeed fragile and unmounted (Fig. 1); their labels are handwritten and sometimes hard to read. Thus, physically lending these specimens is altogether impossible. The authors of this contribution are carrying out an effort to transform the historical herbarium into a modern facility. The main room has been consolidated, and has been exclusively dedicated to specimen preservation, and a communicating working room was created. New instruments are being purchased, and a coordinated effort is ongoing to systematically study collections.The herbarium is actively replying to (photographic) loan requests, providing information to any inquiring botanist, and receiving visiting scholars; local scientists are, as in the past, actively studying collections (e.g., 2, 3, 4), and various recent requests to NAP include DNA extraction from historical specimens for phylogenetic research (e.g., 5, 6).

This is accomplished also thanks to a partial database (7). A further effort is needed in order to make this information compliant with the standards, in order to allow the aggregation of data in national and international networks, in the wait for a possible complete digitization in the framework of an ongoing nation-wide project.

Given the fact that the majority of the loan requests concern type material, with the available human resources, however, we are envisaging a typification project, which would be an excellent starting point in terms of sheer knowledge of the specimens. Our aim in the time being is that of preparing a database listing the published designations for the most important historical collections (e.g., those by Tenore, Gussone, Pasquale and N. Terracciano) and making type specimens available on line, both to highlight the most frequent typification problems at NAP, and to support specialists in typification processes.

Fig. 1. A specimen of the collection "Gussone – Generale" at NAP.

1) A. Santangelo, G. Caputo, V. La Valva (1995) Allionia, 33, 103-120
2) R. Vallariello, D. Iamonico, E. Del Guacchio (2016) Phytotaxa, 263, 131-138
3) A. Santangelo, E. Del Guacchio, P. Cennamo, P, Caputo (2017) Phytotaxa, 298, 119-133
4) E. Del Guacchio, A.R. Bean, G. Sibilio, A. De Luca, O. De Castro, P. Caputo (2019) Taxon, in press
5) O. De Castro, B. Menale (2004) Taxon, 53, 147-151
6) P. Cennamo P., E. Del Guacchio, L. Paino, O. De Castro, B. Menale, M. Vazquez-Torres, P. Caputo (2013) Phytotaxa 141: 40-54
7) G. Moggi (2012) Gli erbari in Italia. In: Taffetani F. (ed.) Herbaria. Firenze, Nardini Editore, 707-814

3.2 = Phycological collections of Herbarium CAG

Annalena Cogoni, Maria Cecilia Loi, Bonaria Mulas
Department of Environmental and Life Science - Botany section, University of Cagliari, Viale S.Ignazio, 13, 09123 Cagliari, Italy

Patrizio Gennari, since the foundation of Hortus Botanicus Karalitanus in 1866, had undertaken the collection and exchange of exsiccata of higher plants, bryophytes, lichens, algae and fungi. The dry collections of the Botanical Garden also included specimens from the "Rabenhorst Algen Europa's" and from the "Erbario Crittogamico Italiano" of the Italian Cryptogamic Society and from other unidentified sources. In these collections the samples are collected both as exsiccata and in slides sometimes containing different taxa. The historical Phycological Herbarium CAG collects 1216 taxa, grouped in 10 divisions and 131 families. Concerning the collection areas, 29 taxa originate from the Americas (Canada and Chile), Asia, and Africa, 308 from different European countries, 279 from Italy, and 259 from Sardinia. while for 341 taxa geographical data are not available. Several specimens are incomplete, and miss some information such as collection date, collection place or collector name. Recent collections amount to 265 taxa, grouped in 4 divisions and 51 families. These samples were collected mainly in Sardinia (250), only 7 come from Friuli Venezia Giulia while 8 do not report locaties. Overall, the most represented divisions in historical herbaria are Rhodophyta (320), Chlorophyta (229), and Charophyta (218), while in the more recent collections the largest number of taxa belongs to Rhodophyta (118). Currently, these collections have been inserted into an spreadsheet; in the context of the rearrangement of the cryptogamic collections of the Herbarium CAG, they will be filed according to the most current herbarium cataloguing criteria.

3.2 = The use of herbarium specimens in evaluating plant extinction risks: some considerations on Sicilian endemics

Gianniantonio Domina, Filippo Scafidi, Emilio Di Gristina
Department of Agriculture, Food and Forest Sciences, University of Palermo, Viale delle Scienze ed.4, 90128 Palermo, Italy, e-mail: gianniantonio.domina@unipa.it

Herbarium specimens provide verifiable and citable evidence of the occurrence of plant taxa at a given place and time. Thus they caΩn be used to identify native ranges, and document which plants are occurring, and where, through time. They provide information on rare, extirpated, or extinct species that can no longer be found in nature. Furthermore, they can serve as a means of locating rare or possibly extinct species, recollecting in the area(s) reported on labels. Thus, herbarium specimens can be used as primary sources of data to have evidence-based extinction risk assessments.

Each extinction risk assessments is an evidence-based hypothesis of the current level of extinction risk of a particular taxon, to be refined, updated, corrected or refused, if more specimens are discovered, or when the scientific identification of one or more specimens is updated in light of new knowledge.

Some data are available from herbarium labels, but often they have to be retrieved *ad hoc*. Most specimen databasing projects aim at including metadata, as coarse-level geographical information, latitude and longitude coordinates (when available), collector name, collection number, date. Finally, the information absent in the label but gleaned directly from the specimen or the label is rarely included in major databases. Spatial data is certainly the herbarium-derived information most widely applied to extinction risk assessments.

Specimens collected less than 30 years old include often latitude and longitude coordinates, providing a best estimate of the collection site. Older specimens are less likely to include coordinates, but latitude and longitude can usually be retrieved from the textual locality information on the specimen. Temporal data are often present on herbarium specimens in the form of collection dates (even if only year for some older specimens) and are usually captured in digitization initiatives. These data are useful for inferring the existence of a particular plant at a particular period. Population size is rarely documented on herbarium labels, aside from generalized descriptions e.g., ‘rare’, ‘common’, ‘100 specimens are to be collected’, which are of use as supporting evidence, but not directly applicable to the criteria.

The main problem with Mediterranean collections is the reduced number of data available in order to make statistical analysis. This happens also with endemics that are usually over-sampled. The combined use of large and local collections, both modern and historical, can be of help. In addition, the presence of a taxon in a herbarium collection or in a floristic list rarely provides information about the number of individuals occurring in the locality. However, herbarium specimens are more reliable in comparison with bibliographic references, because their identification can be checked. The study of historical herbarium specimens can give some indications on their native status, as for *Astragalus thermensis* Vals., *Ipomoea stolonifera* (Cirillo) J.F.Gmel., and *Centaurea acaulis* L. in Sicily and help in distinguishing if they need to be protected or can be considered aliens.

Herbarium studies can be very informative for species with wide distribution that are more easily prone to local extinctions, as for instance *Anacamptis palustris* (Jacq.) R.M.Bateman, Pridgeon & M.W.Chase. For narrow endemics, there are more problems. For some taxa, their distribution has decreased since their description, as for *Erica sicula* Guss. subsp. *sicula* and *Adenostyles alpina* subsp. *nebrodensis* (Wagenitz & I.Müll.) Greuter. For some others, their distribution is increased as for *Orobanche chironii* Lojac. and *Petagnaea gussonei* (Spreng.) Rauschert. These cases does not represent actual enlargements of distribution, but just an increase in knowledge. For *O. chironii*, the new localities have been found in conservative environments and in protected areas, so that is was possible to reduce the risk category to which these plants are assigned; for *P. gussonei*, the new localities were found in human-modified and threatened environments, increasing the level of attention required for conservation.

3.2 = *Erbario Anzalone*: a collection of RO Herbarium with a great importance for the floristic knowledge of Lazio region

Marta Latini, Gianluca Nicolella, Maurizio Pierfranceschi, Giovanna Abbate, Mauro Iberite
Department of Environmental Biology, Sapienza University of Rome, P.le A. Moro 5, 00185 Roma, Italy

The Herbarium Museum of Sapienza University of Rome (RO) hosts over one million specimens, in several historical and contemporary collections. It is divided into six main Herbaria (*Erbario Romano*, *E. Generale*, *E. Cesati*, *E. Montelucci*, *E. Anzalone, E. Bazzichelli*), and several minor collections (1). The computerized cataloging of RO Herbarium collections, carried out for many years (2, 3), has produced a Filemaker Pro relational database (4), which hosts also georeferenced data from literature, and fields surveys. Databases allow various analyses on data, e.g. the taxonomic composition of the collections, their temporal and geographical coverage, and collectors' activity. At the same time, catalogues of the collections and floristic atlases can also be produced. *Erbario Anzalone* is a relevant collection for the knowledge of the flora of central Italy. Bruno Anzalone (1921-2007), full professor from 1974 to 1996 at Sapienza University of Rome, is considered the greatest expert of regional flora in Lazio. The collection, placed in the *Erbario Romano* room of the Museum, hosts about 37,700 specimens of vascular plants. It is organized in 361 parcels, and arranged in alphabetical order according to family, genus, and species; the delimitation of families and genera follows Pignatti (5). The specimens collected in Lazio are separated from those collected in other areas. The specimens collected by Anzalone in the Abruzzo, Lazio and Molise National Park are kept in a separate collection (43 parcels). The computerized cataloging of the materials, carried out from 2003 to 2014 with the supervision of the herbarium staff, was completed also thanks to the work of students, as the collection is also used for educational purposes. The nomenclatural update (6, 7) and the georeferencing of the materials is currently underway.

The specimens belong to 180 families, and 997 genera. The most represented families are Asteraceae (4,539 specimens), Fabaceae (3,305), Apiaceae (2,174), Lamiaceae (1,718), Brassicaceae (1,677), Caryophyllaceae (1,470), Scrophulariaceae (1,373), Rosaceae (1,370), Poaceae (1,226), and Ranunculaceae (1,069). The most represented genera are *Trifolium* (552), *Vicia* (547), *Centaurea* (486), *Ranunculus* (455), *Silene* (452), *Senecio* (448), *Carex* (429), *Euphorbia* (370), *Galium* (355), and *Quercus* (335). Over 98% of the specimens were collected in Italy; 69% were collected in Lazio, 8% Trentino-Alto Adige, 7% Abruzzo, and 3% Marche. Anzalone collected about the 90% of the specimens (33,789); among the other 276 recorded contributors, the most active were Cesare Banchieri (682 specimens from Monte Cairo, Frosinone), Luigi Veri (404 specimens from the Ponziane islands), and Edda Lattanzi (356 specimens from different territories). The oldest specimen dates back to 1920, and the most recent one to 2004.

The composition of the collection reflects the studies carried out by Anzalone throughout his career, testifying his interest in genera such as *Astragalus*, *Centaurea* (Fig. 1), *Ferula*, *Pastinaca*, *Seseli*, and the close relationship between Anzalone and his region. His intense activity, uninterrupted from 1940 to 2004, provided the essential data for drafting the vascular flora of Lazio (8), and for the geolocalised database that RO Museum is preparing (4). Within the database, the *Erbario Anzalone* provided valuable information on species distribution, useful to perform phytogeographical regionalisations (9), and to map rare, endemic, and endangered taxa (10).

Fig. 1. Holotype of *Centaurea scannensis* Anzalone, Soldano & F.Conti

1) G. Abbate et al. (2018) Ann. Bot. (Roma) 8, 59-65
2) A. Millozza et al. (2005) Inform. Bot. Ital. 37(1, A), 354-355
3) M. Iberite et al. (2010) Ann. Bot. (Roma) suppl. 2009, n.s., 79-84
4) M. Iberite et al. (2014) Atti 48° Congr. SISV (Roma, 17-19 sett. 2014), 47
5) S. Pignatti (1982) Edagricole, Bologna
6) F. Bartolucci et al. (2018) Plant Biosyst. 152(2), 179-303
7) G. Galasso et al. (2018) Plant Biosyst. 152(3), 556-592
8) B. Anzalone et al. (2010) Inform. Bot. Ital. 42(1), 187-317
9) M. Latini et al. (2017) Bot. Rev. 83(3), 253-281
10) S. Orsenigo et al. (2018) Biol. Conserv. 224, 213-222

3.2 = Diachronic analysis of pre-Linnean Herbaria: plant travels and historical phytoterapy

Mariacristina Villani
Botanical Garden, University of Padua, Via Orto Botanico 15, 35123 Padua, Italy

Pre-Linnaean botanical collections are precious historical sources of information. They are recognized to be invaluable documents for many reasons: they record the floristic diversity of a certain region in a certain time, they provide information on history of classification, and they are sources of documentation for tracing the travel of alien species, and the history of the pharmaceutical botany. However, ancient herbaria are not easy to access, because during their long history they often changed owners, thus their whereabouts could be unknown, as private properties, or hidden in libraries outside Universities or Museums, or destroyed during the World Wars. Anyway, even the available pre-Linnean plant collections, held in museums or herbaria, need to be managed with attention, because the exsiccata are normally mounted in fragile sheets arranged in volumes.

The identification of the plants collected in the past is made difficult by the extreme reduction of the specimens, often consisting of little fragments (often limited to a single leaf), not showing all the characters needed for the identification, or lying in a bad conservation status. Sometimes, specimens are lost, and their shape alone is still visible on the herbarium sheet. As far as the nomenclature is concerned, in most cases plants are labeled with polynomials, or with vernacular names, often showing an incorrect spelling. The taxonomic identification is thus difficult, because of the lacking of an unambiguous binomial system of classification. In this context, the comparison with herbaria of the same period (or made of specimens collected in the same locations), and with checklist, is an important and powerful tool for achieving an identification.

The Botanical Garden of Padua has a long history in the field of cultivation of medicinal plants, and a historical tradition of exchange or selling herbaria to other Universities or Museums (i.e. the collections of Sloane Herbarium, with a set of over 800 sheets with plants collected by Macchion in the Botanical Garden of Padua), as was in use during the Renaissance. Hence, it offers many items to feed a database of plant taxa of medicinal use in the pre-Linnean period. The database can be used to obtain information about 1) the consistence of the collections, thus highlighting important elements of the biodiversity of past centuries, 2) the identity of ancient polynomials, and 3) botanical exchanges during the Renaissance. Furthermore, the database can provide valuable information to compare this collection with those of other Botanical Gardens of Italy, or Europe (i.e. Leiden), or with other pre-Linnean herbaria (i.e. Clifford's herbarium, Hermann's herbarium, Cupani's herbarium).

3.3 = Databasing, digitizing and data-sharing: the case of the Herbaria of Florence within the Italian network of botanical collections, and the forthcoming European scenery

Lorenzo Cecchi, Piero Cuccuini, Anna Donatelli, Lorenzo Lastrucci, Chiara Nepi
Università degli Studi di Firenze, Sistema Museale di Ateneo, Museo di Storia Naturale, Collezioni botaniche

Natural history museums witness biodiversity through specimens coming from all regions of the world, gathered in over 500 years of scientific exploration. They are a huge archive that no single human being could hope to collect alone even during an entire life devoted to research. Due to the increasing importance that "Big data" retrieved by cumulative analyses can represent for a number of different fields, and applications (from bio-geodiversity and genetic to climate-change studies), the digital sharing of this information is a scientific priority and such repositories are (again) becoming leading-edge, rapidly growing organizations of fundamental significance for contemporary research.

The Florence NHM started contributing to the goal of a "global virtual scientific museum and research platform" since 2007, by joining several herbarium digitization projects funded by The Andrew W. Mellon Foundation (New York). These efforts focused on types (the "original material" according to the The International Code of Nomenclature for Algae, Fungi, and Plants), and has led to the publication of some 19 thousands digital images on public websites.

Today, however, novel technology have incomparably changed the efficiency of the whole digitization process, and may allow the acquisition of high-resolution images, and of related metadata, from millions of specimens in a few months at relatively low costs. This is why Florence NHM promoted the creation of CORIMBO, a network of 50 Italian herbaria globally representing one of the richest scientific collections in the World, and is now leading LEGIT, a national initiative aiming to the complete digitization of over 10 million specimens.

We hope that this could become the initial core of a great (virtual) National Natural History Museum, also including DNA biobanks as strategic partners, to which one day also zoological and anthropological collections may be added. Our goal was inspired by enlightened models as that of the Netherlands (the Naturalis in Leiden), and by a broad pan-European research initiative, the DiSSCo, whose mission is to unify and make bio- and geo-diversity information available at any required scale.

3.3 = Digitization of Michele Guadagno Herbarium. 1. Pteridophytes and Gymnosperms

David Dolci[1], Francesco Roma-Marzio[2], Silvia Loré[2], Simonetta Maccioni[2], Lorenzo Peruzzi[1]
[1]Department of Biology, University of Pisa, Via Derna 1 56126 Pisa, Italy; [2]Sistema Museale di Ateneo dell'Università di Pisa, Orto e Museo Botanico, Via Luca Ghini 13, 56126 Pisa, Italy.

Plant collections are essential elements for botanical research. Herbarium specimens document the world's flora and vegetation, including how they changed in response to human activities, and provide the basis for scientific names (1). Most of herbarium data is accessible to only a small number of botanists, and its consultation requires long times. In the last decades, many Herbaria started digitization projects in order to simplify information retrieval (2, 3, 4). Generally, digitization is a procedure composed by image acquisition and metadata storage (5). Data storage in a database system allows information to be summarised, categorized and manipulated, in order to retrieve specific information, otherwise hidden in an overwhelming pile of specimens.

Among the collections preserved at the *Herbarium Horti Botanici Pisani*, one of the richest is the Herbarium Guadagno, organised between 1900 and 1925 by Michele Guadagno (1878-1930), an engineer and botanist from Naples. It counts 547 packages, with more than 35,000 specimens, and 13,000 species (6). Most of the specimens were collected by Guadagno from central and southern Italy, but a significant portion was obtained through exchanges from foreign countries and collectors. The herbarium Guadagno arrived in Pisa in 1939, bought by Alberto Chiarugi, director of Pisa's Botanic Garden at that time.

At the end of 2018, the Botanic Museum of the University of Pisa started a collaborative project with the Department of Biology, focussed on the digitization of all the specimens of the Michele Guadagno Herbarium. The digitization procedure follows two distinct steps: 1) acquisition of high-resolution digital images (600 dpi, 24-bit colour depth) using a Bookeye® 4 Professional planetary scanner and 2) label data (metadata) acquisition and entry into the Virtual Herbaria JACQ (7) online database.

The digitization of Herbarium Guadagno started with pteridophytes and gymnosperms, which is now completed, while digitization of angiosperms is ongoing. During the digitization, 735 specimens of pteridophytes and 124 specimens of gymnosperms were found. Pteridophytes were mostly (58%) collected in Italy, while only 44% of gymnosperms were collected in Italy. The most represented genera in pteridophytes are *Asplenium* (155), *Cystopteris* (41), *Aspidium* (40), *Equisetum* (36), and *Polypodium* (27); the most represented genera in gymnosperms are *Juniperus* (45), *Pinus* (28), and *Ephedra* (17).

Michele Guadagno used to exchange specimens with other botanists: concerning pteridophytes and gymnosperms the main correspondents were J.R. Landmark (Sweden), A. Noblet (France), J. Rohlena (Montenegro), G. Evers (Austria), C. Bicknell (Italy), G. Rigo (Italy), and A. Mazza (Italy).

1) P.S. Soltis, G. Nelson, S.A. James (2018) Appl. Plant Sci., 6(2), e1028
2) B.M. Thiers, M.C. Tulig, K.A. Watson (2016) Brittonia, 68(3), 324-333
3) A.P. Seregin, D.F. Lyskov, K.V. Dudova (2018) Turk. J. Bot., 42(6), 801-805
4) K.M. Harris, T.D. Marsico (2017) Appl. Plant Sci., 5(4), e.1600125
5) M.G. Simpson (2010). Plant systematics. Academic press
6) D. Mazzitelli (1993) Le collezioni di Michele Guadagno nell'Herbarium Horti Botanici Pisani. Revisione critica delle Monocotiledoni. Master Thesis in Natural Sciences, Università di Pisa
7) Virtual Herbaria JACQ. Available at: https://herbarium.univie.ac.at/database/index.php

3.3 = Herbaria and pollination information: a possible and necessary marriage

Annalisa Managlia[1], Rosa Ranalli[2], Simone Flaminio[3], Umberto Mossetti[1], Laura Bortolotti[3], Denis Filer[4], Marta Galloni[2]
[1]Orto Botanico ed Erbario, Sistema Museale di Ateneo, Università di Bologna, via Zamboni 33, 40126 Bologna, Italy; [2]Dipartimento di Scienze Biologiche, Geologiche e Ambientali, Università di Bologna, Piazza di Porta San Donato 1; [3]Consiglio per la ricerca in agricoltura e l'analisi dell'economia agraria, Centro di Ricerca Agricoltura e Ambiente (CREA-AA), Via di Saliceto 80, 40128, Bologna, Italy; [4]Department of Plant Sciences, University of Oxford, South Parks Road, Oxford OX1 3RB, United Kingdom.

Specimens housed in herbaria are one of the most valuable resources for contemporary plant diversity research in a world which is changing because of pressing environmental issues. This is one reason why museums all over the world are digitalizing their collections. Specimens provide a) direct evidence of biodiversity in time and space, b) data on biogeographical patterns, c) evidence for plant extinction, d) conservation and threat, e) raw material for identification guides, f) comparative data for writing taxonomic accounts, and g) an invaluable resource for understanding the tree of life.

The BOLO herbarium in Bologna (one of oldest in the world, with numerous valuable historical collection) is fully engaged in the digitalization of its collections. Recently our data were migrated to the BRAHMS management system, enabling us not only to curate our data more efficiently, but also to explore its potential for research studies. In 2005, the entire *corpus* of the Ulisse Aldrovandi collection, one of the oldest surviving and wide-ranging of its time (1522-1605), was published using BRAHMS online. These data, aside from their historical interest, are now available for taxonomic, biogeographic and ethnobotanical studies.

The aim of our current study is to associate ecological information with our herbarium specimens, in particular, to link herbarium vouchers to data on flower visitors and pollination using the plant-pollinator observation data collected by the Plant Reproduction Ecology Laboratory of Department BiGeA. We are constructing a database that will integrate data from living plants, herbarium samples, observed pollinators, entomological specimens and palynological data. This database, aside from helping us to catalyse further research initiatives at BOLO, will also be published online, opening up its potential for environment planning and other practical applications.

3.3 = Digitization activities at the *Herbarium Horti Botanici Pisani*

Francesco Roma-Marzio[1], Lucia Amadei[1], Samuele Gerace[2], Simonetta Maccioni[1], Lorenzo Peruzzi[2]
[1]Orto e Museo Botanico dell'Università di Pisa, Sistema Museale di Ateneo, Via L. Ghini 13, 56126 Pisa, Italy; [2]Dipartimento di Biologia, Università di Pisa, Via Derna 1, 56126 Pisa, Italy

The inestimable value of herbaria, like all natural history collections, is widely recognized in the fields of traditional taxonomy, systematics, and biogeography. In the last decades, herbaria turned out also to have great potential in light of the recent attention to pressing environmental and ecological problems. Herbaria are the repositories of nomenclature types, and, sometimes, they preserve useful data to allow studies on endangered species or represent a documentation of taxa that have gone extinct in historical times (1).

Given the high scientific, historical, and cultural value of herbarium collections, in the last 20 years many efforts have been made in order to digitize herbarium collections, and to make them available to the scientific community (2) and the citizens.

The digitization process – i.e. the capture of images and metadata from specimen labels – is an essential first step in transforming this vast amount of data into accessible, usable, and useful information.

In this framework, the Herbarium of the University of Pisa (PI), currently preserving about 350,000 specimens, started a process of digitization in November 2017. This process is preceded by the assignment of a unique numerical code to each specimen. Then, a high-resolution image is obtained using a Bookeye 4 Professional planetary scanner that is capable of scanning a sample in less than 3.5 s, with an optical resolution of 600 dpi. The image of each specimen is stored in a web server provided by the University of Pisa, and the linked label data (metadata) is entered into the Virtual Herbaria JACQ (3). JACQ is an international database project coordinated by the University of Wien, that allows structured registration of metadata, georeferencing of each specimen, and automatic linking of these data to digital images. At the end of the digitization process, each specimen is marked with a stamp (“D”) to mean that it was completely digitized.

One of the strengths of the free project Virtual Herbaria JACQ is the presence of a query system which allows searching simultaneously all the Herbaria that are partner of the project (currently 48). In addition, this system allows to download a .csv file containing all the metadata of the recorded specimens, as well as a .kml file containing the geographical data, useful for GIS-based analyses.As far as type specimens are concerned, the Herbarium of the University of Pisa has also joined the project JSTOR Global Plants, the world’s largest database of digitized plant specimens (4).

Currently, to optimise efforts, the digitization process of the general herbarium is focusing on two main activities: 1) to digitize all the new accessions and revised specimens; 2) to digitize all the type specimens.

In the latter case, a special focus is given to the digitization of one of the most important collection preserved at the Herbarium Horti Botanici Pisani: the pteridophytes collected by Giuseppe Raddi (1770-1829) during his expedition in Brazil (1817-1818), and bought in 1829 by Gaetano Savi (1769-1844). 400 herbarium specimens were digitized, of which 99 are nomenclatural types (5), including 66 holotypes, 32 lectotypes, and one isolectotype. Of the names linked with these types, 59 are currently considered as heterotypic synonyms, while 40 are currently accepted.

To date, over 4,000 georeferenced specimens are digitized, and available on Virtual Herbaria JACQ. Among them, 152 are type specimens, which are also available in JSTOR Global Plants.

1) C. Lavoie (2013) Perspect Plant Ecol. Syst., 15(1), 68-76
2) M. Tulig, N. Tarnowsky, M. Bevans, A. Kirchgessner, B.M. Thiers (2012) ZooKeys, 209, 103-113
3) H. Rainer (2017) Virtual Herbaria JACQ. Retrieved from https://herbarium.univie.ac.at/database/index.php
4) JSTOR Global Plants (2019) JSTOR Global Plants. Available from: http://plants.jstor.org/.
5) R.E.G. Pichi Sermolli, M.P. Bizzarri (2005) Webbia, 60(1), 1-393

3.3 = The Virtual Herbarium of the Botanical Garden of the University of Palermo

Rosario Schicchi[1], Natale Surano[2]
[1]Department of Agricultural, Food and Forest Sciences (SAAF), University of Palermo, Viale delle Scienze, Parco d'Orleans, IT-90128 Palermo, Italy; [2] University of Palermo Museum System, Via Lincoln 2, IT-90133 Palermo, Italy

Herbarium collections are an important tool for scientific research, especially in the fields of systematic botany, and phytogeography (1). In the last decade, the growing development of Internet has allowed the growth of online information, even in areas initially considered less relevant, such as virtual museums. This context includes the development of "virtual herbaria", i.e. high-resolution image databases coupled with metadata.

Currently, in the world there are about 3,000 herbaria and, approximately, 350 million specimens. In Italy there are 70 herbaria registered in the international *Index Herbariorum* catalogue, including private, public non-academic (civic museums, naturalistic museums, regional bodies, etc.), and academic herbaria, which preserve collections of inestimable scientific value.

In line with the Collmap project – a project of the Italian Association of Natural History Museums (ANMS), endorsed by MIUR, for the census of all naturalistic collections – a number of scientific museums have developed a "virtual" section. The Italian network of botanical museums (CORIMBO) as well has as an objective the development of a project for the digitization of herbarium collections.

The Palermo Botanical Garden's *Herbarium Mediterraneum* (PAL), founded at the beginning of the 19th century, preserves collections (vascular plants, ferns, mosses, liverworts, algae, fungi, and lichens) coming from Sicily, as well as from many other parts of the world, including Australia, Africa, Central and South America, and Europe. The current Herbarium was developed by combining the Generale and Siculo Herbaria, which were originally separate. Their collections were estimated to include around 200,000 (Generale) and 60,000 (Siculo) dried specimens. Today, following the addition of new collections and acquisitions, the Herbarium total collection consists of approximately 400,000 specimens. The *Herbarium Mediterraneum*, along with many other important herbaria from across the world, is currently developing a database where images of the specimens will be stored, together with their metadata.

At the moment, the *Virtual Herbarium* is an inventory (2) searchable by label texts. To make the herbarium collections accessible online, in 2009 the creation of a daily updated electronic inventory of the herbarium was started (3). Currently, it consists of approximately 110,000 specimen entries, but only for a selection of families high-resolution digital images are available, in a format that allows zooming in by means of a browser (4). Those specimen images are of sufficient quality to allow the viewing of features which can normally be seen with a hand lens only. For specimens with geographic coordinates, it is possible to display a Google Maps window, that indicates the point of collection and/or the related satellite image. Over the next three years, it is estimated that the entire heritage of the herbarium will be digitized.

1) G. Moggi (2012) Gli erbari in Italia, Nuova Grafica Fiorentina
2) D. Kislov et al. (2017) Botanica Pacifica, 6(2), 59–68
3) M. Schmull et al. (2005) Taxon, 54(1), 251-254
4) M. Tulig et al. (2012) Zookeys, 209, 103-113

3.5 = Population genetics of *Cypripedium calceolus* in Italy

Adamo Martino[1], Fay Mike[2], Mucciarelli Marco[1], Gargiulo Roberta[2]
[1]Dipartimento di Scienze della Vita e Biologia dei Sistemi, Università degli Studi di Torino, Torino, Italy; [2]Jodrell Laboratory, Royal Botanic Gardens, Kew, Richmond, UK

Cypripedium calceolus is a rare orchid species, which could be candidate as flagship species, thanks to its recognizable flowers, and to its rarity, which in some areas is becoming progressively more pronounced (1). One of the areas where *C. calceolus* seems to be progressively disappearing is the Italian peninsula, which during the last glacial maximum (LGM ~22,000 years ago) was probably one of the glacial refugia of the species, and thus one of its differentiation centers afterwards (2). The analysis of population genetics of Italian lady's slipper orchid could be a powerful tool for better understanding the reasons behind its decline. Main factors are essentially of anthropic origin: over-collection in the wild, and habitat loss are the main drivers. On the other side, its peculiar reproductive biology could have a strong impact on population stability, due to clonality and pollination constraints. Otherwise, some protected European populations slowly decline without clear symptoms of stress.

In our study, we focused on the population of *C. calceolus* in Valle Pesio, Western Alps. This population is important for several reasons: it is probably related with the extinct historical population discovered by Clarence Bicknell during his botanical explorations in XIX century, and while it is geographically isolated, it seems to be a sort of repository of a big genetic diversity, probably due to the relatively big number of individuals. Thus, all efforts to preserve this heritage for the future generations are fully justified.

Individuals were genotyped at 11 nuclear microsatellites loci and compared to the data already available for populations occurring in Central Alps, and Central Apennines (2). Genetic structure in Valle Pesio is consistent with the overall structure found in other Italian populations of *C. calceolus.* Genetic diversity in Valle Pesio is comparable to the variation found in populations occurring in Central Alps, and higher than populations occurring in Central Apennines. Future conservation strategies will rely on predictions based on these results, and on the general phylogeographic history of *C. calceolus* in Eurasia.

1) H. Pedersen, H.N. Rasmussen, I. Kahandawala, M.F. Fay (2012) Cons. Genetisc, 13(1), 89-98
2) R. Gargiulo, S. Pironon, E. Zheleznaya et al. (2019) J. Biogeogr., 46(3), 526-538

3.5 = Morphological and molecular identification of wild grasses from Quba and Qusar districts of Azerbaijan

[1]Parvin Aghayeva, [1]Valida Ali-zade, [2]Salvatore Cozzolino, [2]Donata Cafasso
[1]Institute of Botany, Azerbaijan National Academy of Sciences, Badamdar highway 40, Baku AZ1004, Azerbaijan; [2]Dept. of Biology, University of Naples Federico II Complesso Universitario di Monte S. Angelo Via Cinthia, 80126, Napoli, Italy

We are investigating and cataloguing plant diversity in the Quba and Qusar districts of Azerbaijan by coupling morphological identification, and DNA barcoding. These districts are located in the south slope of Great Caucasus, which is a floristically rich region of Azerbaijan, subject to investigation since the beginning of last century (1, 2, 3, 4, 5).

Plants were identified with the use of identification keys, but many specimens were difficult to identify on the basis of morphological characters alone (because of missing floral and/or fruit parts). Thus, an identification metodology based on molecular barcoding was adopted. Total DNA from freshly collected or herbarium specimens were extracted. Samples were amplified using four plant DNA barcode markers: plastidial *rpoB*, accD, *matK*, and nuclear ITS1 ribosomal spacer. Sequences were assembled with BioEdit, and manually edited. Edited sequences were first identified by using BLAST search in NCBI, and the closest reference sequences were chosen for further comparisons. In particular, both *ex novo* generated sequences, and closed reference sequences were aligned by using MUSCLE program in Mega X. Phylogenetic trees were constructed using parsimony method implemented in PAUP. Maximum Parsimony analyses were run using heuristic search option with tree-bisection reconnection (TBR algorithm), using 100 random sequences additions to find global optimal tree for each family. The strengths of internal branches in resulting tree was tested with bootstrap analyses using 1,000 replicates, setting stepwise addition option to simple. We found that several members of the Asteraceae were very close to the reference sequences of *Leucanthemum ageratifolium* Pau, *Bellis margaritifolia* Huter, Porta & Rigo, *B. perennis* L., *Centaurea hedgei* Wagenitz*, C. trinervia* Steven ex Willd*., Psephellus pulcherrimus* (Willd.) Wagenitz*, P. xanthocephalus* (DC.) Boiss*., Senecio leucanthemifolius* subsp. *vernalis* (Waldst. & Kit.) Greuter, and *Taraxacum* F.W.Wigg. sect. *Taraxacum,* (98-99% sequence similarity). Fabaceae included taxa with 99-100% sequence similarity to *Lathyrus odoratus* L*., Trifolium montanum* L., *T. ambiguum* M.Bieb., and *Vicia cracca* L. Among Rosaceae, 98-100% sequence similarity was found with *Potentilla anserina* L*., P. chinensis* Ser., *P. agrimonoides* M.Bieb., and *P. discolor* Bunge. Orchidaceae taxa had 99% blast identity with *Orchis purpurea* Huds*., O. simia* Lam., and *O. mascula* (L.) L*.,* respectively. Finally, Linaceae had 98-99 % sequence similarity with *Linum ciliatum* Hayek, *L. nodiflorum* L., *L. pamphylicum* Boiss. & Heldr. ex Planch. For a better resolution on species identification of some taxa, which still have an ambiguous taxonomic identification (e.g., within Linaceae and Asteraceae), we are now planning to use a multi-gene nuclear approach based on next generation sequencing methods.

1) Aghayeva P., Qarakhani P., Huseynova A., Ali-zade V. (2018) Wild ornamental plants of the family Asteraceae from the northeastern part of Azerbaijan. Chornomors'k.bot.z., 14(3), 204–212

2) Флора Азербайджана. Изд-во АН Азерб. ССР. Баку. 1950, I: 369с.; 1952, II: 317с.; 1952, III: 407с.; 1953, IV: 401с.; 1954, V: 578 с.; 1955, VI: 539с.; 1957, VII: 646с.; 1961, VIII: 688с. (in Russian)

3) Huseynova A., Aghayeva P., Qarakhani P., Ali-zade V. (2019) *Serratula coronata* L. (*Asteraceae*), a new species record for the flora of Azerbaijan. Ukrainian Botanical Journal. 76(1): 67–70.

4) Карягин И.И. (1928). Материалы по растительности Кубинского уезда. Изв. Азерб. Гос. Ун-та. 7: 121-141. (in Russian)

5) Конспект флоры Кавказа. Изд.-во Санкт-Петербургского университета. Товарищество научных изданий КМК, 2008, III (1): 469с.; 2012, III (2): 623с. (in Russian)

3.5 = The new checklist of bryophytes of Italy: methods, purposes and perspectives

Michele Aleffi[1], Roberta Tacchi[1], Silvia Poponessi[2]
[1]School of Biosciences and Veterinary Medicine, Plant Diversity & Ecosystems Management Unit, Bryology Laboratory & Herbarium, University of Camerino, Via Pontoni 5, 62032 Camerino (MC), Italy; [2]Department of Chemistry, Biology and Biotechnology, University of Perugia, Via del Giochetto 6, 06126 Perugia, Italy

Ten years after the publication of the *Check-list of the Hornworts, Liverworts and Mosses of Italy* (1), the authors propose a new edition *(Check-list of the Bryophytes of Italy*). The compilation of a new check-list is necessary not only because in recent years numerous studies have increased the bryological knowledge of the Italian country, but also because the publications of the two Checklist of the Mosses and the Hepatics and Anthocerotes of the Mediterranean basin (2, 3), and the World Checklist of Hornworts and Liverworts (4) have literally revolutionized the systematic, and taxonomy of Europe's bryological flora.

Recent molecular, and taxonomic works have brought several changes to the systematics, and nomenclature of many species, genera, and even entire families, in particular to the pleurocarpous mosses, and the liverworts. Thus, while the general framework of the volume remains unaltered, a comparison of this new edition of the checklist, and the previous would prove difficult, both quantitatively, and qualitatively.

The current publication provides a complete list of the Hornworts, Liverworts and Mosses known to date in Italy (defined by its current political borders). Reports from the Republic of San Marino and the Vatican City State were also included. The checklist of Hornworts and Liverworts lists 292 species, 5 subspecies and 5 varieties, in 87 genera and 42 families. The checklist of Mosses lists 884 species, 12 subspecies and 44 varieties, in 232 genera, and 58 families. The present checklist is based on information from the literature, studies, and revision (in particular for species from taxonomically difficult genera, or groups of species of doubtful occurrence) of bryological collections from several Italian and foreign institutional or private herbaria, from the author's recent observations and reports, and those of numerous European bryologists.

Regional distribution is reported for each taxon; the tabular representation follows that of the previous editions of the checklists. Each report uses the following symbols:

○	report based on collections published before 1968
●	report based on collections published during or after 1968
(○)	doubtful report based on collections published before 1968
(●)	doubtful report based on collections published during or after 1968
+	report without precise collection data or locality

The checklist also provides critical notes on some important misidentifications, nomenclatural, and taxonomical problems, and species for which one report only is known.

Species to be considered absent from the Italian flora, either due to revision of herbarium specimens, or because of their ecology, have been listed alphabetically in an appendix. A second appendix lists taxonomically unclear binomials, and species of doubtful ecology, and chorology for which herbarium specimens could not be traced, and checked. Bibliographic sources have been cited for each species. The checklist is also supplemented by a lists of synonyms.

Furthermore, the Bryological Bibliography of Italy has been re-published with updates for 2018. It covers all the works published in over two centuries by Italian, and foreign scholars reporting on bryophytes for the Italian territory. The total number of bibliographic citations is over 2250. The bibliography provides a general list of the bibliographic entries in alphabetical order by author, an asterisk denoting the general works about Italy overall, and which do not report localities, or molecular, taxonomical, or biogeographic works, etc., which also indirectly concern the Italian bryoflora.

1) M. Aleffi, R. Tacchi, C. Cortini Pedrotti (2008) Bocconea, 22, 1-256
2) R.M. Ros et al. (2007) Cryptogamie, Bryologie, 28, 351-437
3) R.M. Ros et al. (2013) Cryptogamie, Bryologie, 34, 99-283
4) L. Söderström *et al.* (2016) PhytoKeys, 59, 1–828

3.5 = What is *Genista anxantica* Ten? A preliminary study using chloroplast markers

Emanuela Di Iorio[1], Sandro Strumia[2], Maria Rosaria Barane Lumaga[1], Annalisa Santangelo[1], Olga De Castro[1]
[1]Department of Biology, Botanical Garden, University of Naples Federico II, Italy, [2]Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, University of Campania, Luigi Vanvitelli, Italy

Genista anxantica Ten. (Fabaceae) was described by Michele Tenore in “Flora Napolitana” (1) on specimens collected by Giovanni Gussone in the Ansanto Valley, near Rocca San Felice (Avellino, southern Italy) (Fig. 1). This valley, called also Mefite, is an extreme environment, due to the presence of a large non-volcanic gas emission rich in CO_2 (98%), and other gases (< 2%, N_2, CH_4, and H_2S) (2). Currently, G. *anxantica* is a synonym of *Genista tinctoria* L. (3, 4, 5), a very polymorphic species. *G. tinctoria* has an euroasiatic distribution, living in deciduous forest understorey, and colonizing grassland at altitudes between 0 and 1800 m (3). The individuals of *G. tinctoria* near Mefite (thereafter reported as *G. anxantica*), have distinctive morphological characteristics, which make the current systematic placement uncertain.

In order to obtain useful elements to clarify its real systematic position, a molecular study was carried out on both historical specimens stored in NAP Herbarium, and individuals recently sampled (Table 1) in the Ansanto Valley, and surrounding areas. Furthermore, samples of *G. tinctoria* were collected from two populations, one in Torella dei Lombardi (T), which is very close (approximately 3.5-4 Km) to the Ansanto Valley, and another in the more distant (approximately 35 Km) Terminio Mountain (TE) (Table 1).

After a preliminary screening of several molecular markers, the *trn*L$^{(UAA)}$ intron, and the *trn*L$^{(UAA)}$-*trn*F$^{(GAA)}$ intergenic spacer have been employed for the molecular analyses (6). For each individual, the genomic DNA was extracted using a CTAB modified method (7), and then amplified, and sequenced by using well consolidated approaches (8, 9). Seven haplotypes were observed with different frequencies among the populations. All recent specimens belonging to the Ansanto Valley (*G. anxantica*, codes T1, G1, and M) show a single haplotype, not shared with the historical specimens sampled by Gussone, and with neighboring *G. tinctoria* populations (codes T and TE). These latter populations show high chloroplast variability with some haplotypes in common.

Fig. 1. Ansanto Valley (Rocca San Felice, Avellino, Southern Italy)

Table 1. Samples analysed in this study.

HISTORICAL SAMPLE				
CODE	N	LOCALITY	DATE	LEGIT
G_NAP	3	Ansanto Valley	20 May 1831	G. Gussone
T_NAP	1	Ansanto Valley	---	G. Gussone
RECENT SAMPLES				
CODE	N	LOCALITY	DATE	LEGIT
M	30	Ansanto Valley	21 June 2016	A. Santangelo
T	20	Torella dei Lombardi	10 June 2016	A. Santangelo
TE	20	Terminio Mountain	30 May 2017	A. Santangelo

In the future, we aim at expanding the populations dataset, and employing additional molecular markers: Internal Transcribed Spacer (ITS) and Short Sequence Repeats or microsatellites (SSRs).

1) M. Tenore (1812) Flora Napolitana 2. Typographia Fibreni
2) G. Chiodini, D. Granieri, R. Avino, S. Caliro, A. Costa, C. Minopoli, G. Vilardo (2010) Geophys. Res. Letters, 37, L11303
3) S. Pignatti (2019) Flora d’Italia 2. Edagricole
4) F. Bartolucci, L. Peruzzi, G. Galasso, A. Albano, A. Alessandrini et al. (2018) Plant Biosyst., 152, 179-303
5) Euro+Med (2006 onwards) http://ww2.bgbm.org/EuroPlusMed/
6) P. Taberlet, L. Gielly, G. Pautou, J. Bouvet (1991) Plant Mol. Biol., 5, 1105-1109
7) J.J. Doyle, L.L. Doyle (1990) Focus, 12, 13-15
8) O. De Castro, S. Cozzolino, S. Jury, P. Caputo (2002) Plant Syst. Evol., 231, 91-108
9) C. Pardo, P. Cubas, H. Tahiri (2004). Plant Syst. Evol., 244, 93-119

3.5 = A multilocus phylogeny of *Fritillaria* in the Maritime and Ligurian Alps

Francesco Dovana[1], Marco Mucciarelli[1], Lorenzo Peruzzi[2]
[1]Department of Life Sciences and Systems Biology, University of Torino, Viale Mattioli 25, 10125 Torino, Italy; [2]Department of Biology, University of Pisa, Via Derna 1, 56126 Pisa, Italy

Fritillaria L. is the largest genus of the tribe Lilieae (Liliaceae), and includes about 140 species occurring in the temperate regions of the Northern Hemisphere (1, 2). Four taxa are mostly endemic to the Alps: *F. tubaeformis* Gren. & Godr. subsp. *tubaeformis*, *F. tubaeformis* Gren. & Godr. subsp. *moggridgei* (Boiss. & Reuter ex Planch.) Rix, *F. burnatii* (Planch.) Backh., all together referred as the *F. tubaeformis* species complex, and *F. involucrata* All. The latter species occurs in South-eastern France (Prealps and Maritime Alps), and in the Maritime and Ligurian Alps of Italy (3, 4).

In the past literature, the *F. tubaeformis* species complex has been variously treated: a) as a single variable species with no, or at most varietal, infraspecific taxa, b) as a single species with two infraspecific taxa, c) as three distinct taxa belonging to two different species, and d) as three different species: *F. tubaeformis*, *F. moggridgei*, and *F. burnatii*.

To further test species-boundaries within the *F. tubaeformis* species complex, a more extensive intra- and inter-specific sampling was carried out, according to the most recent taxonomic, and biogeographic revision (5, 6). By sequencing a set of plastidial DNA regions (*mat*K, *rpo*C1, *rpl*16, *pet*A and *ndh*F) run on populations sampled in type localities of the Maritime, and Ligurian Alps, we demonstrated that *F. tubaeformis*, and *F. burnatii* are genetically independent taxa, fully confirming previous morphological, morphometric, and genetic results (6, 7). Furthermore, differently from what reported in old literature, *F. burnatii* is not related to the central European *F. meleagris* L. Our study also supported the separation at subspecies level between *F. tubaeformis* and *F. moggridgei* (5). These two taxa show contiguous, partially interdigitated, but not overlapping ranges, and rather different ecological features (6). Genetic isolation by environment or ecology, which seems to be more common than isolation by distance, might have occurred between the two subspecies and this divergence may have had important consequences for both local adaptation and phenotypic diversity at the intraspecific level (8). Our analysis showed that *F. involucrata*, differently from what shown in previous phylogenetic reconstructions, is not related to *F. tubaeformis*. Previous studies in *F.* subg. *Fritillaria* based on large dataset phylogenies (9, 10, 11), although highly informative, have considered alpine taxa only marginally. This resulted in either the synonymization of discrete taxa or the splitting of different variants into separate species, contributing to inaccurate taxonomic circumscriptions. A detailed analysis of genetic variants is crucial to solve the most critical taxonomic cases.

1) E.M. Rix (2001) *Fritillaria*. A revised classification. The *Fritillaria* Group of the Alpine Garden Society, Edinburgh
2) L. Peruzzi (2016) Plant Biosyst., 150, 1341-1347
3) A. Ebone, D. Bombonati, B. Gallino (2009) Riv. Piem. St. Nat., 30, 313-340
4) J.M. Tison, P.J. Jauzen, H. Michaud (2014) Flore de la France Méditerranèenne Continentale. Naturalia Publications Turriers
5) F. Bartolucci, L. Peruzzi (2012) Candollea, 67, 23-29
6) M. Mucciarelli, P. Rosso, V. Noble, F. Bartolucci, L. Peruzzi (2016) Plant. Syst. Evol., 302, 1329-1343
7) M. Mucciarelli M., M.F. Fay (2013) Phytotaxa, 91, 1-23
8) D. Dagnino, L. Minuto, G. Casazza (2017) Plant Biol., 19, 1003-1011
9) N. Rønsted, S. Law, H. Thornton, M.F. Fay , M.W. Chase (2005) Mol. Phylogenet. Evol., 35, 509-527
10) P.D. Day, M. Berger, L. Hill, M.F. Fay, A.R. Leitch, I.J. Leitch, L.J. Kelly (2014) Mol. Phylogenet. Evol., 8, 11-19
11) J. Huang, L.Q. Yang, Y. Yu, Y.M. Liu, D.F. Xie, J. Li, X.J. He, S.D. Zhou (2018) Ann. Bot., 122, 1245-1262

3.5 = Genetic diversity in the threatened *Bellevalia webbiana* (Asparagaceae) parallels functional and reproductive traits

Lorenzo Peruzzi[1], Giovanni Astuti[1], Sabrina Algisi[2], Andrea Coppi[2]
[1]Department of Biology, University of Pisa, Via Derna 1, 56126 Pisa, Italy; [2]Department of Biology, University of Firenze, Via Micheli 1, 50121 Firenze, Italy

Amongst the numerous Italian narrow endemic plants, the Webb's hyacinth (*Bellevalia webbiana* Parl., Asparagaceae; 1, 2, 3) is one of the most evolutionarily relevant, and threatened. According to (4), the range of this bulbous perennial herb is restricted to an area of pre-Apennines (100–700 m a.s.l.) in Tuscany, and Emilia-Romagna (Central Italy), with two disjunct population groups. Typical habitats for Webb's hyacinth are open fields, and meadows, wood margins, olive groves, and vineyards. During the last century, Webb's hyacinth disappeared from several historical localities due to the development of human settlements (4). For these reasons, this species is currently listed in The IUCN Red List of Threatened Species as Endangered (EN A2c) (5). Despite contributions providing important information on the species' reproductive (6), and functional (7) traits, no population genetic information was available so far.

In this study, a DNA fingerprinting approach was applied to study the five richest populations of this species (each with $N > 50$ individuals): three in Tuscany (Pratolino, Uccellatoio, Tavarnuzze), and two in Emilia-Romagna (Casola Valsenio and Faenza). For each population, we sampled leaf tissues in silicagel from 15 individuals. The primers selected for the ISSR analysis were (Hex)ISSR4, (Fam)CT8 and (Tet)ISSR8. A total of 108 loci were detected (100% polymorphic) in the 75 individual profiles. AMOVA highlighted that 71.2% of genetic variation is within populations, and 28.8% among populations, with an average $F_{ST} = 0.29$ (a value only slightly below the mean found in plants using ISSR, see 8). Average gene diversity over loci ranges from 0.083 (Uccellatoio) to 0.116 (Pratolino) in Tuscan populations, while it is slightly higher in the populations from Emilia-Romagna (0.129 for Faenza and 0.131 for Casola Valsenio). Despite the low genetic variability among populations, the individuals from Faenza are neatly differentiated from the others, showing 11 specific loci. Indeed, they group separately both in PCoA and NeighborNet analyses of the molecular binary matrix. The optimum number of genetic clusters (9) inferred from the STRUCTURE analysis (burn-in period of 50,000 and a posterior number of 100,000 MCMC permutations, 15 replicates for each value from K = 1 to K = 10) is K = 2. The individuals from Pratolino, Uccellatoio, Tavarnuzze, and Casola Valsenio either belong to the first K group (33 out of 60 individuals showing a proportion of membership above 80%) or are a genetic admixture among the two K groups (27 out of 60 individuals). On the contrary, the proportion of membership to the second K group is above 80% in all the 15 individuals from Faenza.

Interestingly, the population from Faenza is the only one statistically different as far as CSR strategy is concerned, being more 'competitor' and less 'stress-tolerant' compared to the other four populations (7). As far as reproductive features (6), the same population shows the tallest inflorescences, with the highest number of flowers, and the highest fruit set. This population is also the only actively conserved by the owners of "Apicoltura Lombardi" in their property. In this framework, our idea is that this 'protected' population is allowed to express its competitive potential, by maintaining a number of genetic traits otherwise lost in the other, disturbed (6, 7), populations. Indeed, disturbance may have caused germplasm selection, and genetic drift, in Pratolino, Uccellatoio, Tavarnuzze, and Casola Valsenio. The peculiar, genetically differentiated, population from Faenza may be seen as an Evolutionarily Significant Unit (ESU; 10), deserving special conservation attention, in order to guarantee the survival of this species. Finally, Emilia-Romagna may represent the centre of origin for *B. webbiana*, by considering that the two populations from this region hold the highest levels of intrapopulational variability, and that Faenza population shows the highest levels of specific loci, but it also shares rare fragments with other populations.

1) A. Chiarugi A (1949) Caryologia, 1(3), 362-376
2) A. Borzatti von Loewenstern, T. Giordani, G. Astuti, A. Andreucci, L. Peruzzi (2013) Plant Biosyst., 147, 776-787
3) G. Astuti, S. Brullo, G. Domina, R. El Mokni, T. Giordani, L. Peruzzi (2017) Plant Biosyst., 151(6), 1120-1128
4) G. Gestri, A. Alessandrini, N. Sirotti, A. Carta, L. Peruzzi (2010) Inform. Bot. Ital., 42, 449-455
5) L. Peruzzi, A. Carta (2011) The IUCN Red List of Threatened Species 2011, eT195349A8957996
6) G. Astuti, G. Bedini, A. Carta, F. Roma-Marzio, A. Trinco, L. Peruzzi (2018) Nat. Cons., 24, 81-92
7) G. Astuti, D. Ciccarelli, F. Roma-Marzio, A. Trinco, L. Peruzzi (2019) Plant Biosyst., 153(1), 12-18
8) H. Nybom (2004) Mol. Ecol., 13, 1143-1155
9) G. Evanno, S. Regnaut, J. Goudet (2005) Mol. Ecol., 14, 2611-2620
10) L.P. Casacci, F. Barbero, E. Balletto (2014) Ital. J. Zool., 81(2), 182-193

3.5 = Alien plants new for European and Sicilian flora

Vivienne Spadaro[1], Patrizia Campisi[1], Francesco M. Raimondo[2]
[1]Department STEBICEF/Section of Botany, Zoology and Anthropology, University of Palermo, Via Archirafi 38, 90123 Palermo, Italy; [2]*PLANTA*/Research, Documentation and Training Center, Piazzetta Cairoli 11, 90123 Palermo, Italy

The knowledge about alien flora of Italy has been recently summarised by (1). The list of the taxa referred to in the electronic appendix of that article, provides for each taxon the appropriate alien status and the regions in which each taxon is present, on the basis of literature, or specimens preserved in Italian herbaria. Other contributions as *notulae* have been published in the specific section of the *Informatore Botanico italiano* and, since 2016, of *Italian Botanist*. In this contribution, two new cases of alien plants are presented, one for the Italian, and European territory, the other for the Sicily Region alone. These are respectively *Xanthosoma sorbifolia* Bunge (Sapindaceae), and *Commelina communis* L. (Commelinaceae).

Xanthoceras sorbifolia is the only species of its genus. It is native to China, and introduced in some gardens of different countries, including Italy, where it is present in Sicily only. The plant has a shrubby or arborescent habit, alternate, pinnate leaves, with 10-20 lanceolate leaflets, sessile. The flowers are grouped in axillary racemes and have a pentapartite, white corolla, with petals showing a small red spot at the base. The fruit is a globose, trilocular capsule, with a hook point, and yellowish when ripe. Each loculus contains 1-2 (-3) showy sub-spherical seeds, of dark brown colour. One individual is cultivated in the park of Villa Whitaker to Malfitano (Palermo). A second individual is preserved in the Botanical Garden of Palermo, introduced in 1997 by seed produced from a plant surveyed in a dendrological park by one of the authors (2). The site of spontaneization of this taxon is in the same garden where it was introduced, and cultivated (3), at a distance of about 30 m from the cultivated individual, in the rock garden, where it was neglected for years. There, the population of *X. sorbifolia* – estimated in about one hundred individuals of different ages, and partly already in full fruiting – occupies an area of about 60 m^2. Outside the city of Palermo, to date, there are no reports of the taxon.

Commelina communis has long been known in various Italian and European regions, both as a naturalized, and as a casual alien. In Italy, its presence was known from many regions except Marche, Molise, and Puglia. Known as “Asiatic dayflower”, it is a perennial herbaceous rhizomatous, with fleshy, violet, creeping and ascending stems. The leaves are relatively large, measuring 8-13 cm in length, and are thick and fleshy. They are lanceolate or elliptic in shape with pointed tips and blunt, clasping bases that form a sheath around the stem. The flowers are relatively small, measuring approximately 1.5 cm in width. This bilaterally symmetrical flower shows six irregularly sized anthers and three petals: two, deep blue, upper petals and one lower, highly reduced, white petal. The base of this bloom is enfolded in a green, heart-shaped bract and is supported by three green sepals. Asiatic dayflower grows best in open and disturbed areas, along roadsides, and woodland borders. It was introduced in central and southeastern Europe, and eastern North America, where it has spread to become a noxious weed.

In Sicily it was already collected in 2017 in Palermo, on the edge of a busy city street in the center. At the same time it was observed spontaneous in areas uncultivated within the Botanical Garden.

1) G. Galasso et al. (2018) Plant Biosyst.,152 (3), 556-592
2) F.M. Raimondo (1997) Il Giardino di Villa Malfitano. Fondazione Whitaker, Palermo
3) F.M. Raimondo, P. Mazzola (2001) Quad. Bot. Amb. Appl., 10 (1999), 149-165

3.5 = The use of herbarium specimens in morphometric investigations: the case of the Italian endemic species *Limonium remotispiculum* (Plumbaginaceae)

Adriano Stinca, Melania Eviano, Assunta Esposito
Department of Environmental, Biological and Pharmaceutical Sciences and Technologies, University of Campania Luigi Vanvitelli, via Vivaldi 43, 81100 Caserta, Italy

Herbaria have been natural history collections, and repositories of both plant biodiversity, and botanical knowledge for over four centuries (1). The use of herbarium specimens has changed during time (1), but its role in botanical studies to describe, identify, and evaluate the distribution of plants remains crucial. Since sometimes botanists have based the description of new taxa on the analysis of one to a few specimens, whose collection could have been influenced by some factors (e.g., accessibility of places, low population density, size of individuals, colour of flowers), they probably provided data which are deficient on phenotypic variability, and ecological range of the plants.

In this contribution, we report the results of a morphometric study on *Limonium remotispiculum* (Lacaita) Pignatti (Plumbaginaceae, Fig. 1) to assess its morphological variability. It is a species endemic to the Tyrrhenian coasts of southern Italy (2), from the Sorrento Peninsula (Campania) to Cirella Island (Calabria) (3). In order to analyze its intra- and inter-population morphological variability, 11 populations were sampled: 5 in Campania (3 in Amalfi Coast at Furore, Maiori, and Cetara; 2 in Cilento at Marina di Camerota, and Palinuro), 3 in Basilicata (Maratea), 3 in Calabria (Dino Island, Scalea, and Cirella Island). Twenty to 50 individuals per population were collected. The exsiccata were deposited at the Herbarium Porticense (PORUN-Herb. Stinca), and at Herbarium Flaminio (HFLA).

Fig. 1. *Limonium remotispiculum* on the coastal cliffs in Scalea (Calabria). Photo by A. Stinca

Thirty-seven morphological characters were selected and measured: 11 qualitative (e.g., apex, margin and hairiness of the leaf, form of the inflorescence, hairiness of the calyx), and 26 quantitative (e.g., number and length of sterile branches, length and width of the leaf, length and width of the flower bracts, length and number of flowers of the spikelet, length of the calyx). The data matrix was processed and analyzed using both univariate and multivariate statistics. Overall, approximately 30,000 measurements on 301 individuals have been carried out.

Reproductive characters only are consistent with the geographical distribution of the examined populations. In fact, on the basis of these characters, the multivariate analysis highlighted the separation of two main clusters, the first one including the populations from Amalfi Coast (Furore, Maiori, and Cetara), the second including all other populations. Thus, they could be considered as separate subspecies or, as currently recognized, as a single variable species.

This study confirms that massive exsiccata collections are an indispensable source in morphometric analyses. On the other hand, they can be useful for future molecular investigations.

1) G. Besnard, M. Gaudeul, S. Lavergne, S. Muller, G. Rouhan, A.P. Sukhorukov, A. Vanderpoorten, F. Jabbour (2018) Botany Letters, 165(3-4), 323-327
2) L. Peruzzi, G. Domina, F. Bartolucci, G. Galasso, S. Peccenini et al. (2015) Phytotaxa, 196(1), 1-217
3) S. Pignatti, R. Guarino, M. La Rosa (2017) Flora d’Italia 2. Edagricole, Bologna

4.1 = *In vitro* pollen germination of three endangered terrestrial orchids of Northwestern Italy

Miriam Bazzicalupo[1], Silvia Priarone[1], Annalisa Giovannini[2], Paolo Giordani[3], Laura Cornara[1]
[1]Department of Earth, Environment and Life Sciences, University of Genova, Corso Europa 26, Genova, 16132, Italy; [2]Council for Agricultural Reasearch and Economics (CREA), Ornamental Species research Unit, Corso degli Inglesi, 508, Sanremo 18038 (Im), Italy; [3]Department of Pharmacy, University of Genova, Viale Cembrano 4, 16148 Genova, Italy

Among angiosperm families, Orchidaceae are one of the most numerous, with an estimate of 28.000 species (1). Although only one third of orchids are terrestrial species, about half of extinct species were perennial geophyte tuberous plants. Habitat changes, including degradation and fragmentation, anthropogenic use of land, buildings, harvesting, disturb to symbiont mycorrhizae, invasive species and wild animals diggings are adverse factors all over the globe. Moreover, since orchids are mainly pollinated by insects, a lower presence of pollinators may cause depletion of populations (2). Many studies have been focused on orchid reproductive biology to pursue conservation strategies, also including exchange of genetic material among distinct plant populations. This aspect has been considered also in the Project LIFEorchids (LIFE17NAT/IT/000596), which aims to the reinforcement/reintroduction of selected orchid species in two different ecological contexts of NW-Italy.

As a preparatory step for a program of hand pollination between different orchid populations, in this study we performed *in vitro* pollen germination tests on two food-deceptive (*Orchis patens* Desf. and *Himantoglossum robertianum* (Loisel.) P. Delforge) and one shelter-deceptive (*Serapias neglecta* De Not.) species. Plant materials were collected from Nozarego (GE) and Pompeiana (IM). Pollinoids were removed in the field from flowers at the onset of anthesis and stored in vials at stable temperature conditions (17°C). SEM analyses of pollinia allowed to characterize the pollen morphologies of the three different species. To evaluate pollen germination, emipollinaria were rehydrated and massulae were mechanically divided before incubation for 24-48 hr at 17°C in a germination medium containing 13% sucrose, at pH 5.7. Tests were repeated every 3d-4d for each class of aged pollinia, and the presence/absence of pollen tubes was checked by light microscopy.

In vitro germination tests ($\geq$50 % pollen tube growth, according to Bellusci et al., 3) showed that pollen viabilities lasted for up to 7-15 d, ranging from 65-80% at 7d for *S. neglecta*, and 61-100% at 15 d for *O. patens* and *H. robertianum*. At 20 d pollen viability was < 50 % in *S. neglecta* and *O. patens*, while *H. robertianum* still showed high pollen viability (60-95%).

Data improve the knowledge on pollen morphology, viability, and germination of three endangered terrestrial orchids, and provide information for programming manual pollination activities that are included in the LIFEorchids project.

1) Christenhusz M.J.M., Byng J.W., 2016. The number of known plants species in the world and its annual increase. Phytotaxa, 261, 3
2) Swarts N.D., Dixon K. W, 2009. Terrestrial orchid conservation in the age of extinction. Annals of Botany, 104, 543–556
3) Bellusci F., Musacchio A., Stabile R. and Pellegrino G., 2010. Differences in pollen viability in relation to different deceptive pollination strategies in Mediterranean orchids. Annals of Botany, 106, 769–774

4.1 = Alien-native species continuum approach: invasion dynamics among Asteraceae

Anna Corli[1,2], Christine Sabine Sheppard[2]
[1]Department of Earth and Environmental Sciences, University of Pavia, Via S. Epifanio 14, 27100 Pavia, Italy; [2]Department of Landscape Ecology and Vegetation Sciences, University of Hohenheim, August-von-Hartmann Str. 3, 70599, Stuttgart, Germany

Alien species represent 3.9% of the global flora of the world, that is more than 13,000 species; in a global change scenario this number is constantly increasing and thus threatening native communities (1).

There are several factors influencing their invasion dynamics: functional traits, interactions between invader and native residents, stochastic effects, residence time as well as the number of introduction events.

With regard to the pollination modes, the success and the establishment of alien plants in a new environment is guaranteed either because of auto-fertility or thanks to their capacity of attracting pollinators; both events can possibly occur. Alien plants benefit from auto-fertility to spread over areas where the lack of co-evolved mutualists would otherwise limit invasion success. At the same time, the widespread generalists among mutualists and their large geographical ranges allow aliens to be easily integrated into networks (1,2). Nevertheless, there are conflicting results among the different frequency of pollination modes between native plants, generally specialists-pollinated, and alien plants (3).

The role of residence time has to be accounted for, as it takes time for a species to spread and adapt to a new area; native plants and archaeophytes are more common and have larger range sizes than neophytes, which arrived later (4). In addition, it has been demonstrated that auto-fertility has a positive correlation with the size of the invaded range and increases plant performances such as fruit and seed production as well as fecundity (5).

In 2016, a multi-species common-garden experiment was performed to investigate how auto-fertility, pollinator dependence, species' range sizes, residence time and invasion status could affect reproductive output and invasion success of Asteraceae in Germany, the largest family of flowering plants in the world. Specifically, the effect of flower visitors exclusion was addressed on 42 annual plants characterized by varying residence times, using an alien–native species continuum, including natives, archaeophytes and neophytes (casual and established).

Plants height, number of capitula, flowering and seed starting day were assessed as well as seeds were collected; the number of flower visitors was then counted as a proxy of pollinator visits. Vegetative and reproductive biomass was evaluated; germination trials were performed to study the effects of pollinator exclusion on seed germination.

Pollinator dependence does not play a crucial role in invasion success, where most Asteraceae being able to self-fertilize. Surprisingly, both established neophytes and natives showed higher abilities to self-fertilize, even if auto-fertility reported no apparent reduction in seed quality, while archaeophytes and casual neophytes were more attractive for pollinators. In contrast to casual neophytes, the established neophytes' strategy was associated with a large reproductive output. Yet, auto-fertility was not associated with range size, since archaeophytes reached the largest range sizes; thus, auto-fertility is not necessarily important for plants to be successful at the large scale.

1) D.M. Richardson, N. Allsopp, C.M. D'Antonio, S.J. Milton, M. Rejmánek (2000) Plant invasions - The role of mutualisms. Biol. Rev.,, 75, 65–93

2) P. Pyšek, V. Jarošík, M. Chytrý, J. Danihelka, I. Kühn, J. Pergl, L. Tichý, J.C. Biesmeijer, W.N. Ellis, W.E. Kunin et al. (2011) Successful invaders co-opt pollinators of native flora and accumulate insect pollinators with increasing residence time. Ecol. Monogr., 81, 277–293

3) S. Sutherland (2004) What makes a weed a weed: Life history traits of native and exotic plants in the USA. Oecologia, 141, 24–39

4) D.M. Richardson, P. Pyšek (2006) Plant invasions: Merging the concepts of species invasiveness and community invasibility. Prog. Phys. Geogr. Earth Environ., 30, 409–431

5) P. Pyšek, D.M. Richardson (2007) Traits associated with invasiveness in alien plants: Where do we stand? In Biological Invasions; Nentwig, W., Ed.; Springer: Berlin/Heidelberg, Germany, Volume 193, pp. 97–125

4.1 = Differences in pollinaria reconfiguration in relation to different deceptive pollination strategies in Mediterranean orchids

Pierluigi Cortis[1], Anna Maria Palermo[2], Michele Lussu[1], Giuseppe Pellegrino[2]
[1]Department of Life and Environmental Sciencies, University of Cagliari, Viale Sant'Ignazio 1, 09123, Cagliari, Italy;
[2]Department of Biology, Ecology and Earth Sciences, University of Calabria – Arcavacata di Rende (CS), Italy

The Euro-Mediterranean orchids encompassing several genera, most of which have a food- or sexually deceptive pollination strategy (1). The sexually deceptive pollination is typical of the genus *Ophrys*, whose flowers mimic the female of its own pollinator in shape and scent. Food-deceptive species, such as the widespread genera *Orchis*, *Anacamptis* and *Dactylorhiza*, display large, showy flowers resembling those of rewarding species but that lack the energetic rewards.

The issue of orchid pollen viability has been investigated in terms of its contribution to incompatibility and fertility, but relatively few taxa have been investigated in terms of pollinarium reconfiguration. After removal from the anther, many pollinaria undergo a bending movement localized along the caudicle to correctly orientate the pollinium for insertion into a stigma cavity. The bending movement time was interpreted as a mechanism to prevent geitonogamy among flowers on the same plant (2).

In this study, we proposed to expand upon current information concerning the pollinarium reconfiguration of widespread Euro-Mediterranean orchids characterized by different types of pollination strategies to evaluate the relationships between pollinarium reconfiguration and pollination strategies.

Twenty species of the genera *Anacamptis*, *Dactylorhiza*, *Ophrys* and *Orchis*, all belonging to the sub-tribe Orchidinae, were selected, taking into account their pollination strategies.

The timing of pollinarium reconfiguration varies extensively from 25 to 400 s. Higher values were reported for *Ophrys* than other species. Evidence was found of highly significant differences in pollinarium reconfiguration among species and among pollination strategies; while, there was a positive correlation among pollinarium reconfiguration trends observed in those species with similar pollination strategies.

The findings of this study provide strong empirical support for the idea that promotion of cross-pollination underlies the evolution of pollinarium reconfiguration. The variation in the timing of pollinarium reconfiguration reflects pollination strategies and thus the duration of visits by an orchid pollinator. Indeed, observation of food deceptive orchids indicate that pollinators spend few time on an inflorescence. On the contrary, pollinators visit more flowers and spend more time on the inflorescences of sexually deceptive orchids, such as foraging on the inflorescences of nectariferous orchids.

1) Jersáková J, Johnson SD, Kindlmann P. (2006) Mechanisms and evolution of deceptive pollination in orchids. Biological Reviews, 81, 219–235
2) Darwin C. (1877) The various contrivances by which orchids are fertlized by insects. 2nd edn. John Murray, London

4.1 = A Fiber-optic refractometer for non-destructive, in-vivo monitoring of flower's nectar concentration

Pietro Malara[1], Giovanna Aronne[2]
[1]CNR- Istituto Nazionale di Ottica, via Campi Flegrei 34 (comprensorio A. Olivetti), 80078 Pozzuoli (NA) Italy;
[2]Department of Agricultural Sciences, University of Naples Federico II, Via Università 100, 80055 Portici (NA) Italy

The total amount of sugar contained in floral nectars is a crucial parameter in plant biology as well as in environmental studies, for it is a quantitative indicator of the flower attractiveness to pollinators. Nectar sugar concentration is generally assessed by measuring its refractive index and conventionally expressed in sucrose-equivalent concentration units (BRIX scale). In a typical measurement the nectar is extracted from the flower with a volumetric glass microcapillary tube and spilled on the prism of a calibrated light refractometer. While this procedure is suitable for many common applications, it is invasive (often destructive), and can't be carried out on flowers with scarce or highly-viscous secretion. Measuring the nectar concentration of such low-producing species, which are quite common in the Mediterranean environment, represents to date an open issue: alternatives to nectar extraction (involving filter paper wicks, flame-thinned capillaries or dilutions) are rather complicated and often return much less precise and accurate values.

In this work we harness the microscopic-size, low cost and biocompatibility of glass optical fibers to demonstrate a non-invasive probe that can be inserted directly inside the flower to instantaneously measure the nectar sugar concentration. Indeed, when the end facet of the optical fiber is in put contact with the flower's nectar, it backreflects a fraction of the incident light that depends on the refractive index gap at the glass-nectar interface, according to the Fresnel reflection law. The contact nature of the measurement (no need to extract the sample) along with the extremely small diameter of the optical fiber core (12 microns), allow to correctly measure the refractive index in volume nectars well below 10 microliters.

After a preliminary calibration with known-concentration sucrose solutions, we tested the refractometer in-vivo on a population of 68 *Primula Palinuri* flowers, the only Mediterranean and maritime species of the whole Primula genus. *Primula Palinuri* is a rare and endangered species surviving only along a narrow area along the Tyrrhenian coast of southern Italy. Its flowers produce a small amount of viscous nectar that cannot be sampled using microcapillaries. The Fiber optic refractometer allowed to get an average value of nectar concentration within the species and also to resolve variations throughout the flowers anthesis. Indeed, we observed that the sugar content increases from flower opening to mature stage and subsequently decreases. These data corroborate the observation that insects visit the mature flowers much more frequently than those in the other stages.

The demonstrated method can be applied to analyze the nectar instantaneously and in-vivo of virtually any flower species, and to detect nectar concentration variations due to floral aging, effective pollination events or environmental growing conditions. These fine variations may be in fact very hard to observe with conventional methods on a statistical ensemble of flowers, but the possibility of performing repeated measurements, or even to monitoring in real time the nectar of individual flowers, could allow to highlight them.

Finally, the simplicity of operation, the reduced cost and the intrinsic ruggedness of the fiber-optic refractometric probe make it an excellent candidate to be engineered into a portable instrument, allowing to envisage applications not only related to species biology or biodiversity conservation, but also in the agricultural sector, such as honey production and crop pollination service.

4.2 = Plants and frugivorous birds in the Botanic Garden of Pisa

Lucia Viegi[1], Leonardo Cocchi[2]
[1]Via Trieste 15, 56126 Pisa, Italy (lucia.viegi@alice.it); [2]Orto e Museo Botanico, Sistema Museale di Ateneo, Università di Pisa, Via Luca Ghini 13, 56126 Pisa, Italy

Among close correlations between plants and animals, there is ornithochory, i.e. seed dispersal by birds (1). At least since 1980s, many papers have highlighted the importance of this kind of dispersal in most natural or semi-natural biomes (2, 3, 4, 5), given that fruit pulp is an important nutritional source for many bird species (6, 7).

In this contribution, we report observations on plant-birds interactions, carried out in the Botanic Garden of Pisa from 2017 up to 2019. We have recorded 207 notes related to 18 plant species - mainly trees and shrubs - and to 15 birds species: 89 out of 207 are fruit-eating observations, involving 12 plant species (Table 1) and 7 bird species (Table 2), mainly in autumn and winter. In the Tables we report the number of feeding records, and the number of bird and plant species are respectively reported, in brackets.

The fruits of Persimmon (*Diospyros kaki*) were eaten (Table 1; Fig. 1) by the highest number of bird species: Blackbird, Blackcap, Blue tit, Great tit and Starling. Blackbird and Blackcap are the species showing the broadest dietary spectrum (Table 1), with 10 and 7 out of the 12 plant species, respectively.

Plant species	**N°**
Diospyros kaki Thunb.	23 (5)
Ilex aquifolium L.	17 (2)
Stranvaesia nussia (Buch.-Ham. ex D. Don) Decne.	11 (2)
Ziziphus jujuba Mill.	10 (3)
Diospyros lotus L.	10 (2)
Hedera helix L.	5 (1)
Morus nigra L.	3 (2)
Euonymus europaeus L.	3 (1)
Ligustrum lucidum W.T. Aiton	3 (2)
Carya illinoinensis (Wangenh.) K.Koch	2 (2)
Arbutus unedo L.	1 (1)
Mahonia aquifolium (Pursh) Nutt.	1 (1)
Total	**89**

Table 1. List of plant species by decreasing number of feeding records; number of bird species in brackets.

Bird species	**N°**
Blackcap (*Sylvia atricapilla*)	39 (7)
Blackbird (*Turdus merula*)	37 (10)
Blue tit (*Cyanistes caeruleus*)	8 (2)
Great tit (*Parus major*)	2 (2)
Starling (*Sturnus vulgaris*)	1 (1)
Jackdaw (*Corvus monedula*)	1 (1)
Greenfinch (*Carduelis chloris*)	1 (1)
Total	**89**

Table 2. List of bird species by decreasing number of feeding records; number of plant species in brackets.

Fig. 1. A half-eaten fruit of Persimmon, 20th November 2017

As regards the colour of fleshy fruits, birds prefer red fruits (3, 8), but black, orange and blue are also attractive. Fruits may provide sugars, lipids, proteins, minerals, water (4, 9) and secondary metabolites, some of these potentially toxic (10, 11, 12).

Results are discussed on the basis of some considerations on the preferences of these birds; further surveys are in progress, encouraged by shortage of information about the Italian bird community.

1) B. Snow, D. Snow (2010) Birds and berries, A&C Black
2) K. Hruska (1993) Giornale Botanico Italiano, 127(3), 635
3) A. Caputa, A. Manzi, K. Hruska (1994) Giornale Botanico Italiano, 128(1), 349
4) C. Bello, M. Galetti, D. Montan, M. Pizo, T. Mariguela, L. Culot, F. Bufalo, F. Labecca, F. Pedrosa, R. Constantini, C. Emer, W. Silva, F.R. da Silva, O. Ovaskainen, P. Jordano (2016) Ecology, 98(6), 1729
5) A.G. Castro, S. Yang, A.T. Carlo (2019) Functional Ecology, 33(2), 229-238
6) H.M. Schaefer, A. Valido, P. Jordano (2014) Proc. R. Soc. B, 281, 2013-2516
7) P.G. Blendiger, N.P. Giannini, I. Zampini, R. Ordonez, S. Torres, J.E. Sayago, R.A. Ruggera, M.I. Isla (2015) Ibis, 157(3), 480-495
8) Q. Duan, E. Goodale, R. Quan (2014) Scientific Reports, 4, 5627
9) P. Jordano (1988) Ardea, 76, 193-209
10) F. Bairlein (1996) Comp. Biochem. Physiol., 113A (3), 215-224
11) J.H. Jacobs, S.J. Clark, I. Denholm, D. Goulson, C. Stoate, J.L. Osborne (2010) Arthropod-Plant Interactions, 4, 19-28
12) H. Coklar, M. Akbulut (2017) J Functional Foods, 35, 166-174

4.4 = Plant-pollinators network analysis of the Island of Sálvora – Atlantic Islands of Galicia National Park (Spain)

Daniele Calabrese[1], Iago Izquierdo[2], Marta Galloni[1], Luis Navarro[2]
[1]Department of Biological, Geological and Environmental Sciences, University of Bologna, Via Irnerio 42, 40126 Bologna, Italy; [2] Department Plant Biology and Soil Sciences, University of Vigo, As Lagoas-Marcosende; 36310-Vigo, Spain

Pollination networks represent the interactions between the communities of plants and their floral visitors (potential pollinators) in a given place. The analysis of these networks and associated metrics can revail and highlight important issues in the mutualistic organization of trophic chains in the ecosystem. Comparing pollination networks in time and space may let us to obtain biodiversity information, including species richness and turnover, specialization, environmental quality and species adaptability in response to habitat changes. In this study we studied plant-pollinator interactions in the island of Sálvora, a protected island uninhabited for 70 years belonging to the National Park of the Atlantic Islands of Galicia, located on the Atlantic coast of the province of Pontevedra (Spain). The survey was conducted mainly on dunal habitat and comprised 44 plant species and 121 insect species, with a total of 413 single interactions registered.

The results obtained from the network metrics describe a situation in which almost all species are generalist, with the species most represented in the habitat as the most interactive. The high degree of connectance of the network highlight a generalized system, as well as the values of generality and vulnerability. Moreover, no nesting or isolated groups of species have emerged and no species-specific relationships have been identified.

The high generalization and low nestedness of the network indicate its stability, suggesting that it might be more resilient to possible alterations in plant and animal communities (1,2).

1) Dunne, J. A., R. J. Williams, and N. D. Martinez. (2002) Network structure and biodiversity loss in food webs: robustness increases with connectance. Ecology Letters, 5, 558–567

2) Estrada, E. (2007) Food webs robustness to biodiversity loss: the roles of connectance, expansibility and degree distribution. Journal of Theoretical Biology, 244, 296–307

4.4 = About plant and bird diversity patterns across wetlands of Sardinia and Sicily: implications for conservation

Alba Cuena-Lombraña[1], Mauro Fois[1], Saverio Sciandrello[2], Renzo Ientile[3], Corrado Marcenò[2,5], Pietro Minissale[3], Sergio Nissardi[4], Carla Zucca[4], Gianluigi Bacchetta[1]
[1]Department of Life and Environmental Sciences, University of Cagliari, S. Ignazio da Laconi 11-13, Cagliari, Italy; [2]Department of Biological Geological and Environmental Sciences, University of Catania, Longo 19, Catania, Italy; [3]Centre for the Conservation and Management of Nature and Agroecosystems (CUTGANA), University of Catania, S. Sofia 98, Catania, Italy; [4]Anthus snc, Via L. Canepa 3, 09129 Cagliari, Italy; [5]Department of Plant Biology and Ecology, University of the Basque Country UPV/EHU, Leioa (Bilbao), Spain

Wetlands are global hotspots of biological diversity (1), ecosystem productivity (2) and economic activities (3). These also play fundamental roles in climate change regulation and mitigation (4). Mediterranean wetlands, in particular, are characterized by a long history of anthropization (5); even though they are still very important or vital habitats for many species. Understanding the relationships among different taxa is therefore fundamental for the conservation of these ecosystems. This research was undertaken to improve the knowledge about the floristic and bird composition in different types of wetlands in Sardinia and Sicily in order to evaluate if the richness and co-occurrence of vascular plants and birds vary among the different wetland categories. We compiled a presence/absence matrix of plants and birds for a representative set of wetlands (Fig. 1), classified according to Ramsar categories (6), extent and number of habitats. Original sighting data were improved by all the available information obtained from the literature. The relationships between the richness of plants and birds' presence were studied through regression analyses. A variance-covariance matrix for species was constructed to evaluate the binary correlation of site occupancy between species (7). Our results showed no relationship between plant and bird species richness, while differences among wetland types in both plant and bird richness were significant. Furthermore, plant and bird species richness showed a positive correlation with size and habitat diversity. The variance-covariance matrix showed a significant relationships between bird and plant species whereasthe co-occurrences of endangered bird and plant was not significant . The occurrence of different taxa in wetlands may be related to several reasons (8): random coincidence, interactions between taxa, a similar response to common factors, and response to different environmental factors that are spatially covariant. Poor correlation between bird and plant diversity could be also due to nested systems, which often occur in species-poor sites, more correlated with a determinate type of habitat. These findings provide a baseline to understanding linkages between the diversity of organisms and constitute an important challenge for the knowledge of how ecological communities and processes are determined at both local and regional scales in order to develop an efficient network for the conservation of wetland biodiversity.

Fig. 1. Distribution of the selected representative set of wetlands in Sardinia and Sicily (Italy)

1) J.P. Gibbs (2000). Conser. Biol.,14, 314-317
2) A.V. Rocha, M.L. Goulden (2009). Agric. For. Meteorol.,149, 159-168
3) W.J. Junk, M.T.F Piedade, R. Lourival, F. Wittmann, P. Kandus, *et al.*, (2014). Aquat. Conser., 24, 5-22
4) A.M.R. Petrescu, A. Lohila, J.P. Tuovinen, D.D. Baldocchi, A.R. Desai, *et al.*, (2015). PNAS,112, 4594-4599
5) M. Álvarez-Cobelas, C. Rojo, D.G. Angeler (2005). J. Limnol., 64, 13-29
6) WWT Consulting (2018) Slimbridge, United Kingdom
7) G. Bell, (2005). Ecology, 86, 1757-1770
8) V. Wolters, J. Bengtsson, A.S. Zaitsev (2006). Ecology, 87, 1886-1895

4.5 = Relationships between plant traits, insect herbivores and plant invasion along elevation in a soil disturbance experiment in permanent meadows

Francesco Boscutti[1], Costanza Geppert[2], Daria Corcos[2], Enrico Braidot[1], Elisa Petrussa[1], Antonio Filippi[1], Lorenzo Marini[2]

[1]Department of Agricultural, Food, Environmental and Animal Sciences, University of Udine, via delle Scienze 91, 33100 Udine, Italy; [2]DAFNAE-Entomology, Viale dell'Università 16, 35020, Legnaro, Padova, Italy

Insect-plant interactions drive many ecosystem functions producing significant shifts in species composition, biodiversity and ecosystem services. The effects of insect herbivores on plant community structure and dynamics represent a prominent example of such mechanisms. Many studies already pointed out how plant succession at both early and old stage can be remarkably affected by phytophagous insect community (1).

Moreover, herbivores are generally thought to sustain plant diversity by curbing dominant plant species and affecting plant competition. In turn, plant defensive strategies, encompassing both physiological a morphological traits, can affected the herbivore predation, shaping the insect-plant interactions (2,3).

Exotic plant invasion has become a worldwide threat to ecosystems and biodiversity. Invasive species replace native plant and changes consumer assemblages and trigger a "bottom-up" cascade that reduces biomass and diversity in higher trophic levels (4). Biological invasion has expected to have a huge increase in the next decades also due to climate changes; in this light, some comprehensive studies on the response of plant community to biological invasion under different climate and herbivores pressures can provide new isight in the future invasion scenarios.

We conducted a manipulative experiment on soil disturbance in permanent meadows along an elevation gradient of ca 1200 m to study the interactions between native and exotic plants traits and phytophagous insect community. We selected three transects including five elevation levels, for a total of 15 experimental blocks. In each site, vegetation was completely removed and soil treated with a shallow tillage. Each block included 4 plots treated by insect exclusion and soil fertilization (Fig. 1). For each plot vegetation, spontaneous plant succession was analyzed every 15 days. At the end of the experiment, all plant species were collected in order to evaluate both physiological and morphological functional traits and the community biomass. Our finding will allowed to depict the effect of phytophagous insect along elevation on plant community succession and facilitation/competition mechanisms between native and exotic plant species. These findings will shed light on the future scenarios of plant invasion in relation to climate condition and native insect communities.

Fig. 1. Experiment block setup

1) Brown VK. (1985) Insect Herbivores and Plant Succession. Oikos, 44, 17–22

2) Moreira X, Petry WK, Mooney KA, Rasmann S, Abdala-Roberts L. (2018) Elevational gradients in plant defences and insect herbivory: recent advances in the field and prospects for future research. Ecography, 41, 1485–1496

3) Bennett RN, Wallsgrove RM. (1994) Secondary metabolites in plant defence mechanisms. New Phytol., 127, 617–633

4) Harvey JA, Bukovinszky T, van der Putten WH. (2010) Interactions between invasive plants and insect herbivores: A plea for a multitrophic perspective. Biol Conserv., 143, 2251–2259

4.5 = Relationship between epibenthic meiofauna and production of allelopathic substances by macroalgae

Laura Pezzolesi[1], Denise Lenzo[1], Marina Antonia Colangelo[1], Rossella Pistocchi[1]
[1]Department of Biological, Geological and Environmental Sciences (BiGeA), University of Bologna, Via Sant'Alberto 163, 48123 Ravenna, Italy

Chemicals with allelopathic function produced by marine organisms, in particular by algae, have received increasing interest for the important role in regulating interactions within planktonic communities. These allelochemicals include polyunsaturated aldehydes (PUAs), which are secondary metabolites with inhibitory effects on reproduction and development of marine invertebrates. PUAs production has been deeply studied in microalgal species, mainly in diatoms, and there is little information on the production of PUAs by macroalgae and their effect on associated benthic meiofauna. The aim of this work was to investigated the relationship between the quali-quantitative profile of the PUAs produced by two macroalgae, *Dyctiopteris polypodioides* (DP) and *Cystoseira compressa* (CC) collected in a benthic environment of the north-eastern Adriatic coast (Ancona, Italy) from May to September 2018, and the associated meiobenthic community, with particular attention to the copepoda of the order Harpacticoida. The results evidenced differences in the quantitative and qualitative production of PUAs between the two algae. Higher concentrations of PUAs, particularly those with a long carbon-chain, such as hexadecatrienal (C16:3), hexadecatetraenal (C16:4), tetradecapentaenal (C14:5), and hexadienal (C6:2), were found in DP than in CC which reported only C6:2 as main compound. The meiobenthic communities showed a different temporal evolution on the two algae with a greater abundance on DP compared to CC, both in terms of main taxa (nauplii, nematodes and copepods) and of the different species of Harpacticoida identified (*Porcellidium viride, Ectinosoma melaniceps, Ameira parvula* and *Heterolaophonte minuta*). These trends could be explained either by the complexity of the algae measured as fractal complexity, and by the concentration of the different PUAs, as emerged above all by the responses of the copepod *Porcellidium viride*, whose abundance was significantly negatively correlated with both the fractal complexity and the concentration of the C14:5 aldehyde, the most abundant in DP. The results of this study highlight as PUAs could induce changes in the dynamics and qualitative composition of meiobenthic communities, thus affecting the interaction between algal cells and herbivores.

Fig. 1. The algae A) *Cystoseira compressa* and B) *Dyctiopteris polypodioides*

Fig. 2. The copepod *Porcellidium virid*

1) T. Wichard, S. A. Poulet, C. Halsband-Lenk, A. Albaina, R. P. Harris, D. Liu, G. Pohnert (2005) Journal of Chemical Ecology, 31, 949-958
2) S. Pichierri, L. Pezzolesi, S. Vanucci, C. Totti, R. Pistocchi (2016) Aquat. Toxicol., 179, 125-133
3) Y. Akakabe, K. Matsui, T. Kajiwara (2003) Bioorganic & Medicinal Chemistry, 11, 3607-3609
4) A. Ianora, S. A. Poulet, A. Miralto (2003) Phycologia, 42, 351-363
5) T. Wernberg, M. S. Thomsen, P. A. Staehr, M. F. Pedersen (2004) Helgoland Marine Research, 58, 154-161

5.1 = Phytochemical and biological screening of *Asteriscus graveolens* (Asteraceae) aerial parts

Achoub Hanane,[1] Mencherini Teresa,*[2], Esposito Tiziana,[2] Luca Rastrelli,[2] Rita Aquino,[2] Gazzerro Patrizia,[2] Zaiter Lahcene,[1] Benayache Fadila,[1] Benayache Samir [1]
[1]Unité de recherche Valorisation des Ressources Naturelles, Molécules Bioactives et Analyses Physicochimiques et Biologiques, Université Frères Mentouri, Constantine 1, Route de Aïn El Bey, 25 000 Constantine, Algérie; [2]Dipartimento di Farmacia, Universita degli Studi di Salerno, via Giovanni Paolo II 132, 84084 Fisciano (SA), Italy; *tmencherini@unisa.it

Asteriscus graveolens (Forsk) DC is the predominant specie of Asteraceae family growing in Saharan regions. It is an herbaceous aromatic plant mainly distributed in south-western of Algeria and Morocco (1) where it is used in folk medicine as excellent heating, for the treatment of fever, gastrointestinal tract complaints, cephalic pains, bronchitis, as well as an anti-inflammatory, antimicrobial, antifungal, and hypoglycemic (2) remedy. Recently (3), the selective citotoxic activity of *A. graveolens* fractions, containing sesquiterpene lactone asteriscunolide isomers, was proved against mouse BS-24-1 lymphoma cells thought the induction of caspase-3-dependent apoptosis. Moreover, the ability of the *Asteriscus*-derived fraction to sensitize the cancer cells to chemotherapeutic drugs like cisplatin/etoposide/doxorubicin was demonstrated (4). In the present research, we describe the isolation of 10 secondary metabolites from polar extracts (ethyl acetate and *n*-butanol) of *A. graveolens* aerial parts. The chemical structures of pure compounds were unambiguously elucidated by NMR analysis. A new acyclic sesquiterpene and two new sesquiterpene germacranolide lactones along with known lactone sesquiterpenes, flavones, a C-13 type nor-isoprenoid, and a quinic acid derivative were identified. On the basis of the activity reported for *A. graveolens* fractions and sesquiterpenes, the extracts and isolated compounds were tested for the ability to decrease the cell viability on two different human cancer cell lines, colon cancer (HCT116) and colorectal adenocarcinoma (DLD1). Extracts and compounds were able to reduce *in vitro* the cell viability with IC_{50} ranging from 89.4 to 296.0 µg/mL (for the extracts) and from 0.1 to 1.7 µM (for compounds). No cytotoxic effect was observed in a normal cell line (Primary Human Dermal Fibroblast, HDFa) up to 300 µg/mL (for the extracts) and 2 µM (for compounds). Thus, our preliminary findings highlight the derivatives of *A. graveolens* aerial parts as potential antiproliferative agents.

1) L. Hammoud, F. León, I. Brouard, J. Gonzalez-Platas, S. Benayache, P. Mosset, F. Benayache. (2018). Tetrahedron Letters, 59(27), 2668-2670
2) B.E.C. Ziani, R.C. Calhelha, J.C.M. Barreira, L. Barros, M. Hazzit, I. Ferreira (2015) Industrial Crops and Products, 77, 582-589
3) Z. Tayeh, R. Ofir (2018) Int J Mol Sci, 19(8), 2219
4) Z. Tayeh, N. Dudai, A. Schechter, V. Chalifa-Caspi, S. Barak, R. Ofir (2018). Int J Mol Sci, 19(8), 2162

5.1 = *In vitro* citotoxicity evaluation of natural compounds from *Punica granatum* L. and *Vitis vinifera* L. on leukemia and multiple myeloma cell lines

Stefano Acquadro[1], Arianna Marengo[1], Carlo Bicchi[1], Barbara Sgorbini[1], Cecilia Cagliero[1], Patrizia Rubiolo[1], Thomas Efferth[2]

[1]Department of Drug Science and Technology, University of Turin, Via Pietro Giuria 9, 10125 Torino, Italy; [2] Institute of Pharmacy and Biochemistry, Department of Pharmaceutical Biology, Johannes Gutemberg University, Mainz, Germany

Hematopoietic cancers (HCs) are malignancies of immune system cells. Leukemia and multiple myeloma represent the most common hematologic malignancy and they are characterized by the clonal expansion of plasma cells. Despite dramatic improvements in patients′ survival over the past decade, due to advances in therapy, exploiting novel molecular targets (immunomodulatory drugs, proteasome inhibitors and monoclonal antibodies), the treatment of relapsed and refractory disease remains challenging (1). A main issue to overcome is the development of drug-resistance in the clinical oncology, which makes it necessary to search for new cytotoxic agents. Plants can be considered a good source of diverse and unique bioactive compounds in the drug discovery field. Although numerous studies have been conducted on the chemical composition and beneficial properties of pomegranate and grapevine edible parts (i.e. juice, grape seeds and skins) (2, 3), little information is available on low food interest parts (i.e. leaves) (4). In this work, two plant extracts (*Punica granatum* L. leaves, *Vitis vinifera* L. leaves) were analyzed to test their potential cytotoxic effect on different cell lines by *in vitro* studies. The HPLC-PDA-MS/MS analysis of the investigated hydroalcoholic extracts reveals the presence of interesting compounds, in particular polyphenols and terpenoids, which are known for their anticarcinogenic properties (5,6,7). Each extract was therefore preliminarily tested, at the final concentration of 10 μg/mL, on two acute lymphoblastic leukemia (ALL) cell lines, a drug-sensitive CCRF-CEM and a doxorubicin-resistant CEM/ADR5000. Further analyses were performed on some enriched fractions and pure compounds identified and isolated from the two investigated plant species. Among all the tested compounds/fractions, ursolic acid exhibited the highest cytotoxic activity that was therefore deeply investigated on nine multiple myeloma cell lines and a normal lymphocytes culture as a control. One of the most responsive cell line, RPMI8226 was selected for RNA extraction and microarray analyses. The obtained data on ursolic acid revealed low IC_{50} values on all multiple myeloma cell lines confirming its ability to promote the inhibition of proliferation in according to the literature data (8). Moreover the normal lymphocytes control was not affected by its citotoxicity. These findings suggest a potential therapeutic activity of this compound for the treatment of multidrug resistant tumors and may be further investigated in order to evaluate its effective mechanism of action.

1) W. Willenbacher, A. Seeber, N. Steiner, E. Willenbacher, Z. Gatalica, J. Swensen, J. Kimbrough, S. Vranic (2018) Int. J. Mol. Sci., 19, 2087
2) S. Sreekumar, H. Sithul, P. Muraleedharan, J. Mohammed Azeez, and S. Sreeharshan, (2014) BioMed Research International, Article ID 686921, 12 pages
3) S. Amor, P. Châlons, V. Aires, D. Delmas (2018) Diseases, 6, 106
4) Y. Kiraz, V. S. Neergheen-Bhujun, N. Rummun, Y. Baran, (2016) Tumor Biology, Volume 37, Issue 2, pp 1803–1815
5) Dahlawi H, Jordan-Mahy N, Clench MR, Le Maitre CL, (2012) Nutr. Cancer, 64(1), 100-110
6) A. Niedzwiecki, M. Waheed Roomi, T. Kalinovsky, M. Rath, (2016) Nutrients, 8(9), 552
7) Huang M, Lu JJ, Huang MQ, Bao JL, Chen XP, Wang YT., (2012) Expert Opinion on Investigational Drugs, 21(12), 1801-1818
8) A. K. Pathak, M. Bhutani, A. S. Nair, K.Seok Ahn, A. Chakraborty, H. Kadara, S. Guha, G. Sethi, B. B. Aggarwal, (2007) Mol Cancer Res, 5, 943-955

5.1 = Potential health promoting properties of *Orobanche crenata* Forssk. extract

Rosaria Acquaviva[1], Giuseppe Malfa[1], Carlo Genovese[2], Barbara Tomasello[1], Floriana D'Angeli[2], Alfonsina La Mantia[1], Rosa Tundis[3], Salvatore Ragusa[4]
[1]Department of Drug Science - Biochemistry Section, University of Catania, Viale A. Doria 6, 95125, Catania, Italy; [2]Department of Biomedical and Biotechnological Sciences - Microbiology Section, University of Catania, Via Androne 81, 95124, Catania, Italy; [3]Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, Via P. Bucci, 87036 Rende (CS), Italy; [4]Department of Health Sciences, University "Magna Graecia" of Catanzaro, Viale Europa, Località Germaneto, 88100, Catanzaro, Italy

Orobanche crenata Forssk. (Orobanchaceae), commonly called "broomrape", is a holoparasitic plant that attacks legumes (1). The broomrape can cause severe damage to the most important crops in the Mediterranean region and the Middle East (2). In Sicily 23 species of *Orobanche* were identified including *O. crenata* Forssk. and *O. ramosa* L. that are responsible of several damage to agriculture. As a holoparasitic plant, it is unable to synthesize nutrients for its survival and it uses metabolites that it obtains from the host plant (3).

The broomrape lacks any chlorophyll then gets carbohydrates from the host's phloem, and water and minerals from the host's xylem.

Orobanchaceae species produce different types of phytoconstituents as flavonoids, iridoides, phenolic compounds, and phenyl-propanoides. Furthermore, for their nutritional and organoleptic properties, in many countries these plants are frequently used to prepare several traditional dishes. Even broomrape has interest as an edible plant due to the high content in antioxidant phenols present in its tender shoots, the only edible parts of the plant (4). *Orobanche crenata* was historically used by some North American tribes for topic treating ulcers and cancerous growths on the skin, and orally for treating some bowel disorders (5). Our precedent studies demonstrated that *O. crenata* leave extract contains polyphenolic compounds with *in vitro* antioxidant and antimicrobial (against Gram-positive strains) activities (6). Since there is an increasing interest in the *in vivo* protective effects of natural compounds contained in plants against oxidative damage involved in several human diseases such as cancer, the aim of the present research was to test the effects of aqueous extract of *O. crenata* leaves on a viability of human breast cancer cell line (MCF-7). In addition, in order to elucidate mechanisms of action of this extract some biochemical parameters (lactic dehydrogenase release, non-proteic thiol groups, ROS) were evaluated. MCF-7 cells were treated for 24h with different concentrations of aqueous extract of *O. crenata* leaves.

Results obtained in the present study showed that aqueous extract of *O. crenata* leaves reduced cell viability of MCF-7 cells in a concentration-depending manner together with a decrease in ROS levels and an increase in free thiols non-protein groups. Since it has been reported (7) that MCF-7 cells depend, for their survival and proliferation, on high levels of ROS, our data suggest that the necrotic cell death observed might be correlated with the anti-oxidant action aroused by the extract.

These results confirm the health promoting properties of *O. crenata* and its potential as chemotherapeutic/chemopreventive agent.

1) J.C. Sillero, M.T. Moreno, D. Rubiales (2005) Plant Dis., 89, 23–27
2) I. Ghannam, M. Al-Masri, R. Barakat (2012) American Journal of Plant Sciences, 3, 346–352
3) H.J. Harloff, K. Wegmann (1987) Proceedings of the 4th ISPFP, 295-309
4) V.V. Bianco (1989) Proc. Int. Symp. Hort. Germplasm cultivated and wild, 55-64
5) S.A. Nada, G.A. El-Chaghaby (2015) J Chem Bio Phy Sci Sec. 5, 171–180
6) C. Genovese, R. Acquaviva, S. Ronsisvalle, G. Tempera, G.A. Malfa, F. D'Angeli, S. Ragusa, D. Nicolosi (2019) Natural Product Research doi: 10.1080/14786419.2018.1552697
7) I. Isnaini, N. Permatasari, K. Mintaroem, B. Prihardina, M.A. Widodo (2018) Oxidants-Antioxidants Profile in the Breast Cancer Cell Line MCF-7, 19, 3175-3178

5.1 = *Plantago major* L.: assessment of phytochemical composition and pancreatic lipase inhibitory potential

Valentina Amodeo, Mariangela Marrelli, Veronica Pontieri, Giancarlo Statti, Filomena Conforti
Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, Via Pietro Bucci, 87036 Rende (CS), Italy

Obesity is the most prevalent nutritional disease and it is reaching epidemic proportions all over the world. Currently, botanical sources receive much attention as an alternative to modern, synthetic anti-obesity drugs. Medicinal plants can reduce body weight through different mechanisms of action, among which the inhibition of pancreatic lipase, a key enzyme for dietary fats absorption (1).

The purpose of this study was to investigate the anti-obesity potential of *Plantago major* L. (Plantaginaceae). The leaves of this plant can be eaten raw in salads or boiled, as soups (2).

Aerial parts of *P. major* were extracted through maceration using methanol (plant to solvent ratio 1:10 g/mL). The raw extract was then sequentially partitioned between *n*-hexane, dichloromethane, ethyl acetate and water.

Phytochemical constituents were identified by means of GC-MS. Total phenols and flavonoids content was also determined (3). The anti-obesity potential of the raw extract and its fractions was verified *in vitro* by testing the ability to inhibit pancreatic lipase using *p*-nitrophenyl caprylate as substrate (4).

The ethyl acetate fraction showed a strong lipase inhibitory activity, with an IC_{50} value equal to 0.86 ± 0.02 mg/ml. Very interesting results were also observed for the raw extract and the dichloromethane fraction (IC_{50} = 2.04 ± 0.07 and 1.77 ± 0.05 mg/mL, respectively).

The antioxidant activity was also verified by means of DPPH and β-carotene bleaching assays (3).

According to obtained *in vitro* results, *P. major* could be a promising source of active principles with anti-obesity potential.

Fig. 1. *P. major* L.

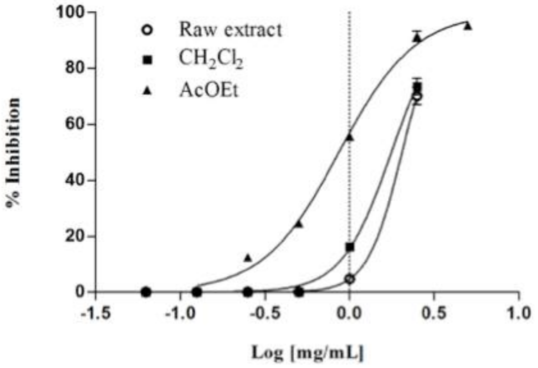


Fig. 2. Lipase inhibitory activity of *P. major* L. extract and fractions.

1) M. Marrelli, F. Conforti, F. Araniti, G.A. Statti (2016) Molecules, 2, 1404
2) S. Gonçalves, A. Romano (2016). Industrial Crops and Products, 83, 213-226
3) M. Marrelli, G. A. Statti, F. Menichini, F. Conforti (2017) Plant Biosystems, 151, 1073-1081
4) M. Marrelli, F. Morrone, M. Argentieri, L. Gambacorta, F. Conforti, P. Avato (2018) Molecules, 23, 2829

5.1 = *In vitro* anti-influenza activity of *Cistus creticus* L. subsp. *eriocephalus* (Viv.) Greuter & Burdet growing in Maremma (Tuscany)

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

Cistus creticus L. subsp. *eriocephalus* (Viv.) Greuter & Burdet (formerly *Cistus incanus* L.) is a perennial shrub, common on sunny or rocky soils in Mediterranean area. Leaves and aerial parts of *C. creticus* have been traditionally used in Greece and Italy as digestive, tonic and to treat respiratory infections (1). Also in Tuscany, in the sea coast area of Maremma, *C. creticus* subsp. *eriocephalus* (CIS) is used as herbal tea to treat cold symptoms and flu. By means of validated *in vitro* tests, in this study we investigated the effect of two samples of CIS aerial parts collected in two sites of Maremma against an influenza A H1N1 strain in pre- and post cell infection. CIS samples were also analysed in order to characterize polyphenols profile. Ethanolic extracts (ethanol 65% V/V) of two CIS samples resulted the most active and gave the best yield in polyphenols (4.5%-5.5% of dry material). These extracts were efficacious in the same manner in *in vitro* inhibiting influenza virus entry in MDCK cells; toxic/effective concentration ratio was >4. Chemical analyses showed that flavan-3-ols oligomers mainly occur in CIS samples and these constituents are likely to contribute more to the antiviral efficacy (2). Our findings suggest that *C. creticus* subsp. *eriocephalus* is worth to be better investigated for its anti-influenza efficacy and ongoing researches are aiming to investigate molecular mechanisms of CIS constituents on viral targets such as the influenza virus glycoproteins, emoagglutinin and neuraminidase.

1) C. Ehrhardt, E.R. Hrincius, V. Korte, I. Mazur, K. Droebner, A. Poetter , S Dreschers, M. Schmolk, O. Planz, S. Ludwig (2007) Antiviral Res., 76(1), 38-47
2) S. Quosdorf, A. Schuetz, H. Kolodziej (2017) Molecules, 22(11), pii: E1989

5.1 = Abietane Diterpene constituents from the roots of *Salvia tingitana* Etl. (Lamiaceae)

Angela Bisio[1], Francesca Pedrelli[1], Martina Fabiano[1], Roméo Arago Dougué Kentsop[1], Gian Carlo Schito[2], Anna Maria Schito[2], Nunziatina De Tommasi[3]
[1]Department of Pharmacy, University of Genova, Viale Cembrano 4, 16148 Genova, Italy; [2]Department of Pharmacy, University of Salerno, Via Giovanni Paolo II 132, 84084 Salerno, Italy; [3]Department of Surgical and Diagnostic Sciences, University of Genova, Largo Rosanna Benzi 8, 16145 Genova, Italy

Salvia tingitana Etl. is an aromatic woody-based perennial shrub original from the Arabian region (1, 2). The analysis of the surface extract of the aerial parts afforded eight new and five known sesterterpenes and one new norsesterterpene, along with other known compounds including five labdane and one abietane diterpenes, one sesquiterpene and four flavonoids, that showed moderate (MIC values ranging from 32 to 128 µg/mL) antimicrobial activity against several Gram positive multi-resistant clinical bacterial strains, also of marine origin (*S.aureus*, *S.epidermidis*, *S.haemoliticus*, *S.hominis*, *S.capitis*, *S.lugdunensis*, *S. saprophyticus*, *S.mitis*, *E.faecium*, *E.faecalis*, *E.durans*, *E.gallinarum*, *E.casseliflavus*). Manool and sclareol exhibited MIC values ranging from 4 to 64 µg/mL. The methanolic extract of the roots, was investigated following a bioassay-oriented fractionation approach. The antimicrobial activity of the methanolic extract against several Gram positive clinical isolates including multiresistant strains of *Staphylococcus aureus*, *S.epidermidis*, *Enterococcus faecium*, *E.faecalis* and *Micrococcus luteus* displayed MIC values ranging from 8 to 32 µg/mL. The extract was then subjected to column chromatography on silica gel, affording eleven semi purified fractions, that were analyzed against several other Gram positive multi-resistant clinical bacterial strains, also of marine origin (*S.aureus*, *S.epidermidis*, *S.capitis*, *S.lugdunensis*, *S.varneri*, *S.simulans*, *S.saprophyticus*, *E.faecium*, *E.faecalis*, *E.durans, E.gallinarum*, *E.avium*). All fractions were active against the tested strains with MIC values ranging from 2 to 64 µg/mL, with exclusion of fraction1 and fraction 11. The most active fraction was fraction 3, displaying MIC values ranging from 2 to 8 µg/mL against both Staphylococci and Enterococci. Fraction 3 was subsequently purified, by means of semi-preparative RP-HPLC, affording six abietane diterpenes, identified by IR, NMR, including TOCSY, COSY, HSQC, HMBC and ROESY experiments, ESI-MS and HR-MS analysis. Hypargenin C isolated in suitable amounts, showed very low MIC values ranging from 2 to 8 µg/mL against the same Gram positive pathogens.

1) M.J.Y. Foley, I.C. Hedge, M. Möller (2008) Willdenowia, 38, 41-59
2) I.C. Hedge (1974). Notes from the Royal Botanic Garden. Vol. 33. Edinburgh

5.1 = Nutritional Evaluation and Bioactive Compounds of Three Italian Edible Wild Species

Annarita Bufano, Bruno Paura, Gianfranco Panfili, Serena Niro, Elisabetta Brugiapaglia
Department of Agricultural, Environmental and Food Sciences, University of Molise, Via Francesco De Sanctis snc, 86100 Campobasso, Italy

This study intends to promote the use of edible wild species (or alimurgical species) in the field of nutrition. The interest in food consumption of spontaneous edible plants in the last decade has grown exponentially. These vegetables have been an important part of the Mediterranean diet and a large number of studies on their therapeutic and nutritional properties (1,2,3) was carried out. These papers highlight the beneficial effects of the alimurgical plants due to the high fiber content, polyphenols, vitamins and minerals. The edible wild species studied for this research were *Crepis vesicaria, Sonchus asper,* (Asteraceae), *Blitum bonus-henricus* (Chenopodiaceae), collected in two sites of the Molise Region (fields near Ripalimosani, pastures near S. Massimo - Matese massif). To date, the knowledge of nutritional and bioactive compounds profile of alimurgical plants is limited and there are no scientific studies for these three species, with the exception of *Blitum bonus-henricus*. In this work, we evaluated composition analysis AOAC (4), the content of liposoluble vitamins such as vitamin A (carotenoids) and vitamin E (tocopherols and tocotrienols) and idrosoluble vitamins: B1 and B2 (thiamine and riboflavin). The extraction of carotenoids and tocols were carried out with the saponification method described by Panfili et al., (5, 6) and determined in HPLC. The activity of vitamin A and E were expressed as Retinol Equivalent (R.E.), and Tocopherol Equivalent (T.E.), calculated as in EFSA, (7) and Sheppard et al. (8), respectively. Thiamine and riboflavin were extracted as in Hasselman et al., (9) before HPLC analysis. Three species under study showed no significant differences for proximate composition except for a highest protein content in *Blitum bonus-henricus.* Analyzed plants were confirmed as an important source of bioactive compounds. As regards carotenoids profile all species were an important presence of xanthophylls fraction (violaxanthin, neoxanthin, anteraxanthin, lutein, zeaxanthin and b-cryptoxanthin) and carotenoids (α-carotene and b-carotene with its isomers 9cis-bcarotene and 13cis-bcarotene). Moreover, the analyzed plants were a source of tocopherols, in particular the α-tocopherol. Taking into account the Recommended Daily Allowance (RDA) for vitamin A and E established by Regulation EU (10), analyzed plants can be declared as a source of vitamin A and E. All plants showed a good thiamine amount, in particular, *Crepis vesicaria* can be considered a source of thiamine, while they are secondary for the riboflavin content. These data can be useful to contribute in the databases on the nutritional and bioactive compounds profile of such alimurgical plants and can contribute to promoting the use of these neglected and underused resources. Moreover, the alimurgical plants could also represent a niche market for the development of functional foods.

1) L. Łuczaj, A. Pieroni (2016) Nutritional ethnobotany in Europe: From emergency foods to healthy folk cuisines and contemporary foraging trends. In: Mediterranean Wild Edible Plants: Ethnobotany and Food Composition Tables
2) NL. Etkin (1994) The cull of the wild. In: Etkin NL(ed) Eating on the wild side. The University of Arizona Press, Tucson, 1–21
3) S.Vitalini, S. Grande, F. Visioli, E. Agradi, G. Fico, F. Tome (2006) Antioxidant activity of wild plants collected in Valsesia, an alpine region of Northern Italy. Phytother Res, 20, 576 –580
4) AOAC. (2000) “Official Methods of Analysis” 17th Ed. Association of Official Analytical Chemists, Washington, DCa
5) G. Panfili, A. Fratianni, M. Irano, (2003) Normal phase high-performance liquid chromatography method for the determination of tocopherols and tocotrienols in cereals. J Agr Food Chem, 51(14), 3940
6) G. Panfili, A. Fratianni, M. Irano, (2004) Improved normal-phase high performance liquid chromatography procedure for determination of carotenoids in cereals. J Agric Food Chem, 51, 3940-3944
7) EFSA, Scientific Opinion on Dietary Reference Values for vitamin A. EFSA Journal 2015, 13(3), 4028
8) AJ. Sheppard, JAT. Pennington, JL. Weihrauch (1993) Analysis and distribution of vitamin E in vegetable oil and foods. In: Vitamin E in health and disease. Packer L, Fuchs J (Eds) Marcel - Dekker New York
9) C. Hasselman, D. Frank, P. Grimm, PA. Diop, C. Soulrs (1989) High-performance liquid chromatographic analysis of thiamin and riboflavin in dietetic foods. J Micronutr Anal, 5, 269-279
10) Regulation EU. No (1169/2011) of the European Parliament and of the Council of 25 October 2011 on the provision of food information to consumers. Official Journal of the European Union

5.1 = Sustainable strategies for cereal by-products valorization

Ilaria Burlini[1], Belén Huerta Lorenzo[2], Immacolata Maresca[1], Massimo Tacchini[1], Alessandro Grandini[1], Alessandra Guerrini[1], Gianni Sacchetti[1]
[1]Department of Life Sciences and Biotechnology, University of Ferrara, P.le Chiappini 2, 44121 Ferrara, Italy; [2]Departamento de Sanidad Animal, Facultad de Veterinaria, Campus Universitario de Rabanales, 14071 Cordoba, Spain

Triticum durum (Desf.), *Oryza sativa* L. and *Zea mais* L. are the so-called "big three" cereal plants as they represent the largest productions worldwide. Their large amounts of by-products suggest the possibility of recovering them as they are important source of health-promoting phytochemicals. According to FAO, one-third of the edible parts of food produced for human consumption gets lost or wastes globally; italian food wastes have been counted around 5.1 MT per year and, beside the mere economic loss, these numbers are closely related to environmental risks (1, 2). The purpose of the present project is to recover wastes from the cereal industry in order to reinsert them within the dermo-functional cosmetic market as high-added value materials, embracing the concept of circular economy. Phenolic acids, mostly bound to cell-walls, are the most representative bioactives in cereals brans and germs; ferulic acid, the main phenolic in literature, demonstrated several applications in both health and food industries as antioxidant, UV absorber, photoprotective and preservative (3, 4). For these reasons, we have developed more effective and greener extraction strategies, compared to those commonly used. The plant materials used have been selected as the main by-products obtained from the milling industry: brans of wheat and rice and germs of yellow and white corn. To develop the project, we extracted ferulic acid both through chemical and enzymatic hydrolysis, verifying the contribution of ultrasound waves to extractive efficacy (alkaline hydrolysis, alkaline hydrolysis coupled with sonication, enzymatic hydrolysis and enzymatic hydrolysis on sonicated material). Therefore, we performed an extraction efficacy screening with HPTLC to evaluate yields and the phenolic molecules extracted. It has followed a RP-HPLC-DAD analysis and quantification of ferulic acid in all obtained extracts and a spectrophotometric quantification of total phenolic content which allowed us to identify the most performing extractions; analyses have confirmed ferulic acid as the main compound in the extracts. In particular, by associating ultrasound waves to yellow corn germ alkaline hydrolysis, we have obtained the best results regarding yields (6.22 ± 0.22%), content of ferulic acid (636.53 ± 3.71 µg FA/mg dried extract), and total polyphenols (844.46 ± 64.60 mg GAE/g dried extract). A screening test was then performed to evaluate the antioxidant activity and the main molecules involved through the DPPH bioautographic assay. The same activity has been investigated through the *in vitro* DPPH spectrophotometric assay evaluating the IC_{50} of the extracts: results obtained with alkaline hydrolysis coupled with sonication, gave the lowest IC_{50} values, in particular with wheat bran (3.61 ± 0.09 µg/mL).

Finally the antimicrobial activity was tested on the most performing extracts, calculating the MICs on three clinical isolated bacterial strains (*S. aureus* MRSA 185087 and MSSA 185960, *S. epidermidis* 185240) and a reference strain of *S. aureus* (ATCC 25923): wheat and rice bran extracts gave the lowest MIC values against the *S. aureus* MRSA strain (32 µg/mL and 16 µg/mL respectively).

The results of this research project allow us to hypothesize possible cosmetic applications of the phenolic extracts obtained: the formulation of one or more dermo-functional products with antioxidant, anti-inflammatory and dermo-protective activities is, in fact, our next research goal.

1) FAO (2011)
2) D. Fattibene and M. Bianchi (2017) IAI, 17
3) N. Kumar, & V. Pruthi (2014) Biotechnol Rep, 4, 86-93
4) F-H., Lin, J-Y. Lin, R. D. Gupta, J.A. Tournas, J.A. Burch, M.A. Selim, N.A. Monteiro-Riviere, J.M. Grichnik, J. Zielinski and S.R. Pinnell (2005). J. Invest Dermatol, 125 (4), 826-832

5.1 = Fruits of *Vitex agnus-castus* vs *V. rotundifolia*: what the differences?

Rosy Caniato[1], Anna Rita Bilia[2], Raffaella Filippini[1], Anna Piovan[1]
[1]Department of Pharmaceutical Science, University of Padova, Via Marzolo, 5 35131 Padova, Italy; [2]Department of Chemistry, University of Firenze, Via Ugo Schiff, 650019 Sesto Fiorentino (Fi), Italy

Vitex agnus-castus L, and *V. rotundifolia* L. have been recently moved from Verbenaceae to the family Lamiaceae. The first is a small deciduous tree whose fruits have been used for more than 2500 around the Mediterranean area for a variety of gynecologic problems. The major compounds most suitable for standardisation are the lipophilic flavonoid casticin, and the iridoid glycoside agnuside (1). The German health authorities have approved the use of *V. agnus-castus* for menstrual cycle irregularities, premenstrual syndrome, mastalgia, and menopausal disorders. Its monograph is also reported in European Pharmacopeia and in EMA monographs.

V. rotundifolia native to seashores throughout the Pacific is a sprawling shrub used as a traditional medicine for treating inflammation, headache, migraine, chronic bronchitis, eye pain, and gastrointestinal infections. Also this species contains casticin and agnuside as main compounds. In view of a possible realization of an European Pharmacopeia monograph of this species, a chemical and morphological study has been made with the aim of highlighting any morphological differences between the two fruits useful for their correct identification.

Ten different samples of *V. rotundifolia* fruits provided by EDQM and samples of *V. agnus castus* L. fruits provided by the seed bank of the Botanical Garden of the University of Padua (Index Seminum 646), have been analysed by HPLC. The phytochemical profile of both species was very similar and main and characteristic constituents content did not show significant differences. A morphological characterization was carried out by the stereo microscope and environmental scanning electron microscopy (ESEM). The average diameter of *V. agnus-castus* and *V.rotundifolia* fruits were 3.2mm and 4.7mm, respectively. In *V. agnus castus* the persistent calyx was more adherent to the fruit, difficult to remove and covering a greater surface area of the fruit than in *V. rotundifolia*. In both species the calyx was very hairy. In *V. agnus castus* trichomes were short, bent or undulate and uni-pluricellular uniseriate. Otherwise in *V. rotundifolia* trichomes were shorter, more numerous and warty-walled with micropapillary cuticle. On the fruit teguments of *V. agnus-castus* numerous glandular peltate trichomes with a unicellular stalk and a uni or multi-cellular head were present next to the calyx. In *V. rotundifolia* the glandular trichomes were in much smaller quantities and less preserved (Fig. 1-2).

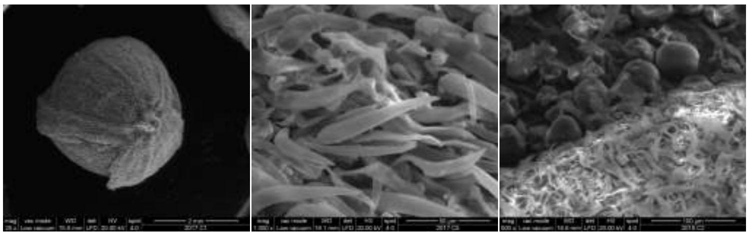


Fig 1. *V. agnus-castus*

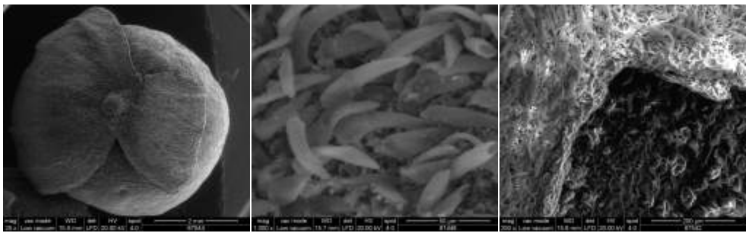


Fig. 2. *V. rotundifolia*

1) M. Cheraghi Niroumand, F.Heydarpour, M.H. Farzae (2018) Plant Review, 12, 103-114
2) Cousins, M.M., Briggs, J., Whitwell, T. (2017) Journal of Environmental Horticulture, 35, 128-137

5.1 = Antimicrobial and Phytotoxic activity of *Origanum heracleoticum* and *O. majorana* essential oils growing in Cilento

Lucia Caputo[1], Teresa Della Pepa[2], Hazem Elshafie[3], Raffaele Capasso[2], Ippolito Camele[2], Maria Rosa Scognamiglio[1], Filomena Nazzaro[4], Vincenzo De Feo[1]
[1]Department of Pharmacy, University of Salerno, via Giovanni Paolo II, 132, 84084 Fisciano, Salerno, Italy; [2]Department of Agricultural and Food Science, University of Naples, via Università, 100, 80055 Portici, Napoli, Italy; [3]School of Agricultural, Forestry, Food and Enviromental Science, University of Basilicata, viale dell'Ateneo Lucano, 85100 Potenza, Italy; [4]Institute of Food Science, CNR-ISA, via Roma 64, 83100 Avellino,Italy

There is a growing interest in essential oils (EOs) as possible alternatives for traditional chemical pesticides and herbicides (1,2). *Origanum heracleoticum* L. and *O. majorana* L. are two aromatic plants belonging to Labiatae family, native to the Mediterranean area. *O. heracleoticum* (syn. *Origanum vulgare* L. ssp. *hirtum*) is also known as 'Greek oregano'; its essential oil, rich in phenols, showed antioxidant, antimicrobial, cytotoxic, antifungal proprieties. Moreover, it is used in aromatherapy as a coadiuvant in treatment of bronchitis, rheumatisms, gastroenteritis (3). *O. majorana* is usually used as a culinary additive but it is used also in cosmetic industry and in phytotherapy due to its numerous emotional, neurological and health benefits (4). This study was carried out to: i) identify the chemical composition of the essential oils (EOs) of *O. heracleoticum* and *O. majorana* ; ii) evaluate the *in vitro* antifungal activity of the EOs against some postharvest phytopathogens (*Botrytis cinerea*, *Penicillium expansum*, *Aspergillus niger* and *Monilinia fructicola*); iii) evaluate the *in vitro* antibacterial activity against *Bacillus megaterium*, *Clavibacter michiganensis*, *Xanthomonas campestris, Pseudomonas fluorescens* and *P. syringae* pv. *phaseolicola*; iv) evaluate the effect of both EOs on the spore germination percentage and their minimum inhibitory concentration (MIC) of *M. fructicola*; v) study the possible phytotoxicity of essential oils and their major constituents, carvacrol for *O. heracleoticum* and terpinene-4-ol for *O. majorana*, against the germination and the initial radicle growth of *Raphanus sativus* L. (radish), *Lactuca sativa* L. (lettuce), *Lepidium sativum* L. (garden cress), *Solanum lycopersicum* L. (tomato). *O. heracleoticum* and *O. majorana* EOs demonstrated promising *in vitro* antimicrobial and antifungal activity against all tested microorganisms. EOs showed high inhibition of spore germination percentage at the minimal inhibitory concentration. Moreover, both germination and radical elongation of selected plants were sensitive to the essential oils.

1) E. Mancini, I. Camele, H. S. Elshafie, L. De Martino, C. Pellegrino, D. Grulova, V. De Feo (2014) Chem Biodivers, 11, 639-651
2) H.S Elshafie, S. Sakr, S.M. Mang, V. De Feo, I. Camele (2016) J Med Food, 19, 1096-1103
3) I. Jerković, J. Mastelić, M. Miloš (2001) Int J Food Sci Technol, 36, 649-654
4) B. Tripathy, S. Satyanarayana, K. Abedulla Khan, K. Raja (2017) Int J Pharma Res Health Sci, 5, 1717-1723

5.1 = Set up of an HPLC-UV/PAD method and microwave-assisted extraction procedure to assess the quality of *Corylus avellana* L. leaves treated with different fertilizer supply

Valeria Cavalloro[1], Mirko U. Granata[1], Rosangela Catoni[2], Francesco Bracco[1], Emanuela Martino[1]
[1]University of Pavia, Department of Earth and Environmental Sciences, Via S. Epifanio, 14, 27100 Pavia, Italy; [2]Sapienza University of Rome, Department of Environmental Biology, P.le A. Moro, 5, 00185 Rome, Italy

Corylus avellana L. (hazelnut) is one of the major nut crops. To date, in Italy a total area of 75,050 ha is invested in hazelnut cultivation, and this make Italy second only to Turkey in this field (1). Nut cultures are increasing worldwide with a highly positive economic impact.

Recent literature evidenced that hazelnut leaves are an interesting source of bioactive secondary metabolites (2). In fact, extracts from leaves of the plant are used in traditional medicine mainly for their antioxidant, antimicrobial, vasoprotective, and antiedema properties (3). Taking into account these considerations, we decided to investigate the effect of different fertilizer supply on the production of secondary metabolites in the leaves of *C. avellana* (CaN) occurring in nature and in *C. avellana* cv. 'Tonda Gentile Trilobata' (CaTGT), one of the most valuable cultivar worldwide (4).

As first part of our work, we set up a proper HPLC-UV/PAD method to obtain the chromatographic fingerprints of the extracts and to quantify quercitrin and myricitrin, the main secondary metabolites. To this aim, different elution conditions on a Chromolith Performance RP-18 endcapped column, under gradient conditions, have been experimented. With the analytical method in hands, we studied the extraction conditions, in terms of extracting solvent, temperature and time, applying a Microwave-Assisted Extraction (MAE) methodology. The best methodology has then been applied to CaN and CaTGT grown under three different nutritional supply: no nutrient -, low nutrient - and high nutrient treatments.

Results highlighted that the treatment with the fertilizer influences the metabolite production, even if in different way. In wild *C. avellana*, the concentration of both the considered compounds significantly decreases by increasing the nutritional supply. A different behavior was observed in cultivated hazelnut: myricitrin concentrations are not influenced by the treatments, whereas the quercitrin amount increases with the fertilizer increase. It is worth noting that the concentration of both quercitrin and myricitrin (by 26% and 10%, respectively) remains significantly higher in wild hazelnut compared to CaTGT, independently from the amount of fertilizer supply.

1) FAO Production Yearbook, 2016
2) A. Gallego, I. Meton, I.V. Baanante, J. Ouazzani, E. Adelin, J. Palazon, M. Bonfill, E. Moyano (2017) Biomed Pharmacother, 89, 565-572
3) A. Cerulli, M. Masullo, P. Montoro, J. Hošek, C Pizza, S Piacente (2018) J. Pharm Biomed. Anal., 160, 168-178
4) M. Oddone, M. Aceto, M. Baldizzone, D. Musso, D. Osella (2009) J. Agric. Food Chem., 57, 3404-3408

5.1 = Chestnut shells (italian cultivar "Marrone di Roccadaspide" PGI): antioxidant activity and chemical investigation with in depth LC-HRMS/MS^n rationalization of tannins

Antonietta Cerulli[1], Assunta Napolitano[1], Milena Masullo[1], Jan Hošek[2], Cosimo Pizza[1], Sonia Piacente[1]
[1]Dipartimento di Farmacia, Università degli Studi di Salerno, via Giovanni Paolo II 132, 84084 Fisciano (SA), Italy; [2]Regional Centre of Advanced Technologies and Materials, Faculty of Science, Palacký University in Olomouc, Šlechtitelů 27, 78371 Olomouc, Czech Republic

Castanea sativa Mill. (Fagaceae) is a deciduous tree growing in Southern Europe, especially in the Mediterranean region and Balkans (1, 2). The nut, commonly named chestnut or marron, represents a traditional basic food and can be processed into different elaborated and diversified food products. Reports on chestnut by-products, such as leaf, shell and bur, revealed that they can be considered a good source of phenolic compounds with marked biological activity, mainly antioxidant properties (1). The 'Marrone di Roccadaspide', a labelled PGI (Protected Geographical Indication) product, represents an important economic resource for the Campania market.

In order to achieve deeper insights into the chemical composition of the waste products of the 'Marrone di Roccadaspide'and to highlight the occurrence of biologically active compounds, the phytochemical investigation of a MeOH extract of the shells of *C. sativa* cultivar 'Marrone di Roccadaspide' has been carried out.

Thus, the methanol extract of shells was investigated for the phenolic content by the Folin-Ciocalteu method and for the antioxidant activity by DPPH and TEAC assays along with a cell-based antioxidant *in vitro* test. To correlate the high phenolic content and the strong antioxidant activity to the chemical composition, a metabolite profile of the MeOH extract of the shells was acquired by high-performance liquid chromatography coupled to multiple-stage linear ion-trap and orbitrap high-resolution mass spectrometry (LC-ESI/LTQOrbitrap/MS/MS^n) along with NMR characterization of isolated compounds.

The identification of a wide range of hydrolysable tannins (e.g. galloyl glucose derivatives, ellagitannins and flavanoellagitannins) and condensed tannins (e.g. proanthocyanidins), along with flavonoids, phenol glucosides, ellagic acid derivatives, and triterpenoids was carried out.

Furthermore, the quantitative determination of the most representative compounds occurring in the methanol extract of *C. sativa* shells was carried out by LC-ESI/QTrap/MS/MS, using the mass tandem experiment called Multiple Reaction Monitoring (MRM).

With the aim to explore the possibility to use chestnut shells as a suitable source of bioactives for the preparation of functional ingredients, the chemical composition and the antioxidant activity of "eco-friendly" extracts (EtOH and EtOH:H_2O 7:3) have been evaluated. The results, and in particular the high superimposability between qualitative analysis and antioxidant activity of MeOH and EtOH:H_2O extracts, suggest for chestnut shells a role of co-product with possible applications in nutraceutical and cosmetic formulations able to protect from oxidative damage.

1) N. Braga, F. Rodrigues, P. P. M. B. Oliveira (2015) Natural Product Research, 29, 1, 1-18
2) A. Cerulli, M. Masullo, A. Mari, A. Balato, R. Filosa, S. Lembo, A. Napolitano, S. Piacente (2018) Natural Product Research, 32, 10, 1170–1175

5.1 = Anti-bacterial prenylated phenols from the Kurdish medicinal plant *Onobrychis carduchorum* (Fabaceae)

Marco Clericuzio[1], Hawraz Mohammed Amin[1,4], Faiq Hussain[3], Laura Cornara[5], Elisa Bona[2], Elisa Gamalero[1], Novello Giorgia[1]

[1]DISIT, Università del Piemonte Orientale, Via T. Michel 11, 15121 Alessandria (Italy); [2]DISIT, Università del Piemonte Orientale, Piazza San Eusebio 5, 13100 Vercelli (Italy); [3]Tishk International University, Erbil, Iraq; [4]Salahaddin University-Erbil, Erbil, Iraq; [5]Department of Earth, Environment and Life Sciences (DISTAV), University of Genova, Corso Europa 26, 16132 Genova, Italy

Onobrychis carduchorum C.C. Towns. is a plant widely employed in the Kurdish traditional medicine, to cure inflammations and other skin diseases. We isolated ten different phenolic metabolites from an acetone extract of leaves and flowers. The phenolic compounds belong to three different classes, i.e.: 1. iso-flavones, having a genistein skeleton; 2. flavanones, bearing a naringenin skeleton; 3. dihydro-stilbenes. Many of them have a prenyl unit on an aromatic ring. The above compounds have been found to date mostly in other Fabaceae, as *Glycyrrhiza glabra* L. (liquorice)[1]. However, their bioactivities are largely unknown. In this work we reported a strong inhibition activity on the growth of *Staphylococcus aureus*, a well-known human pathogen. In particular, compound (Fig. 1) shows an inhibitory activity on growth, comparable to that of vancomycin, using the agar-diffusion standard method.

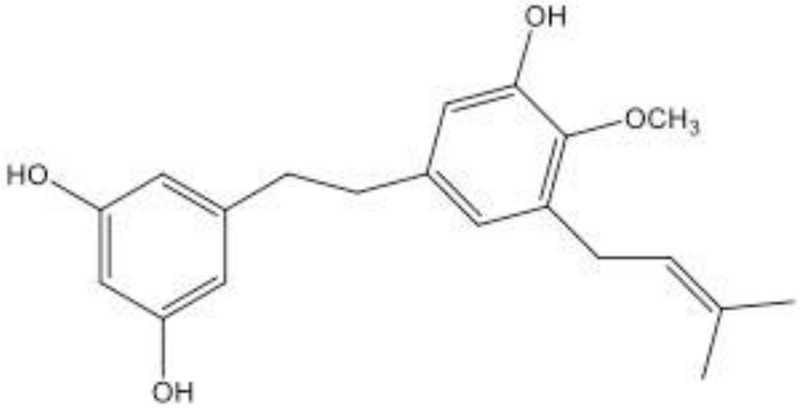


Fig. 1.

1) D.M. Biondi, C. Rocco, G. Ruberto (2003) New dihydrostilbene derivatives from the leaves of Glycyrrhiza glabra and evaluation of their antioxidant activity. J. Nat. Prod., 66, 477-480

5.1 = Metabolite profile of *Symphytum officinale* L. BY LC-MS and NMR analysis

Gilda D'Urso[1], Milena Masullo[1], Alberto Plaza[2], Sonia Piacente[1]
[1]Department of Pharmacy, University of Salerno, Via Giovanni Paolo II, 132, 84084 Fisciano (SA), Italy; [2]R&D, P&G Health Germany GmbH, Alsfelder Str. 17, 64289 Darmstadt, Germany

Symphytum officinale L. (Boraginaceae). commonly known as comfrey, is a perennial herbaceous plant, commonly found in Europe, Asia and North America. It has been used in folk medicine for the treatment of diarrhea, bronchitis, tuberculosis, ulcers and hemorrhoids (1-2). Like other species belonging to the Boraginaceae, comfrey contains hepatotoxic and carcinogenic pyrrolizidine alkaloids (PAs). The LC-MS profile of an ethanolic extract of *S. officinale* performed in positive ion mode highlighted the presence of 10 PAs and their N-oxides. Therefore, since PAs-depleted extracts are used as topical formulations to reduce inflammation, particularly in the treatment of broken bones, tendon damages, painful joints and muscles (3), for a better understanding of the chemical composition and to get preliminary insights on the contribution of the metabolites that may play a role in the anti-inflammatory activity, a phytochemical investigation of a PA-depleted ethanolic extract was performed. LC-ESI/MS and LC-ESI/MS/MS guided the identifcation of 6 compounds including: allantoin (**1**), protocatechuic acid (**2**), *p*-hydroxybenzoic acid (**3**), caffeic acid (**4**), rosmarinic acid (**5**) and globoidnan A (**6**). Noteworthy, compounds **1-5** were previously isolated in comfrey roots (2,4) while this is the first report of globoidnan A (**6**), an arylnaphtalene lignan. The molecular formula and fragmentation pattern of **6** perfectly matched that of salvianolic acid C, previously identified in roots of *S. officinale* by LC-MS (2), but isolation followed by NMR analysis of the pure compound allowed us to unambiguously assign the structure of this compound as globoidnan A. Moreover, a quantitative determination by LC-MS was carried out, using a very sensitive and selective mass tandem experiment such as Multiple Reaction Monitoring (MRM). The quantitative results highlight that globoidnan A (**6**) and rosmarinic acid (**5**) exhibit the highest concentrations of 20.02 mg/100 g and 14.03 mg/100 g of dried extract, respectively (5).

Moreover, this work highlights once again that the only MS information is not sufficient to determine the stucture of plant constituents and extends and reinforces the notion that the combination of LC-MS and NMR techniques is a powerful tool to achieve the truly chemical structure of naturally occurring molecules in plants.

1) C. Staiger (2012) Phytother. Res., 26, 1441-1448
2) A. Trifan, S. E. W. Opitz, R. Josuran, A. Grubelnik, N. Esslinger, S. Peter, S. Bram, N. Meier, E.Wolfram (2018) Food Chem. Toxicol., 112, 178-187
3) I. Sowa, R. Paduch, M. Strzemski, S. Zielinska, E. Rydzik-Strzemska, J. Sawicki, R. Kocjan, J. Polkowski, A. Matkowski, M. Latalski, M. Wojciak-Kosior (2018) Nat. Prod. Res., 32, 605-609
4) B. Grabias, L. Swiatek (1998) Pharm. Pharmacol. Lett., 8, 81-83
5) J. Seigner, M. Junker-Samek, A. Plaza, G. D'Urso, M. Masullo, S. Piacente, Y. M. Holper-Schichl, R. de Martin (2019) Front. Pharmacol. 10, 289. doi: 10.3389/fphar.2019.00289

5.1 = Micromorphological features of feijoa fruit and evaluation of phytochemical profile and biological properties of its essential oil

Marcella Denaro[1], Antonella Smeriglio[1,2], Clara De Francesco[1,2], Laura Cornara[3], Davide Barreca[1], Ersilia Bellocco[1], Giovanna Ginestra[1], Giuseppina Mandalari[1], Domenico Trombetta[1]
[1]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Viale Palatucci, 98168 Messina, Italy; [2]Foundation Prof. Antonio Imbesi, University of Messina, P.zza Pugliatti, 1, 98122 Messina, Italy; [3]Department of Earth, Environment and Life Sciences, University of Genova, Italy

Acca sellowiana (O. Berg) Burret (commonly known as feijoa) is an evergreen shrub belonging to the *Myrtaceae* family, native to the highlands of southern Brazil, northern Argentina and some areas of Colombia and Uruguay. Today it is widely cultivated in Italy as a garden plant, for its beautiful flower, and as a fruit tree. The cultivation of tropical fruits in Italy is increasing, thanks to the particularly favourable climate, especially in southern regions, like Sicily and Calabria.

The aim of this study was to investigate the micromorphological features of the feijoa fruit peel (*A. sellowiana var. coolidge*) by scanning electron microscopy (SEM) and then to evaluate the phytochemical profile as well as the antioxidant (1), cytoprotective (2) and antimicrobial properties (3) of the feijoa essential oil (EO) by several *in vitro* cell-free and cell-based assays.

SEM analysis showed that the mature fruit pericarp is differentiated into three regions: exocarp, mesocarp and endocarp. An outer epidermis and a hypodermis compose the exocarp. A thin cuticle covers the epidermis, presenting on its surface scattered stomata, openings of secretory cavities, and simple unicellular trichomes. Transversal sections show an epidermal layer and a mesocarp composed by many layers of parenchyma, rich in schizogenic secretory cavities, near the epidermis, and by stone cells, and vascular bundles (4).

GC-FID and GC-MS analyses allowed identifying and quantifying a total of 40 compounds belonging to four classes: monoterpenes, oxygenated monoterpenes, sesquiterpenes and other non-terpenic compounds. The main compounds were β-selinene (17.39%), cariophyllene (16.74%), germacene D (5.32%), methyl benzoate (4.46%) and 3-octanone (4.28%). Sesquiterpenes were the most abundant compounds (76.89%), followed by monoterpene hydrocarbons (3.26%) and oxygenated monoterpenes (0.34%).

The EO showed a strong and dose-dependent ($R^2 > 0.99$) antioxidant and free-radical scavenging activities, with the following order of potency (IC_{50}): TEAC (3.11 µg/ml) > FRAP (3.87 µg/ml) > Iron-chelating activity (4.21 µg/ml) > Folin-ciocalteu (4.66 µg/ml) > ORAC (7.65 µg/ml) > DPPH (77.58 µg/ml).

The EO (1.25-40 mg/ml) was tested for cytoprotective properties on isolated lymphocytes pre-treated with 100 mM *tert*-butyl-hydroperoxide (t-BOOH). It significantly counteracted the activity of this strong oxidant in the range 20-40 mg/mL, while below these values the effect was negligible. Moreover, the EO (5-40 mg/mL) was able to reduce the ROS production induced by t-BOOH on erythrocytes.

A preliminary antimicrobial screening against GRAM+ (*Staphylococcus aureus* ATCC 6538P and *Staphylococcus epidermidis* ATCC 35984), GRAM- (*Pseudomonas aeruginosa* ATCC 9027 and *Escherichia coli* ATCC 10536) bacteria and fungi (*Candida albicans* ATCC 10531) highlighted that EO showed a weak bacteriostatic activity against *S. epidermidis* (MIC value of 5.3 mg/ml), whereas no effect was observed against *P. aeruginosa* and *E. coli* (MIC ≥ 5.3 mg/ml). Lower MIC values were detected against *S. aureus* and *C. albicans* (MIC of 2.7 mg/ml).

Based on these results, we decided to investigate the activity of EO against clinical strains of *S. aureus, C. albicans, C. glabrata,* and *C. parapsilosis*. The effect was bacteriostatic rather than bactericidal, *S. aureus* clinical strains being more sensitive than *Candida* ones. Data indicate that feijoa fruit EO, due to its particular phytochemical profile, antioxidant and antimicrobial properties, could find various application, especially in the food, nutraceutical and pharmaceutical field.

1) Smeriglio A, Alloisio S, Raimondo FM, et al. (2018) Food Chem Toxicol., 119, 407-416
2) Smeriglio A, Cornara L, Denaro M, et al. (2019) Food Chem., 279, 47-355
3) Clinical and Laboratory Standards Institute (CLSI) Clinical and Laboratory Standards Institute Performance Standards for Antimicrobial Susceptibility Testing. CLSI; Wayne, PA, USA (2012) Twentieth Informational Supplement
4) Esemann-Quadros K, Mota AP, Barbante Kerbau G, et al. (2018) Rev. Bras. Frutic., 30(2), 296-302

5.1 = Safety control of edible flowers: microbial contamination and toxic trace elements

Giuliana Drava[1], Francesca Pedrelli[1], Vincenzo Minganti[1], Barbara Ruffoni[2], Massimiliano D'Ambola[3], Nunziatina De Tommasi[3], Angela Bisio[1], Anna Maria Schito[4]

[1]Department of Pharmacy, University of Genova, Viale Cembrano, 4, 16148 Genova, Italy; [2]Consiglio per la Ricerca e la Sperimentazione in Agricoltura – CREA, Centro di ricerca Orticoltura e Florovivaismo, Sanremo (IM), Italy; [3]Dipartimento di Farmacia, University of Salerno, Via Giovanni Paolo II, 132, 84084 Salerno, Italy; [4]Dipartimento di Scienze Chirurgiche e Diagnostiche Integrate, Sezione di Microbiologia, University of Genova, Largo Rosanna Benzi, 8, 16145 Genova, Italy

The present study is part of the INTERREG-ALCOLTRA project "ANTEA" (http://www.interregantea.eu/), aimed to increase the supply chain of edible flowers cultivated in Liguria (Italy) and PACA (France). The consumption of various edible flowers is at present increasingly used in gourmet cuisine and demand for these products has increased (1, 2). Edible flowers are a good source of phenolic compounds thus providing health benefits to consumers (3). No official lists of edible and non-edible flowers were emitted by any international body until now, as well as legal requirements for edible flowers marketing (4). The European Regulation (EC) No. 258/97 provides some information on the safety of these flowers (4). Rapid Alert System for Food and Feed (RASFF) reported foodborne-disease caused by edible flowers mainly associated with the presence of unauthorized chemical compounds and/or pathogens (5). The present study focused on microbial contamination and trace element content, in order to evaluate the safety of this niche food product.

Amounts of edible flowers comparable to those used by chefs for recipes were collected from 4 species of flowers grown in pots (*Begonia semperflorens*, *Salvia elegans*, *Tagetes lemmonii*, *Viola odorata*) and analyzed for the presence of *Listeria monocytogenes* and *Salmonella* spp., according to the standard protocols EN/ISO 11290-1 and EN/ISO 6579, respectively, as required by the Italian safety standards depicted by the Reg. CE 1441/2007. Moreover, for 5 species (*Agastache aurantiaca*, *Anthirrinum majus*, *Begonia semperflorens*, *Tagetes patula*, *Tulbaghia cominsii*) prepared as "ready to eat" food (*i.e.* "IV gamma" on the Italian market) the presence of *Escherichia coli* β-glucuronidase positive strains was also evaluated, according to the standard protocol EN/ISO16649-2 of the Reg. CE 1441/2007. As additional investigation, for all kind of samples of edible species the total bacterial and fungal loads were calculated. The most representative bacterial species were identified by means of Matrix-Assisted Laser Desorption-Ionization Time Of Flight Mass Spectrometry (MALDI/TOF MS) at San Martino Polyclinic Hospital in Genova. Petals of the 8 floral species were freeze dried and grinded; the concentrations of 10 trace elements (Cd, Co, Cu, Fe, Mn, Ni, Pb, Sr, V, Zn) were measured using Optical Emission Spectrometry with Inductively Coupled Plasma source (ICP-OES).

None of the analyzed edible species showed contamination by *L. monocytogenes*, *Salmonella* spp. or *E.coli* β-glucuronidase positive. The bacterial and fungal loads ranged from 3×10^3 to 6×10^5 ufc/g and from $< 1\times10^2$ to $4{,}7\times10^3$ ufc/g, respectively. The most abundant bacterial colonies identified with MALDI/TOF MS belonged to the widespread/ubiquitous in nature *Bacillus* and *Pseudomonas* genera that, in some circumstances, may be responsible for local or systemic infections in humans, particularly in hospital environments (6, 7).

Determining the mineral composition of edible flowers is also important since some elements (*e.g.* As, Cd, Pb) are toxic even at low concentrations, and other elements (*e.g* Cu, Fe, Mn, Ni, Zn), although essential, become toxic or may induce allergic reactions when threshold levels are exceeded (8). The present study did not point out any metal hyperaccumulator species.

In conclusion, none of the studied samples showed health risk related to microbial contamination and trace elements.

Acknowledgement: The EU INTERREG ALCOTRA Project n°11039 "ANTEA" is kindly acknowledged

1) T.C.S.P. Pires, M.I. Diasa, L. Barrosa, R.C. Calhelha, M.J. Alvesa, M.B.P.P. Oliveira, C. Santos-Buelga, I.C.F.R. Ferreira (2018) Food Research International, 105, 580–588

2) R. González-Barrio, M.J. Periago, C. Luna-Recio, F.J. Garcia-Alonso, I. Navarro-González (2018) Food Chemistry, 252, 373–380

3) T.K. Lim (2014) Edible Medicinal and Non Medicinal Plants - Volume 8, Flowers. Springer, New York

4) L. Fernandes, S. Casal, J.A. Pereira, J. A. Saraiva, E. Ramalhosa (2017) Journal of Food Composition and Analysis, 60, 38–50

5) RASFF Portal, Rapid Alert System for Food and Feed. https://webgate.ec.europa.eu/rasff- window/portal

6) A. Peix, M.H. Ramírez-Bahena, E. Velázquez (2018) Infection, Genetics and Evolution, 57, 106–116

7) F. Celandroni, S. Salvetti, S.A. Gueye, D. Mazzantini, A. Lupetti, S. Senesi, E. Ghelardi (2016) PLoS One 11(3): e0152831

8) A. van der Ent, A.J.M. Baker, R.D. Reeves, A.J. Pollard, H. Schat (2013) Plant Soil, 362, 319–334

5.1 = Phytochemical investigation of *Cornus sanguinea* L. fruits

Anna Maria Iannuzzi[1], Fabiano Camangi[2], Marinella De Leo[1,3], Alessandra Braca[1,3], Nunziatina De Tommasi[4]

[1]Dipartimento di Farmacia, Università di Pisa, Via Bonanno, 33, 56126 Pisa, Italy; [2]Scuola Superiore Sant'Anna di Studi Universitari e di Perfezionamento di Pisa, Piazza Martiri della Libertà, 33, 56127 Pisa, Italy; [3]Centro Interdipartimentale di Ricerca "Nutraceutica e Alimentazione per la Salute", Università di Pisa, Via del Borghetto, 80, 56124 Pisa, Italy; [4]Dipartimento di Farmacia, Università degli Studi di Salerno, via Giovanni Paolo II, 132, 84084 Fisciano (SA), Italy

In Italy, over 800 spontaneous plants are used as popular traditional food (1). Among them, "common dogwood" or "bloody dogwood" (*Cornus sanguinea* L.) fruits were once used to make jams, juices, and sometimes an oil for frying. The plant is a species native to most of Europe and western Asia and it is a member of the Cornaceae family. It is a deciduous shrub which grows 2-4 m tall (sometimes reaching 6 m); it is diffused at an altitude of 0-1500 m asl. It is found in different mixed temperate broadleaved forests dominated by oaks, limes, maples, ashes, elms, and hornbeam, along with other mesophile shrub species. This dogwood spreads by seeds and stolons. The young twigs are dark red and hairy. The leaves are opposite, 6-8 cm long and 3-5 cm wide, broadly ovate or elliptical, acuminate, pubescent, with 3-5 pairs of veins and with short stalks and their colour is pale green, turning to reddish in autumn. The flowers are hermaphrodite, with four white or creamy-white petals (4-7 mm long); they are produced in corymbs. Flowering occurs in May-June and fruits mature in September-October. The fruit is a globose drupe, 5-8 mm wide, initially reddish, turning purplish-black at maturity and containing a single seed (2,3). The fruits are not toxic and have high concentration of vitamin C, however they have an unpleasant taste. Only preliminary studies about their secondary metabolites content are previously reported, indicating the presence of quercetin glycosides (4). Thus, a complete chemical investigation of *C. sanguinea* fruits was performed.

Lyophilized fruits of *C. sanguinea*, collected in Livorno (Italy), were defatted at room temperature with *n*-hexane and then extracted with MeOH by exhaustive maceration. The obtained extract was dissolved in water and partitioned firstly with EtOAc and then with *n*-BuOH. The *n*-BuOH extract was finally chromatographed on Sephadex LH-20 column and subsequently by RP-HPLC to obtain pure compounds, that were identified by mono- and bidimensional NMR and MS analyses. As results, the phytochemical investigation of the *n*-BuOH extract of *C. sanguinea* fruits led to the isolation of seven flavonoid glycosides, including the new compound myricetin 3-*O*-α-L-arabinopyranoside 4'-*O*-β-D-glucopyranoside, together with three new cyclohexanone derivatives. Isolated flavonoids showed quercetin, myricetin, dihydromyricetin, and isorhamnetin as aglycones, according to previous studies on fruits belonging to *Cornus* genus. On the other hand, the presence in the *Cornus* genus of very polar small molecules having cyclohexanone skeletons is reported in this study for the first time.

1) Camangi F., Guarrera P.M., Camarda I., Tomei P.E., Lentini F., Stefani A., Leporatti M.L., Pieroni A. (2013) Usi alimentari in Etnobotanica. Conservazione di un patrimonio culturale come risorsa per uno sviluppo sostenibile. Centro Universitario Europeo per i Beni Culturali di Ravello, IV, 113-142. ISBN 978-88-7228-724-8

2) Tutin T.G., Heywood V.H., Burges N.A., Valentine D.H. (1976) Flora Europaea, Rosaceae to Umbelliferae, II, 313, Cambridge University Press, Cambridge

3) Pignatti S. (2018) Flora d'Italia. III, 2-3, Edagricole, Bologna

4) Popovic Z., Matic R., Bajic-Ljubicic J., Tesevic V., Bojovic S. (2018) Geographic variability of selected phenolic compounds in fresh berries of two *Cornus* species. Trees, 32, 203-214

5.1 = Quality control of oregano by NMR-based metabolomics and morphological analysis

Lorenzo Marincich[1], Manuela Mandrone[1], Ilaria Chiocchio[1], Immacolata Maresca[2], Alessandra Petroli[3], Ferruccio Poli[1]
[1]Department of Pharmacy and Biotechnology (FaBit), University of Bologna, Via Irnerio 42, 40126, Bologna, Italy; [2]Department of Life Science and Biotechnology (SVeB), University of Ferrara, Piazza Luciano Chiappini 2, 44123 Boara - Ferrara, Italy; [3]Department of Industrial Chemistry "Toso Montanari", University of Bologna, Viale del Risorgimento 4, 40136, Bologna, Italy

Oregano is one of the world's best-selling herbs, and consequently it is often subject to frauds (1,2). Therefore, developing new methods for oregano quality control is of great importance to guarantee quality and consumer trust.

Despite the large heterogeneity of *Origanum* genus, only two species are recognized on European market as 'true' oregano, namely: *Origanum vulgare* L. and *Origanum onites* L., and 2% w/w is the maximum degree of impurity allowed (3,4).

In this work, morphological analysis was coupled with ^{1}H NMR-based metabolomics in order to develop a new method for broad spectrum oregano quality control, able to discriminate between different oregano species and to detect adulterations.

Twenty-four samples of oregano were provided by two different Companies, while commonly adulterant plants were harvested in the Botanical Garden of Bologna or in wild populations, retaining voucher specimen.

For NMR analysis, 30 mg of grounded sample were extracted in duplicates, using 1 mL of a bland (1:1) of CD_3OD:D_2O phosphate buffer pH 6.0 (0.1 M containing 0.1% TMSP standard), sonicated for 20 min and centrifuged for 15 min. Then, 600 µL of supernatant were transferred in NMR tubes for the analyses. The obtained spectra were opportunely processed to be treated by multivariate data analysis (PCA, OPLS-DA, O2PLS).

Firstly, PCA and OPLS-DA proved validated to discriminate among different oregano species. Samples of *O. vulgaris* were discriminated from the ones of *O. onites*, and not marketable oregano species were clearly detected. For instance, *O. majorana* ^{1}H NMR profile resulted strongly different from the two-marketed species of oregano, showing large quantity of tyrosine and lacking thymol (5). Especially in this case, the metabolomic analysis lead to more satisfactory results than the morphological one, in fact, one sample rating high quality (99% pure oregano) by morphological analysis, resulted, by metabolomic analysis, pure *O. majorana,* which is non-marketable as oregano.

Consequently, two different models were developed to detect impurities in *O. onites* and *O. vulgaris*, respectively. In agreement with the morphological analysis, the developed NMR-based PCA were able to differentiate oregano samples with high degree of purity from the ones containing impurities. Thymol and rosmarinic acid emerged as the most important biomarkers for the discrimination, and, on their diagnostic signals, it was built an O2PLS model able to predict the degree of oregano impurity.

In conclusion, rapidity and accuracy of this technique, together to the broad-spectrum analysis of metabolites make this approach interesting to detect oregano frauds based on ^{1}H NMR profile, allowing also to detect specie-specific biomarkers. Further studies are ongoing on adulterants, in order to develop models endowed with the required sensitivity, accuracy and predictability, able to detect adulterants presence and quantify their percentage in the samples.

1) Marieschi, M., Torelli, A., Poli, F., Sacchetti, G., Bruni, R. (2009) A RAPD-based method for the quality control of Mediterranean oregano and its contribution to pharmacognostic techniques. Journal of Agricultural Food and Chemistry, 57, 1835-1840

2) Galvin-King, P., Haughey, S. A., & Elliott, C. T. (2017) Herb and spice fraud; the drivers, challenges and detection. Food Control, 88, 85-97

3) Oregano Monograph 01/2011:1880 (2017) In European Pharmacopoeia 9th ed., 1464-1466

4) Lohumi, S., Lee, S., Lee, H., & Cho, B. (2015) A review of vibrational spectroscopic techniques for the detection of food authenticity and adulteration. Trends in Food Science & Technology, 46(1), 85-98

5) Busatta, C., Barbosa, J., Cardoso, R. I., Paroul, N., Rodrigues, M., Oliveira, D. D., Oliveira, J. V. D, Cansian, R. L. (2017) Chemical profiles of essential oils of marjoram (Origanum majorana) and oregano (Origanum vulgare) obtained by hydrodistillation and supercritical CO2. Journal of Essential Oil Research, 29(5), 367-374

5.1 = Phytochemical and biological characterization of a *Cannabis sativa* L. variety with high cannabidiol content

Claudia Muscarà[1,2], Antonella Smeriglio[1,2], Domenico Trombetta[1], Giuseppina Mandalari[1], Erminia La Camera[1], Gianpaolo Grassi[3], Clara Circosta[1]

[1]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, V.le SS. Annunziata, 98168 Messina, Italy; [2]Foundation Prof. A. Imbesi, University of Messina, Italy; [3]CRA-Research Institute for Industrial Crops (ISCI), 45100 Rovigo, Italy

Non-drug *Cannabis sativa* L. (Cannabaceae), also known as hemp or industrial hemp as it contains in the whole plant less than 0.2% of Δ^9-tetrahydrocannabinol (Δ^9-THC), exhibits, in the different varieties, a high titer of non-psychoactive cannabinoids such as cannabidiol (CBD)/cannabidiolic acid (CBDA) or cannabigerol (CBG)/cannabigerolic acid (CBGA) (1). In light of this, recently, it has aroused a growing interest for its possible use in therapy. Cannabinoids represent the specific compounds of *C. sativa* but other important constituents are also terpenes and polyphenols. Among the non-psychotropic cannabinoids, CBD is the one of greatest interest from the pharmaceutical point of view since it has been found to possess attractive activities such as antioxidant, anti-inflammatory, neuroprotective, anticancer and antimicrobial.

The aim of this study was to evaluate and compare the phytochemical profile as well as the antioxidant and antimicrobial properties of two different hexane extracts obtained from flowering tops dried of *C. sativa var. fibrante* with high CBD content and Δ^9-THC content <0.2%. These two cannabis hexane extracts were obtained from the flowering tops dried as such and after hydrodistillation of the essential oil and have been named CFHE1 and CFHE2, respectively.

A preliminary phytochemical screening highlighted a high total phenols content in both extracts investigated (19108 ± 1504.96 mg GAE/100 g FW for CFHE1 and 8587 ± 676.20 mg GAE/100 g FW for CFHE2). These results were corroborated by HPLC-DAD analysis, which showed a high titer of both acid and neutral phytocannabinoids, with CBD as the most abundant compound (275.69 and 392.78 mg/100 g FW). The greater presence of CBD in CFHE2 is due to the decarboxylation of the acid form (CBDA), which occurs during the hydrodistillation process.

Moreover, GC-MS analysis leaded to identification of 88 and 56 compounds into CFHE1 and CFHE2, respectively. Cannabinoids are the most abundant compounds in both extracts investigated, although a statistical significant difference in the relative abundance (80.83% and 82.92%, respectively) was highlighted. This difference is mainly attributable to the loss of sesquiterpenes (2.98% *vs* 0.05%, respectively) and sesquiterpene oxides (2.12% *vs* 0.04%, respectively) during the hydrodistillation process.

Both extracts showed remarkable antioxidant activity, with the following order of potency: TEAC (EC_{50} 4.17 and 5.65 μg/ml) > β-carotene bleaching (EC_{50} 18.05 and 17.28 μg/ml) > ORAC (EC_{50} 12.51 and 56.73 μg/ml) > Iron-chelating activity (EC_{50} 63.43 and 33.02 μg/ml) > FRAP (EC_{50} 80.21 and 144.86 μg/ml) > DPPH (EC_{50} 254.10 and 317.23 μg/ml) for CFHE1 and CFHE2, respectively.

A preliminary antimicrobial screening against GRAM+ (*Staphylococcus aureus* ATCC 6538P), GRAM- (*Pseudomonas aeruginosa* ATCC 9027 and *Escherichia coli* ATCC 10536) bacteria and fungi (*Candida albicans* ATCC 10231) highlighted that both extracts did not show any activity against *P. aeruginosa* and *C. albicans*. On the contrary, both extracts showed activity against *E. coli* and *S. aureus* (MIC values in the range between 4.88 and 9.77 μg/ml for both extracts).

Based on these results, we decided to focus our attention on *S. aureus* clinical strains. Both extracts showed bacteriostatic and bactericidal activity against 19 *S. aureus* clinical strains (MIC in the range between 4.88 and 9.77 μg/ml and MBC in the range between 9.77 and 156.26 μg/ml, for both CFHE1 and CFHE2).

These findings suggest that most likely the remarkable antioxidant and antimicrobial properties highlighted by cannabis extracts are mainly due to phytocannabinoids, but it is also possible that minor compounds present in the extracts such as terpenes might be involved in some type of complementary effect to that of the phytocannabinoids (2).

1) A. Smeriglio, S.V. Giofrè, E.M. Galati, M.T. Monforte, N. Cicero, V. D'Angelo, G. Grassi, C. Circosta (2018) Fitoterapia, 127, 101-108
2) E.B. Russo (2011) Br. J. Pharmacol., 163, 1344-1364

5.1 = *Citrus bergamia* Risso & Poit. (Rutaceae) polyphenols improve dyslipidemia and pathophysiological features in a mouse model of non-alcoholic fatty liver disease

Vincenzo Musolino[1,2,3], Micaela Gliozzi[1,2,3], Federica Scarano[1,2,3], Ernesto Palma[1,2,3], Vincenzo Mollace[1,2,3], Salvatore Ragusa[1,2,3].

[1]Institute of Research for Food Safety & Health IRC-FSH, University *Magna Graecia*, Catanzaro, Italy; [2] Nutramed Consortium, Catanzaro, Italy; [3]Department of Health Sciences, University *Magna Graecia*, Catanzaro, Italy

Despite NASH is rapidly emerging as a major public health problem, currently, no approved drug therapy exists. Bergamot (*Citrus bergamia* Risso et Poiteau) is a species growing in Calabria (Southern Italy), whose fruit juice is enriched with flavonoids and phenolic compounds, which improves dyslipidemia and markers of systemic inflammation in patients with Metabolic Syndrome (1,2). Although different mechanisms have been proposed to explain the possible role of polyphenols in preventing NAFLD, no evidence exists regarding molecular mechanisms able to counteract the degeneration of steatosis to NASH. The aim of this study was to perform a preclinical "proof of concept" study of Bergamot polyphenolic formulation (BPF99®) for the treatment of NASH.

A disease reversal study was performed in the diet-induced animal model of NAFLD (DIAMOND) (3). DIAMOND mice were fed a normal chow diet or a high fat diet plus high fructose-glucose water solution (WD SW) for up to 27 weeks. WD SW animals were treated with vehicle or BPF99® (50mg/kg/day gavage, once daily) starting from week 16. The effect of BPF on glucose tolerance and insulin sensitivity was assessed by glucose tolerance test (GTT) and insulin tolerance test (ITT). The degree of steatosis, inflammation and hepatocellular ballooning were assessed using the NASH CRN and SAF score. The index of the oxidative stress was evaluated by assessing serum lipid peroxidation product malondialdehyde (MDA) and serum total antioxidant status (TAS). The effects of BPF® on lipogenic, inflammatory and pro-apoptotic pathways were assessed by western blot analysis. Treatment of WD SW-fed mice with 50mg/kg/d BPF® significantly improved glucose tolerance ($p<0.05$ vs vehicle) and insulin resistance ($p<0.05$ vs vehicle). BPF99® reduced ALT (mean 71.6 vs 44.6 IU/l, $p< 0.01$), triglycerides (38.8 vs 28.1 mg/dl, $p< 0.05$), LDL-C (39.2 vs 23.7 mg/dl, $p< 0.001$). It significantly improved NASH resolution ($p< 0.001$) and the SAF scores ($p< 0.05$) while the NAS improvement approached significance. BPF99® reduced markers of oxidative stress, along with reduced JNK and p38 MAP kinase activity. Finally, BPF99® significantly reduced PARP expression levels in mice with NASH. BPF99® did not reduce the number of mice with fibrosis but improved collagen proportional area ($p<0.04$) and procollagen I and III expression.

In conclusion, BPF99® resolves NASH and ameliorates key histological and pathophysiological features of NASH through the modulation of MAPKs and PARP-1, along with improvement in ALT and dyslipidemia in a mice fed a hypercaloric diet.

1) Mollace V, Sacco I, Janda E, et al. (2011) Hypolipemic and hypoglycaemi activity of bergamot polyphenols: from animal models to human studies. Fitoterapia

2) Gliozzi M, Walker R, Mollace V (2014) Bergamot Polyphenols: Pleiotropic Players in the Treatment of Metabolic Syndrome. Journal of Metabolic Syndrome

3) Asgharpour A, Cazanave SC, Pacana T, Seneshaw M, Vincent R, Banini BA, et al. (2016) A diet-induced animal model of non-alcoholic fatty liver disease and hepatocellular cancer. Journal of hepatology

5.1 = Quali-quantitative analysis of *Astragalus membranaceus* roots extracts

Valentina Parisi[1], Massimiliano D'Ambola[1], Nunziatina De Tommasi[1], Andrea Ariano[2], Lorella Severino[2], Rosario Russo[3]

[1]Dipartimento di Farmacia, Università di Salerno, Via Giovanni Paolo II 132, 84084 Fisciano (SA), Italy; [2]Dipartimento di Medicina Veterinaria e Produzioni Animali, Università di Napoli Federico II, via Delpino 1, 80137 Napoli, Italy; [3]Giellepi S.p.A. Health Science, via B. Cellini 37, 20851 Lissone (MB) Italy

Astragalus membranaceus Bunge (Fabaceae) is one of the most widely used traditional chinese herbal medicines. The plant is a perennial herb characterized by a straight, long, cylindrical, roots, erect steams and yellow corolla. *A. membranaceus* prefers hilly or wooded areas and mainly grown in Northeast, North, and Northwest China as well as in Mongolia and Korea (1). It is used in traditional medicine as an immunostimulant, tonic, antioxidant, hepatoprotectant, diuretic, antidiabetic, anticancer, and expectorant (2). More than 100 specialized metabolites, such as isoflavonoids, flavonoids, triterpenes, saponins have been identified in *A. membranaceus*, and various biological activities of both extracts and isolates have been reported (3). Phytochemical investigations on *A. membranaceus* roots reported the presence of a variety of specialized metabolites mainly belonging to the cycloartane-type and oleanane-type triterpenoid, cycloartane-type and oleanane-type saponin, isoflavonoid, flavonoid and fenolic acid classes (4).

The purpose of our project consists in a quali-quantitative analysis of hydroalcoholic extracts of *A. membranaceus* roots, supplied by Giellepi S.p.A. ^{1}H-NMR and LC/ESI/MS experiments were first performed in order to have a complete view on the *A. membranaceus* roots metabolome and to evaluate the quality of different extracts. The quantitative analysis of extracts was performed by ultra-high-performance liquid chromatography tandem with mass spectrometry. The determination of isoflavonoids (daidzein, formonetin,wogonin, ononin) and astragalosides (astragaloside I and astragaloside IV) content, was obtained on an HPLC Phenomenex Gemini C_{18} Column (50x2.00mm), of Shimadzu ultra-high-performance liquid chromatography (UHPLC) system. The compounds were quantified by using an AB sciex QTRAP 5500 triple quadruple mass spectrometer. MRM modes in negative scan mode were employed to quantify isoflavonoid derivatives, instead SIM modes in positive scan was used to analyze astragalosides. The results of the qualitative-quantitative study showed qualitative similarities in all the studied extracts and higher amount of isoflavonoids compared to the astragalosides.

1) J. Fu, Z. Wang, L. Huang, S. Zheng, D. Wang, S. Chen, H. Zhang, S. Yang (2014) Phytother. Res., 28, 1275–1283
2) F. Wu and X. Chen (2004) J. Chin. Med. Mater, 27, 232–234
3) Q. Xu, X. Ma, X. Liang, (2007) Phytochem. Anal., 18, 419–427
4) Y. Li, S. Guo, Y. Zhu, H. Yan, D. Qian, H. Wang, J. Yu, J. Duan (2019) Molecules, 24, 434

5.1 = *Juniperus macrocarpa* Sm. and *J. oxycedrus* L. (Cupressaceae) essential oils and extracts as inhibitors of carbohydrates-hydrolysing enzymes

Salvatore Ragusa[1], Marco Bonesi[2], Monica R. Loizzo[2], Rosaria Acquaviva[3], Maria C. Tenuta[2], Mariarosaria Leporini[2], Nicodemo G. Passalacqua[4], Rosa Tundis[1]

[1]Department of Health Sciences, University *Magna Graecia* of Catanzaro, Viale Europa-Località Germaneto, Catanzaro, Italy; [2]Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, Via P. Bucci, 87036 Rende (CS), Italy; [3]Department of Drug Science-Biochemistry Section, University of Catania, Viale A. Doria 6, Catania, Italy; [4]Natural History Museum of Calabria and Botanic Garden, University of Calabria, Rende (CS), Italy

Diabetes mellitus is a group of metabolic diseases characterized by chronic hyperglycaemia resulting from defects in insulin secretion, insulin action, or both. This condition is associated with long-term damage, dysfunction, and failure of various organs (1). Type 2 diabetes is the most common type of diabetes, accounting for around 90% of all diabetes cases. Post-prandial hyperglycaemia plays an important role in the development of this type of diabetes. Therefore, the regulation of plasma glucose level is crucial for its delay or prevention. One of the most common approaches to reduce or delay the intestinal absorption of glucose is the inhibition of carbohydrates-hydrolysing enzymes such as a-amylase and a-glucosidase (2). During the last years, several studies investigated the potential role of natural compounds as a-amylase and a-glucosidase inhibitors.

In this context, this study aimed to investigate the potential inhibition of carbohydrates-hydrolysing enzymes a-amylase and a-glucosidase of essential oils and extracts of *Juniperus macrocarpa* Sm. and *J. oxycedrus* L.

The genus *Juniperus* (Cupressaceae) includes many native plants of the Mediterranean regions. *Juniperus oxycedrus* L. (prickly juniper, plum juniper, cade juniper, red-berry juniper, cada) is a shrub or small tree native in the West Mediterranean region from Morocco and Portugal east to southern Italy (3), growing on a variety of rocky sites from sea level up to 1600 m altitude (4). *J. macrocarpa* Sm., often treated as an ecologically vicariant subspecies of *J. oxycedrus*, occurs across the northern Mediterranean region from south-western Spain east to western Turkey and Cyprus, growing on coastal sand dunes from sea level up to 75 m altitude (5).

In this work, the aerial parts of *J. macrocarpa* Sm. and *J. oxycedrus* L. were collected from plants cultivated in the Botanical Garden, University of Calabria, whose provenance was in Calabria (Southern Italy), and authenticated by Dr. NG Passalacqua. The essential oils were obtained by hydrodistillation from dry aerial parts, using a Clevenger-type apparatus. Solvent at different polarity, such as *n*-hexane, dichloromethane, ethyl acetate, and methanol, were used to obtain by maceration four extracts. All samples were able to inhibit both enzymes in a concentration-dependent manner. The most interesting activity was found against a-glucosidase. The essential oils were the most active with IC_{50} values of 141.6 and 150.4 mg/mL, for *J. oxycedrus* and *J. macrocarpa*, respectively. Ethyl acetate extracts showed the best a-glucosidase inhibition with IC_{50} values of 192.4 and 200.3 mg/mL, for *J. oxycedrus* and *J. macrocarpa*, respectively.

The obtained data provide a rationale to further investigate *J. macrocarpa* and *J. oxycedrus* as source of compounds for the development of products for treating and/or preventing diseases with high social impact such as type 2 diabetes.

1) Shaw JE, Sicree RA, Zimmet PZ (2010) Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract, 87, 4-14

2) Tundis R, Loizzo MR, Menichini F (2010) Natural products as α-amylase and α-glucosidase inhibitors and their hypoglycaemic potential in the treatment of diabetes: an update. Mini Rev Med Chem, 10, 315-331

3) Roma-Marzio F, Najar B, Alessandri J, Pistelli L, Peruzzi L. (2017) Taxonomy of prickly juniper (Juniperus oxycedrus group): A phytochemical-morphometric combined approach at the contact zone of two cryptospecies. Phytochemistry, 141, 48-60

4) Orhan N, Orhan IE, Ergun F (2011) Insights into cholinesterase inhibitory and antioxidant activities of five Juniperus species. Food Chem Toxicol, 49, 2305-2312

5) Adams RP (2004) Junipers of the World. Trafford

5.1 = The Prickly Pear peel (*Opuntia ficus-indica* (L.) Mill.): an example of source of bioactive compounds obtained using an eco-innovative extraction technology, extractor Naviglio

Pierpaolo Scarano[1], Daniele Naviglio[2], Antonello Prigioniero[1], Rosaria Sciarrillo[1], Carmine Guarino[1]
[1]Department of Science and Technology, University of Sannio, Via Port'Arsa 11, 82100 Benevento, Italy; [2]Department of Chemical Science, University of Naples 'Federico II', Via Cupa Nuova Cintia 21, Napoli, Italy

The Prickly Pear (*Opuntia ficus-indica L. Mill.*) belongs to the Cactaceae family and grows in different parts of the world such as: in North and South America (Mexico and the United States, Argentina, Peru, Bolivia and Brazil), in Africa (Morocco, Tunisia, Eritrea, Ethiopia and South Africa), in Europe and Asia (Spain, Italy, Israel and Iran). Among these, Mexico is the world's largest producer of prickly pear (more than 400,000 tons/year) (1). The prickly pear shows an enormous genetic variability being a polyploid, in particular octoploid; this variability could reflect the diversity of the colors of the prickly pear fruit that varies between red, purple, green, orange and yellow (2). Prickly pear has a high quantity of peel (between 40-45% of the total weight of the fruit), which generally represents the discarded fruit processing. However, this by-product can be a good source of bioactive compounds (3) that could be obtained using different technologies and eco-innovative extraction techniques such as the Extractor Naviglio® (4). This is one of the techniques that has itself best to solid-liquid extraction in the field of bioactive molecules and compounds. In fact, from the peel can be extracted natural pigments, which, in modern industry, have attracted the attention of both producers and consumers thanks to their proven safety with respect to synthetic dyes. Natural pigments are generally compounds that promote beneficial health effects, positively influencing biological activities due to their antioxidant potential, showing preventive effects against various diseases such as cancer, neurodegenerative and cardiovascular diseases (5). The color stability and antioxidant activity of these pigments, however, are limited due to their rapid degradation in the presence of factors such as oxygen, light, pH or temperature (6). The peel of prickly pears is rich in betalain, which are generally classified into two groups, betacyanins and betaxantines, based on their structural characteristics and light-absorbing properties. Both are water-soluble pigments: the betacyanins give the red-violet color and the betaxantines confer the yellow-orange color (7). All betalain are based on a common structural unit, betalamic acid, which condenses with various amino acids or groups of free amines, or structures containing indoline to form betaxanthins or betacyanins respectively (8). The present study deals with the evaluation of the application, for the first time, of the dynamic solid-liquid extraction method of the Extractor Naviglio®, of the effects of the pH ($pH \leq 5.0$), of the extraction solvent (water:ethanol mixture in 80:20 ratio) and storage conditions (environment and refrigeration) on the content of betacyanin and betaxantine in prickly pear skin extracts. It was noted that factors such as pH, storage time and temperature influenced color stability, according to literature. Operative condition and the use of lower temperatures (≈4°C) could be applied to extract a more interesting quantity of betacyanins and betaxantines from prickly pear skin. A good recovery of these colors of prickly pear peel, which today is a waste product of the food industry, could allow an interesting use as an alternative to synthetic dyes.

1) M. P. Cano, A. Gómez-Maqueo, T. García-Cayuela, J. Welti-Chanes (2017) Food Chem., 237, 612–622
2) D. M. Jimenez-Aguilar, J. M. Lopez-Martınez, C. Hernandez-Brenes, J. A. Gutierrez-Uribe, J. Welti-Chanes (2015) J. Food Compos. Anal., 41, 66–73
3) F. J. Barba, P. Putnik, D. Bursa (2017) 67, 260–270
4) D. Naviglio (2003) Anal. Lett., 36(8), 1647–1659
5) M. Carocho, P. Morales, I. C. F. R. Ferreira (2018) Trends Food Sci. Technol., 71, 107–120
6) G. B. Celli, M. S. Brooks (2017) Food Res. Int., 100, 501–509
7) C. Betancourt, M. J. Cejudo-bastante, F. J. Heredia, N. Hurtado (2017) Food Res. Int., 101, 173–179
8) F. Gandía-Herrero, J. Escribano, F. García-Carmona, J. Escribano, F. García-Carmona, F. Gand (2016) 8398

5.1 = Chemical composition of the non-volatile metabolites of Rhus coriaria drupes from Madonie Mounts

Rosario Schicchi[1], Anna Geraci[2], Massimo Geraci[3], Maurizio Bruno[2], Giovanna Valentino[4], Severina Pacifico[4]
[1]Department of Agricultural, Food and Forest Sciences (SAAF), University of Palermo, Viale delle Scienze, Parco d'Orleans, 90128 Palermo, Italy; [2]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo, Viale delle Scienze, Parco d'Orleans, 90128 Palermo, Italy; [3]Nebrodi Regional Park (Sicily) Piazza Duomo, 98076 S.Agata Militello (ME), Italy; [4]Dipartimento di Scienze e Tecnologie Ambientali, Biologiche e Farmaceutiche - Università degli Studi della Campania "Luigi Vanvitelli" via Vivaldi 43, 81100 Caserta, Italy

Rhus coriaria L. (Anacardiaceae), known as "Sommacco siciliano" (Sumac), is a deciduous shrub 1 to 4 meters high. It has imparipinnate leaves, 10-20 centimetres long with 9-15 leaflets. It flowers in May-August and the flowers are small, white-greenish coloured. The fruit is a red-brown spherical drupe, poisonous if consumed fresh (1). The species grows in southern Europe and the Middle East. It is considered a cryptogenic taxon for Italian Flora (2), of ancient introduction in Sicily (3).

Sumac contains colouring substances and tannins used in dying and tanning fine leather (4). It has a high fungal resistance property, and it is significantly effective against wood decay (5).

The plant is used for its relevant properties in the folk medicine in the Middle East and fruits are used as a spice in many traditional dishes (4, 6).

Rhus coriaria was largely cultivated in Sicily for its tanning properties in the nineteenth century (3, 7).

The chemical composition of the pericarp of Sumac drupes collected on the calcareous substrate of the Madonie Mounts, is thoroughly investigated using an integrated approach employing NMR and HRMS metabolic profiling tools. For this purpose, Sumac fruits, firstly divided in pericarp and seed integument and endosperm, underwent ultrasound assisted maceration using an hydroalcoholic solution (MeOH:H_2O, 1:1, v:v) as extractant. Obtained extracts were investigated by means of ^{1}H-NMR spectroscopy highlighting the pericarp diversity in gallic acid and other phenol and polyphenol compounds, beyond essential amino acids, saccharides and small organic acids. Then, solid phase microextraction, using C18 reverse phase cartridges, was employed to achieve the (poly)phenol rich fraction. UHPLC-HRMS/MS chemical profiling revealed twenty-seven secondary metabolites, mainly gallotannin, differing in the number of galloyl moieties – from three to seven - and galloyl flavonol glycosides.

1) S. Pignatti (2017) Flora d'Italia, Edagricole
2) F. Bartolucci et al. (2018) Plant Biosystems, 152, 179-303
3) G. Inzenga (1874) Manuale pratico della coltivazione del sommacco in Sicilia. Pedone-Lauriel, Palermo
4) A. Shabbir (2012) Journal of Animal and Plant Sciences, 22(2), 505-512
5) S. Sen, C. Tascioglu, K. Tırak (2009) Int. Biodet. Biodeg., 63, 135-141
6) S. Giovanelli, G. Giusti, P.L. Cioni, P. Minissale, D. Ciccarelli, L. Pistelli (2017) Industrial Crops and Products, 97, 166–174
7) G. Barbera, M. Bazzi (1987) Informatore Agrario, 20, 57-61

5.1 = Triterpenoid derivatives with anti-inflammatory activity from *Vitellaria paradoxa* C. F. Gaertn

Carmina Sirignano[1], Daniela Rigano[1], Tommaso Venneri[1], Barbara Romano[1], Ferruccio Poli[2], Orazio Taglialatela-Scafati[1]
[1]Department of Pharmacy, University of Naples Federico II, School of Medicine and Surgery, Via D. Montesano 49, 80131 Naples, Italy; [2]Department of Pharmacy and Biotechnology, University of Bologna, Via Imerio 42, Bologna, Italy

Vitellaria paradoxa C. F. Gaertn, belonging to the Sapotaceae family, is a plant commonly known as Shea butter tree for its valuable shea butter, an edible fat extracted from the fermented kernel, which is widely used in the production of cosmetics and to protect the skin against sunburn (1).

Stem bark and leaves of *V. paradoxa* are used in traditional medicine for the treatment of various ailments; for instance, a bark decoction of the plant is used as an anti-inflammatory remedy and to facilitate childbirth. Moreover, in the West African countries, the infusion of leaves is traditionally employed to treat malaria (2,3).

The phytochemical investigation of the EtOAc extract of *V. paradoxa* stem bark led to the isolation of triterpene alcohols, such as α- and β-amyrin, lupeol, butyrospermol, and different acetyl (Fig. 1) and cinnamyl (Fig. 2) ester derivatives, for which the stereostructures were elucidated on the basis of HR-ESIMS and 1D and 2D NMR experiments.

The isolated compounds were evaluated *in vitro* for their anti-inflammatory properties on murine macrophages J774 stimulated or not with the lipopolysaccharide (LPS).

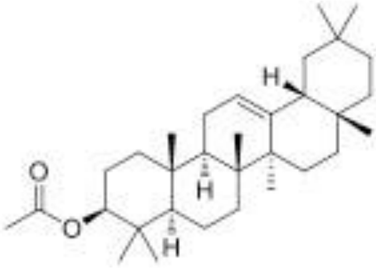


Fig. 1.

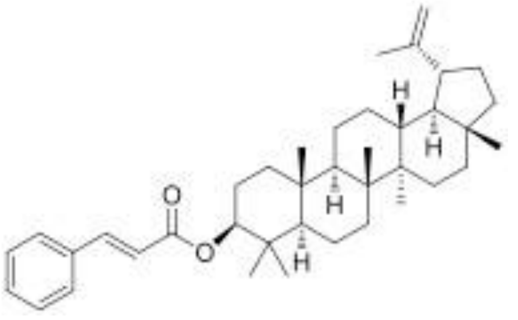


Fig. 2.

1) J. Zhang, M. Kurita, T. Shinozaki, M. Ukiya, K. Yasukawa, N. Shimizu, H. Tokuda, E.T. Masters, M. Akihisa, T. Akihisa (2014) Phytochemistry, 108, 157-170

2) K. O. Eyong, H. S. Foyet, G. Baïrys, G. N. Folefoc, E. A. Asongalem, A. Lagojda, M. Lamshöft (2015) J Ethnopharmacol, 174, 277-286

3) O. Jansen, L. Angenot, M. Tits, J. P. Nicolas, P. De Mol, J. B. Nikiéma, M. Frederich (2010) J Ethnopharmacol, 130(1), 143-150

5.1 = The hull of ripe pistachio nuts (*Pistacia vera* L.) as a source of new promising tyrosinase inhibitors

Antonella Smeriglio[1,2], Valeria D'Angelo[1], Marcella Denaro[1], Domenico Trombetta[1], Maria Paola Germanò
[1]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, Viale Palatucci, 98168 Messina, Italy; [2]Foundation Prof. Antonio Imbesi, University of Messina, P.zza Pugliatti 1, 98122 Messina, Italy

Pistachio nuts (*Pistacia vera* L., Anacardiaceae) are a rich source of many important biofunctional compounds, which possess a broad range of health-promoting properties. In a previous study, we have reported the antioxidant and cytoprotective activity of extracts from the ripe pistachio hull (1). Moreover, the RP-HPLC-DAD analysis on a pistachio hull acidified extract showed a very interesting anthocyanin profile, where ideain (cyanidin-3-*O*-galactoside) represents the main compound identified (2). Since recent studies have shown the inhibitory effects of anthocyanins on tyrosinase enzyme (3), the aim of our study was to evaluate the anti-tyrosinase activity and the antioxidant properties of a pistachio hull extract (PHE, methanol/water/acetic acid; 70/29.5/0.5, v/v/v) by several *in vitro* assays.

Results showed that PHE strongly inhibits the mono and diphenolase activity of mushroom tyrosinase (IC_{50} 141.07 and 116.08 μg/ml, respectively), the rate-limiting enzyme in melanin biosynthesis as compared to kojic acid (IC_{50}= 2.24 μg/ml), a known whitening agent. In addition, PHE was able to hinder L-DOPA auto-oxidation in a concentration dependent manner (0.125- 5.0 mg/ml).

Furthermore, PHE demonstrated a strong and dose-dependent (R^2> 0.99) antioxidant and free-radical scavenging activities, with the following order of potency (IC_{50}): TEAC (3.95 μg/ml) > ORAC (6.18 μg/ml) > FRAP (9.00 μg/ml) > DPPH (17.81 μg/ml) > Folin-Ciocalteu (74.79 μg/ml) > Iron-chelating activity (78.18 μg/ml).

These findings suggest that the ripe pistachio hull may be considered a promising source of antioxidants and tyrosinase inhibitors, which could find employment in the food, cosmetic and pharmaceutical field.

Fig. 1. Hull of ripe pistachio nuts (*Pistacia vera* L.)

1) Barreca D, Laganà G, Leuzzi U, Smeriglio A, Trombetta D, Bellocco E. (2016) Evaluation of the nutraceutical, antioxidant and cytoprotective properties of ripe pistachio (Pistacia vera L., variety Bronte) hulls. Food Chem., 196, 493-502
2) Bellocco E, Barreca D, Laganà G, Calderaro A, El Lekhlifi Z, Chebaibi S, Smeriglio A, Trombetta D. (2016) Cyanidin-3-O-galactoside in ripe pistachio (Pistachia vera L. variety Bronte) hulls: Identification and evaluation of its antioxidant and cytoprotective activities. Journal of Functional Foods. 27, 376-385
3) Jhan JK, Chung YC, Chen GH, Chang CH, Lu YC, Hsu CK. (2016) Anthocyanin contents in the seed coat of black soya bean and their anti-human tyrosinase activity and antioxidative activity. Int J Cosmet Sci., 38(3), 319-24

5.1 = *Ocimum campechianum* Mill. leaves: an Amazonian promising drug

Massimo Tacchini[1], Laura Scalvenzi[2], Matteo Radice[2], Ilaria Burlini[1], Immacolata Maresca[1], Gianni Sacchetti[1], Alessandro Grandini[1], Alessandra Guerrini[1], Mónica Paulina Echeverría Guevara[2]
[1]Department of Life Sciences and Biotechnology (SVeB), UR7 Terra&Acqua Tech Lab., University of Ferrara, Piazzale Chiappini 3, 44123 Ferrara (Malborghetto di Boara), Italy; [2]Department of Earth Sciences, Amazonian State University, km. 2 ½ vía Puyo a Tena (Paso Lateral), 160106 Puyo, Ecuador

Leaves of *Ocimum campechianum* Mill. (syn. *Ocimum micranthum* Willd.) were collected from a wild population in the Ecuadorian Amazonia, authenticated by Dr. David Neill and a voucher specimen was deposited at the Herbarium ECUAMZ of the Amazonian State University (UEA) in Ecuador. *O. campechianum* is a plant species of the Lamiaceae family, widespread across Central and South America, and it was chosen because of its broad spectrum of biological activities. It is traditionally used as ingredient for infusions and beverages or as flavouring agent for foods, against cough, bronchitis and general infections or as anti-inflammatory, antipyretic, to treat conjunctivitis and even as diuretic and emmenagogue (1, 2, 3). Literature reported also a good antioxidant capacity (4) and, recently, a larvicidal activity *in vitro* (5) for its essential oil.

The plant crude drug was characterized based on essential oil, methanolic and hydro-alcoholic (ethanol 70%) extracts composition. The chemical characterization of essential oils, performed with GC-MS analyses, revealed a chemical profile characterized mainly by eugenol (44.1%), β-caryophyllene (10.4 %), β-elemene (6.9%) and 1,8-cineole (7.7%).

The methanolic and hydro-alcoholic extracts, chemically characterized for the first time in this research, showed a similar fingerprinting, with the main presence of rosmarinic acid, followed by caftaric, chlorogenic acids as minor components (RP-HPLC-DAD, NMR). The ethanolic extract exhibited the greatest abundance of every compound. In particular, the quantities of caftaric and chlorogenic acid were about twice as much as in the other extract, while rosmarinic acid was nearly four times more abundant. A preliminary screening of the extracts against A549 cell line were performed but with negative outcomes. On the other hand, extracts did not show any cytotoxicity against HaCat cell line, giving indication of safety of use of the extracts. The antibacterial activity against *Pseudomonas syringae* pv. *syringae* was also tested, and essential oil resulted the most active preparation with a MIC of 2.5 mg/ml. All extracts exhibited a noteworthy antioxidant capacity (DPPH). The highest activity was showed, once again, by the essential oil with an IC_{50} of 7.7±0.1 µg/ml, close to the value of the positive control (Trolox). The hydroalcoholic extract exhibited a bioactivity close to the one of essential oil (11.1±0.01µg/ml), while the methanolic displayed a weaker activity when compared to the previous extracts (52.3±2.7 µg/ml). Rosmarinic acid and eugenol, tested as main components of extracts and essential oil, respectively, showed values close to Trolox. The results confirmed very promising properties for the formulation of cosmetic and food supplements products.

1) L.C. Di Stasi, G.P. Oliveira, M.A. Carvalhaes, M. Queiroz-Junior, O.S. Tien, S.H: Kakinami, M.S. Reis. (2002) Fitoterapia, *73*, 69-91
2) R.E. Schultes, R. Raffauf (1990) *The Healing Forest*, Dioscorides Press: Portland, OR
3) R.F. Vieira, J.E. Simon. (2000) Econ. Bot., 54, 207-216.
4) G. Sacchetti, A. Medici, S. Maietti, M. Radice, M. Muzzoli, S. Manfredini, E Braccioli, R. Bruni. (2004) J. Agric. Food Chem., 52, 3486-3491
5) L. Scalvenzi, M. Radice, L. Toma, F. Severini, D. Boccolini, A. Bella, A. Guerrini, M. Tacchini, G. Sacchetti, M. Chiurato, R. Romi, M. Di Luca (2019) Parasite 26, 23

5.1 = Phenolic profile, antioxidant properties and brine shrimp toxicity of a hydroalcoholic extract obtained from the leaves of *Brassica incana* Ten. (Brassicaceae) growing in Sicily

Maria Fernanda Taviano[1], Emilia Cavò[2,1], Salvatore Ragusa[3], Paola Dugo[1], Francesco Cacciola[4], Andreana Marino[1], Manuela D'Arrigo[1], Natalizia Miceli[1]

[1]Department of Chemical, Biological, Pharmaceutical and Environmental Sciences, University of Messina, S.S. Annunziata, 98168 Messina, Italy; [2]Foundation "Prof. Antonio Imbesi", University of Messina, Piazza Pugliatti 1, 98122 Messina, Italy; [3]Department of Health Sciences, University *Magna Graecia* of Catanzaro, Viale Europa, Località Germaneto, 88100 Catanzaro, Italy; [4]Department of Biomedical and Dental Sciences and Morphofunctional Imaging, via C. Valeria, 98125 Messina, Italy

Brassica incana Ten. (Brassicaceae), is a suffrutex up 100 cm high, woody at the base, branched, glabrous except at base. Basal leaves (8-15 x 12-25 cm) with petiole 5-15 cm long with 2 irregularly dentate wings (5-15 mm width), are pubescent to tomentose especially on the lower surface and along the veining, ovate to lanceolate, lyrate; the lamina has margin entire and irregularly crenate or 1-2 lobes in the lower half, usually obtuse; upper leaves (1 x 4 cm) are denticulate, with amplexicaule basal auricles, gradually smaller. The flowers are gathered in racemes many-flowered with yellow spatulate petals (6-7 x 16-18 mm). The fruit is a siliqua patent (40-80 x 2-3 mm), constricted at intervals, terete, gradually attenuate into beak (1-3).

In Italy, this species grows in Tuscany, Lazio, Campania, Puglia, Basilicata, Calabria and Sicily, where it mainly occurs on the cliffs and the calcareous rocky slopes, from sea level up to about 600-800 m of altitude (2,4). *B. incana* is an edible plant; in Sicily, it is used for the preparation of omelettes and, together with other wild herbs, of the "Frascatula", a traditional Sicilian polenta.

Although several species belonging to the *Brassica* genus have been the subject of numerous phytochemical and biological investigations, information regarding *B. incana* is very limited. In continuation of our researches on species of the Brassicaceae growing in Sicily (5-8), this study was undertaken to investigate the phenolic composition and the antioxidant properties of a hydroalcoholic extract (70% methanol) obtained from the leaves of *B. incana* grown wild around Capo D'Orlando (Messina).

The total phenolic content of *B. incana* extract, determined spectrophotometrically by the Folin-Ciocalteu assay, was equal to 37.20 ± 0.93 mg GAE/g extract. By HPLC-PDA-ESI-MS analysis, a total of 17 polyphenolic compounds were positively identified. Among the detected compounds isorhamnetin-glycoside turned out to be the most abundant one (4.28 mg/g ± 0.54 %RSD), followed by quercetin-3-hydroxyferuloylsophoroside-7-glucoside (3.91 mg/g ± 0.52 %RSD) and isorhamnetin-3-glucoside-7-glucoside (3.33 mg/g ± 0.77 %RSD).

In order to broadly characterize the antioxidant potential of the extract, different "in vitro" methods were utilized: DPPH, reducing power and ferrous ions chelating activity assays (6). Butylated hydroxytoluene (BHT) and ethylenediaminetetraacetic acid (EDTA) were used as reference standards. *B. incana* extract exhibited moderate radical scavenging activity in the DPPH test (IC_{50} = 1.31 ± 0.05 mg/mL) and low reducing power. By contrast, the extract exhibited good chelating properties, reaching nearly 80% activity at the highest tested concentration (2 mg/mL).

Finally, aimed at establishing the potential safety of the extract, brine shrimp (*Artemia salina* Leach) lethality bioassay was performed (6). The median lethal concentration value indicated that *B. incana* extract did not display any toxicity against brine shrimps ($LC_{50} > 1000$ µg/mL).

Altogether, our findings enrich the knowledge about the phytochemical composition and the biological properties of *B. incana,* and highlight that this edible plant species represents a safe source of antioxidants.

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

1) Heywood V.H. (1964) *Brassica* L. In: Flora Europaea, Vol. I, p. 335-339
2) Pignatti S. (2017) *Brassica* L.. In: Flora d'Italia, Vol. 2, p. 1016-1028
3) Raimondo F.M., Mazzola P., Ottonello D. (1991) Flora Mediterranea, l, 63-86
4) Castellano G., Bazan G. (2009) Quaderni di Botanica Ambientale e Applicata, 20(1), 263-268
5) Taviano M.F., Melchini A., Filocamo A., et al. (2017) Pharmacognosy Magazine, 13, 738-743
6) Taviano M.F., Filocamo A., Ragusa S., et al. (2018) Plant Biosystems, 152, 795-803
7) Miceli N., Filocamo A., Ragusa S., et al. (2017) Chemistry & Biodiversity, 14(e 1700073), 1-11
8) Miceli N., Cavò E., Ragusa S., et al. (2019) Chemistry & Biodiversity, 16(e1800677), 1-11

5.1 = Screening of one hundred eighty-five plant extracts as tyrosinase and elastase inhibitors

Tomasi Paola[1], Mandrone Manuela[1], Lianza Mariacaterina[1], Chiocchio Ilaria[1], Marincich Lorenzo[1], Sanna Cinzia[2], Maxia Andrea[2], Ferruccio Poli[1]
[1]Department of Pharmacy and Biotechnology, University of Bologna, Via Irnerio, 42, 40126 Bologna, Italy; [2]Department of Life and Environmental Sciences, University of Cagliari, Via Sant'Ignazio da Laconi 13, 09123 Cagliari, Italy

One hundred eighty-five plant extracts were tested *in vitro* against two enzymes of dermatological relevance: tyrosinase and elastase. Inhibitors of these enzymes find application for skin disorders as well as in cosmetic field as anti-ageing, anti-wrinkles and whitening agents (1,2).

In this work, hydro-alcoholic extracts from 185 plant samples were screened against the two above mentioned enzymatic targets. The tested plants are used in different traditional medicine systems such as: Ayurveda, African traditional medicine, Chinese traditional medicine, Mediterranean traditional and folk medicine, and different traditional medicines from America. Among the plants used in the Mediterranean tradition, particular attention was given to plants from Sardinia (Italy), in fact, 35% of the Mediterranean plants were harvested in this area, and 12% of them were endemic.

Sardinia, due to its geographical isolation and high geological and geomorphological diversification, represents a hotspot for biodiversity within the Mediterranean basin. However, despite Sardinian endemic plants resulted interesting for their phytochemical and biological features, yielding also new molecular scaffolds [3], the majority of them remains still scantly investigated.

Among the tested extracts, twenty-seven samples resulted endowed with promising enzymatic inhibitory activity. In particular, sixteen extracts were active against both enzymes, nine were able to inhibit only tyrosine, and only two were selectively inhibiting elastase. The IC_{50} values of the selected samples ranged from 5 to 150 µg/mL and from 10 to 250 µg/mL against elastase and tyrosinase respectively. *Rhodiola rosea* L. (root) resulted the most active plant against both enzymes, *Cytinus hypocistis* L.(aerial parts) and *Glycyrrhiza glabra* L. (root) were the most active among the plants selectively inhibiting tyrosinase, while *Pistacia lentiscus* L. (leaves) and *Myrtus communis* L. (fruits) were the most active plants endowed with selectivity for elastase. Noteworthy, the most promising plants emerged from this study are all used in Mediterranean traditional medicine, and some of them were endemic from Sardinia (*Limonium morisianum* Arrigoni and *Hypericum scruglii* Bacch.) (4).

Furthermore, total phenolic and flavonoid content of all plants were analyzed and correlated to the percentage of enzymatic inhibition at 50 µg/mL, by Pearson correlation coefficient analysis.

A positive correlation was observed between the potency of enzymatic inhibition of tyrosinase and the total phenolic content of samples. This data is consistent also with the role of this enzyme as polyphenol oxidase (1). A positive correlation was also observed between the potency of elastase inhibition and the total phenolic content, which is supported by previous reported evidences of polyphenols as elastase inhibiters (2,5).

Moreover, the high content of aromatic compounds found in the most active plants (ranging from 37.4 to 147.0 mg GAE/g for phenolic and from 1.5 to 86.6 mg RE/g for flavonoids), confers them also additional value as skin protectors from oxidative damage. The selected plants might have an importance to develop phytocosmetics and in order to identify the metabolites responsible for the activities, further biological and phytochemical studies are ongoing on the selected plants.

1) T. Pillaiyar, M. Manickam, V. Namasivayam (2017) J. Enzyme Inhib. Med. Chem., 32, 403-425
2) T.S. Thring, P. Hili, D.P. Naughton (2009) BMC Complement. Altern. Med., 9, 27
3) C. Sanna, M. Scognamiglio, A. Fiorentino, A. Corona, V. Graziani, A. Caredda, P. Cortis, M. Montisci, E.R. Ceresola, F. Canducci, F. Poli, E. Tramontano, F. Esposito (2018) PLoS One 13, e0195168
4) I. Chiocchio, M. Mandrone, C. Sanna, A. Maxia, M. Tacchini, F. Poli (2018) Ind. Crops. Prod., 122, 498-505
5) J. Wittenauer, S. Mäckle, D. Sußmann, U. Schweiggert-Weisz, R. Carle (2015) Fitoterapia, 101, 179-187

5.1 = Multidisciplinary evaluation of *Nigella sativa* L. oil: phytochemical study, antioxidant activity and antimutagenicity properties

Toniolo Chiara[1], Di Sotto Antonella[2], Di Giacomo Silvia[2], Serafini Mauro[1]
[1]Department of Physiology and Pharmacology, Sapienza University, P.le Aldo Moro 5, 00185, Rome, Italy; [2]Department of Environmental Biology, Sapienza University, P.le Aldo Moro 5, 00185, Rome, Italy

Recently *Nigella sativa* L. (Ranunculaceae), commonly known as black cumin, has aroused great interest for its beneficial effects on the organism. Studies have shown that Thymoquinone, the main bioactive constituent of the seeds oil, has a potential anticancer property (1-3).

The aim of the present work was to carry out a multidisciplinary evaluation of five marketed seeds oils of *N. sativa* from different countries. Using different analytical techniques the content of thymoquinone, antioxidant activity and antimutagenicity properties were determined.

The analysis of thymoquinone was performed using High Performance Thin Layer Chromatography (HPTLC) system and quantitative determination by spectrodensitometric scanning (4,5). The evaluation of the antimutagenicity properties and the antioxidant activity were carried out respectively by *in vitro* assays Ames test and DPPH test (6-8).

The HPTLC analysis showed the concentration of thymoquinone was over the range 2.31-14.66 μg/mg oil, thymoquinone percentage was between 0.23-1.46% for 1 mg of oil.

In preliminary toxicity assay all samples did not produce any precipitate up to 500 μg/ml. Cytotoxicity tests demonstrated the dose-dependent toxic effect start at 250 μg/ml. *Nigella sativa* seeds oils exhibited strong antimutagenicity against tert-butylhydroperoxide in all treatment protocols. The sample C1 (500 μg/ml) inhibited the oxidative mutagenicity of tBOOH in a value 60 and 70% in the pre-treatment and co-treatment, respectively.

These samples also showed antioxidant properties, recording the maximum effect at 5 mg/ml, with an inhibition of 68.3% (sample C3).

The HPTLC method was found to be efficient and selective to determine thymoquinone in *N. sativa* seeds oils. Toxicological tests have confirmed the reported antimutagenic and antioxidant properties.

1) Ali B. H. and Blunden G. (2003) Pharmacological and Toxicological Properties of Nigella sativa Phytotherapy Research, 17, 299–305 DOI: 10.1002/ptr.1309
2) Gali-Muhtasib H., El-Najjar N., Schneider-Stock R. (2006) The medicinal potential of black seed (Nigella sativa) and its components Advances in Phytomedicine, 2, 133-153 https://doi.org/10.1016/S1572-557X(05)02008-8
3) Md Asaduzzaman K., Han-chun C., Mousumi T., Dian-zheng Z. (2011) Anticancer Activities of Nigella Sativa (Black Cumin) African Journal of Traditional, Complementary and Alternative Medicine, 8(5), 226–232 DOI: 10.4314/ajtcam.v8i5S.10
4) Velho-Pereira R.M., Barhate C.R., Kulkarni S.R., Jagtap A.G. (2011) Validated high-performance thin-layer chromatographic method for the quantification of thymoquinone in Nigella Sativa extracts and formulations Phytochemical Analysis, 22(4), 367-373 DOI: 10.1002/pca.1289
5) Sobolev AP, Mannina L, Capitani D, Sanzò G, Ingallina C, Botta B, Fornarini S, Crestoni ME, Chiavarino B, Carradori S, Locatelli M, Giusti AM, Simonetti G, Vinci G, Preti R, Toniolo C, Reverberi M, Scarpari M, Parroni A, Abete L, Natella F, Di Sotto A. (2018) A multi-methodological approach in the study of Italian PDO "Cornetto di Pontecorvo" red sweet pepper. Food Chemistry., 30(255), 120-131 doi: 10.1016/j.foodchem.2018.02.050
6) Di Sotto A., Di Giacomo S., Toniolo C., Nicoletti M., Mazzanti G. (2016) Sisymbrium Officinale (L.) Scop. and its Polyphenolic Fractions Inhibit the Mutagenicity of Tert-Butylhydroperoxide in Escherichia Coli WP2uvrAR Strain. Phytother Res., 30(5), 829-34, doi: 10.1002/ptr.5588
7) Blois M.S. (1958) Antioxidant determinations by the use of stable free radical Nature, 181, 1199-1200
8) Nicoletti M., Toniolo C., Venditti A., Bruno M., Ben Jemia M. (2015) Antioxidant activity and chemical composition of three Tunisian Cistus: Cistus monspeliensis Cistus villosus and Cistus libanotis Natural Product Research, 29(3), 223-230, DOI:10.1080/14786419.2014.947486

5.2 = The effect of light intensity on plant growth and essential oil composition of rosemary

Claudio Cervelli[1], Antonio Raffo[2], Stefano Ferrari Nicoli[2], Eric Mozzanini[1], Elisabetta Lupotto[2]
[1]CREA – Centro di Ricerca Orticoltura e Florovivaismo, Corso Inglesi 508, 18038 Sanremo, Italy; [2]CREA - Centro di Ricerca per gli Alimenti e la Nutrizione, Via Ardeatina 546, 00178 Roma, Italy

Rosemary (*Rosmarinus officinalis* L., synonym of *Salvia rosmarinus* Schleid.) is a perennial aromatic herb native of the Mediterranean Basin and widely cultivated in the world. It is traditionally used as a spice herb for culinary purposes (1) and, more recently, for essential oil (EO) production.

It is well established that not only plant growth and morphology but also EO production are strongly influenced by climatic conditions (2-5). The composition of EO is primarily under genetic control (6), but it is also highly dependent on environmental conditions, such as daylength, irradiance, temperature and water supply (7).

In the frame of the national project on "Implementation of the FAO International Treaty on Plant Genetic Resources for Food and Agriculture" the effect of the light intensity on plant growth and EO yield of rosemary were investigated at 100%, 50% or 25% of natural sunlight.

Light intensity (LI) reduction determined a dry weight decrease of both the rosemary aerial part (- 57 %) and roots (-72%). Reduction of LI also decreased both the stem diameter, the number of total shoots and the specific leaf dry weight, while increased the leaf area.

EO was obtained by steam distillation and was analysed by GC-MS, obtaining 28 constituents mainly belonging to monoterpene hydrocarbons and oxygenated monoterpenes. Major constituents were a-pinene, 1,8-cineole, camphene, camphor and borneol. LI conditions affected the percentage of 27 out of the 28 EO constituents. Major differences were observed between plants subjected to 50% LI and the other two groups of plants.

A group of compounds, such as a-pinene, 1,8-cineole, camphene, b-pinene, myrcene, a-phellandrene, a-terpinene and b-caryophyllene showed enhanced levels in plants exposed to 50% sunlight, whereas a second group of compounds, such as 3-carene, camphor, borneol, 1-terpinen-4-ol, a-terpineol, verbenone and humulene lowered their content in these plants. Major changes due to reduction from 100% to 50% of light intensity were observed for a-pinene, camphor, borneol and verbenone.

These results show that the light intensity is very important in rosemary to increase biomass production but also have implications on EO composition, potentially changing its sensory qualities and biological properties (antimicrobial and antioxidant activities).

1) Sasikumar, B. (2012) Rosemary, in Peter, K.V. (Ed.), Handbook of herbs and spices. Second Edition. Volume 1. Woodhead Publishing Limited, Cambridge, UK, 452-468
2) Conover, C. (1990) The climate indoors, in: Novak J., Rudnicki R.M. (Eds.), Postharvest handling and storage of cut flowers, florist's green and potted plants. Timber Press, Portland, OR
3) Gratani, L., Covone F., Larcher, W. (2006) Leaf plasticity in response to light of three evergreen species of the Mediterranean maquis. Trees, 20 (5), 549–558
4) Gregory, P.J., Palta, J.A., Batts, G.R. (1997) Root systems and root: mass ratio - carbon allocation under current and projected atmospheric conditions in arable crops. Plant and Soil, 187, 221-228
5) Kozlowski, T.T., Pallardy, S.G. (1997) Growth control in woody plants. Academic Press, San Diego, CA
6) Figueiredo, A.C., Barroso, J.G., Pedro, L.G., Scheffer, J.J.C. (2008) Factors affecting secondary metabolite production in plants: volatile components and essential oils. Flavour and Fragrance Journal, 23, 213–226
7) Franz, C., Novak, J. (2009) Sources of Essential Oils, in: Başer, K.H.C., Buchbauer, G. (Eds.), Handbook of Essential Oils: science, technology, and applications. CRC Press, Boca Raton, FL, 39-82

5.2 = Can agriculture be eco-friendly? Plant extracts as grapevine defense inducers

Antonio Filippi, Elisa Petrussa, Zhoreh Rabiei, Marco Vuerich, Enrico Braidot
Department of Agri-Food, Animal and Environmental Sciences, University of Udine, via delle Scienze 206, 33100 Udine, Italy; antoniofilippi@yahoo.it

The reduction of phytochemical application in vineyard is a principal aim in the modern viticulture. In this perspective, alternative strategies could be the biological control by microorganisms or application of defense elicitors, capable to induce the expression of resistance genes (e.g. chitinases, β-1,3-glucanases) (Yakhin et al., 2016; Krzyzaniak et al., 2018; Kulbat, 2016). In the present study, hydroalcoholic extracts (B.A.) from *Vitis vinifera* L. by-products and *Olea europaea* L. and *Ailanthus altissima* (Mill.) leaves were tested on both grapevine suspension cell cultures and plantlets as possible elicitors of pathogen-related chitinases. When the B.A. were incubated with cells at 1% no significant cell death in the following 48h was observed despite an increase in chitinase activity, particularly using B.A. from grape marcs. Immunochemical analysis with anti-chitinase antibody revealed that this protein was expressed at a constitutive level in cell cultures and its expression did not change after treatment, suggesting that the bioextract could directly modulate chitinase rather than induce gene expression. In plantlets, the most active bioextract (grape marcs) was foliar sprayed at different doses and leaf chitinase was followed after 8, 24 and 48 h. As a result, 0.1%, as well as higher doses (2 and 20%, respectively), enhanced chitinase activity.

Fig. 1. *Vitis vinifera* L. plants in field for treatment

Fig. 2. *Vitis vinifera* L. leaf from untreated plant

1) Yakhin, O.I., Lubyanov, A.A., Yakhin, I.A., Brown, P.H., (2017) Biostimulants in Plant Science: A Global Perspective. Front Plant Sci 7. https://doi.org/10.3389/fpls.2016.02049

2) Krzyzaniak, Y., Trouvelot, S., Negrel, J., Cluzet, S., Valls, J., Richard, T., Bougaud, A., Jacquens, L., Klinguer, A., Chiltz, A., Adrian, M., Héloir, M.-C. (2018) A Plant Extract Acts Both as a Resistance Inducer and an Oomycide Against Grapevine Downy Mildew. Front. Plant Sci. 9. https://doi.org/10.3389/fpls.2018.01085

3) Kulbat, K. (2016) The role of phenolic compounds in plant resistance. Biotechnology and Food Science Vol. 80

5.3 = Metabolomic and physiological changes induced by the holoparasitic species *Cuscuta campestris* Yunck. on *Artemisia campestris* subsp. *variabilis* (Ten.) Greuter

Araniti Fabrizio, Abenavoli Maria Rosa
Department AGRARIA, University Mediterranea of Reggio Calabria, Locality Feo di Vito, 89122, Reggio Calabria (RC), Italy

Cuscuta campestris Yunck. is a stem holoparasitic plant species widespread in the Mediterranean area, which parasitizes several wild and crop species causing yield and economic losses (1,2). Among them, *Artemisia campestris* subsp. *variabilis* (ten.) Greuter is one of the most affected and, because of this massive parasitization, several Calabrian populations have been undermined.

Although some information is available concerning host recognition and anatomical alterations induced to the host plants (3,4), no data are reported on *C. campestris* and *A. campestris* interaction mechanisms and on the effects induced on the primary and secondary metabolism of host plants after the parasitization.

In the present study, the composition of volatile organic compounds (VOCs), involved in the host-parasite interaction, and the changes induced on primary metabolism as well as on several physiological parameters of host species *A. variabilis* have been deeply studied.

The VOC analysis, carried out using the HS-SPME-GC/MS technique, highlighted a reduction of some molecules involved in plant defense against herbivores and cuscuta parasitization. In particular, a general reduction in sequiterpene content, which plays a pivotal role in plant defense against cuscuta (5), was observed. On the contrary, the monoterpene profile, signal molecules involved in host recognition, was not affected by the parasitization. Finally, cuscuta induced a drastic reduction in the 3-hexen-1-ol acetate production, which, currently, is the only known naturally molecule with a repellent action against cuscuta (3).

These results suggested that cuscuta infection breaks down the plant's defenses, favoring further parasitization.

At physiological level, cuscuta parasitization altered the photosynthetic machinery of the host inducing alterations of several parameters connected to photosystem II (PSII) activity. Among them, physical damages to the antenna complexes (reduction in Fv/Fm parameter) and a general reduction of the light adapted PSII efficiency (ϕ_{II}), were observed.

Moreover, significant changes in leaf osmotic potential, in hormonal (IAA reduction and ABA increase) and protein content as well as alterations in fresh and dry biomass were observed.

In addition, metabolomic analysis highlighted that parasitization significantly altered the amino acidic and glycogen metabolism strongly increasing the osmoprotectant production (Eg. proline, GABA, galactinol etc.), which generally accumulate in plants as strategy defense from oxidative stress.

Finally, a significant accumulation of two sugars, trehalose and 2-deoxygucose, in parasitized plants was observed. These sugars are known to interfer with the cell wall polysaccharides of cuscuta vines tip causing necrosis and reducing the parasitization process (6).

Concluding, we hypothesize that cuscuta parasitization induces an increase of internal plant defenses (primary metabolites fundamental for plant survival) at the expense of external ones (secondary metabolites) in host species *Artemisia*, limiting plant defense against further parasitizations.

1) E. Le Floc'h (1991) Biogeography of Mediterranean Invasions, 67-80
2) A.F. Farah, M.A. Al-Abdulsalam (2004) Scientific Journal of King Faisal University, 5(1), 103-113
3) J.B. Runyon, M.C. Mescher, C.M. De Moraes (2006) Science, 313(5795), 1964-1967
4) L. Hong, H. Shen, H. Chen, L. Li, X. Hu, X. Xu, Z. Wang (2011) Pakistan Journal of Botany, 43(4), 1853-1859.
5) J.B. Runyon, M.C. Mescher, C.M. De Moraes (2008) Plant Physiology, 146(3), 987-995
6) K. Veluthambi, S. Mahadevan, R. Maheshwari (1982) Plant Physiology, 70(3), 686-688

5.3 = Improving knowledge of the interactions between endophytic bacteria and phytoplasmas in tomato to ameliorate the plant health status and control the phytoplasma disease

Simone Cantamessa[1], Elisa Gamalero[1], Nadia Massa[1], Guido Lingua[1], Graziella Berta[1], Patrizia Cesaro[1]
[1]DISIT, Università del Piemonte Orientale, Viale T. Michel 11, 15121 Alessandria, Italy, simone.cantamessa@uniupo.it

Stolbur or big bud is an epidemic yellows disease of many important plant species, including tomato, caused by a phytoplasma (*Candidatus Phytoplasma solani*), for which there is currently no cure. Plant Growth-Promoting Bacteria (PGPB) have been shown to improve plant resistance against phytoplasma disease. In this research project, possible plant protection induced in tomato by the endophyte *Pseudomonas migulae* 8R6 was investigated. 8R6 is able to synthesize 1-aminocyclopropane-1-carboxylate (ACC) deaminase, that cleaves ACC to α-ketobutyrate and ammonia and thereby decreases ethylene levels in host plants. Low level of ethylene can alleviate the stress symptoms. Three experiments were performed: first,in a the short time experiment the degree of colonization of mutant 8R6 expressing GFP and its localization in the plant tissues of 3 different tomato cultivars was evaluated by confocal fluorescence microscopy and image analyse techniques. The effects of 8R6-GFP on seed germination was carried on. In a second experiment the effects of 8R6-GFP on growth and health of the tomato cvs, in the presence/absence of '*Ca. P. solani*' were evaluated by microbiological, molecular, and physiological techniques. The possible resistance induced will be correlated to 8R6-GFP localization inside the host tissues by confocal and image analyses techniques. Finally, the production of seeds containing 8R6-GFP was explored, to allow a simple and direct use of the endophyte.

5.3 = Volatile analysis of essential oils extracted by plant resins and preliminary study of their antimicrobial activity against *Agrobacterium tumefaciens* and *Pseudomonas savastanoi*

Stefania Caparrotta[1], Elettra Marone[2], Diego Comparini[1], Cosimo Taiti[1], Stefano Mancuso[1]
[1]DAGRI, Dipartimento di Scienze e Tecnologie Agrarie, Alimentari, Ambientali e Forestali, University of Florence, Viale delle idee 30, Sesto Fiorentino, 50019, Florence, Italy; [2]Faculty of Biosciences and Technologies for Agriculture, Food and Environment - University of Teramo, Via R. Balzarini, 1, 64100 Teramo, Italy

Plant secondary metabolites obtained by natural resins exudated from trees, in the last two decades have intensively investigated due to their pharmacological activities including anti-inflammatory, anti-carcinogenic and antiviral action (1,2). Antifungal activity of essential oils extracted from *Boswellia* plants as well as the antimicrobial activity against human pathogens as *Staphylococcus aureus, Escherichia coli* and *Pseudomonas aeruginosa* (3,4) have been tested. Although the medicinal-pharmaceutical proprierties of natural resin are well known, few studies deal with the action of these mixtures against plant pathogens. Recenlty, the insecticidal activity of *B. carterii* essential oil against insect pests of legume seeds (5) and the antimicrobial activity of *B. ovalifoliolata* against *Xanthomonas citri* (6) were reported. In this study, essential oils from eleven resins belonging to Burseraceae families (*Boswellia*, C*ommiphora*, *Canarium* and *Bursera* genus) and one to Fabaceae family (*Hymenea* genus) (Fig. 1A) have been extracted using a Clevenger-type hydrodistillation. Subsequently, the essential oil of each resin was recovered in an hermetic glass bottle and stored at 4°C. Then, each oil was used i) to perform the Volatile Organic Compound (VOC) profile using the Proton Transfer Reaction time-of-flight Mass Spectrometry (PTR-ToF-MS) instrument and ii) to test the antimicrobial activity *in vitro* against *Agrobacterium tumefaciens* and *Pseudomonas savastanoi* pv. savastanoi.

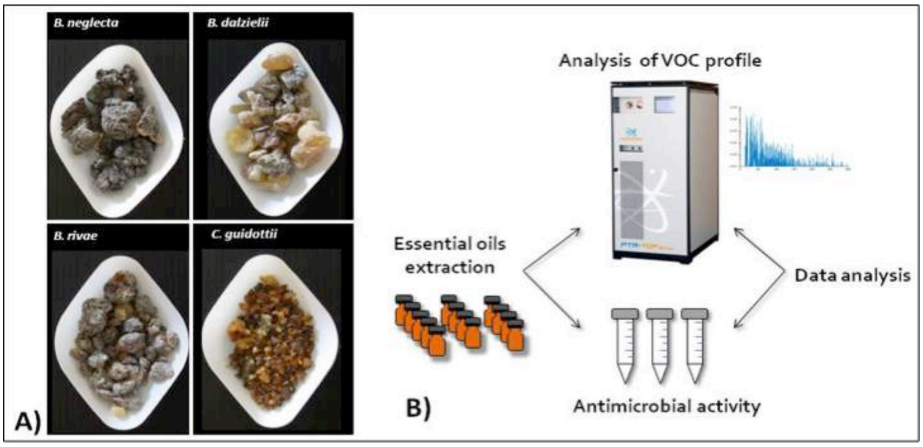


Fig. 1. A) Some resins as *Boswellia neglecta*, *B. dalzielii*, *B. rivae*, *Commiphora guidotti* used to extract essential oils by clevenger-type water hydrodistillation.
B) Schematic representation of the experimental phases followed in this work.

The volatile profile analysis shown that essential oils obtained by *Boswellia papyrifera*, *B. dalzielii*, *B. frereana* and *B. rivae* resins were the most abundant in term of signal intensity and number VOC detected. Interestingly, after 24h *B. papyrifera*, *B. frereana* and *B. rivae* essential oils decreased the bacterial growth of *A. tumefaciens* (the percentage of growth inhibition was respectively of 94.94%, 92.40% and 91.30% in comparison to control conditions) while the strong effect against *P. savastanoi* was observed by essential of *B. frereana* (66.67%), followed by *B. papyrifera* (42.13%), *B. neglecta* (33.64%), *Commiphora guidotti* (30.01%) and *Canarium madascariensis* (26.07%). The statistical analysis showed a positive correlation among terpenes and terpenoid compounds with the bacterial growth inhibition, suggesting the potential of these complex mixtures as new source of active compounds useful for plant disease treatment.

1) Bakkali F., Averbeck S., Averbeck D., Idaomar M. (2008) Food Chem. Toxicol., 46(2), 446-475
2) Qurishi Y., Hamid A., Zargar M.A., Singh S.K., Saxena A.K. (2010) J. Med. Plant Res., 4(25), 2778-2786
3) Camarda L., Dayton T., Di Stefano V., Pitonzo R., Schillaci D. (2007) Ann. Chim., 97(9), 837-844
4) Raja A.F., Ali F., Khan I.A., Shawl A.S., Arora D.S., Shah B.A., Taneja S.C. (2011) BMC microbiol., 11(1), 54
5) Kiran S., Kujur A., Patel L., Ramalakshmi K., Prakash B. (2017) Pestic. Biochem. Phys., 139, 17-23
6) Anitha G., Sudarsanam G. (2013) Int. J. Appl. Biol. Pharm. Technol., 4(1), 243-247

5.3 = A preliminary screening of fungal extracts and of metabolites derived from them, on the toxicity toward *Tetranychus urticae* (Acarinae)

Marco Clericuzio[a], Roman Pavela[b]

[a]DISIT - Università del Piemonte Orientale, Via Teresa Michel 11, 15121 Alessandria (Italy); [b]Crop Research Institute, Drnovska 507 - 16106 Prague 6, Ruzyne (Czech Republic). Supported by the Ministry of Agriculture of the Czech Republic, institutional support MZE-RO0418

One of the most important challenges, the fruiting bodies of higher mushrooms have to face in nature, is that with animal predators as insects or other arthropods, both in the larval state and in the adult. It is very likely that fungi have selected several secondary metabolites during natural evolution, in order to fight predators and parasites. However, little is known about this kind of interaction, for the thousands of fungal metabolites isolated so far. Here we present a preliminary screening of some crude extracts of Basidiomycota (directly obtained from basidiomes collected in the wild), and of some chromatographic fractions derived from them; also a few pure compounds could be tested in this assay. We found that the raw extract of *Cortinarius percomis* (Agaricales, Cortinariaceae) caused high mortality (100%) in adults of *Tetranychus urticae* and at the same time inhibited egg laying, showing a 100% ovicidal effect at a dose of 100 $\mu g.cm^{-2}$. From this extract we could isolate phlegmacin-8’-methyl ether (Fig. 1), the main yellow-orange pigment[1] of the fungus. **1** showed toxicity data very close to those of the crude extract, and therefore can be considered a major contributor in the anti-arthropod activity of *C. percomis*.

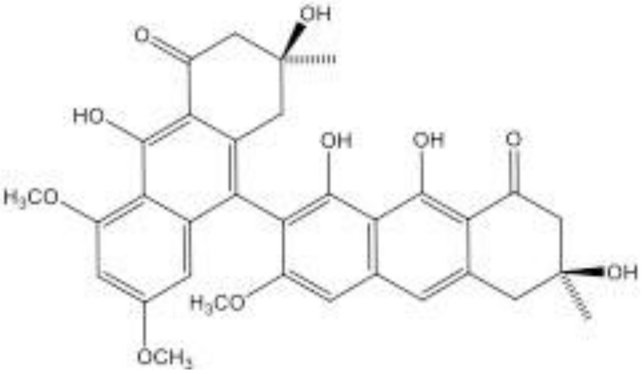


Fig. 1.

Also the raw extract of *Tricholoma pardinum* (Agaricales, Tricholomataceae) was very effective, causing 83.3 and 100% mortality of *T. urticae* adults, 24 and 96 hours after the application 100 $\mu g.cm^{-2}$ dose, respectively. A third effective fungal sample was the lipophilic sub-extract of *Russula olivacea* (Russulales, Russulaceae), which showed significant ovicidal effects in our tests. The metabolites responsible of the toxicity of these latter two extracts are still under investigation.

1) W. Steglich et al. (1973) Neue Phlegmacine-Derivate aus *Cortinarius percomis* (Agaricales), Z. Naturforsch, 28c, 354-355

5.3 = Polyketide synthases in the ericoid endomycorrhizal fungus *Oidiodendron maius*

Elena Martino[1], Stefania Daghino[1], Simone Belmondo[1], Domitilla Meloni[1], Shingo Miyauchi[2], Sabrina Collin[3], Annegret Kohler[2], Christophe Jacob[3], Silvia Perotto[1]

[1]Department of Life Sciences and Systems Biology, University of Turin, v.le Mattioli 25, 10125 Turin, Italy; [2]INRA, UMR INRA-Université de Lorraine 'Interactions Arbres/Microorganismes', Laboratoire d'Excellence ARBRE, INRA-Nancy, 54280 Champenoux, France; [3]UMR 7365, Ingénierie Moléculaire et Physiopathologie Articulaire (IMoPA), CNRS-Université de Lorraine, Biopôle de l'Université de Lorraine, Campus Biologie Santé, 9 Avenue de la Forêt de Haye, BP 20199, 54505, Vandœuvre-lès-Nancy Cedex, France

Fungi produce a wide variety of biologically active secondary metabolites, a large proportion of them being polyketides, a group of compounds characterized by a great complexity and structural diversity. Polyketides are produced by polyketide synthases (PKSs), very large multifunctional iterative enzymes that operate as "assembly lines" to bind together basic units of acyl-coenzyme A. Several functions have been attributed to fungal polyketides. Most of them play a role in the ecological and evolutionary adaptation of fungi (Fujii, 2010), but they are involved in plant-fungal interactions in some phytopathogenic fungi.

Potential roles of polyketides have not been investigated so far in mycorrhizal fungi, although a recent investigation of the genomic features of 60 mycorrhizal fungi with different taxonomy and ecology (Martino et al., 2018) has revealed in *Oidiodendron maius*, an ericoid endomycorrhizal fungus, the highest number of genes coding for PKSs. Transcriptomic data indicate that some *O. maius* PKSs encoding genes are upregulated in symbiosis.

We are currently investigating the potential role of PKSs in *O. maius* following different approaches that involve: i) the generation of an inventory of the different types of PKSs in the genome of *O. maius* and of other taxonomically diverse ericoid mycorrhizal fungi, ii) a comparison of the protein sequences of *O. maius* PKSs with functionally characterized enzymes identified in other fungal species, iii) the generation of mutants lacking PKSs genes highly expressed in symbiosis, in order to analyze their mycorrhizal phenotype, and iv) the heterologous expression of these *O. maius* PKSs in yeast, in order to identify the polyketide produced.

As *O. maius* is one of the very few genetically tractable mycorrhizal fungi, it represents an interesting model system to investigate with a molecular approach the role of polyketides in the mycorrhizal symbiosis. No data are currently available on the polyketides produced by this symbiotic fungus, and our investigation should provide a better picture of its genetic potential in the polyketide biosynthetic pathway, as well as to the characterization of some of these compounds and potential role in symbiosis.

1) Fujii I. (2010) Functional analysis of fungal polyketide biosynthesis genes. The Journal of Antibiotics, 63, 207–218
2) Martino E., et al. (2018) Comparative genomics and transcriptomics depict ericoid mycorrhizal fungi as versatile saprotrophs and plant mutualists. New Phytologist, 217, 1213–1229

5.3 = Comparative investigation on *Anthemis tinctoria* var. *pallida* and *A. cretica* subsp. *tenuiloba* by pharmacological and phytochemical approaches

Luigi Menghini, Maurizio Ronci, Lucia Recinella, Giustino Orlando, Annalisa Chiavaroli, Sheila Leone, Matteo Politi, Simonetta Di Simone, Maria Chiara Ciferri, Pamaela Taddei, Claudia Luffarelli, Luigi Brunetti, Claudio Ferrante
Department of Pharmacy, University “G. d’Annunzio” di Chieti, Via dei Vestini 31, 66100 Chieti, Italy

Anthemis L. is the second largest genus in the family Asteraceae, comprising more than 210 species, which are distributed in western Eurasia, the Mediterranean Area and small part of eastern Africa (1). The species of the *Anthemis* genus are known to possess various biological properties and have broad use in pharmaceutics, cosmetics and food chemistry. The flowers of the *Anthemis* species are well documented for their usage as antiseptic and healing herbs, with flavonoids and essential oils being their main active components (2). The ethyl acetate, methanolic and aqueous extracts of aerial the parts of *Anthemis tinctoria* var. *pallida* (ATP) and *A. cretica* subsp. *tenuiloba* (ACT) were investigated for their phenol and flavonoid content and their antioxidant and key enzyme inhibitory potential. The extracts showed strong antioxidant abilities, with the MeOH and aqueous extracts being a superior source of antioxidant compounds. For both species, only the EtOAc and MeOH extracts were potent against AChE and BChE (only EtOAc extract for ATP). The extracts showed remarkable enzyme inhibitory effects against tyrosinase and α-glucosidase, as well. We also studied *Anthemis* extracts in an experimental model of cortical spreading depression (CSD) (3). We assayed the influence of extracts on oxidative stress and neurotransmission biomarkers, including lactate dehydrogenase (LDH) and serotonin (5-HT), in isolated rat cortex challenged with K+ 60 mM Krebs-Ringer buffer (excitotoxicity stimulus). An untargeted proteomic profile was finally performed, in order to explore the putative mechanism. The pharmacological study highlighted the capability of A. *cretica* water extract to blunt K+ 60 mM increase in LDH activity and 5-HT turnover, and restore physiological activity of specific proteins involved in neuron morphology and neurotransmission, including NEFMs, VAMP-2 and PKCγ, thus further supporting the neuroprotective role of A. *cretica* water extract.

Fig. 1. *Artemia salina* cysts for brine shrimp test

Fig. 2. Allelopathy assays on 3 varieties of lettuce

1) J.D. Staneva, M.N. Todorova, L.N. Evstatieva (2008) Phytochemistry, 69, 607-618
2) O. Kilic, A. Kocak, E. Bagci (2011) Zeitschrift für Naturforschung C, 66, 535-540
3) L. Menghini, L. Leporini, G. Vecchiotti, M. Locatelli, S. Carradori, C. Ferrante, G. Zengin, L. Recinella, A. Chiavaroli, S. Leone (2018) Food research international, 109, 91-98

5.3 = Treatments influence plant growth and microbiota in soils affected by apple replant disease

Lidia Nicola[1,2,4], Elena Turco[1], Martin Thalheimer [2], Heribert Insam[3], Ilaria Pertot[1,5]
[1]Research and Innovation Centre, Fondazione Edmund Mach, Via E. Mach 1, 38010 San Michele all'Adige (TN), Italy; [2]Department of Earth and Environmental Sciences, University of Pavia, via Sant'Epifanio 14, 27100 Pavia, Italy; [3]Laimburg Research Centre for Agriculture and Forestry, Laimburg 6, I-39040 Post Auer (BZ), Italy; [4]Department of Microbiology, University of Innsbruck, Technikerstrasse 25d, A-6020 Innsbruck, Austria; [5]Center Agriculture Food Environment, University of Trento, Via E. Mach 1, 38010 San Michele all'Adige (TN), Italy

Apple Replant Disease (ARD) is a key problem in apple production, occurring when apple trees are replanted in the same soil, but its aetiology is still uncertain. This is why different treatments are applied in field to reduce ARD symptoms with varying degrees of success. The aim of this work was to monitor the effects of specific soil treatments (fumigation, soil washing, addition of a fungal biocontrol agent and of compost) on M9 rooted cuttings and on the microbiota in ARD-affected soil and the rhizosphere, using metabarcoding. The soil used was sampled along the rows of an apple orchard in northern Italy, which had been continuously cultivated with apple trees for several decades and was affected by ARD. The soil was sieved and divided into lots for the different treatments. The first lot was fumigated with dazomet, the second one was washed with half of its volume in water every day for ten days and left to dry; the third one was amended with conidia of *Trichoderma atroviride* SC1 (Vintec; Belchim Crop Protection); the fourth one was amended with 1:5 (v:v) of organic compost, while the remaining lots (fifth and sixth) were left untreated. The soil lots were placed in pots and a rooted cutting of M9 rootstock was planted in each pot. The pots were kept at 20 °C in a greenhouse for three months, at the end of which soil and rhizosphere sampling took place. Total DNA was extracted and the fungal ITS and bacterial 16S regions were pyrosequenced with the Roche's 454 Platform. The experiment was repeated twice in the same period of two following years. Addition of compost or of a biocontrol agent (*Trichoderma atroviride* SC1) did not reduce ARD symptoms in either year, but changed the microbial communities in bulk soil. Soil washing and fumigation restored plant growth, however with a different degree of success in the two years. The microbial communities in fumigated soils were significantly different from those in the untreated control in both years, while soil washing did not modify them. A number of OTUs known to promote plant growth (*Pseudoxanthomonas* sp., *Sphingomonas* sp., *Sphingobium* sp., *Sphingopyxis* sp., *Nocardioides* sp.) were found to be positively correlated with shoot growth. In conclusion, the soil treatments modified the microbial communities, but a positive effect on ARD symptoms was detected only with fumigation and soil washing, with different success in the two years. These results indicate that although microorganisms have a role in ARD development, there could be other contributing factors in the complex ecology of soil, as supported in the multivariate ecological ARD hypothesis (1).

1) L. Nicola, H. Insam, I. Pertot, B. Stres (2018) Appl. Soil Ecol., 129, 24-33

5.3 = Endophytes in *Echinacea* Moench (Asteraceae) change their activity after seed germination

Alessio Papini[*,1], Marian Viola[1], Elisangela Miceli[2], Anna Maria Puglia, Teresa Faddetta, Valentina Maggini[°,§], Corrado Tani[1], Maria Antonietta Viscillo[1], Fabio Firenzuoli[°], Silvia Schiff[1], Patrizia Bogani[2], Alessio Mengoni[1], Renato Fani[*,2], Massimiliano Cardinale[3]
[1]Laboratory of Plant Biology, Department of Biology, University of Florence, Via Madonna del Piano 6, 50019 Sesto Fiorentino, Italy, [§]Department of Experimental and Clinical Medicine, University of Florence, Largo Brambilla 3, 50134 Florence, Italy; [°]Referring Center for Phytotherapy, Tuscany Region, Careggi University Hospital, Largo Brambilla 3, 50134 Florence, Italy; [2]Laboratory of Microbial and Molecular evolution, Department of Biology, University of Florence, Via Madonna del Piano 6, 50019 Sesto Fiorentino, Italy; [2]Laboratory of Plant Genetics, Department of Biology, University of Florence, Via Madonna del Piano 6, 50019 Sesto Fiorentino, Italy; [3]Department of Biological and Environmental Sciences and Technologies, University of Salento. *Corresponding authors alpapini@unifi.it; renato.fani@unifi.it

Echinacea Moench is a north American genus of tribe Heliantheae (Asteraceae). This tribe is characterized by a modified achene, named cypsela, and, within it, by an internal phytomelanin layer. Despite its north American origin, *E. purpurea* (L.) Moench is widely cultivated worldwide for its pharmaceutical properties. The cypselas of *E. purpurea* were examined in order to investigate the presence and localization of endophytic microorganisms in the embryo. The presence of endocellular bacteria was observed in the parenchyma of the cotyledons, enclosed in a membrane structure similarly to the situation observed for other endocellular bacteria such as mycobacteria. The endophytic bacteria in *Echinacea* were enclosed within the host plasma membrane, with only a narrow space between this last and the bacterial membrane. No clear evidence of the bacterial wall was observed, as in other endocellular bacteria, but the shape of the bacterium was maintained, suggesting that a bacterial wall might be present. Also fungi were recorded in the perianth, the most external component of the cypsela. The presence of fungi in the cypsela was apparently arrested at the level of the pericarp, containing phytomelanin.

Three different components of microorganisms accompany the embryos before their dispersal: i) one endocellular bacterial component in the cotyledons; ii) another more generic bacterial component adhering to the external side of the perianth, and a iii) fungal component contained inside the porous layer of the perianth, whose remarkable structure may be considered as an adaptation for fungal transportation.

The observation of the seedling leaves after seed germination, showed that the bacteria had moved in a space between the plasma membrane and the wall of the parenchyma beneath the epidermis.

In *E. purpurea* the presence of endophytes has recently been related to the increase of alkilamides content and to the higher expression level of the valine decarboxylase (*VDC*) gene (Maggini et al. 2017), involved in the biosynthesis of the amine moieties of alkylamides.

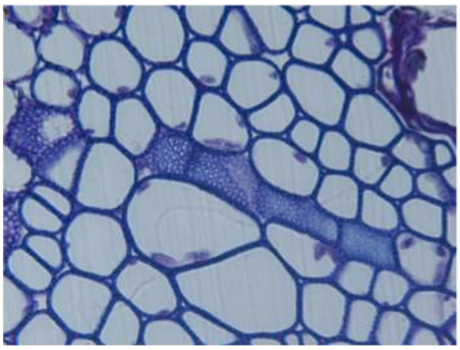


Fig. 1. Fungi in the pericarp

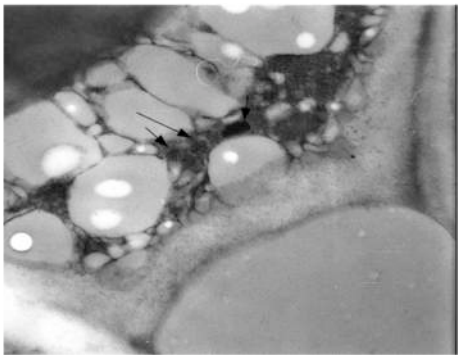


Fig. 2. Endophytic bacteria (arrows) in the cotyledon

1) V. Maggini, M. De Leo, A. Mengoni et al. (2017) Plant-endophytes interaction influences the secondary metabolism in *Echinacea purpurea* (L.) Moench: an in vitro model. Sci Rep 7, 16924

5.3 = Allelopathic effects of aqueous extracts from old durum wheat (*Triticum durum* Desf.) genotypes on seed germination of *Portulaca oleracea* L. and *Stellaria media* (L.) Vill.

Alessia Restuccia *, Aurelio Scavo, Paolo Caruso, Paolo Guarnaccia, Giovanni Mauromicale
Department of Agriculture, Food and Environment (Di3A), University of Catania, Via Valdisavoia 5, 95123 Catania, Italy; *Corresponding author: a.restuccia@unict.it

Weeds represent the most important pest in agroecosystems, causing quantitative and qualitative damages on the agricultural production. In the last years, the utilization of allelopathic mechanisms, such as the isolation of allelochemicals for the production of bioherbicides (1), offered a new important tool for a chemical-free weed management (2).

The objective of this study was to evaluate the allelopathic effects of aqueous extracts (50 and 100%) obtained from *Triticum durum* Desf. roots, stems and ears on seed germination and mean germination time of *Portulaca oleracea* L. and *Stellaria media* (L.) Vill. In particular, the allelopathic potential of three *T. durum* ancient varieties ('Timilia', 'Russello' and 'Farro lungo') with a modern one ('Mongibello') were tested, compared with distilled water as control.

Overall, *T. durum* extracts reduced the germination percentage by 36%. The allelopathic activity was plant part-, species- and concentration-dependent (Fig. 1). *S. media* was more sensitive to *T. durum* aqueous extract than *P. oleracea*, and the inhibitory activity increased with increasing concentration. Moreover, ear extracts were the most efficient (−41% of seed germination), followed by stem (−35%) and root ones (−32%). 'Timilia' and 'Mongibello' showed the highest allelopathic potential. Nevertheless, all extracts significantly increased the mean germination time by 2.1 days with respect to control.

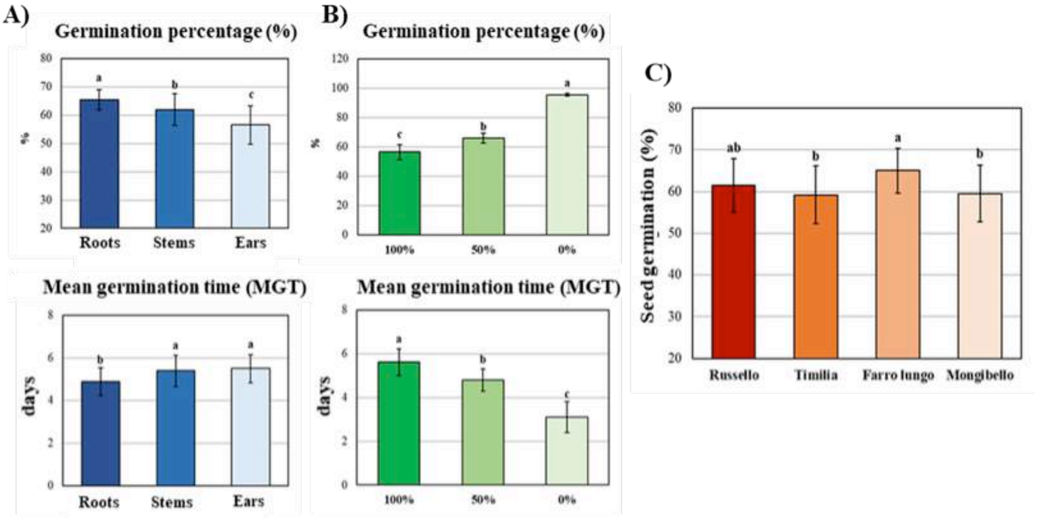


Fig. 1. Effect of plant organ (A), extract concentration (B) and genotype (C) on the allelopathic activity of *T. durum* aqueous extracts. Values are expressed as mean of the target species. Different letters indicate significant for $p \leq 0.05$. Each bar means ± standard error

These results represent a first step in the understanding of the allelopathic activity of old *T. durum* genotypes and are very promising for the future development of a chemical-free weed management based on the utilization of living or dead mulch, bioherbicides and crop rotation under an Integrated Weed management System (IWMS).

1) Scavo A., Restuccia A., Pandino G., Onofri A., Mauromicale G. (2018) Allelopathic effects of *Cynara cardunculus* L. leaf aqueous extracts on seed germination of some Mediterranean weed species. Italian Journal of Agronomy, DOI: 10.4081/ija.2018.1021

2) Scavo A., Restuccia A., Mauromicale G. (2018) Allelopathy: Principles and Basic Aspects for Agroecosystem Control. In: Gaba S., Smith B., Lichtfouse E. (eds) Sustainable Agriculture Reviews 28. Sustainable Agriculture Reviews, vol 28. Chapter 2: 47-101. Springer, Cham. DOI https://doi.org/10.1007/978-3-319-90309-5_2

5.3 = Biological activities of methanol extract from *Sclerocarya birrea* (A. Rich.) Hochst. leaves

Daniela Russo[a], Rocchina Miglionico[a], Monica Carmosino[a], Faustino Bisaccia[a], Immacolata Faraone[a], Chiara Sinisgalli[a], Maria Francesca Armentano[a], Luigi Milella[a]
[a]Dipartimento di Scienze, Università degli Studi della Basilicata, viale dell'Ateneo lucano, 10, 85100 Potenza, Italy

Plants produce an enormous variety of natural products with diverse chemical structures. Natural products in plants are involved in diverse functions, providing protection against pests, diseases, ultraviolet-B damage and other environmental stresses. In the last years, they were investigated for their biological activities (1, 2) and the development of modern technology allowed more and more plant extracts to be useful to medical practice.

Sclerocarya birrea (A. Rich.) Hochst, more commonly known as marula, belongs to the Anacardiaceae family. It is an indigenous, fruit-bearing tree native of sub-Saharan Africa. Its fruits are eaten or processed to make beer or jam, while kernels are eaten or used to extract oils, and leaves are used as forage for livestock (3). Bark, leaves, and roots of *S. birrea* have attracted attention because they are traditionally used to treat several human ailments such as dysentery, fevers, malaria, diarrhoea, stomach ailments, rheumatism, sore eyes, gangrenous rectitis, infertility, headaches, toothache, and body pains, while the methanol extract of *S. birrea* root and bark showed antioxidant and proapoptotic activity (3, 4).

In this study the LC-MS analysis, total content of polyphenols, flavonoids, and tannins of the methanol extract from *S. birrea* leaves (MLE) as well as the *in vitro* biological activity. The antioxidant activity was measured using four different *in vitro* assays to measure the inhibition of lipid peroxidation β-carotene bleaching (BCB), radical scavenging activity (2,2-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid)), (ABTS), superoxide anion radical (SO), and nitric oxide (NO) (3).

MLE showed high content of flavonoids (132.7 ± 10.4 mg of quercetin equivalents/g). The extract reported dose-dependent antioxidant efficacy comparable with used standards (Fig. 1). MLE reported no cytotoxic effect on fibroblast cell line showing an $IC_{50} > 400$ µg/mL.

The antioxidant activity can be attributed to the chemical profile of leaf extract. LC-MS analysis showed the presence of several flavonoids, mainly quercetin, kaempferol and their derivatives (5). The dietary intake of flavonoids has beneficial health effects due to their antioxidant and radical-scavenging properties.

Our findings allow us to hypothesize a future use of leaf extract from *Sclerocarya birrea* in nutraceutical, pharmaceutical and cosmeceutical fields. Further studies will be directed to the characterization of unknown compounds and to confirm its activity in preclinical models.

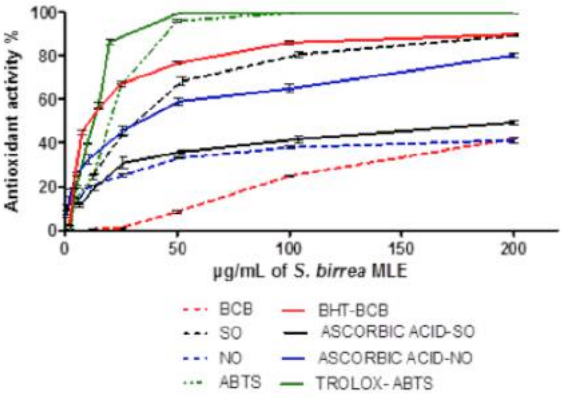


Fig. 1. Antioxidant activity of methanol extract from *Sclerocarya birrea* leaves

1) Milella, L. (2016) α-Glucosidase and α-Amylase Inhibitors from Arcytophyllum thymifolium. J. Nat. Prod. 79:2104–2112

2) Lamorte, D., (2018) Future in the Past: Azorella glabra Wedd. as a Source of New Natural Compounds with Antiproliferative and Cytotoxic Activity on Multiple Myeloma Cells. IJMS, 19(11), 3348

3) Russo, D. (2013) Profiling of Phytochemicals in Tissues from Sclerocarya birrea by HPLC-MS and Their Link with Antioxidant Activity. ISRN Chromatography (Article ID 283462)

4) Armentano, M.F. (2015) Antioxidant and proapoptotic activities of Sclerocarya birrea [(A. Rich.) Hochst.] methanolic root extract on the hepatocellular carcinoma cell line HepG2. BioMed Res. Int. 2015

5) Russo, D. (2018) A comparative study on phytochemical profiles and biological activities of Sclerocarya birrea (A. Rich.) Hochst leaf and bark extracts. IJMS, 19(1), 186

5.3 = *In silico* characterization of fungal small RNA reveals putative plant mRNA targets in the symbiosis between an arbuscular mycorrhizal fungus and its host plant

Alessandro Silvestri[1], Valentina Fiorilli[1], Laura Miozzi[2], Gian Paolo Accotto[2], Massimo Turina[2], Hailing Jin[3], Luisa Lanfranco[1]
[1]Department of Life Sciences and Systems Biology, University of Torino, Viale P.A. Mattioli 25, 10125 Torino, Italy; [2]Institute for Sustainable Plant Protection, CNR, Strade delle Cacce 73, 10131 Torino, Italy; [3]Department of Microbiology and Plant Pathology, Center for Plant Cell Biology, Institute for Integrative Genome Biology, University of California, Riverside, CA 92521, USA

Small RNAs (sRNAs) are short non-coding RNA molecules that regulate gene expression at transcriptional or post-transcriptional levels through a mechanism known as RNA interference (RNAi). Recent studies have highlighted that they are also involved in cross-kingdom communication: sRNAs can move across the contact surfaces from “donor” to “receiver” organisms where they can target specific mRNAs, leading to a modulation of host metabolic pathways and defense responses (1). Very little is known about RNAi mechanism and sRNAs occurrence in Arbuscular Mycorrhizal Fungi (AMF), an important component of the plant root microbiota (2).

Taking advantage of the available genomic resources for the AMF *Rhizophagus irregularis* we described its putative RNAi machinery, which is characterized by a single *Dicer*-like (*DCL*) gene and an unusual expansion of *Argonaute*-like (*AGO*-like) and *RNA-dependent RNA polymerase* (*RdRp*) gene families. Focusing on the symbiosis between *R. irregularis* and the model plant *Medicago truncatula*, we characterized the fungal sRNAs population, highlighting the occurrence of an active sRNA-generating pathway and the presence of microRNA-like sequences. *In silico* analyses revealed that several fungal sRNAs have the potential to target *M. truncatula* transcripts (3). Co-expression experiments in *Nicotiana benthamiana* leaves showed that a fungal sRNA could suppress, in a sequence-specific manner, the accumulation of the protein encoded by the putative plant target gene.

The identification of RNAi-related genes, together with the characterization of the sRNAs population, suggest that *R. irregularis* is equipped with a functional sRNA-generating pathway. Moreover, the *in silico* analysis predicted several plant transcripts as putative targets of specific fungal sRNAs suggesting that cross-kingdom post-transcriptional gene silencing may occur during AMF colonization.

1) M. Wang, A. Weiberg, F-M. Lin, B.P.H.J. Thomma, H-D Huang, H. Jin (2016) Nature Plants, 2, 16151
2) L. Lanfranco, V. Fiorilli, C. Gutjahr (2018) New Phytologist, 220(4), 1031-1046
3) A. Silvestri, V. Fiorilli, L. Miozzi, G.P. Accotto, M. Turina, L. Lanfranco (2019) BMC Genomics, 20, 169

6.1 = Towards the (re)discovery of Italian popcorns (*Zea mays* L. subsp. *mays* Everta Group): a conservation and cultural mission by the University of Pavia Germplasm Bank and Botanical Garden

Nicola M. G. Ardenghi[1], Marco Canella[1], Paolo Cauzzi[2], Graziano Rossi[1]
[1]Department of Earth and Environmental Sciences, University of Pavia, Via S. Epifanio 14, 27100 Pavia, Italy; [2]Botanical Garden, University Museum System, University of Pavia, Via S. Epifanio 14, 27100 Pavia, Italy

Popcorn is one of the most popular and distinctive snack foods in the world. It derives from the explosion of the caryopses (“kernels”) of maize (*Zea mays* L. subsp. *mays*) upon exposure to heat and the subsequent expansion of starch to form “flakes” (1). This trait, known as “popping expansion” (1), can be found mainly in a single group of maize culta (Everta Group), distinguished for a specific set of botanical characters: presence of tillers; multiple and small catoclesia (“ears”) per culm; caryopses minute with highly corny (“flint”) endosperm. The Everta Group is ancient and basal in the domestication history of maize, as recently revealed by the strict phylogentic relationship between some Mexican popcorn culta (e.g., ‘Palomero Toluqueño’) and teosinte taxa [e.g., *Z. mays* subsp. *mexicana* (Schrad.) Iltis] (2, 3). It was widley employed in the Americas already in Pre-Columbian times (3) and currently is cultivated on large scale in Argentina, United States, France, South Africa, and Hungary (4).

Popcorns were among the earliest maize groups to land and spread in Italy in the 16th century (5), however, in the course of centuries, due to their small-scale production and their genetic isolation mechanisms (which prevented crossings with other groups), remained confined to familiar gardens, mostly as a horticultural crop to serve as comfort food (5, 6). Although some of them probably contributed to the genesis of important flint landraces and cultivars (such as Rostrata and Microsperma Groups: 5, 7), popcorns did not play any role in the Italian breeding programs of the 20th century and thus they do not appear in the major literature sources of that time. The only available data regarding Italian traditional popcorns come from A. Brandolini (5), who mentions some accessions (no more conserved in his institution, CREA MAC in Bergamo) from an incomplete census conducted mainly in northern Italy during the 1950s.

On the basis of this scenario, we aimed to continue the research through ethnobotanical surveys across the whole country, in order to compile a comprehensive and updated inventory of the Italian traditional popcorns, their uses and their history, besides conserving those still surviving on-farm.

Between 2017 and 2019, we conducted interviews with different farmers; for the purpose of data collection a Facebook page was also created (https://www.facebook.com/mais.popcorn.italiani/). 15 seed collections were acquired from six different Italian regions (Piemonte, Lombardia, Veneto, Trentino-Alto Adige, Emilia-Romagna, Campania), now stored at the University of Pavia Germplasm Bank, currently the only germplasm bank preserving Italian traditional popcorns. They belong to the two main types of popcorn (“rice”, with pointed kernels; and “pearl”, with rounded kernels) mentioned by (5). Within these two categories, a wide range of variability has been observed, especially regarding the color of the pericarp, and the length and the shape of the ears. Yet, further characters (both morphological and phenological), for a preliminary classification of the germplasm material, need to be evaluated on complete individuals that are being cultivated at the Pavia Botanical Garden. In addition, we collected 26 oral testimonies from nine regions (from Piemonte southward to Calabria), evidencing a wide distribution of local popcorn culta in the past, handed from generation to generation at least since the late 19th century. On linguistic grounds, more than a dozen of different dialect names were recorded, revealing how this crop, despite having been poorly documented, had been incorporated in the Italian rural culture in the last five centuries.

Although the present data are preliminary, the quantity of oral testimonies not supported by seed samples (higher than the number of the acquired seed accessions) reveals the relict character of this crop in Italy, that has undoubtedly been affected from the late 1940s by the spread of commercial cultivars and the growth of the popcorn industry (1, 8). Since the extinction risk appears remarkable, location and collection of germplasm, together with scientific divulgation, are urgently needed.

1) K.E. Ziegler (2001) In: Hallauer, A.R. (ed.), Speciality corns. 2nd ed. Boca Raton, CRC Press, 199–234
2) Y. Matsuoka et al. (2002) Proc. Natl. Acad. Sci, U.S.A, 99(9), 6080−6084
3) A. Santacruz-Varela et al. (2004) Crop Sci, 44, 1456-1467
4) G. Meleán (2013) http://ithappenedinlatinamerica.blogspot.com/2013/05/the-business-of-popcorn-maize.html.
5) A. Brandolini, A. Brandolini (2006) Il Mais in Italia. Storia naturale e Agricola. Bergamo, Crf Bergamo
6) A. Brandolini, A. Brandolini (2001) Pl. Genet. Resources Newslett, 126, 1-11
7) N.M.G. Ardenghi et al. (2018) PeerJ 6:e5123
8) A. Brandolini, E. Lucconi (1979) Italia Agricola, 116(4), 256–261

6.1 = Fascination of Plants Day 2019

Valeria Bianciotto[1], Luisa Lanfranco[2]
[1]Istituto per la Protezione Sostenibile delle Piante (IPSP) Sede di Torino – CNR; [2]Dipartimento di Scienze della Vita e Biologia dei Sistemi, Università degli Studi di Torino, Viale Mattioli 25, 10125 Torino, Italy

On 18 May 2019, the International Fascination of Plants Day (FoPD) was organized for the fifth time by plant scientists across the world, under the umbrella of the European Plant Science Organization (EPSO). Since the first edition in 2012, the mission of this activity has been to get as many people as possible around the world fascinated by plants and enthused over the importance of plant science for agriculture, in sustainability producing food, as well as for horticulture, forestry, and all of the non-food products such as paper, timber, chemicals, energy, and pharmaceuticals.

In 2019, during the Fifth International Fascination of Plants Day, over 850 events in 52 countries of five continents were organised by scientific institutions, universities, botanical gardens, and museums, together with farmers and companies.

In Italy the event has been a big success. In more than 70 Italian cities, from North to South, scientific institutions and privates opened their doors, for the whole month of May 2019, with a variety of interactive plant-based events for all the family and insiders. Over 170 events - conferences, workshops, shows, exhibitions, visits guided tours and walks - were organized to present the beauty of plants and the most recent discoveries in plant biology research. The complete program of the Italian FOPD 2019 is available at www.plantday.it and in this poster we will present some of these fascinating events organized in marvelous frames as botanical gardens, private villas or museum town such as Matera (European Capital of Culture 2019).

6.1 = Valmalenco (SO): new Botanic Gardens for science and people

Martina Bottoni[1,2], Claudia Giuliani[1,2], Lorenzo Colombo[1,2], Fabrizia Milani[1,2], Paola Sira Colombo[1,2], Patrizia Berera[3], Piero Bruschi[4], Gelsomina Fico[1,2]
[1]Department of Pharmaceutical Sciences, University of Milan, Via Mangiagalli 25, 20133 Milan, Italy; [2] Ghirardi Botanic Garden, Department of Pharmaceutical Sciences, University of Milan, Via Religione 25, 25088 Toscolano Maderno, Brescia, Italy; [3]Rete degli Orti Botanici della Lombardia, Piazza Matteotti 27, 24122 Bergamo, Italy; [4]Department of Agricultural, Environmental, Food end Forestry Science and Technology, University of Florence, Piazzale delle Cascine 28, 50144 Florence, Italy

The triennial project Interreg B-ICE Italy-Switzerland *Bernina Terra Glacialis. Study and exploitation of a precious natural and cultural heritage in an open Alpine region, with innovative approaches to the future* started in December 2018. The project is aimed to create a management model for the ongoing climate change. Within this context, we are performing the census of the autochtonous flora and an ethnobotanical investigation aimed at the study and enhancement of the plant and cultural biodiversity in Valmalenco (SO), in particular in the municipal territories of Chiesa in Valmalenco, Caspoggio, Lanzada, Spriana e Torre di Santa Maria.

The following phases of the project concern the planning and the realization of Botanic Gardens of different types for the preservation of species of interest, selected on the basis of their traditional and potential uses, ancient crops or species threatened by the actual climate change. In detail:

(i) Didactic Botanic Garden at Caspoggio, near the Mountain Centre Zenith. Educational paths and guided tours aimed at children will be developed by means of communication tools based on the game and also on the scientific deepening through innovative iconographic and caption devices. It is planned to create *ad hoc* botanic labels and digital sheets that will be accessible with tablets and electronic frames. The data provided will include pictures of the plants, information on their morphological and structural complexity, as well as on the traditional uses, recipes or anecdotes related to their uses. The Garden will become a reference point and a focal junction of tourist-ricreative and sports routes, in order to implement the attractiveness of the Valley.

(ii) High-rise Botanic Garden in Sant'Antonio di Caspoggio, near the chair lift. The communicative actions are going to be developed through a dual approach: iconographic devices devoted to the scientific research (floristic and chorological survey of native species, ethnobotanic survey, bibliographic survey on the biological activity) and exhibition panels on topics related to global warming. Several categories of visitors will be involved with a fruition of the contents at multiple levels of technological expertise, from reading (*ad hoc* botanic labels) to the use of tablets.

In both the Gardens we are going to realize: *(a)* caption devices and panels with trilingual description (Italian/English/German), in order to welcome an audience that becomes mainly touristic during spring and summer; *(b)* training courses devoted to educators with botanical/herbal and communication expertise.

Finally, among the species of interest, we are going to select target species in order to activate their cultivation and to support the creation of shared Gardens in residential areas and individual homes.

As a whole, these activities will enhance the knowledge about the importance of the plant biodiversity conservation, also in view of the actual scenario of climate change, and on the value of the cultural heritage connected to the plant traditional uses and their sustainable exploitation.

At the end of the project the Botanic Gardens will become repositories of the culture of the territory and places of transmission of the intergenerational knowledge, accessible to everybody. This will allow the local communities to acquire a wider awareness of the cultural heritage of the Valley, generating new perspectives for the involvement of the local people and positive effects on the tourist attractiveness.

6.1 = "Peppers of the World" at the Botanical Garden of the University of Pavia

Paolo Cauzzi[1], Francesco Perez[2], Matteo Manidi[1], Francesco Bracco[1,3], Nicola M.G. Ardenghi[3]
[1]Botanical Garden, University Museum System, University of Pavia, via S. Epifanio 14, 27100 Pavia, Italy; [2]Associazione Amici dell'Orto Botanico, via S. Epifanio 14, 27100 Pavia, Italy; [3]Department of Earth and Environmental Sciences, University of Pavia, via S. Epifanio 14, 27100 Pavia, Italy

Capsicum L. (Solanaceae), a small genus of less than 40 species native to the Americas (1), is one of the most economically important crops in the world, cultivated across the tropical and temperate regions mainly as a spice and vegetable, both hot (under the vernacular names "ají", "chile", "chili", "chilli", "peperoncino", "pimiento") and sweet ("bell pepper", "paprika", "peperone"), but also for ornamental, medicinal and even defense purposes (2, 3); currently, China and India are the world's largest producers of fresh and dried peppers respectively (4). In the course of millennia, five species of *Capsicum* were domesticated independently in various areas of the Americas: *C. annuum* L., *C. baccatum* L., *C. chinense* Jacq., *C. frutescens* L., and *C. pubescens* Ruiz & Pav. (3). *Capsicum* domesticates are interested by an extreme variability in fruit morphology and piquancy, that led botanists, in the course of centuries, to adopt various and conflicting classification schemes (5). Culta of the five domesticated species are currently recognized within well-delimited cultivar groups, colloquially known as "pod types", that are defined on the basis of berry morphology, piquancy, flavour, and culinary uses (2, 5, 6).

At the Botanical Garden of the University of Pavia (where *Capsicum* was cultivated already in the 18th century), a new collection of chile peppers was established in 2018, after Francesco Perez, oil geologist, former Eni employee, and hot peppers enthusiast, donated, in 2005, 79 seed samples to the University of Pavia Germplasm Bank. Perez had acquired the seeds through his travels around the world and from his colleagues involved in international missions, hence the name of the collection: "*Peperoncini dal Mondo*" ("Peppers of the World"). Each seed accession was numbered and stored within a specific electronic database, together with associated information such as vernacular name, geographical provenance, and name of the donor; data regarding the taxonomic and cultonomic classification are progressively added upon identification of the propagated individuals at flowering and fruiting stages. In 2018, 29 seed accessions, pertaining to two species (*C. annuum* and *C. chinense*) and 10 pod types, were grown at the Botanical Garden and exhibited to the public, receiving an instant success, which led to further spontaneous donations and a sensible englargement of the collection itself (5). Nowadays, "*Peperoncini dal Mondo*" includes 345 seed accessions from 51 countries, donated by 67 different donors. Between March and May 2019, 72 accessions, coming from four continents (Africa, America, Asia, Europe) and probably pertaining to at least four species (taxonomic identification will be carried out from the late summer on) were grown, expecting to be exhibited in the next September.

On the basis of the results achieved in 2018, the role of "*Peperoncini dal Mondo*" at the Botanical Garden of the University of Pavia extends far beyond the limits of the ornamental collection or the "world's hottest pepper" tag commonly associated with most exhibitions of chile peppers. Thanks to its unique variety of taxa, culta, and geographic provenances, this collection reached the dignity of a practical mean of scientific divulgation, allowing to spread concepts such as biological and horticultural diversity, domestication, ethnobotany, conservation, and the relationships between plants and cuisine.

1) G.E. Barboza et al. (2016) In: Kadereit, J.W., V. Bittrich (eds.), The Families and Genera of Vascular Plants. Flowering Plants. Eudicots 14. Springer International Publishing, Cham, 295–357
2) J. Andrews (1998) The Pepper Lady's Pocket Pepper Primer. Austin, University of Texas Press
3) C.R. Clement et al. (2016) Bol. Mus. Paraense Emilio Goeldi. Ci. Humanas, 11(1), 193-195
4) FAOSTAT (2019) http://www.fao.org/faostat/en/#data/QC
5) N.M.G. Ardenghi et al. (2019) Notiz. Soc. Bot. Ital.: in press
6) P.W. Bosland, E.J. Votava (2012) In: Bosland, P.W., E.J. Votava (eds.), Peppers: Vegetable and Spice Capsicums. Wallingford, New York, CABI Publishing, 13-38

6.1 = *Fagus sylvatica* planted at high altitude (Alpine Botanical Garden of Campo Imperatore)

Loretta Pace[1], Federica De Braco[1], Beatrice Farda[1], Marialaura Fidani[1], Irene Petrucci[1], Giovanna Pannunzio[1]
[1]Department of Life, Health and Environmental Sciences University of L'Aquila, Piazzale Salvatore Tommasi 1, 67100 L'Aquila, Italy

The Alpine Botanical Garden of Campo Imperatore (GACI) is located along the southern slopes of Mount Aquila (at 2117 m asl) on the western aspect of Gran Sasso d'Italia (Fig. 1). It is in the heart of Gran Sasso and Laga Mountains National Park. GACI was found in the 1952 by Vincenzo Rivera (botanical professor and the first Rector of the University of L'Aquila), with the aim to study the high altitude vegetation in a natural laboratory (1,2). In Apennines the arboreous vegetation is limited to an altitudinal zone up to 1800 m. *Fagus sylvatica* L. (beech tree) is the major component of deciduous forests (both in Apennines and Alpes) and it has colonized various altitudinal levels, from 600 m to 1800 m asl (3). The experiment conducted in the GACI (1954) was about examining the woody plants' growth at higher altitude (about 2000 meters of elevation) and their adaptations to the different metereological conditions. Different specific taxa of different genera (*Abies, Ailanthus, Cedrus, Cupressus, Fraxinus, Larix, Ostrya, Picea, Pinus, Populus, Robinia, Ribes, Thuja*) were implanted at the GACI as but with no success. Beech seedlings borned by seeds gathered under high-altitude shrubs (1500-1800 m) were implanted in apposite big tanks and their development was controlled. After a few years Rivera discovered that the primary bud had not survived because of severe weather conditions, but he observed that some secondary buds were developed (4). The extreme weather conditions, caused by strong and frequent winds, very low average temperatures and intense UV radiation, influence the growth and metabolism of plants. Still today, after more 60 years, in the Alpine Botanical Garden of Campo Imperatore it is possible observe the high altitude adaptation of two beech plants. The most conspicuous difference between these survival specimens and the beeches grown at lower altitudes are the limited height and the smaller size of the leaves. The original tanks where beech trees were implanted from Rivera provide them with protection and were therefore decisive for their survival (Fig. 2).

Fig. 1. A view of the GACI

Fig. 2. *Fagus sylvatica* in big tanks

1) L. Pace, C. Catonica (1998) Aree Protette in Abruzzo, Carsa Edizioni, 204-209
2) L. Pace, G. Pacioni (2002) CAI Bol. IV GTE, 9, 33-50
3) F. Tammaro (2019) Alberi crocevia tra natura, arte e letteratura Vertigo, 215-233
4) V. Rivera (1956) Annali di Botanica XXV fasc. I-II

We are grateful for support received from Gran Sasso and Laga Mountains National Park.

6.1 = Genova Botanical Garden as resource for scientists and citizens

Ester Rosa[1,3], Grazia Cecchi[1], Simone Di Piazza[1], Paola Malaspina[2,3], Mauro Mariotti[1], Elena Mora[1], Enrica Roccotiello[1], Stefano Rosatto[1], Claudia Scopesi[3], Claudia Turcato, Mirca Zotti[1]
[1]Department of Earth, Environment and Life Sciences, University of Genoa, C.so Europa 26, 16136 Genoa, Italy; [2]Department of Pharmacy, University of Genova, Viale Cembrano 4, 16148 Genova, Italy; [3]A.D.M. Associazione Didattica Museale, c/o Natural History Museum "G. Doria", Via Brigata Liguria 9, 16121 Genoa, Italy

The Botanical Garden of University of Genova is located within the historic center of Genova. The botanical gardens are places of extraordinary importance for scientific research, biodiversity conservation, education and environmental divulgation, so they must be preserved, valorized and made known to citizenship (1). In fact, among the activities carried out by botanical gardens it is important to highlight the role in making known and understanding the importance of the diversity of the plant kingdom and to raise public awareness on the conservation and protection of plant species (2).

The Botanical Garden of Genova has always been a promoter of events and activities, direct to citizenship and to scholastic world, aimed at promote the botanical garden and more generally at stimulating attention on the environmental issues. From several years, the botanical garden participates to different manifestations, such as *Euroflora* and *Festival della Scienza*, to cultural events, by hosting exhibitions and performances, and to educational and outreach activities for students or citizens. Since many years, the volunteers of the association *Amici dell'Orto Botanico* contribute to the preservation and enhancement of the Botanical Garden of Genova and organize guided tours and public seminars. Additionally, since two years the association *ADM (Associazione Didattica Museale)*, in collaboration with mycologists and botanists of the University of Genova, has increased and improved the activities for school, families and citizenship.

Thanks to the contribution of all these different and complementary realities, to involve different type of public, the following activities are carried out in the botanical garden:

- laboratory and guided tours for all types of school,
- workshops for adults or children, such as photography, creation of closed terrariums, tastings, theatrical performances, book presentations, laboratories or manual activities on botany;
- cycles conferences, field trips and thematic guided tours for general public.

Finally, to increase the possibilities of transmitting and spreading scientific culture within the Botanical Garden, the internal spaces have been recently improved with the realization of a teaching aula to accommodate the visitor audience.

1) E. C. Moskwa, and C. Gary (2012) Recreation, education, conservation: the multiple roles of botanic gardens in Australia, Annals of Leisure Research, 15.4, 404-421
2) V. Kuzevanov, & S. Sizykh, (2006) Botanic gardens resources: tangible and intangible aspects of linking biodiversity and human well-being, Hiroshima Peace Science, 28, 113-134

6 = From *in-vivo* to *in-vitro*: linking beneficial effects of *Terminalia macroptera* leaf extracts on mice to specific secondary metabolites

Negri Stefano[1], Ior Lydia[2], Poletti Stefania[1], Gambini Sofia[1], Avesani Linda[1], Guzzo Flavia[1]
[1]Department of Biotechnology, University of Verona, 15 Strada Le Grazie, Verona, Italy; [2]Department of Pharmacology, University of Jos, 1 Bauchi Road, Jos, Nigeria

The genus *Terminalia* (Combretaceae) includes several medicinal species that are widely exploited in traditional folk medicine in many African and Asian countries[1]. Local healers from Northern Nigeria rely on the use of different preparations from *Terminalia macroptera* Guill. organs to treat the symptoms of various infectious diseases (e.g. hepatitis, malaria and enterocolitis) and CNS-related disorders (e.g. epilepsy, anxiety and depression) together with other minor ailments[2,3].

In this work we elucidated the phytochemical composition of methanolic and ethylacetate fractions of *T. macroptera* leaf extracts, which exhibited *in-vivo* protective and beneficial behavioural effects in mice models for anxiety and depression. The untargeted metabolomics analysis, performed by RP-UPLC coupled to high resolution ESI-qTOF, revealed a notable composition in polyphenols belonging mostly to the classes of gallic acid esters, ellagic acid derivatives, chebulic and non-chebulic ellagitannins and, in minor amounts, flavonoids. Moreover, in order to investigate the mechanism by which *T. macroptera* metabolites could be responsible for the observed biological activities, we performed preliminary *in-vitro* screening for the evaluation of both antioxidant capacity and the ability to inhibit human monoamine oxidases (MAO) enzymes, involved in the homeostasis of neurotransmitters. The high phenolics abundance resulted in great antioxidant activity of *T. macroptera* leaf extracts tested through ABTS and FRAP *in-vitro* assays. Furthermore, the aqueous extract showed good MAO-A inhibition properties, suggesting a potential relation between specific metabolites of this plant and its ascribed antidepressant and anxiolytic effects.

1) Fahmy N, Al-Sayed E, Singab AN. (2015) Med Aromat Plants, 4, 1-22

2) Pham AT, Dvergsnes C, Togola A, Wangensteen H, Diallo D, Paulsen BS, Malterud KE. (2011) J. Ethnopharmacology, 137, 1486-1491

3) Bum EN, Neteydji S, Djafsia G, Njifutie N, Taiwe GS, Rakotonirina Sv, Rakotonirina A. (2012) Asian J. Pharm. Health Sci., 2, 555-561

6 = A step forward with wastewater treatment: using *Phragmites australis* as an eco-friendly biosorbent in the view of circular economy

Elisabetta Bianchi[1], Ilaria Colzi[1], Andrea Coppi[1], Chiara Berardi[2], Donatella Fibbi[2], Cristina Gonnelli[1]
[1]Department of Biology, Università degli Studi di Firenze, via Micheli 1, 50121 Florence, Italy; [2]Gestione Impianti Depurazione Acque SpA, via di Baciacavallo 36, 59100 Prato, Italy

The increasing use of heavy metals has inevitably led to an increased flux of metallic substances in ground and surface water, posing serious ecological and health risks. Among numerous clean-up techniques for water treatment, adsorption methods are widely used for the removal of heavy metals. Alternative non-conventional low-cost adsorbents have been investigated and natural, industrial as well as synthetic materials have been tested. Nonetheless, plant-derived materials are one of the favorite substrates used for metal removal from contaminated water, because they are renewable, abundant, easy to handle, cost-effective and present minimal problems for their final disposal. Reed (*Phragmites australis* (Cav.) Trin. ex Steudel) is a macrophyte commonly used in the wetlands constructed for water purification, and in the last decades its use as biosorbent has attracted increasing attention. Therefore, in the view of a circularly economy policy, in this study we investigated how to improve the removal of contaminants recycling the biomass of *P. australis* already present in a constructed wetland (CW), which operates as post-treatment of effluent wastewater from an activated sludge plant serving the textile industrial district of Prato (Italy). After the annual mowing of the reed plants, the biomass was dried and blended to derive a sustainable and eco-friendly biosorbent and its adsorption capacity for Fe, Cu and Zn was investigated comparing the most commonly used batch system with column system, easier to handle as biofilters for CWs. In addition, we evaluated the effect to add other sustainable materials in the column, able to favor the flow of the effluent, to avoid expected limits of the packed beds, easily clogged and susceptible to high-pressure drops. The possibility of regeneration and biosorbent reuse was also evaluated. To the best of our knowledge, this is the first report of a study on the biosorption ability of *P. australis* dried biomass from its same CW and on the generation of a hybrid column to optimize its use. The results will be of fundamental importance to suggest an easy, effective and "green" strategy that any CW-based treatment plant could experience for the cleaning of the most precious resource of the Earth, water.

6 = Aerobiological monitoring of the conservation showcases of the Benedictine Chorals of San Pietro in Perugia (Italy)

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

Inside museums, the objects are often showed in special showcases with the aim of protecting the artworks from dust, theft, vandalism or involuntary damages caused by visitors. The museum showcases must also respond to another fundamental conservative requirement: to stabilize their microclimate as a combination of air temperature and relative humidity, concentrations of gaseous and airborne pollutants (1). If it is possible to control the environment of the museum at the rooms level and of the entire building, then the conditioning of the inside showcases areas will not be critical. However, in most museums, it is not possible to achieve a sufficient control of temperature, HR and in particular gaseous and biological pollutants, and therefore it is necessary to consider the showcases as a secondary level of environmental control where check directly T, HR, lighting, air pollutants and parasites (2,3).

The preventive conservation aims of the research were to characterize the quality of the air inside the showcases of the Chorals of the Monastery of San Pietro in Perugia for analysing the risk of contamination and alteration of the artworks by microorganisms of fungal origin present in the conservation microenvironments. The Chorals are large Codes, made, between 1471 and 1529, in parchment, wood and leather with sacred texts in Latin and Gregorian music, used for the Conventual Mass sung. They are maintained in showcases located in museum spaces of the «Tesori d'Arte» Gallery owned by the Foundation for Agricultural Education, which has been administering the ancient Abbey complex of San Pietro for about 120 years. Aerobiological monitoring was carried out on a weekly basis, both in five showcases and, for comparison, in the same exhibition gallery. The objectives of the present work have been achieved through the study of the non-viable airborne fungal component (non-cultivable fraction) and viable (cultivable fraction). The non-viable aerospore was monitored with the Personal Volumetric Air Sampler Spore Trap, the viable one with the sedimentation technique on Petri dishes. The combined aerobiological sampling methods allowed us to provide a reliable description of the variability over time of potentially biodeteriogen aerosporological particles in the studied environments (showcases and Gallery), to monitor the different spore distribution in the showcases, to identify their kind and to highlight situations of risk.

The results showed that the concentration of the non-cultivable aerosporological fraction (n° of spores/m^3 of air) increased in relation to the seasonal trend, both inside the showcases and inside Gallery. A variability of bioaerosols and various concentration peaks both in the single showcases and in the area of the Gallery also emerged. The results obtained with the sedimentation method also showed a total fungal load (CFU/cm^2) very variable in the showcases and within the single considered months. Positive correlation coefficients evidenced significant relationships both between the average spore concentration inside the Gallery and all the showcases and between the two monitored environmental variables (Temperature and Relative Humidity). As far as the qualitative investigation is concerned, the sampling methods allowed to detect a constant presence of spores belonging to fungal groups indicated by the literature as biodeteriogen agents in libraries and archives.

In conclusion, the different entity of the fungal particles, viable and non-viable, monitored inside the showcases, even if reduced, suggests the need to perform periodic checks of aerosporological pollution also in these micro-environments, in order to highlight any excessive values and thus preventing biodeterioration risk situations.

1) Schieweck A., Salthammer T. (2011) Indoor air quality in passive-type museum showcases Journal of Cultural Heritage, 12, 205–213
2) Carlini L., De Nuntiis P., Guaraldi C., Mandorli P., Monco A. (2007) Oggetti nel tempo. Principi e tecniche di conservazione preventiva, a cura dell'Istituto per i Beni Artistici Culturali e Naturali della Regione Emilia-Romagna, Servizio Musei e Beni Culturali
3) Merzenich C. (1991) Vetrine ad impianto passivo di climatizzazione: una bibliografia ragionata, Kermes, 11, 3-11

6 = Genetic studies on *Citrus lumia* (Rutaceae)

Roberta Russo[1], Francesco Maria Raimondo[2], Vivien Spadaro[3], Franco Palla[3]
[1]Istituto di Biomedicina e Immunologia Molecolare "A. Monroy", Consiglio Nazionale delle Ricerche, Via U. La Malfa 153, 90146 Palermo, Italy; [2] PLANTA/Centro di Ricerca, Documentazione e Formazione, P.zzetta Cairoli 11, 90123 Palermo, Italy; [3]Dipartimento di Scienze e Tecnologie Biologiche, Chimiche e Farmaceutiche, sezione di Botanica, Via Archirafi 38, 90123 Palermo, Italy

Citrus lumia is a taxon described by Risso (1, 2), following the publication of his renowned *Citrus* treatises in which he had referred to the "lumies" in rather general terms, whereas the binomial *Citrus lumia* was formally published later (3). Previously, Risso (1) had already described *C. limetta*, including both the lime and the "lumia"; that is still accepted in some works on *Citrus* (4); moreover, "limetta" and "lumia", resemble the lemon, are distinct from it and from each other. The main distinctive features are flower color and the taste of the pulp, which is acid in *C. limetta* but sweet in *C. lumia,* that is of old origin, (5) and being known as "lumia", especially in Italy and France. Although plenty has been written on the differences and affinities between Risso's two taxa, lumie and limetta are sometimes considered the same, but the differences were clear from the onset: the lumie is a sweet lime, the limetta is acid and is well illustrated in the third volume of the earliest, pre-Linnean citrics treatise, published in Italy (5).The hybrid nature of *C. lumia* was already noted by Gallesio (1811) and accepted by Risso & Poiteau (2), that described the essential features of "lumies", highlighting that the "lumies" are not the same mentioned in the 12th century by Falcando – i.e., lemons; they mentioned no less than twelve "lumies". Among them is a *Citrus lumia dulcis* (sweet-fruited lumia), which coincides with the *Limon dulci medulla vulgaris* of Ferrarius (5), with pleasant, sweet juice and 9-11-locular fruit. Their other "lumias" resemble the orange (*C. sinensis*), lime (*C. limetta*), lemon (*C. limon*), and citron (*C. medica*); the first two being characterized by reddish flower color and the last two by sweetish fruits, clearly distinguishable from true lemon and citron, similar in appearance, leaves and flowers but with very acid fruits. As proposed by Raimondo (6), the genetically characterization of *Citrus lumia* is needed, in order to define the genetic structure of the real *Citrus lumia* Risso. In this study preliminary results on genetic relationship based on Inter-Simple Sequence Repeats (ISSR) molecular markers, representing an efficient system to investigate at intraspecific level (7), are presented. Particularly, genomic DNA was extracted from six *Citrus* samples, grinding fresh leaves in liquid nitrogen according to the modified protocols of GenElute genomic DNA Miniprep Kit (Sigma Chemical): 1) *C. limon* cv *dulcis*; 2) *C. limetta* cv Palestina; 3) *C. limetta* cv Petrarca; 4) Citrus aff. grandis (Magione); 5) Citrus aff. (HBP); 6) Citrus aff. lumia (Cefalù). Genomic DNA molecules, quantified by Eppendorf bio-photometer, were utilized as template in ISSR-PCR *in vitro* amplifications. Eight ISSR primer, performing the annealing step at temperature between 46,5 and 48,5°C, were utilized and the product of PCR reactions resolved by 2.0% agarose gel electrophoresis (1X TAE).

Fig 1. PCR products by ISSR primers. A = 5'-CAACAACAACAACAA- 3'; B = 5'GACAGACAGACAGACA- 3'. C = control. M= 100 bp DNA ladder (Sigma)

As showed for two of the ISSR primers (Fig.1 A-B) specific profiles are distinguishable for each of the *Citrus* samples (1-6) as well as for the Control.
Further analyses with different ISSR marker are on-going in order to perform UPGMA clustering analyses, as well as the botanical features and also evaluating the essential oils chemical composition.

1) A. Risso (1813) Annales du Muséum national d'Histoire naturelle, 20, 169-212, 401-431
2) A. Risso, A., Pouteau (1818-1822) Histoire naturelle des Orangers. Herissant le Doux, Paris, 278, tabb. 109
3) A. Risso (1826) *Citrus lumia*, Citre lumie. In: Histoire naturelle des principales productions de l'Europe méridionale et particulièrement de celles des environs de Nice et des Alpes Maritimes, 414-418
4) F. Calabrese, F. Barone (2009) Lime e limette. In: Vacante V. & Calabrese F. Trattato di Agrumicoltura. Edagricole, Bologna, 123-124
5) J.B. Ferrarius (1646) Sumptibus Hermani Scheus. Romae. Pp. 230 + 96 gravures
6) F.M. Raimondo, L. Cornara, P. Mazzola, A. Smeriglio (2018) Quad. Bot. Amb. Appl., 26(2015), 69-76
7) A.A. Sharafi, A.A. Abkenar, A. Sharafi (2017) Journal of Science and Research, 2, 22-27
8) G. Lombardo, Schicchi R., Marino M., Palla F. (2012) Plant Biosystem, 146, 19-26

6 = Interactions of *Ailanthus altissima* (Mill.) Swingle with the Aurelian Walls in Rome (Italy)

Guido Trotta[a]; Valentina Savo[a]; Emanuela Cicinelli[a], Giulia Caneva[a]
[a]Department of Science, University Roma Tre, Viale G. Marconi 446, 00146 Rome, Italy

Ailanthus altissima Mill. (Swingle) is an extremely aggressive and invasive exotic species, which often finds ideal growing conditions in archeological areas in Italy (1,2). In archeological contexts, the tree can grow in close proximity or directly on walls or other masonry structures, damaging them with a mechanical or chemical action of their roots (2). The species is also resistant to almost every method of eradication, from manual cutting to herbicides use (3). For instance, it has been noted that cutting the trees is a trigger for the sprouting of numerous suckers. We quantitatively measured the diameter and height of individuals, the dimension of patches and the number of suckers originating from adult individuals and stumps. We also qualitatively estimated the damages (4) inflicted by *Ailanthus altissima* on the Aurelian Walls in Rome. We did these analyses to identify the areas along the walls that are currently at risk and those that will likely be at risk in the future.

Along the Aurelian Walls, this species is quite widespread (Fig. 1) and already altering the masonry structures (Fig. 2). We performed 48 surveys analyzing all the accessible *Ailanthus altissima* individuals and patches. We noted 577 individuals, including seedling and suckers. The majority of plants were small, although we observed several large developed trees, with an average height of about three meters and an average diameter of about 3.5 cm. Among the 48 surveys, we identified 16 situations where trees were causing visible damage to the wall structures (Fig. 2, 3), to which we added 173 plants that were likely causing damage to underground structures. We noted both adult plants growing adjacent to the walls or suckers sprouting from the base of the walls originating from trees on the other side. Considering the management of the areas, which includes periodical mechanical cutting, the majority of trees causing damages are two years old and with a diameter above 5 cm, although we observed that even very young and small trees are capable of producing damages. We mapped the areas that are more at risk of damages, also highlighting potential future risks. We suggest monitoring actions to contain the growth and expansion of this species along the Aurelian Walls in Rome, avoiding uncontrolled cutting that can only trigger the sprouting of new individuals from the stumps.

Fig. 1. Individuals along the walls

Fig. 2. A young tree growing among the bricks of the wall

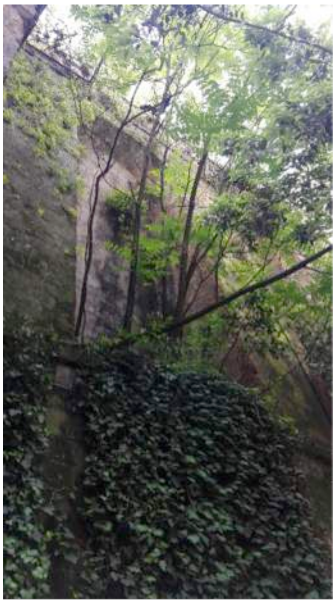


Fig. 3. Developed trees growing on the walls

1) L. Celesti-Grapow, C. Blasi (2004) Weed Technology, 18(1), 1508-1514
2) F. Casella, M. Vurro (2013) Arboricultural Journal: The International Journal of Urban Forestry, 35(3), 172-181
3) S. Constán-Nava, A. Bonet, E. Pastor, M.J. Lledó (2010) Forest Ecology and Management, 260(6), 1058-1064
4) B. Fitzner, K. Heinrichs (2001) Acta-Universitatis Carolinae Geologica, 1, 12-13

6 = First study of air pollution in Umbria Region by way of moss bag biomonitoring

Silvia Poponessi[1], Annalena Cogoni[2], Michele Aleffi[3], Daniela Gigante[4]
[1]Department of Chemistry, Biology and Biotechnology, University of Perugia, Via del Giochetto 6, 06126 Perugia, Italy; [2]Department of Environmental and Life Science, University of Cagliari, Viale S. Ignazio 13, 09123 Cagliari; [3]School of Biosciences and Veterinary Medicine, University of Camerino, Via Pontoni 5, 62032 Camerino (MC), Italy; [4]Department of Agricultural, Food and Environmental Sciences, University of Perugia, Borgo XX giugno 74, 06121 Perugia, Italy

Biological monitoring is based on the indirect analysis making use of particular "bioindicator" organisms or communities, in order to assess the changes in some environmental variable from situations considered unaltered (natural). In particular, bioaccumulation allows the concentration of pollutants in plant tissues to be correlated with the levels of pollution present in the environment.

The methodology adopted in the present study is classified as "active bio-monitoring", which consists in transplanting organisms -in this specific case, bryophytes- properly treated, to monitor target places. Goodman and Roberts introduced the 'Moss bag technique' in 1971 (1). Mosses are indeed suitable biomonitors to entrap air pollutants. Before a moss bag can be prepared for the analysis, an appropriate selection of the used mosses is needed. The old vs. young shoots of the moss should be separated. It is well known that older parts of the stem contain different amounts of elements than younger tissues (3). Different substrates and exposure to the atmosphere should be considered too. In fact, the elemental deposition on mosses growing on soil and rock dust is quite different from those growing on tree trunks (4). It is very important to use homogeneous samples. The technique is based on the fact that dehydrated bryophytes do not die but go into latent life and, if wet again, return to perform their vital functions.

This protocol has been here used for conducting a detailed survey of some polluted urban areas, where native mosses are usually absent because of predominantly paved constructions (2). The study areas are located in the Provinces of Perugia and Terni (central Italy) in areas subject to high pollution from industrial sources and vehicular traffic. The exposure of the moss bags took place in three phases, each lasting 3 months, from April 2017 to January 2018. Among the most commonly used species of mosses, *Hypnum cupressiforme* Hedw. was chosen, being well suited to the needs of the research. The moss bags were located in proximity of the Regional ARPA Instrumental measurement stations, with the aim to highlight any correlation between directly and indirectly measured data. The first outcomes are here described and commented.

The fundamental characteristic of bryophytes, which makes them excellent candidates for biomonitoring, is the fact that they are able to accumulate heavy metals in their tissues even in conditions of dehydration. The advantages consist above all in the ability of these organisms to act as "data integrators" and in the low costs of the methods used. Indeed, a remarkable benefit from the moss-bags use is that instrumental direct measurements are usually carried out within short time periods, providing punctual values of concentration of pollutants, whereas moss bags act as a long term integrator of air pollutants providing cumulative concentrations. Long-term sampling is a prerequisite for the assessment of cumulative exposure to a certain pollutant with hazardous effect on human health. Additionally, moss bags do not require power supply and maintenance. For these reasons they might represent a valuable, more efficient alternative to the standard instrumental direct measurements.

1) Goodman, T.G., Roberts, T.M. (1971) Plants and soils as indicators of metals in the air. Nature, 231, 287–292
2) Kosior, G., Ciężka, M., Górka, M., Samecka-Cymerman, A., Kolon, K., Kempers, A.J., Jędrysek, M.-O. (2015) δ34S values and S concentrations in native and transplanted Pleurozium schreberi in a heavily industrialized area. Ecotoxicol. Environ. Saf., 118, 112–117
3) Fernández, J.A., Aboal, J.R., Carballeira, A., 2010. Testing differences in methods of preparing moss samples. Effect of washing on Pseudoscleropodium purum. Environ. Monit. Assess., 163, 669–684
4) Adamo, P., Giordano, S., Sforza, A., Bargagli, R., 2011. Implementation of airborne trace element monitoring with devitalised transplants of Hypnum cupressiforme Hedw.: assessment of temporal trends and element contribution by vehicular traffic in Naples city. Environ. Pollut., 159, 1620–1628

6 = On the bryophyte flora of the Tuscan-Emilian Apennine (N Italy)

Marta Puglisi[1], Michele Aleffi[2], Ilaria Bonini[3], Annalena Cogoni[4], Sandro Perego, Alessandro Petraglia[5], Silvia Poponessi[6], Francesco Sguazzin

[1]Department of Biological, Geological and Environmental Sciences, University of Catania, via A. Longo 19, 95125 Catania, Italy; [2]School of Biosciences and Veterinary Medicine, University of Camerino, Via Pontoni 5 I-62032 Camerino (MC), Italy; [3]Department of Life Science, University of Siena, via P.A. Mattioli 4, 53100 Siena, Italy; [4]Department of Scienfe of Life and Environment, University of Cagliari, v.le S. Ignazio 13, 09123 Cagliari, Italy; [5]Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Parco Area delle Scienze 11/a, 43124 Parma, Italy: [6]Department of Chemistry, Biology and Biotechnology, University of Perugia, v. del Giochetto 6, 06126 Perugia, Italy

The excursion of the Bryological Group of the Italian Botanical Society was organized in July 2018 in the National Park of the Tuscan-Emilian Apennines, where many different types of mountain habitats were investigated. The field work was carried out in areas fall within the Emilia-Romagna region in the localities Passo della Scalucchia, Passo Pradarena, Lago Bargetana, at altitudes between 1350 and 2050 m a.s.l. The collections were made from soil, rocks and tree bark in different habitats (beech woods, heathlands with *Vaccinium myrtillus* L., snow valleys). The result of this survey is a checklist of 113 species (22 liverworts and 91 mosses) including 15 new records for the Emilia Romagna region. some of which of phytogeographical interest and deserving a special protection over time. Among these we quote, for istance, *Scapania uliginosa* (Lindenb.) Dumort., a liverwort rare in Italy as well as in Europe (1), *Grimmia elongata* Kaulf., a moss species rare in Italy, *Orthotrichum pulchellum* Brunt., candidate in the forthcoming European Red List. In addition to the new records, the occurrence of 7 species is confirmed for Emilia Romagna region. Among these, there are some remarkable species for the Italian bryophyte flora and worthy to be safeguarded too, as *Brachythecium cirrosum* (Schwägr.) Schimp., rare in Europe where it is considered as Critically Endangered, Endangered, Vulnerable, according to the IUCN categories and criteria, in many Countries (1). Overall, the most widespread species in the investigated area were *Plagiochila porelloides* (Torrey ex Nees) Lindenb., *Homalothecium sericeum* (Hedw.) Schimp., *Hypnum cupressiforme* Hedw. var. *cupressiforme*, *Polytrichastrum alpinum* (Hedw.) G.L.Sm., *Polytrichum piliferum* Hedw., *Pterigynandrum filiforme* Hedw.

Based on the results of this investigation, the bryophyte flora of investigated area shows a ratio liverworts/mosses equals to 0.242, with a scarce liverwort component, comparable with that of Monte Bondone (Trentino Alto Adige) which is 0.256 (Privitera et al., 2010).

As regards the phytogeographical analysis, the Boreo-temperate species prevail (30.7%), followed at a distance by Boreo-arctic montane (16.2%), Boreal-montane (11.7%) and Temperate species (10.8%); the less represented categories are the Mediterranean (5.4%) and Wide Temperate (2.7%).

In conclusion, this study has pointed out a rich bryophyte diversity in the investigated area, with a significative set of species rare in Italy. For this reason the Apennines can be considered an important site for the biodiversity conservation, surely less affected by human disturbance which is one of the most dangerous cause of biodiversity loss.

1) N. G. Hodgetts (2015) Irish Wildlife Manuals, No. 84. National Parks and Wildlife Service, Department of Arts, Heritage and the Gaeltacht, Ireland, 1-125

2) M. Privitera, M. Aleffi M., G. Bertani, P. Campisi, A. Carratello, M. Codogno, A. Cogoni, L. Miserere, S. Perego, M. Puglisi, F. Sguazzin, R. Tacchi, A. Zimbone (2010) Fl. Medit., 20, 193-209

6 = Screening of mosses for potential application in urban ecosystems

Sara Romeo*[1], Paola Castellari[2], Katia Perini[2], Andrea Giachetta[2], Claudia Turcato[1], Mauro Mariotti[1], Enrica Roccotiello[1]
[1]Department of Earth, Environment and Life Sciences, University of Genoa, Corso Europa 26, 16132 Genova, Italy;
[2]Department of Architecture and Design, University of Genoa, Stradone S. Agostino 37, 16123, Genova, Italy;
*Corresponding author: sara.romeo.genova@gmail.com

Mosses can survive in unfavorable environmental conditions thanks to their ecological needs in terms of growing substrates, low amount of water and nutrients required, ability to absorb liquids up to 20 times their weight and vegetative desiccation tolerance. Although they were considered main buildings' enemy, recent studies (1, 2) highlighted their potential use to cover and protect buildings and other urban surfaces.

Several studies are needed to clarify this point and to understand the most performing species able to sustain stressful conditions as the ones given by building surfaces of cities (e.g., wind, solar radiation, air pollution, etc.). Therefore, the main aim of this work is to select the best species able to survive to severe desiccation, and to grow on main building surfaces.

Several *taxa* were collected from different substrata and in different environmental conditions (e.g., from soil to plaster, from low to high water availability, from shadow to full sunlight): *Barbula unguiculata* Hedw., *Brachythecium salebrosum* (Hoffm. ex F. Weber & D. Mohr) Schimp., *Hypnum jutlandicum* Holmen & Warncke, *Isothecium myosuroides* Brid., *I. alopecuroides* (Lam. ex Dubois) Isov., and *Rhynchostegium confertum* (Dicks.) Schimp. At the end of the screening phase, *B. unguiculata* was the species chosen for subsequent growing experiment.

A mixture of gametophytes of *B. unguiculata* and deionized water was placed on different materials used as vertical or horizontal growing support: lime plaster or cement plaster, bricks, slate, quartzite and irrigation felt. Half of them were covered with cheese cloth, 8 replicates each growing surface (covered or uncovered).

Gametophytes mixture was incubated in a growth chamber at 18±2°C, 20 μM/m²s light intensity, 60% of relative humidity, both on horizontal and vertical support. Mixture was daily hydrated by spraying 6,5 ml of water each spot and submitting them to weekly dehydration of two days.

After two months of incubation new biomass was produced. The first results shown that the growing support is important for physical but not chemical characteristics because of its high-water retention and homogeneous water distribution ability.

This interdisciplinary study between plant biology and architecture provide a more comprehensive way to identify new low-cost materials for greening urban surfaces.

1) Kaufman, M.A. (2016) A Feasibility Growth Study of Native Mosses Associated with Self-Sustaining Flora on Vertical Infrastructure. In: International Conference on Transportation and Development 2016: Projects and Practices for Prosperity - Proceedings of the 2016 International Conference on Transportation and Development, 683–95
2) Park, J.-E., and H. Murase (2008) Evapotranspiration Efficiency of Sunagoke Moss Mat for the Wall Greening on the Building. In American Society of Agricultural and Biological Engineers Annual International Meeting 2008, ASABE, 6, 3612–21

6 = Policy species monitoring and conservation measures in the Dolomiti Bellunesi National Park (N-E, Italy)

Marco Canella[1], Thomas Abeli[2], Simone Orsenigo[1], Andrea Mondoni[1], Gianni Poloniato[3], Cesare Lasen[3], Graziano Rossi[1]
[1]Department of Earth and Environmental Sciences, University of Pavia, Via S. Epifanio, 14, 27100 Pavia, Italy; [2]Department of Science, University of Roma Tre, Viale Guglielmo Marconi 446, 00146, Roma, Italy; [3]Dolomiti Bellunesi National Park, Piazzale Zancanaro, 1 - 32032 Feltre, Italy

The Dolomiti Bellunesi National Park (PNDB), was founded in 1993 and belongs to Nature 2000 as SAC (WDPA ID 6181). One of the main scientific reasons leading to the institution of the Park was the great richness and rareness of its flora. Besides the alpine, boreal and temperate - Eurasian species, there are also many species gravitating around the eastern regions (Illyrian, south-eastern European species), and Mediterranean-orophilous species. The area has also been included in the UNESCO World Heritage List, due to his "spectacular landscapes of international significance for geomorphology". During February 2018, over twenty operators including National Park staff, researchers of the University of Pavia, rangers (*Carabinieri - Comando per la Tutela della Biodiversità e dei Parchi*) and volunteers were involved in a training course for the monitoring of several Habitat Directive 92/43/EEC plant species (1) and other entities considered rare or endangered at local level. The aim of the course was to prepare operators for the next Habitat Directive field monitoring session. Permanent plots were established in several populations of the policy species *Adenophora liliifolia* (L.) Ledeb. *ex* A.DC, *Cypripedium calceolus* L. and *Gladiolus palustris* Gaudin and of the species of local conservation interest in the PNDB *Campanula thyrsoides* L. subsp. *carniolica* (Sund) Podlech, *Hypochaeris facchiniana* Ambrosi, *Lilium carniolicum* Bernh. *ex* W.D.J.Koch and *Pinguicula poldinii* J.Steiger *&* Casper. In the case of *Gladiolus palustris* vegetation surveys were performed on Mount Grave to evaluate the effect of management (periodical mowing) realized by PNDB to avoid the encroachment of abandoned grasslands (3). Data analysis revealed that mowed plots were significantly richer in number of species and supported a higher number of ramets of *G. palustris* than control (un-mowed) ones. Seed collecting following ENSCONET protocols (4) was also performed in late summer, as an *ex-situ* conservation measure. Over 50 accessions have been conserved at the Plant Germplasm Bank of the University of Pavia and the Millennium Seed Bank of Kew. Some species have already been employed in germination studies, such as *H. facchiniana*, to investigate seed quality and conditions for subsequent plant propagation. Furthermore, a hydrothermal-time model of germination is currently in progress for *C. thyrsoides*. Propagation actions are planned in 2019-2020, in collaboration with the PNDB alpine botanic garden for acclimation, before reintroduction.

1) European 'Habitat' directive 92/43/CEE (1992) Official journal of European Communities, L 206, 35, 22
2) http://www.dolomitipark.it/it/page.php?id=123
3) Tasser E., U. Tappeiner (2002) Impact of land use changes on mountain vegetation, International Association of Vegetation Science, 5(2),173-184
4) Seed Collecting Manual for wild species (2009) Royal Botanic Gardens, Kew (UK) and Universidad Politécnica de Madrid (Spain)

6 = Five years of monitoring of flora and vegetation within the Life project LIFE13 NAT/IT/000471 "Island conservation in Tuscany, restoring habitat not only for birds": was it worth it?

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

The Project RESTO CON LIFE "Island conservation in Tuscany, restoring habitats not only for birds" is a Life Natura project co-funded by the European Commission (www.restoconlife.eu). Within this project, over the last five years, the Tuscan Archipelago National Park has undertaken, together with the other partners of the project, several actions to improve the conservation status of habitats and rare species of animals and plants on the islands of Pianosa, Elba, Montecristo and Giannutri (Tuscan Archipelago, Italy). As Department of Biology of the University of Florence, we have been involved in the planning phase of the whole project, in the preliminary actions on flora and vegetation, mostly linked to the mapping of alien plants and the testing for best procedures for their control (1) and on studies on native ones (2). In addition, we have been responsible for the whole duration of the project of the scientific monitoring of flora and vegetation linked to the main actions. We present here the most significant results of these five years of monitoring, starting from the pre-intervention context to the one after the first years after the main practical interventions.

On the Island of Elba the project aimed at the restoration of the complex of dunal habitats at the Lacona site, throughout bio-structures to avoid the dune erosion and to facilitate the establishment and protection of the incipient dune. The data collected up to 2018 highlighted the benefit effect of the structure already in the first year after its establishment, with an increase of specie richness and cover in the area of the incipient dune. However, a catastrophic storm in winter 2018 almost erased all the results obtained.

On the island of Giannutri the project aimed at the eradication of the invasive *Carpobrotus* spp. as well as at the restoration of the invaded coastal habitats, by planting native species. The data on the monitoring elucidated the dramatic effects of *Carpobrotus* spp., which get to outcompete all native species in the areas completely invaded. After the eradication, we recorded a first recovery of native species and the successful establishment of native species planted during the project.

On the Island of Pianosa we aimed at the restoration of the *Quercus ilex* forests, almost vanished from the island and replaced by pine plantations. The main actions involved the sowing of holm oak acorn in pine plantations and in the control *Pinus halepensis,* through the plantation thinning out. The data showed a moderate success of the sowings and particularly the rapid colonization of the pure pine plantations by heliophilous species. These results should represent a first step towards the establishment of a more dynamic understory (with Mediterranean scrubs), more likely to be colonized by *Q. ilex* in the future, and to guarantee the survival of the small plants deriving from our sowings.

Fig. 1. Monitoring plot at Giannutri

Fig. 2. Monitoring transect along the dune at Lacona

1) L. Lazzaro, G. Ferretti, E. Bianchi, R. Benesperi (2018) Plant Biosystems, DOI: 10.1080/11263504.2018.1536083
2) L. Lazzaro, E. Sarracco, R. Benesperi, A. Coppi (2018) Caryologia, 71(1), 50-57

6 = Genetic diversity of Sicilian common bean germplasm

Anna Scialabba[1], Francesco Maria Raimondo[2], Ignazio Digangi[3], Maria Carola Fiore[4]
[1]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo, Via Archirafi 38, 90123 Palermo, Italy; [2]*PLANTA*/Research, Documentation and Training Center for the Mediterranean and Tropical regions, Palermo, Italy; [3]Living Plants Germplasm Bank of Nebrodi, Contrada Pirato, 98060 Ucria (ME), Italy; [4]CREA Research Centre for Plant Protection and Certification, 90011 Bagheria (PA), Italy

In Italy, common bean is the major cultivated legume, with a dry beans production of 11,176 tons and over 6,000 cultivated hectares (1). Many Sicilian bean landraces are now endangered as they became obsolete due to the spread of commercial varieties. Italian farmers preserved traditional local landraces, which usually have local names, are well adapted to specific pedo-climatic conditions and are appreciated by consumers valuing their better taste and higher nutritional properties (2,3,4). Despite being poorly known, these landraces represent a genetic heritage to be preserved and promoted.

Biobanks play a major role in preserving genetic resources that need extensive characterization such as genomic and proteomic profiling (5).

The "Sicilian Plant Germplasm Repository" of STEBICEF Department of Palermo University (SPGR/ PA) and the "Living Plants Germplasm Bank" founded by the Nebrodi Regional Park (ME-Italy) collected and stored a germplasm collection of fifty-seven Sicilian common bean (*Phaseolus vulgaris* L.) landraces. Morphological characterization of these seeds has showed a redundancy of some accessions, and supporting the hypothesis that local farmers name the same cultivar in multiple ways (6).

The present study reports the genetic characterization of common bean landraces with the aim to define a core collection and to develop a comprehensive strategy for the conservation of these crop genetic resources.

Leaf materials of five plants collected from each accession, were lyophilized and used for DNA extraction. Eight SSR loci, used in Italian common bean genetic studies, were selected according to their Polymorphic Information Content (PIC) values and dispersed map locations (7,8). The PCR fragments were genotyped on an ABI 3130 XL sequencing machine, using as standard varieties controls the common bean genotype BAT 93, representative of Mesoamerican gene pool, together with JALOEP558 and MIDAS genotypes, representative of the Andine gene pool.

Genetic relationships among local common bean cultivars, as determined by UPGMA cluster analysis, highlighted a high level of diversity between local cultivars, evidencing a genetic segregation of all studied landraces. These results demonstrate the importance of including all studied accessions in the core collection, highlighting the weakness of phenotypic characterization as accurate criterion for planning and setting-up *ex situ* collections with high intrinsic value. Indeed, genetic markers should be used to define more accurately the range of inter- and intra-landraces diversity.

Besides playing a major role in distributing biospecimens and associated data for research purposes (9), Biorepositories promote conservation policies and the sustainable use of intrinsic crop diversity. These policies and information can and should be shared with local farmers, who can directly benefit from these fundamental research efforts.

1) FAOSTAT 2017. http://www.fao.org/faostat
2) V. Montesano, D. Negro, G. Sarli, G. Logozzo, Zeuli P. Spagnoletti (2012) Genet. Resour. Crop Evol., 59(5), 701-716
3) L. Lioi, A. Nuzzi, B. Campion, A.R. Piergiovanni (2012) Genet. Resour. Crop Evol., 59(3), 455-464
4) L. Raggi, B. Tiranti, V. Negri (2013) Genet. Resour. Crop Evol., 60(4), 1515–1530
5) M. Govindaraj, M. Vetriventhan, M. Srinivasan Hindawi (2015) Genetics Research International Volume 2015, Article ID 431487, 14 pageshttp://dx.doi.org/10.1155/2015/431487
6) A. Scialabba, F.M. Raimondo, A. Messina, M.C. Fiore (2018) V International Plant Science Conference, 12-15 September, 97, Fisciano (SA)
7) M. Kwak, P. Gept (2009) Theor. Appl. Genet., 118(5), 979-992
8) K. Yu,S.J. Park, V. Poysa, P. Gepts (2000) J. Hered., 91, 429-434
9) Y. De Souza, J. Greespan (2013) AIDS, 27(3), 303-312

6 = A quantitative approach for habitat monitoring at national scale

Cervellini Marco[1], Chiarucci Alessandro[1], Nascimbene Juri[1], Angelini Pierangela[2], Casella Laura[2], Maccherini Simona[3], Angiolini Claudia[3], Cerabolini Bruno[4], Dalle Fratte Michele[4], Fattorini Lorenzo[5]
[1]Department of Biological, Geological, and Environmental Sciences, Alma Mater Studiorum, University of Bologna, Bologna, Italy; [2]ISPRA - Italian National Institute for Environmental Protection and Research, via V. Brancati, 60, 00144 Roma, Italy; [3]Department of Life Sciences, University of Siena, Via Mattioli 4, 53100 Siena, Italy; [4]Department of Theoretical and Applied Sciences, University of Insubria, via J.H. Dunant, 3, I-21100 Varese, Italy; [5]Department of Economics and Statistics, University of Siena, P.za S.Francesco, 8, 53100 Siena, Italy

Improving the conservation status of species and habitats targeted by the “Directive 92/43/EEC” means to achieve an effective and standardized monitoring program of Natura 2000 sites, so that this approach fits well with the aims of European 2020 Biodiversity Strategy (1). This goal has to be accomplished by each EU member state. We present a sampling strategy we are developing for monitoring all the habitat types on the Italian territory, based on a multi-phase monitoring programme. General aim of this sampling scheme is to generate a standardised, reliable and comparable approach for the quantitative assessment of the habitat amount and conservation status. Monitoring is based on three sampling phases: I) for each habitat type, presence absence data on the geographical grid of 10 km x 10 km cells (2) are used to select a spatially balanced sample of cells containing the habitat by means of one-per-stratum sampling, with sampling fractions ranging from 100%, for rare habitat types to 10%, for common habitat types; II) Each cell selected in the first phase is partitioned into sub-quadrats exploited as sampling units to select habitat patches by means of network sampling in order to estimate the habitat cover and/or the number of patches; III) for some habitat types, point, plot or transect sampling are performed in a third phase to estimate some vegetation attributes. This monitoring program allows achieving statistically sound estimates of habitat cover and status based on a sampling scheme that can be easily standardised and repeated over time. After the validation of this multi-phase habitats monitoring on the Italian territory, the possibility to maintain at European scale a favourable conservation status of the Nature 2000 habitats network, could be a reliable, applicative and shareable achievement by all member States.

1) EEA, EU 2010. Biodiversity Baseline (Tech. Rep. No. 12/2010, EEA, Copenhagen, 2010)
2) EC, 2006. Assessment, monitoring and reporting under Article 17 of the Habitats Directive: Explanatory notes and Guidelines, Final draft, October 2006

6 = Exploring the response of grassland vegetation to global change: a resurvey study in the Dolomiti Bellunesi National Park (Italy)

Juri Nascimbene[1], Chiari Lelli[1], Cesare Lasen[2], Gianni Poloniato[2], Chiara Vallese[1], Marcello Tomaselli[3], Alessandro Chiarucci[1]

[1]Department of Biological, Geological and Environmental Sciences, Alma Mater Studiorum University of Bologna, Via Irnerio 42, 40126 Bologna, Italy; [2]Dolomiti Bellunesi National Park, Piazzale Zancanaro 1, 32032 Feltre, Italy; [3]Department of Chemistry, Life Sciences and Environmental Sustainability, University of Parma, Parco Area delle Scienze11/a, 43124 Parma, Italy

Global change, including climate and land-use change and eutrophication, is rapidly impacting mountain ecosystems causing major concerns for biodiversity conservation even in protected areas. In this context, vegetation resurvey is increasingly addressed as a powerful approach for quantifying and monitoring the effects of global change, since plant communities may inform on general patterns of biodiversity across time slices. In this study, we applied this approach to explore the response of grassland vegetation to global change in the Dolomiti Bellunesi National Park that is among the floristically more relevant regions of the Eastern Italian Alps. In this area, the long tradition of botanical studies allowed us to retrieve a huge pool of historical vegetation data, ranging between 1975 and 2003, and representing different grassland types. On this basis, in 2018 we started a resurvey study focused on a selected pool of vegetation plots distributed across the main grassland types according with an elevational and management gradient. Plot selection was also based on the possibility to accurately relocate the sampling site. During the first year of fieldwork we resampled 69 vegetation plots spanning an elevational gradient between 430 m and 2190 m. Preliminary results indicated a general increase in species richness along the whole gradient (original plots: mean = 34.9 ± 13.8 species; resurvey: mean = 41.3 ± 15.2 species), with higher beta diversity at increasing elevation. Beta diversity from the original to the resurveyed plots was mostly related to species turnover, with a small component of nestedness. These preliminary results likely reflect both the effects of climate change (i.e. an upslope species migration due to climate warming, with a competitive replacement at the expenses of the most sensitive and narrow range species) and land-use change related to a decreasing grazing activity.

6 = Effects of environmental heterogeneity on phenotypic variations of *Lilium pomponium* L.

Luigi Minuto[1], Carmelo, N. Macrì[1], Davide Dagnino[1], Maria Guerrina[1], Frédéric Médail[2], Gabriele Casazza[2]
[1]Department of DISTAV, University of Genoa, Corso Europa 26, 16132, Genoa, Italy; [2]Aix Marseille Université, Avignon Université, CNRS, IRD, IMBE. Technopôle de l'Arbois-Méditerranée, BP 80, 13545 Aix-en-Provence cedex 4, France

Species response to environmental changes is mainly determined by the response of ecologically marginal populations. Thus, to quantify the variation in the ecological traits across a species' geographic range is crucial to understand factors shaping the distributional patterns. In this study, we compared phenotypic variation in climatically marginal and central populations of *Lilium pomponium* L., a plant endemic to the Maritime and Ligurian Alps, spanning from Mediterranean to Alpine habitats. We used bioclimatic variables to group the populations into marginal Mediterranean (MM), marginal continental (MC), central (CC) and marginal subalpine (MS), based on their distance from the climatic optimum of the species. Phenotypic traits related to plant-pollinator interaction and to reproductive capacity (seed production and germination) were considered to verify differences in species performance from the center towards the margins. In particular, we evaluated: (I) the presence of the pollen limitation; (II) seeds production; (III) the capacity for self-fertilization; (IV) seed germination capacity; (V) flower dimension; (VI) the number of flowers per scape; (VII) the relative position of the sexual organs.

In *L. pomponium* geographic and environmental gradients was not concordant, in fact MC populations occupied the southern climatic margin, although they were not on the geographical periphery but closed to the centre of the distributional range. The MM and MC groups were more pollen-limited than CC and MS, no significant differences in seed production and seed set were detected among groups. This result may be explained by different factors like resources limitation, seed predation and herbivory can reduce seed production in CC and MS groups. Moreover, marginal groups did not show an increase in self-fertilization rate. Seed size increased from warm (MM and MC) to cold (MS) edges and in all groups seed germination decreased at high temperature, especially in CC and MS groups. These differences may be a strategy to increase the possibilities of germination, in fact in warm edge, seeds are small because they need less water to germinate than cold edge ones. Moreover, the exposure to warmest summer temperatures could make warm edge seeds more resistant to temperature increase. Flower dimension decreased from warm to cold edge likely because populations in warm edge (MM and MC) presents a short flowering period and require large flowers to attract pollinators. Moreover, in MC group plants had more flowers par scape to increase the level of geitonogamy, as the ability to carry out self-pollination is less than 0.1%. The results of the traits associated with the interaction plant-insect revealed that in *L. pomponium* the separation of sexual organs varied between individuals and populations, nevertheless the proportion of flower showing this separation was significantly higher in MM group. The difference detected in phenotypic traits suggests that populations occurring under different climatic conditions are exposed to different environmental pressure.

6 = Melanization in parmelioid lichens as a passive defense mechanism against microbiological degradation

Lorenzo Fortuna[1], Silvia Ongaro[1], Alessandro Foscari[2], Guido Incerti[2], Stefano Martellos[1], Mauro Tretiach[1]
[1]University of Trieste, Department of Life Sciences, Via Licio Giorgieri, 10, Trieste, Italy; [2]University of Udine, Department of Agricultural and Environmental Sciences, Via delle Scienze, 206, Udine, Italy

The family Parmeliaceae is one of the most evolved, diversified and widespread groups of lichenized ascomycetes. It includes more than 2000 species, nearly all forming foliose or fruticose stratified thalli, corticated on both upper and lower surfaces. Most of the parmelioid genera share a common trait: a well-developed lower cortex, which is brown to black due to the heavy deposition of melanin-like pigments. Melanins are present in all life kingdoms, mostly as hetero-polymers composed by a plurality of chemical moieties, which make them excellent scavengers of reactive oxygen species (ROS), and adsorbent of both UV and high PAR radiations. Though it is generally recognized that a melanized upper cortex can protect the underlying layers from intense solar radiation, the role(s) of a melanized lower cortex in parmelioid lichens remains to be fully understood. In this study, we hypothesized that the heavily melanized lower cortex of *Hypogymnia physodes* (L.) Nyl. (Parmeliaceae) could act as a passive defense mechanism against degradation processes. To test this hypothesis, *H. physodes* samples with (M+) or without (M-) melanized lower cortex were exposed to degradation by soil micro-flora of spruce and chestnut forests. Lichen samples, as well as soil samples of spruce forest, were collected in the eastern Alps (Casera Razzo, NE Italy), whereas soil samples of chestnut forest were collected in the Tuscan pre-Appennines (Mount Amiata, central Italy). Lichen samples were cleaned from extra-debris, devitalized by heat shock treatment (60°C for 24 h) to inactivate the metabolic anti-microbial defenses, and washed in distilled water. M- samples were prepared by removing the lower cortex under a stereomicroscope by means of a precision cutter. Afterwards, 30 M+ and 30 M- samples (each of 150 mg) were enveloped, one by one, in a 2×2 cm^2 plastic mesh of 40 µm, and stored over silica for 48 h. The initial weight was determined by gravimetric measurements with a sensitivity of ±0.5 mg. Then, both M+ and M- samples were singularly buried in plastic vessels containing 60 g of either soil type, previously sifted at 3 mm, dried at 40 °C for 4 d, and then hydrated with distilled water up to 30% of their dry weight. Plastic vessels containing lichen samples were dark-incubated for five months in plastic boxes, which were ventilated at 2.5 l min^{-1} with water-saturated air at room temperature, in order to maintain an aerobic environment, and a water potential of both forest soils in a range between -1 and 0 Mpa. After 50, 100 and 150 d ($T_{1\text{-}3}$), five M+ and M- samples were retrieved from the vessels, gently cleaned with a brush and stored over silica for 48 h before being weighed. At each retrieval time, soil water potential was monitored with a dew point meter (Model WP4, Decagon Devices Inc.). Loss of lichen biomass was expressed as relative difference with respect to the initial weight. Finally, three out of the five samples were analyzed with a CHN analyzer (Elementar Vario Micro) after being pulverized with a mixer mill (30 Hz for 4 min).

Lichen samples showed a significant biomass loss and a C/N decrease throughout the experiment in all treatment combinations. Independent of the soil forest type, mass loss in M- samples was higher (+15%). However, lichen samples buried in chestnut forest soil were more degraded (+10%) suggesting that, under experimental conditions, its soil micro-fauna was more active than that of spruce forest. This could explain why the difference between the C/N ratio of M- and M+ samples were higher in samples buried in the chestnut forest soil (~40%) than the other (~5%). At each retrieval time, the non-melanized thalline surfaces (upper cortex and medulla) of both M- and M+ samples were more fragmented in comparison to the melanized lower cortex. This evidence, coupled with biomass loss measurements, clearly indicate that a melanized lower cortex is more resistant to degradation processes, and that it is probably involved in preserving the integrity of the thallus.

6 = Climatic factors and functional traits determine the climatic niches of lichen genus *Peltigera* Willd. in Europe

Chiara Vallese[1], Juri Nascimbene[1] , Gabriele Casazza[2], Paolo Giordani[3], Renato Benesperi[4]
[1]Department of Biological Geological and Enviromental Sciences, University of Bologna, Piazza di Porta San Donato 1, 40126 Bologna, Italy; juri.nascimbene@unibo.it; chiara.vallese2@unibo.it; [2]Aix Marseille Univ, Avignon Université, CNRS, IRD, IMBE, Marseille, France, gabriele.casazza@imbe.fr; [3]DIFAR, University of Genoa, Viale Benedetto XV 3, 16132 Genova, Italy; giordani@difar.unige.it; [4]Department of Biology, University of Florence, Via Madonna del Piano 6, 50019 Sesto Fiorentino, Firenze, Italy; renato.benesperi@unifi.it

Ecological niche modelling is powerful tool to estimate a set of suitable environmental conditions for a species or a community, also to identify possible risks for conservation. Due to their poikylohydric nature, lichens distribution is strongly influenced by climate. By means of "ecospat", "vegan" and "ade4" packages running on the R environment, we calculated the climatic niche of 23 species of the lichen genus *Peltigera* (Willd.) occurring in Italy across multiple environmental conditions, using bioclimatic variables, i.e. temperature and precipitation, at the European scale. Variables have been priorly selected, by excluding those resulting autocorrelated. A final dataset of 7 bioclimatic predictors was maintained. We assessed the rate of overlapping between niches, the niche breadth, and the distance between average habitat suitable condition for the species, and average habitat conditions of the study area (marginality). We also assessed the role of 11 selected functional traits in promoting the species in their niches. In accordance with literature, we found that the highest niche overlapping between the two tripartite species, *Peltigera leucophlebia* (Nyl.) Gyeln. and *Peltigera apthosa* (L.) Willd (i.e. species with both green algae and cyanobacteria as photobiont), while the niche of *Peltigera collina* (Ach.) Schrad, the only epiphytic species among Italian *Peltigera*, has the lowest niche overlap with the others. In addition, *P. collina* has a restricted and less represented (high value of marginality) niche across Europe. Our analysis on niches breadth and marginality shows that there is a difference between the two tripartite species probably due to different substrata and climatic preferences. The hierarchical partition of functional traits shows that 34% of variance of niche breadth is explained by traits strictly associated with the Water Holding Capacity of the thallus (e.g., veins and thallus thickness). Our results corroborate the hypothesis that climatic factors strongly influence the distribution pattern of *Peltigera* species across Europe, helping to identify which species would require more conservation efforts in a climate change perspective. A future work including a species distribution modelling of the potential effects of climate change on these species would provide additional information.

6 = Molecular and morphological characterization of fungi on the bark of *Castanea sativa* in Azerbaijan

Dilzara N. Aghayeva[1], Elnara Isgenderli[1], Bianca Elena Maserti[2], Lamiya Abasova[1], Paola Bartolini[2], Alessia Pepori[2]
[1]Institute of Botany, Azerbaijan National Academy of Sciences, Badamdar highway 40, Baku, AZ1004, Azerbaijan; [2]National research Council, Institute of Sustainable Plant Protection (CNR-IPSP), Area della Ricerca di Firenze, via Madonna del Piano 10, 50019 Sesto Florentino, Firenze, Italy

Castanea sativa Mill. is a valuable tree among the species belonging to the family *Fagaceae*. The tree has big population in the northern part of Azerbaijan and is believed to be native for the Great Caucasus region. Natural and planted stands of *C. sativa* form small patches growing with other broadleaves species in the country. Pests and pathogens pose a significant threat to chestnut tree worldwide. Chestnut tree hosts many co-occurring species, among which fungi dominate in number. Recently diversity of fungi occurring in chestnut tissues has been reported from various countries including Azerbaijan (1, 2, 3, 4, 5, 6). The aim of the study was to identify the fungi associated with chestnut trees, especially those affected by *Cryphonectria parasitica* (Murrill) M.E.Barr. This work was done partly in the frame of the bilateral projects supported by Science Development Foundation of Azerbaijan in 2015 and by CNR-ANAS in 2016-2017.

About 20 bark and leaf samples have been collected in one chestnut growing site of each three districts (Zagatala, Shaki, Qabala). All samples were wrapped up separately into pieces of paper, put into the plastic bag and kept in the fridge after delivery to the laboratory. Samples were surface sterilized with 70% ethanol and plated into the Petri dishes (90 mm) containing PDA (Difco™, USA) based on standard fungal isolation techniques. In total 23 fungal cultures were obtained and incubated at room temperatures, then were grouped based on morphology first, later isolates displaying different morphotypes were selected for ITS sequencing. The nuclear ribosomal internal transcribed spacer (ITS1/2) regions, including 5.8S rRNA and parts of 18S and 28S rRNA genes were amplified using the primers ITS1 and ITS4 (7) PCR, purification and sequencing of the PCR products were carried out by using the E.Z.N.A. ® Tissue DNA Kit (Omega Bio-Tek) following manufacturer protocol.

All obtained sequences were compared to those in the NCBI database. Comparative bioinformatics analysis among the sequenced fragments displayed similarity with the following species, identified as *Botryosphaeria dothidea*, *Sirococcus castanea*, *Jattaea aurea, Phomopsis capsici* and few isolates belonged to the genus *Trichoderma*, and identified as *T. lixii*, *T. harzianum Trichoderma* sp. of the phylum Ascomycota. Other isolates were identified as *Hyphodermella rosea*, *Phanerochaete chrysosporium* and *Schizophyllum commune* of the phylum Basidiomycota. *B. dothidea*, *J. aurea*, *Ph. capsici* and two *Trichoderma* species represent new records on chestnut bark in Azerbaijan, which extend our knowledge on fungi related to chestnut.

1) Adamčíková, K., Juhásová, G., Kobza, M., and Ondruškova, E. (2013) Diversity of microfungi on branches of *Castanea sativa* in Slovakia. Pol. Bot. J., 58(2), 741–746

2) Aghayeva D.N. (2001). Mycobiota of *Castanea sativa* Mill. in Azerbaijan. For. Snow Landsc. Res., 76, 3, 405–408

3 Aghayeva D.N., Rigling D., Meyer J.,Mustafabeyli E. (2018) Fungal diversity on the bark of the chestnut tree affected by *Cryphonectria parasitica.* Acta Horticulture P. 79-86. DOI10.17660/ActaHortic.2018.1220.12

4) Akilli, S., Katircioğlu, Y.Z., Maden, S. (2009) Chestnut cankers in Black Sea region of Turkey. Acta Hortic. 815, 247–252 https://doi.org/10.17660/ActaHortic.2009.815.32

5) Visentin, I., Gentile, S., Valentino, D., Gonthier, P., Tamietti, G., Cardinale, F. (2012) *Gnomoniopsis castanea* sp. nov. (*Gnomoniaceae*, *Diaporthales*) as the causal agent of nut rot in sweet chestnut. J. Plant Pathol., 94(2), 411–419

6) Maresi, G., Oliveira Longa, C.M., and Turchetti, T. (2013) Brown rot on nuts of *Castanea sativa* Mill: an emerging disease and its causal agent. iForest – Biogeosciences and Forestry, 6(5), 294–301

7) White T.J., Bruns T., Lee S., Taylor J. (1990) Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In PCR Protocols: A guide to Methods and Applications, M.A. Innis, D.H. Gelfand, J.J.Sninsky, T.J. White, eds. (San Diego, USA: Academic Press), 315–322

6 = Characterization and biosecurity implications of fungi in an open irrigation system and rice field water

Grazia Cecchi[1], Danilo Manzi[1], Mirca Zotti[2], Anna Maria Picco[1]
[1]Department of Earth and Environmental Sciences, University of Pavia, Via Ferrata 1,27100, Pavia, Italy; [2]Department of Environmental Earth and Life Sciences (DISTAV), University of Genoa, Corso Europa 26,16132, Genoa, Italy

Waterborne fungi, in particular in open irrigation system, are still little investigated and, to date, the major of the studies carried out deals with the possible diffusion in to the irrigation waters of plant pathogens (1). However, water for irrigation can be acquired from natural water sources such as lakes, rivers, rainfall and groundwater, or from artificial sources such as reservoirs and dams and it influences the fungal diversity in the irrigation system. In fact, not only pathogens strains can be transported, but also saprotrophs and potential biological control agents (BCA). Survival in these waters is influenced by many factors such as temperature, pH, solar radiation (visible and UV), turbidity, organic material concentration, and the presence of competing, antagonistic, parasitic and/or predatory organisms. Fungi are extremophile organisms able to colonize also aquatic environments (2). In particular, in the irrigation waters, they can be metabolically active or can survive in latent forms thanks to the production of resting structures (i.e. sclerotia, chlamydospores, spores; 1). As concern rice field, many studies were carried out regarding fungal pathologies and biosecurity (3; 4;5), but to date, there is a lack of information about the role played by the paddy waters network in the diffusion and dispersion of fungi and about the ecology of these waterborne fungal strains. In this work, we investigated the fungal characterization of vital strains in a rice field located at Vistarino (Pavia, Lombardia, Italy). During the summer 2018, the complete irrigation system (canals, ditches, and paddy) was sampled in June, July and August. These waters were both filtered on acetate filters and inoculated as is on plates of PDA (Potato Dextrose Agar), SAB (Saburaud) and CZ (Czapek Dox Agar) amended with antibiotics (Chloramphenicol, Streptomycin and Penicillin G) in order to limit bacteria growth. Each sample was plated in three Petri dishes (Ø15 cm) for colture medium. The plates were incubated in the dark at 20°C and checked weekly. In total, 876, 561 and 1797 CFUs (Colonies Forming Units) were counted in canals, ditches and paddy samples, respectively. The most representative counted genera were: *Aspergillus*, *Penicillium*, *Rhizopus* and *Cladosporium* from the canals; *Cladosporium*, *Rhizopus* and *Penicillium* from the ditches; *Cladosporium*, *Fusarium*, *Trichoderma* and *Alternaria* from the paddy. However, some interesting species were also isolated such as *Epicoccum nigrum* Link, *Aureobasidium pullulans* (de Bary & Löwenthal) G. Arnaud, *Bipolaris* sp. and *Drechslera* sp.. In particular, *Fusarium*, *Bipolaris* and *Drechslera* can cause rice diseases (i.e. root rots, seed-rot and seedling disease, stackburn and brown leaf spot disease), *Cladosporium* and *Penicillium* species are common saprotrophic fungi, while *Trichoderma* species, together with *E. nigrum* and *A. pullulans*, can act as competitor and biocontrol agents against *Fusarium* genus (6). These results imply that some potential pathogenic fungi can be really diffuse to the rice plants by the irrigation system, but also confirm that other rice pathogens, such as *Pyricularia oryzae* Cavara (1981) (anamorph of *Magnaporthe oryzae* B. Couch sp. nov., 7), prefer airborne dispersion (4,8).

1) R. E. Zappia, D. Huberli, G. E. St. J. Hardy, K. L. Bayliss (2014) Plant Pathology, 63, 961-972
2) C. K. Tsui, C. Baschien, T. K. Goh (2016) In Biology of Microfungi (pp. 285 – 313). Springer, Cham
3) S. H. Ou (1985) Rice diseases. IRRI
4) A. M. Picco, M. Rodolfi (2002) Aerobiologia, 18(2), 163-167
5) M. Almaguer, T. I. Rojas, F. J. Rodríguez-Rajo, M. J. Aira (2012) European journal of plant pathology, 133(2), 473-482
6) F. Sempere, M. P. Santamarina (2009) Annals of Microbiology, 59(2), 259-266
7) B.C Couch., L.M. A Kohn (2002) Mycologia, 94(1), 683-693
8) C. M. Leach (1980) Ecology and Epidemiology, 70(3), 201- 205

6 = Mycoremediation of soil polluted by insecticides and fungicides

Chiara Daccò[1], Alessia Manassero[1], Giulio Pasinetti[1], Solveig Tosi[1]
[1] Mycology Laboratory, Department of Earth and Environmental Sciences, University of Pavia, Via S. Epifanio 14, 27100 Pavia, Italy

Bioremediation is a technique that mainly uses microorganisms (both fungi and bacteria) to degrade pollutants, such as plant protection products and hydrocarbon residues, which persist in soil, in the atmosphere or in water, exploiting their ability to use these compounds as a source of nutrients for their growth. The degradation of these substances leads to a partial or total reduction of their toxicity for the benefit of man and the environment. Mycoremediation consists in using fungal organisms to carry out the detoxification of the environment. The aim of the present research was to isolate and select fungal strains able to grow in the presence of fungicides and insecticides used in agriculture and to assess their potential in mycoremediation *in vitro*. The evaluation of the effective detoxification of the medium was possible thanks to the use of the fungus *Botrytis cinerea* MA 2753 which proved to be an excellent bioindicator for monitoring the bioremediation process[1]. More than 70 fungal strains capable of tolerating high concentrations of insecticides and fungicides were isolated (Fig. 1) from soil using the bait technique[2]. The screening process was performed using multi-well plates (Fig. 2)[3] to evaluate the growth of every strain on different fungicides and insecticides as the only carbon source (Figg. 3-4). The most tolerant strains capable of using pesticides for their growth were selected for detoxification tests in liquid substrates.

Fig. 1. Fungal isolation process

Fig. 2. Multi-well plates for growth test on pesticides

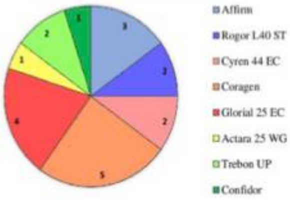


Fig. 3. Susceptibility of each insecticide to fungi (n° of strains for each compound)

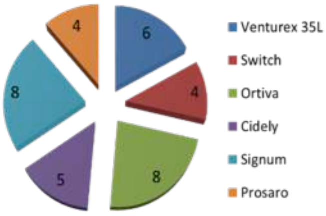


Fig. 4. Susceptibility of each fungicide to fungi (n° of strains for each compound)

The removing of toxic compounds by fungi was tested using the growth of *B. cinerea* as bioindicator. After determination of the MIC and MFC for each toxic compound on *B. cinerea*, the bioindicator was inoculated on the samples treated by mycoremediation and its growth was considered as proof of lowering of toxicity. Among the tested strains, species belonging to *Paecylomyces*, *Trichoderma*, *Chaetomium*, and *Actinomucor* showed the best results of mycoremediation. Detoxification was recorded above all on insecticides and Cidely was the most susceptible fungicide to mycoremediation. Although literature reports many data on the *Basidiomycota* species[3] able to degrade pesticides, in this research most of the active fungi belong to *Ascomycota*. The results obtained analyzing the selected strains indicate them as potentially very useful for mycoremediation processes.

1) G. M. Gadd (1986) Toxicity screening using fungi and yeast. In "Toxicity testing using microorganisms", vol. II, Dutka BJ & Bitton G (Eds.), CRC Press, Florida, 43-77
2) G. Caretta, E. Piontelli (1975) Isolation of keratinophilic fungi from soil in Pavia, Italy. Sabouraudia, 13, 33-7
3) F. Spina F., G. Cecchi, A. Landinez-Torres, L. Pecoraro, F. Russo, B. Wu, L. Cai, X. Z. Liu, S. Tosi S., G. C. Varese, M. Zotti, A. M. Persiani (2018) Fungi as a toolbox for a sustainable bioremediation of pesticides in soil and water. Journal of Plant Biodiversity, 152, 474-488

6 = From conservation to utilization: the value of *ex-situ* collections of microorganisms

Valeria Prigione, Iolanda Perugini, Luisella Reale, Giovanna Cristina Varese
Department of Life Sciences and Systems Biology, University of Torino, Viale P.A. Mattioli 25, 10125 Torino, Italy

Nowadays, microbial diversity is viewed as a valuable resource for science and industry with implications for economy and finance. The growing awareness of the importance of microbial biodiversity has resulted in an increased recognition of the value of *ex-situ* collections of microbial strains. Biological Resource Centres (BRCs) are, in fact, the pillars of biotechnological research, since they provide expertise, and specialised structures for the acquisition, preservation, identification, characterisation, and secure distribution of cultivable organisms. In recent years, the number of new microorganisms preserved in the BRCs has greatly increased, thanks to the development of initiatives for the isolation of strains (often belonging to species never described before) from extreme and unexplored environments. This led to an increase in the biotechnological value of BRCs for the development of a sustainable bio-economy.

At the Department of Life Sciences and Systems Biology, University of Torino, is active the *Mycotheca Universitatis Taurinensis* (MUT) that currently preserves more that 6,000 fungal strains ascribable to about 1,400 species. The MUT, one of the most important BRCs in Italy, can be considered a fungal biodiversity bank of particular systematic, ecological and applicative interest, and a point of reference for the Italian and international scientific, industrial, and biotechnology communities. Today, indeed, fungi are considered among the most promising organisms in biotechnological applications.

Since 2006, the MUT has been working according to the ISO 9001 standards and since 2008 is member of ECCO (European Culture Collections' Organisation) and WFCC (World Federation for Culture Collections). It also takes part to the European project MIRRI (Microbial Resources Research Infrastructure, www.mirri.org). MIRRI allowed the creation of an infrastructure that involves the main European BRCs aiming to provide high-quality services such as deposit, storage and access to samples of viable microorganisms and their derivatives. In fact, BRCs have little relevance unless the organisms they hold are recognized by the users as a high quality product in terms of purity, viability, genetic identity, and are accompanied by a maximum of relevant bioinformation.

More recently (September 2017), a Joint Research Unit for the implementation of the Italian node of MIRRI (MIRRI-IT JRU - http://www.mirri-it.it) has been founded with the aim of developing a tight network of Italian collections of microbial resources. The mission is to overcome fragmentation in availability of resources and services, enhancing the quality management system of the collections while focusing on needs, and challenges of the stakeholders interested in the biotechnological transfer of these resources.

6 = The fungal component associated with *Cannabis sativa* L. samples for therapeutic use

Temporiti Marta Elisabetta Eleonora[1], Calvi Lorenzo[2], Tosi Solveig[1]
[1]Department of Earth and Environmental Sciences, University of Pavia, via Sant'Epifanio 14, 27100 Pavia, Italy; [2]Department of Agricultural and Environmental Science University of Milan, Via Celoria 2, 20133 Milan, Italy

Inflorescences of *Cannabis sativa* L. contain cannabinoids and they can be used in preparations as drugs for the treatment or mitigation of different human health disorders (1). These preparations are usually subjected to gamma radiation to lower their microbial contamination, because they have to comply with the limits of microbial concentration reported by pharmacopoeia (2). The aim of this research was to investigate which growing and drying conditions were contributing to a lower fungal contamination of the inflorescences of *Cannabis* plants (Fig. 1) in order to avoid the gamma radiation step of the preparations. The inflorescences of *Cannabis* plants were examined after the cultivation in two different conditions (indoor and outdoor). After the growth, three different drying locations were tested: 1) closed and ventilated room [in according with Good Agricultural and Collection Practices, GACP (3)]; 2) closed room without any ventilation, 3) a porch; 4) a barn dedicated to straw storage. All dried inflorescences were plated on rich medium in Petri dishes and, after incubation, the colony forming units (CFU; Fig. 2) of fungi were counted and identified. As control samples were analyzed commercial gamma radiated inflorescences used to therapeutic purpose. The results obtained showed that counted CFU for inflorescences grown indoor were less than ones found in inflorescences cultivated outdoor. This difference was observed in all samples independently from drying conditions. Among the samples grown indoor, those dried in the barn were the ones with higher CFU counting, but the contamination was always lower than limit reported by European Pharmacopoeia (4). On the other hand, the samples cultivated outdoor presented differences in CFU amount according to the drying method, and the number ranged from 10^2 (inflorescence drying in the porch) to 10^5 (unventilated closed room). The results showed that the presence of aeration (artificial ventilation in one of the rooms or natural ventilation in the porch) was important for an efficient drying and resulted also in a lower number of CFU when compared to the samples dried in the closed room and in the barn. Gamma radiated samples showed 10^2 CFU that was in accordance with European Pharmacopoeia (4). The isolated fungi (Fig. 3) belonged to three different genera: *Aspergillus*, *Cladosporium* and *Penicillium*. In samples drying in the ventilated closed room, only *Penicillium griseoroseum* was found, while in the ones drying in the unventilated room there were *P. griseoroseum*, *P. fannelliae* (indoor grown samples) and *Aspergillus ochraceus* (outdoor grown samples). From samples drying in the porch or in the barn, *Cladosporium cladosporioides* and *A. ochraceus* were isolated. *A. ochraceus* was also found in the inflorescences grown outoor and dried in the unventilated room. It is important to pay attention to the presence of *A. ochraceus* because of his ability to produce ochratoxin A. This mycotoxin is nephrotoxic, carcinogenic, teratogenic, and immunotoxic and it can contaminate cereal and agricultural products (5). From gamma-radiated inflorescence only *Cladosporium cladosporioides* and *C. sphaerospermum* were isolated. In conclusion, all of samples grown indoor presented a CFU number that was lower than the limit concentration reported by European Pharmacopoeia for non gamma-radiated samples (4). These results suggest that attention to culture and drying method could be a possible alternative to radiation of inflorescences.

Fig. 1. Inflorescence of *Cannabis sativa*

Fig. 2. Colony forming units

Fig. 3. Isolated fungi

1) Kumar R.N., Chambers W.A., Pertwee R.G. (2008). Pharmacological actions and therapeutic uses of cannabis and cannabinoids. Anaesthesia, 56(11), 1059–1068

2)Hazekamp, A. (2016). Evaluating the Effects of Gamma-Irradiation for Decontamination of Medicinal Cannabis. Frontiers in Pharmacology, 7

3) World Health Organization Geneva, 2003. WHO guidelines on good agricultural and collection practices (GACP) for medicinal plants

4) European Pharmacopoeia 8. Article 5.1.8

5) Wang, Y., Wang, L., Wu, F., Liu, F., Wang, Q., Zhang, X., et al. (2018) A consensus ochratoxin A biosynthetic pathway: insights from the genome sequence of *Aspergillus ochraceus* and a comparative genomic analysis. Applied and environmental microbiology, AEM.01009–18.

6 = Analyzing the relationship between yield and pollen concentrations in chilean hazelnut plantations

Lorenzo Ascari[1,*], Consolata Siniscalco[1], Giovanna Palestini[1], María José Lisperguer[2], Eloy Suarez Huerta[3], Tommaso De Gregorio[3], Laura Giustarini[3], Simone Bregaglio[4]
[1]Department of Life Sciences and Systems Biology, University of Turin, Viale Mattioli, 25,10125 Torino, Italy; [2]Hazelnuts Company Division, Ferrero Trading Luxembourg, Rue de Trèves, L 2632 Findel, Luxembourg; [3]CREA - Council for Agricultural Research and Economics, Research Center for Agriculture and Environment, I-40128 Bologna, Italy; *Corresponding Author, E-mail: lorenzo.ascari@unito.it, Tel.: +39 011 6705985

Chilean hazelnut industry has been recently experiencing a solid development and nut production has consistently increased. *Corylus avellana* is subjected to strong inter-annual yield fluctuations, which raise the issue of price predictability. In alternate bearing, a high fruit load hinders flower initiation thus leading to a poorer fruit production in the following year. This suggests the use of pollen data as a proxy for flower number in the prediction of hazelnut irregular bearing. We addressed this question using high-resolution yield data and airborne pollen concentrations coming from four hazelnut plantations located in Central Chile and three hazelnut cultivars. Pollen parameters were calculated from modelled and non-modelled pollen seasons, after selecting for the best modelling approach. An alternate bearing index was calculated in order to characterize hazelnut biennial bearing and to select orchards with stronger yield variability. The calculated pollen parameters were then correlated to selected and non-selected yield data. Significant correlations were found between pollen parameters and nut yields and the relationship was stronger when alternate bearing was higher. Among parameters describing pollen season intensity, the modelled pollen season peak had the highest coefficient of variation (CV) and its positive correlation with crop production was consistent across the studied sites and cultivars. Our data strongly support the integration of airborne pollen data in harvest prediction systems of hazelnut as a predictor of alternate bearing and annual yield.

6 = Archaeobotanical analysis from the long-term rural settlement of Contrada Castro (Corleone, Palermo): preliminary data

Giuseppe Bazan[1], Claudia Speciale[2], Angelo Castrorao Barba[3], Stefano Vassallo[4], Pasquale Marino[5],
[1]Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF), University of Palermo, Italy; [2]National Institute of Geophysics and Volcanology, Napoli, Italy; [3]CSIC (Consejo Superior de Investigaciones Científicas-Spanish National Research Council), EAA, LAAC, Granada, Spain; [4]"Soprintendenza BB.CC.AA" of Palermo, Italy; [5]Bona Furtuna LLC, Los Gatos, CA, USA.

The project "Harvesting Memories: Ecology and Archaeology of Monti Sicani landscapes" aims to analyse the long-term relationship of landscape dynamics and settlement patterns in a Mediterranean inland of Central-Western Sicily. The project combined different interdisciplinary approaches of vegetation science, landscape ecology, archaeobotany, history and archaeology in order to diachronically understand and reconstruct the human-society-environment interactions.

From 2017 to 2019 a new rural settlement has been investigated in Contrada Castro (Corleone, Palermo). The excavation in Contrada Castro (1) showed a clear case of long-term occupation of a hill-top site during Late Archaic/Classical age (6th-5th c. BC) and the Byzantine and Islamic period (7th-11th c. AD).

Soils from the archaeological excavation were sampled to obtain evidences about paleo-vegetation and vegetal paleo-diet. Archaeobotanical data (seeds and charcoal remains) represent an informative source in human-environmental dynamics to collect specific data on a small scale in terms of chronology and topography (2). Furthermore, presence of edible plants as cereals, pulses and fruit characterize their use as economical resources. Unfortunately, archaeobotanical analyses from archaeological sites in Sicily are still not very common (3). For the reconstruction of the paleo-environment and the use of woody resources from the three chronological phases of the site, a total of 239 liters of soils were analysed. Taxonomical identification was made by optical microscopy through the comparison with the reference collection and specific atlases (4). More than 400 wood charcoals were observed, about 80% of them was identified. Thanks to comparisons with the current vegetation, so far 9 species have been identified: *Quercus ilex* L., *Quercus* cfr. *pubescens* Willd., *Pistacia terebinthus* L., *Rhamnus alaternus* L., *Fraxinus ornus* L., *Ulmus minor* Mill. subsp. *canescens* (Melville) Browicz & Ziel., *Acer campestre* L., *Ostrya carpinifolia* Scop., *Populus nigra* L. Identification reached the detail of genus or family in 5 cases, *Phillyrea* sp., *Sorbus* sp., *Pyrus* sp. and maybe one species belonging to the family of Moraceae. The woody vegetation is therefore represented by evergreen oaks, semi- and deciduous oaks, maples, ash trees, associated with riparian species such as elm, poplar and hornbeam, and shrub species such as backthorn, terebinth, sorb and plum. Cultivated species are mostly not represented. Despite the widespread presence of the evergreen oaks in the whole record, differences between the three chronological phases were identified, highlighting a selective use of the wild species present in the area and a specific collection of wood for the hearths. Archaeological layers from last phase of occupation of the site are characterised by the presence of pulses and cereal kernels, notably concentrated in some hearths. Their analyses allow to identify staple sources for the village and agricultural techniques in the area.

1) A. Castrorao Barba, R. Miccichè, F. Pisciotta, P. Marino, G. Bazan, C. Aleo Nero, S. Vassallo (2018) The Journal of Fasti Online, 1-12
2) C.A. Hastorf, V.F. Popper (1988) Chicago. ISBN: 978022631893
3) BRAIN Network (2018) http://brainplants.successoterra.net/sites.html
4) D.M Pearsall. (2009), Walnut Creek. ISBN-10: 1598744720

6 = Characterization of phychoactive plant pollen: a new tool in forensic investigations

Clara Conte[1]; Laura Cornara[1].
[1]Department of Earth, Environment and Life Sciences, University of Genova, Italy
clara.conte93@hotmail.it

Palynology, based on the micromorphological characterization of pollen and spores, can be a valid ally for the resolution of forensic cases. Since its first application in 1959 for a murder in Austria, this discipline has been applied to cases of illegal smuggling involving drugs, weapons, medicines, and money (1). Also in Italy, several examples of palynology applied to kidnapping and homicide crime solving have been reported. In particular, a close connection was found between forensic palynology and aerobiology, as a reference tool for establishing the season when unknown death occurred (2-4).The use of palynology is also useful to trace possible pollen contamination of drugs: e.g. in a seizure of a batch of drugs in New York in 1991 (5). It is also possible to solve cases of murder, rape, theft, and food fraud (6). Pollen can adhere firmly to any surface and resist decomposition, while its morphology can be a distinctive feature for the identification of plant species and habitats (1,4,6). The present study concerns the pollen of 12 species of psychoactive plants belonging to 9 families, including illegal drug plants (e.g. *Erythroxylum coca*; *Lophophora williamsii*), and plants used as legal alternatives to drugs (e.g. *Leonotis leonurus*; *Brugmansia suaveolens*), some of which are currently marketed on internet. Given the lack of literature in this study, we started to compile a palynological atlas of psychoactive plants, aimed at providing support in the forensic field. These data can been useful to trace the origin of illegal drugs, and to identify the most likely route of drug shipping (2). The selected pollen grains was sampled from herbarium specimens, the Reference Pollen Collection (Palinoteca), or prepared as fresh samples collected from the Botanical Garden of Genoa University and the Hanbury Botanical Garden of Ventimiglia. Unacetolyzed pollen was prepared in glycerin jelly containing basic fuchsin dye. The analysis was carried out on 40 pollen grains for each species at the magnification of 40x.The diagnostic features of pollen grains studied were: length of polar axis (P) and equatorial axis (E), pollen shape (P/E ratio), number and type of apertures, exine thickness and sculpture. For the two species belonging to Convolvulaceae (*Ipomoea violacea* and *Argyreia nervosa*), also spine length, interspinal distance, pore diameter, exine and nexine thickness, have been considered. Observations were carried out by using light and scanning electron microscopy. In some cases, the analyses showed slight morphological differences compared to literature data, probably due to a different number of measurements, while in other cases new morphological features were recorded. A web repository is being created to make morphometric data and pictures of psychoactive plant pollen available, thus providing a useful tool for forensic investigations.

1) DC. Mildenhall (2008) SIAK-Journal − Zeitschrift für Polizeiwissenschaft und polizeiliche Praxis, 4, 35-52
2) A.M. Mercuri, A.Travaglini (2009) GEA-European journal of aerobiology and environmetal medicine, 2, 3-6
3) Montali E., Mercuri A.M., Trevisan Grandi G., Accorsi C. A. (2006) Towards a “crime pollen calendar” Pollen analysis on corpses throughout one year. Forensic Science International, 163, 211-223
4) A.M. Mercuri (2015) Applied palynology as a trans-disciplinary science: the contribution of aerobiology data to forensic and palaeoenvironmental issues. Aerobiologia, 31, 323-339
5) E. A. Stanley (1992) MICROSCOPE, 40, 149-152
6) V.M. Bryant, G.D. Jones (2006) Forensic Science International, 163, 183–197

6 = Preliminary phenological study and seasonal variability of alkaloids in *Senecio inaequidens* DC.

Paola Fortini, Luana Aquilano, Maria Iorizzi, Piera Di Marzio
Department of Bioscience and Territory, University of Molise, Pesche (IS), Italy

Allochthonous species are one of the main environmental emergencies and are considered the second leading cause of biodiversity loss on a global scale. Molise is one of the Italian regions with the lowest number of allochthonous species, but the phenomenon is constantly growing and needs to be monitored. *Senecio inaequidens* DC., originating in Southern Africa, after its arrival (about 20 years ago) and subsequent stabilization, shows signs of expansion in recent years. *S. inaequidens* is a perennial herbaceous species with a bushy appearance, favouring anthropized environments such as road margins, escarpments and railway embankments. *S. inaequidens* produces, as secondary defence compounds, the pyrrolizidine alkaloids (PA) which can reach 18% of the dry weight of the plant. These alkaloids are accumulated mostly in the vacuoles of the cells of the inflorescences and peripheral tissues of the stem. PAs are the most common toxins among those that constitute a serious danger to the health of wild and farmed animals (1) and they also represent a significant carcinogenic danger for mammals, including humans, through the consumption of contaminated cereals, honey, milk and eggs. The aim of this work was to study the phenological behavior of *S. inaequidens*, following the phenological key proposed by Ellenberg (2), and to measure how the content of the PAs inside the plant varies throughout the annual cycle in four stations in the province of Isernia. Synthetically, *S. inaequidens* individuals tend to be fully developed plants almost all year round, showing only a short period of yellowing between December and February, which is never total, since green stems are always present growing at the base. The flowering period covers the period from May to mid-November and in this period we can observe peaks in June and late September-early October. Even plants with dry branches showed the presence of flower buds. Yellowing in the coldest months is accompanied by fruit ripening and seed dispersion. The highest PAs concentration is obviously in the inflorescence and in June it reaches the highest values when there is a 50% greater flowering. Throughout the entire growing season, the PAs content is variable in the stems but fairly constant in the leaves. These preliminary results are useful for a subsequent qualitative-quantitative evaluation of the alkaloids contained in *S. inaequidens* and for its management.

Fig. 1. Different phenological phases in *Senecio inaequidens* following Ellenberg's indices

1) EFSA (2007) EFSA Panel on Contaminants in the Food Chain (CONTAM) (2011) Scientific opinion on pyrrolizidine alkaloids in food and feed. EFSA J., 9, 2406
2) Ellenberg H. (1974) Zeigerwerte der Gefäbpflanzen Mitteleuropas. Scripta Geobot, 9, 1-97

6 = Study of a little known landrace of *Fagopyrum tataricum* (L.) Gaertn. of Valtellina (Northern Italy)

Annamaria Giorgi[1,2], Gigliola Borgonovo[3], Valeria Leoni[2], Giulia Ceciliani[2], Luca Giupponi[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, University of Milan, Via Celoria 2, 20133 Milan, Italy; [3]Department of Food, Environmental and Nutritional Sciences, University of Milan, Via Celoria 2, 20133 Milan, Italy

Conservation and valorization of landraces are topics of great interest since many are endangered genetic resources that could be useful not only in crop improvement plans but also to support the economy of territories where they are (or were) traditionally cultivated. This research analyzed the characteristics of a little known landrace of *Fagopyrum tataricum* ("Grano Siberiano Valtellinese") (Fig. 1) introduced at the end of the 18th century in the mountain areas of Valtellina (SO), a valley in the Northern Italy, and which has now almost disappeared (1). Agronomical, ecological (functional strategy) and phytochemical (rutin, quercetin and fagopyrin content) analyses were carried out in order to characterize this landrace and compare it with other genotypes of *F. tataricum* and *F. esculentum*. Analysis showed that the "Grano Siberiano Valtellinese" is the genotype best adapted to the environmental conditions of Valtellina. In fact, in the experimental fields set up in a mountain area of Valtellina, it developed taller plants with a high number of flowers. Furthermore, it was found to be the most tolerant to stress (functional strategy: CS/CSR). Phytochemical analyses showed that this genotype, compared to other local landraces of *F. esculentum*, has a higher concentration of rutin in the seeds (764 ± 39 µg/g) and in the shoots (370 ± 66 µg/g) which make it interesting for the production of nutraceutical foods. "Grano Siberiano Valtellinese" could also be used to make herbal teas or cosmetics (*F. tataricum* is included in the European Cosmetic Ingredient database) as occurred for decades in Eastern countries.

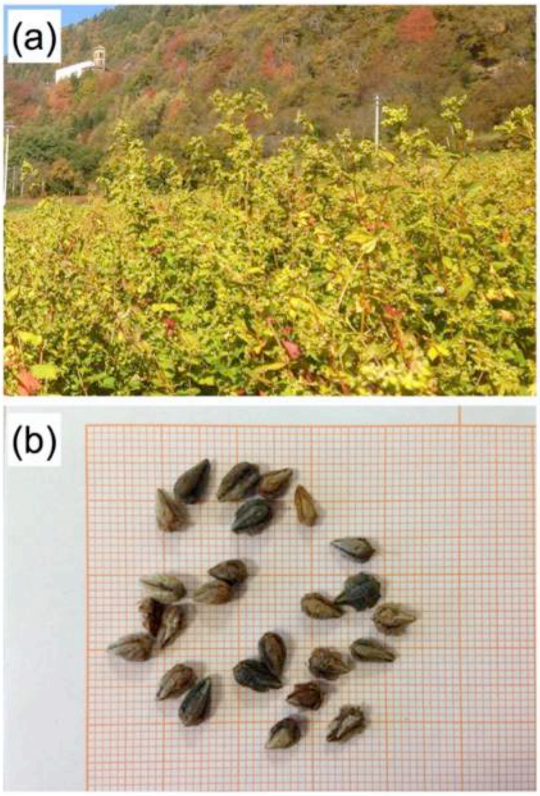


Fig. 1. "Grano Siberiano Valtellinese" landrace: plants in the experimental field (a) and seeds (b)

1) L. Giupponi, G. Borgonovo, S. Panseri, A. Giorgi (2019) Multidisciplinary study of a little known landrace of *Fagopyrum tataricum* Gaertn. of Valtellina (Italian Alps). Genetic Resources and Crop Evolution, 66, 783-796

6 = Quality traits of saffron (*Crocus sativus* L.) produced in Italy

Luca Giupponi[1], Valeria Leoni[1], Giulia Ceciliani[1], Sara Panseri[2], Radmila Pavlovic[1,2], Guido Lingua[3], Alfredo Di Filippo[4], Annamaria Giorgi[1,5]

[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 Health, Animal Science and Food Safety, University of Milan, Via Celoria 10, 20133 Milan, Italy; [3]Dipartimento di Scienze ed Innovazione Tecnologica, Università degli Studi del Piemonte Orientale, Via Bellini 25, 15121 Alessandria, Italy; [4]Department of Agriculture and Forest Sciences, Via de Lellis, 01100 Viterbo, Università degli Studi della Tuscia; [5]Department of Agricultural and Environmental Sciences - Production, Landscape and Agroenergy, University of Milan, Via Celoria 2, 20133 Milan, Italy

Saffron (*Crocus sativus* L.) is considered the most expensive spice in the world (1). This plant is an autumn-flowering geophyte and is believed to have originated in Greece, Asia Minor and Persia. It is mainly produced in Iran but also in some European countries such as France, Greece, Spain and Italy (2, 3). This research is the first to investigate the quality of saffron produced in Italy. Quality analysis was performed according to ISO 3632 1,2:2010-2011. In particular, moisture content and the amount of picrocrocin (flavour strength), safranal (aroma strength) and crocins (colouring strength) of about 400 samples collected from throughout Italy were evaluated using a spectrophotometric methodology. Qualitative analysis was carried out from 2015 to 2018 considering about 100 samples per year.

Results show that more than 84% of samples belong to the first quality category, about 10% belong to the second quality category, approximately 2% belong to the third quality category and the rest of the samples are outside all categories. Exclusion of samples from the first category was mainly due to high moisture content (>12%) and lower coloring strength (crocin < $200\, A^{1\%}_{1\,cm}$). This research shows that high quality saffron can be produced all over Italy from plain to mountain areas. In conclusion, some good practices for the production of this precious spice are provided to further improve the quality of Italian saffron. This research was supported by “FISR-MIUR Italian Mountain Lab” project.

Fig. 1. *Crocus sativus* flowers

Fig. 2. Spice

1) A. Giorgi, D. Bertoni, A. Manzo, S. Panseri, (2015) L’Oro Rosso delle Alpi. Manuale tecnico-scientifico di produzione dello zafferano. Biblion ed., Milano

2) A. Manzo, S. Panseri, D. Bertoni, A. Giorgi, (2015) Economic and qualitative traits of Italian Alps saffron. Journal of Mountain Science, 12, 1542-1550

3) Giorgi A., Pentimalli D., Giupponi L., Panseri S. (2017) Quality traits of saffron (*Crocus sativus* L.) produced in the Italian Alps. Open Agriculture, 2, 52-57

6 = Evaluation of the invasiveness of non-native plant species in the "Bosco Siro Negri" Natural Reserve (Pavia, Lombardy, Italy)

Carlo Maria Cusaro, Martina Rogledi, Maura Brusoni, Francesco Bracco
Department of Earth and Environmental Sciences, University of Pavia, Via S. Epifanio 14, 27100 Pavia, Italy

The "Bosco Siro Negri" Integral natural Reserve covers 11 ha and is situated along the Ticino river in the Po Plain, in the municipality of Zerbolò, Pavia. It is an alluvial forest inserted in a rural ecosystem characterized by rice, corn and poplar cultivation. The Reserve is managed by the Department of Earth and Environmental Sciences of the University of Pavia, which organizes scientific research, educational and dissemination. Being a wild area close to human activities, mostly related to agriculture, it is subject to anthropic disturbance that affects the spread of invasive alien species (IAS).

The aim of this study was to evaluate the invasiveness of IAS within and along the perimeter of the Reserve.

Five different habitats have been identified within and around the Reserve: wood, wood clearings, wood perimeter, poplar cultivation and uncultivated meadows. In these habitats, ten IAS have been identified and detected: *Ambrosia artemisifolia* L., *Amorpha fruticosa* L., *Artemisia verlotiorum* Lamotte, *Erigeron annuus* (L.) Pers., *Oenothera biennis* L., *Phytolacca americana* L., *Robinia pseudoacacia* L., *Solidago gigantea* Aiton, *Sorghum halepense* (L.) Pers. and *Sycios angulatus* L.

The coverage percentage value according to Braun-Blanquet method (1964) and the invasiveness index, as proposed by Tyler (2015), have been calculated for each IAS. Tyler Index (TI) ranges from 0 to 40,1 (1).

The abundance values and the Tyler Indices obtained for each plant in the different surveys have then been compared and studied using ANOVA. The analysis has been performed with R-software (2).

The results of ANOVA show that the most distributed IAS within the entire reserve are *A. artemisifolia* (TI = 16,1)*, A. fruticosa* (TI = 20)*, A. verlotiorum* (TI = 18)*, R. pseudoacacia* (TI = 15,5) and *S. gigantea* (TI = 23)*,* that are also the species with the highest mean values of invasiveness index.

A. artemisifolia has been detected in wood clearings, wood perimeter, poplar cultivation and uncultivated meadows: the highest mean coverage percentage values have been registered in the wood perimeter (10,5%) and in the poplar cultivation (20,5%). *A. fruticosa* has been found only in the habitat of wood clearings, with a high mean coverage percentage value (75,38%). *A. verlotiorum* has been recorded in the habitats of wood perimeter, poplar cultivation and uncultivated meadows: the highest mean coverage percentage values have been registered in the wood perimeter (15,22%) and in the poplar cultivation (51,6%). *R. pseudoacacia* has been detected in wood clearings (40%) and wood perimeter (37%). Finally, *S. gigantea,* the species with the highest value of invasiveness index, has been observed in wood clearings, wood perimeter, poplar cultivation and uncultivated meadows: the highest mean coverage percentage values have been registered in uncultivated meadows (67%).

Our results show that *S. gigantea* and *A. fruticosa* represent the most spread and dangerous IAS within and around the Reserve "Bosco Siro Negri" and control measures are needed. Moreover, the obtained results highlight that a thick autochthonous vegetation represents an obstacle to the spread of IAS into the Reserve: in fact, *S. gigantea* and *A. fruticosa* are present only where the vegetation is sparse and discontinuous.

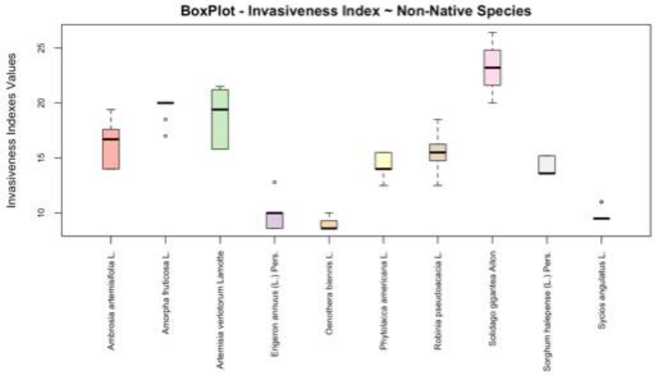


Fig. 1. Values of Invasiveness Indexes for each species

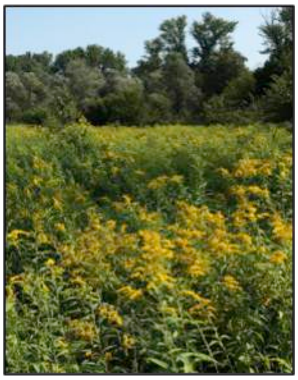


Fig. 2. *S. gigantea*

Fig. 3. *A. fruticosa*

1) Tyler T, Karlsson T, Milberg P, Sahlin U, Sundberg S (2015) Invasive plant species in the Swedish flora: developing criteria and definitions, and assessing the invasiveness of individual taxa. Nordic Journal of Botany 33: 300–317, 2015
2) Rcore Team (2013) R: A language And Environment For Statistical Computing. R Foundation For Statistical Computing, Vienna, Austria.Isbn3-900051-07-0, Url Http://zww.r-project.org/

Work funded by Ministry of the Environment and Protection of Land and Sea of Italy - Natural Reserve "Bosco Siro Negri"

6 = Alien flora across European sand dunes

Silvia Giulio[1], Alicia Teresa Rosario Acosta[1], Marta Carboni[1], Juan Antonio Campos[2], Milan Chytrý[3], Javier Loidi[2], Jan Pergl[4], Petr Pyšek[4,5], Maike Isermann[6], John A. M. Janssen[7], John S. Rodwell[8], Joop H. J. Schaminée[7], Corrado Marcenò[2]

[1]Roma Tre University, Department of Science, Rome, Italy; [2]University of the Basque Country (UPV/EHU), Bilbao, Spain; [3]Department of Botany and Zoology, Faculty of Science, Masaryk University, Brno, Czech Republic; [4]Department of Invasion Ecology, Institute of Botany, The Czech Academy of Sciences, Průhonice, Czech Republic; [5]Department of Ecology, Faculty of Science, Charles University, Prague, Czech Republic; [6]Department of Ecology, Bremen University, Bremen, Germany; [7]Wageningen Environmental Research, Wageningen, The Netherlands; [8]Independent Researcher, Lancaster, UK

The introduction of alien species in new environments is one of the main drivers of biodiversity decline in a growing number of ecosystems worldwide, and in Europe it is expected to increase with intercontinental trade, socioeconomic activities and the ongoing climate change, especially in coastal areas (1). The coastal dunes of Europe are an ideal model system to analyse invasion processes in habitats across varying disturbance regimes, because they display contrasting diversity patterns along the seashore-inland ecological gradient, and the gradual differences in macro-bioclimatic conditions and socioeconomic backgrounds across European regions are related to variations in patterns of invasion (2). In this context we analysed, for the first time, the composition of the alien flora of the sand dune belts surrounding Europe, using data from the European Vegetation Archive (EVA), a large consolidation consortium of multiple vegetation-plot databases (3). We asked the following questions: 1) What is the level of invasion across the sand dunes surrounding Europe? 2) What invasion trajectories do alien plants follow to reach these dunes? 3) What life forms are most prominent among the alien plants? 4) What is the degree of overlap in alien species across the main gradients? For each of these questions, and through different quantitative metrics, we first considered patterns of (a) the sand dunes system of all the European coastal belts as a whole. Then we inspected how each pattern varied across (b) the two most pioneer coastal sand dune habitats, i.e. shifting dunes and grey dunes, and across (c) the main European biogeographical coastal regions, i.e. Atlantic, Baltic, Black Sea and Mediterranean bioregions. We observed high levels of invasion, above all by annual herbs, and by species with a broad niche, and at the same time subtle adaptations to specific positions in the gradients of habitats and bioregions. Invasion levels didn’t vary sensibly across the habitats but did so across specific biogeographical regions, where invasions were also different in nature, with highest alien species diversity on Atlantic dunes but highest relative frequency of few alien species on Black Sea dunes. North America resulted as main donor of alien plants, and the Mediterranean basin was reconfirmed as being more of a donor than a recipient of alien plants. Overall, the study provides a comprehensive assessment of alien plant invasions on the sand dunes of European coastal bioregions, and may represent a contribution for priorities establishment in invasion management strategies, both at European and inter-regional level.

(1) Janssen J.A.M., Rodwell J.S., Criado M.G., Arts G.H.P., Bijlsma R.J., Schaminee J.H.J. (2016) European Red List of Habitats : Part 2. Terrestrial and freshwater habitats. European Union

(2) Chytrý M., Pyšek P., Wild J., Pino J., Maskell L.C., Vilà M. (2009) European map of alien plant invasions based on the quantitative assessment across habitats. Diversity and Distributions, 15(1), 98-107

(3) Chytrý M., Hennekens S.M., Jiménez Alfaro B., Knollová I., Dengler J., Jansen F., et al. (2016) European Vegetation Archive (EVA): an integrated database of European vegetation plots. Applied Vegetation Science, 19(1), 173-180

6 = The BambApp project: a citizen science network for monitoring and defining the distribution of bamboos in Piedmont and Aosta Valley (NW Italy)

Andrea Mainetti[1], Enri Simone Ravetto[1], Valerio Mezzasalma[2], Fabrizio De Mattia[2], Jessica Frigerio[2], Roberto Damilano[3], Sandra Buzio[3], Michele Lonati[1]

[1]Department of Agriculture, Forest and Food Sciences, University of Torino, Largo Paolo Braccini 2, 10095 Grugliasco (TO), Italy; [2]FEM2-Ambiente srl, Piazza della Scienza 2, 20126 Milano (MI), Italy; [3] Ente di gestione delle Aree protette del Po torinese, Corso Trieste 98, 10024 Moncalieri (TO), Italy

The subfamily Bambusoideae comprises the least-understood and most diverse group of plants in Poaceae (1). Three tribes belong to this subfamily: Bambuseae (tropical woody bamboos), Olyreae (herbaceous bamboos), and Arundinarieae (temperate woody bamboos), all of them not native to Europe. However, in the last years several bamboo species (mainly Arundinarieae) have been spread for both ornamental and productive purposes among plant nurseries and private citizens in Europe and Italy as well. In Piedmont and Aosta Valley (north-western Italy) some species belonging to the genus *Phyllostachys* have been included in local black lists (2, 3) due to their vegetative expansion often remarkable, but the issue of bamboo invasiveness and threat to natural environment is still uncertain (4). The 'BambApp' project (December 2017-April 2019) aimed to fill the knowledge gap about the presence and distribution of bamboo species naturalized in Piedmont and Aosta Valley, as a preliminary step toward the definition of bamboo invasiveness. The project was based on the participation of private citizens using the iNaturalist mobile application (5) for the monitoring. Nowadays, citizen sciences projects are considered suitable approaches to increase species data collection and monitoring, while ensuring generally lower costs than traditional research. Within the project, contributors were asked to provide latitude/longitude, altitude, and stand extension for every record of naturalized bamboo. Moreover, four pictures (i.e. a plant basis, an intermediate node, leaves, and entire stand) and two specimens (i.e. one branch and some leaves) were required. The leaf specimens were used to identify 100 records at species level through DNA barcoding and RAPD methods. The resulting species represented the reference base to recognize the morphological characters used for the visual identification all other records, from the respective pictures. The contributors recorded 937 stands in the survey area, belonging to nine different species. Only two records were not bamboos while only nine stands were recorded two times. An estimate of the cost of two researchers monitoring the same stands (distributed across 14,500 km) reached 27,000 €. Therefore, citizen science has proved to be a reliable and cost-effective tool for monitoring also in the case of the present project. The most frequent and widespread species were *Phyllostachys aurea* Carrière ex Rivière & C.Rivière (n=620, 67%), *Phyllostachys viridiglaucescens* (Carrière) Rivière & C.Rivière (n=148, 16%), and *Pseudosasa japonica* (Siebold & Zucc. ex Steud.) Makino ex Nakai (n=56, 6%), respectively. Furthermore, one bamboo species new for the Italian alien vascular flora and two species new for both Piedmont and Aosta Valley were found. Only 0.3% of the stands were reported above 800 m a.s.l., up to 1034 m a.s.l. for a *Phyllostachys viridis* (R.A.Young) McClure stand. Stand size ranged between few square meters to more than one hectare but more than 73% covered less than 100 m^2. As additional outcome of the project, a descriptive photographic guide for the identification of naturalized bamboo species of Piedmont and Aosta Valley was released in March 2019 (6).

1) Soderstrom T., Calderon C. (1979) A Commentary on the Bamboos. Biotropica, 11(3), 161-172
2) Reg. Piemonte (2015) Black list-Management list. Gruppo di lavoro specie esotiche RP, Torino, Italy
3) Bovio M. (2016) Agg. Lista Rossa e Nera della flora vascolare della Valle d'Aosta. Rev. Vald. Hist. Nat., 70, 57-74
4) Pagad S. (2016) Bamboos and Invasiveness. INBAR Working Paper 77
5) https://www.inaturalist.org/
6) Lonati M. et al. (2019) Guida all'identificazione dei bamboo naturalizzati in Piemonte e Valle d'Aosta. DISAFA, Grugliasco (TO)

6 = The riparian vegetation of Pioverna river (Lecco, Italy): preliminary analysis

Paolo Fogliata[1], Luca Giupponi[2], Alessio Cislaghi[1,2], Annamaria Giorgi[1,2], Gian Battista Bischetti[1,2]
[1]Department of Agricultural and Environmental Sciences (DiSAA), University of Milan, Via Celoria 2, 20133 Milan, Italy; [2]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

This research was performed as part of a cooperation agreement between Lombardy Region and the University of Milan to provide scientific support for the river maintenance of the territory and the management of riparian vegetation. To obtain information about river vegetation for management purposes, it is important to understand its ecological succession, identifying the possible dynamic stages and the characteristics of the mature vegetation that can grow in the region.

The Pioverna river, Lecco province (Italy), is one of the areas studied as part of the cooperation agreement. Phytosociological relevés were carried out along the river from April 2019 and assessment will continue until September 2019. The relevés were analysed using cluster analysis and CSR functional strategy and other ecological features of the species of each group were also evaluated (1 - 2 - 3).

This abstract shows the preliminary results of the first surveys. Cluster analysis showed 3 groups: a) herbaceous vegetation (Figure 1a), b) vegetation dominated by *Buddleja davidii* Franch. (Fig. 1b), c) woody vegetation with *Salix* spp. and *Robinia pseudoacacia* L. (Fig. 1c). These are three stages in the ecological succession of this riparian ecosystem. Cluster *a* represents the first stage mainly composed by pioneer herbaceous species. The second (*b*) is a community dominated by one exotic and invasive plant (*Buddleja davidii*), which is widespread near Italian rivers. The last (*c*) represents vegetation closer to maturity where the coverage of *Salix* spp. has partially prevented the spread of *Buddleja davidii.* Overall strategy abilities indicate that group *b* has the highest value of competitive species, underlining that invasive plants can block community assembly through succession. The other two groups (*a* and *c*) have a higher proportion of stress-tolerant and ruderal species.

For a complete description of the vegetation of the Pioverna river, the assessment will continue through summer 2019.

Fig. 2. Type of vegetation: a) herbaceous vegetation, b) vegetation dominated by *Buddleja davidii,* c) woody vegetation with *Salix* spp. and *Robinia pseudoacacia*

1) Hunt R., Hodgson J. G., Thompson K., Bungener P., Dunnet N. P., Askew A. P. (2004) Applied Vegetation Science, 7, 163-170
2) Kyle G. & Leishman M. R. (2009) River Research and Application, 25, 892–903
3) Giupponi L., Corti C., Manfredi P., Cassinari C. (2013) Plant Sociology, 50(2), 47-56

POST CONGRESS

Hortus botanicus Leiden: 429 years of stability and change

Gerda A. van Uffelen
Hortus botanicus Leiden, g.a.van.uffelen@hortus.leidenuniv.nl

Founded in 1590, the Hortus botanicus Leiden has always been part of Leiden University. Right from the start it was a garden equipped for research and education, but also open to the public – and therefore a true museum, even by today's standards.

Its position in the university has varied over the centuries. The latest big change was the separation of the Hortus and the National Herbarium of the Netherlands (formerly Rijksherbarium), when the herbarium was moved to Naturalis Biodiversity Center in Leiden. The Hortus now only keeps a living plant collection plus a small library.

Our long history and original location in the heart of an old Dutch city are among our unique selling points; they have caused a spectacular increase in the number of visitors (around 175.000 annually), bringing in more money, but also demanding new investments. Funds raised from sources outside the university organization nowadays give us more financial possibilities.

Currently one of the main challenges is to find the optimal balance between the upkeep of our large scientific plant collection and the university research and teaching depending on it, and the pressure on our resources caused by such a large number of visitors and public-related activities.

Firmly embedded in the structure of Leiden University the Hortus can be sure of its future, but of course we have to meet with standards and demands of Leiden University and the Faculty of Science. However, we are expected to raise a large part of our funds from our activities in the public and scientific sector.

The Largest Global Network of Botanic Gardens and Setting International Standards for Botanic Gardens through Accreditation

Brian Lainoff
Botanic Gardens Conservation International, Descanso House, 199 Kew Road, Richmond, Surrey, TW9 3BW, United Kingdom

Botanic Gardens Conservation International (BGCI) is a membership organisation, representing botanic gardens in more than 100 countries around the world. We aim to support and empower our members and the wider conservation community, so that our knowledge and expertise can be applied to reversing the threat of extinction facing plants. Our vision is a world in which plant diversity is valued, secure and supporting all life, and our mission is to mobilise botanic gardens and engage partners in securing plant diversity for the well-being of people and the planet.

In 2018, BGCI announced the launch of an international Botanic Garden Accreditation scheme. The BGCI Botanic Garden Accreditation is aimed at botanical institutions wishing to establish their credentials as botanic gardens and reach international standards. Organisations applying for accreditation will be assessed on criteria encompassing leadership, collections management, horticulture, public education, community / cultural activities, conservation actions, scientific research, staff, networking and sustainability.

The BGCI Conservation Practitioner Accreditation recognises botanic gardens with a conservation-oriented approach. Advanced Conservation Practitioner Accreditation recognises botanic gardens with a focus on conservation actions that support local, national or global conservation goals. Institutions may apply for more than one accreditation.

Activities that botanic gardens do uniquely well – such as documenting, understanding, growing and conserving plant diversity across the taxonomic array – are not sufficiently recognised by policymakers and funders. This has led to the erosion of the values and activities that define a botanic garden compared to a public park, for example. The BGCI Botanic Garden Accreditation assesses and places a high value on the unique skills, knowledge and data in botanic gardens, thus setting a global standard for botanic gardens.

BGCI's Botanic Garden Accreditation is designed to be as inclusive as possible, but still ensure a level of international standards across botanic gardens. For this reason, the criteria it measures will assess and value the fundamental components of a botanic garden. BGCI defines these components in the *BGCI Technical Review: defining the botanic garden and how to measure performance and success*[1] and in the *BGCI Accreditation Standards Manual*[2]. These international standards and targets include:

- A reasonable degree of permanence
- Proper documentation of the collections, including wild origin, and adequate plant labelling
- Monitoring and long-term maintenance of plants in the collections
- Adequate labelling of plants
- Open to the public
- Communication of information to other gardens, institutions and the public
- Promoting conservation through extension and environmental education activities
- Exchange of seed or other materials with other botanic gardens, arboreta or research institutions
- Undertaking of scientific or technical research on plants in the collections
- Conserving rare and threatened plants in *ex situ* collections and, wherever possible, in their natural habitats
- Compliance with international and national regulatory frameworks
- Adoption and promotion of sustainable and ethical practices

References
1) Smith, P., & Harvey-Brown, Y. (2017). Defining the botanic garden, and how to measure performance and success (Tech.). Botanic Gardens Conservation International. https://www.bgci.org/resources/bgci-tools-and-resources/bgci-technical-reviews/
2) Botanic Gardens Conservation International. (2018). BGCI Accreditation Standards Manual. https://www.bgci.org/resources/bgci-tools-and-resources/bgci-accreditation-standards/

Elenco completo degli abstract in ordine alfabetico