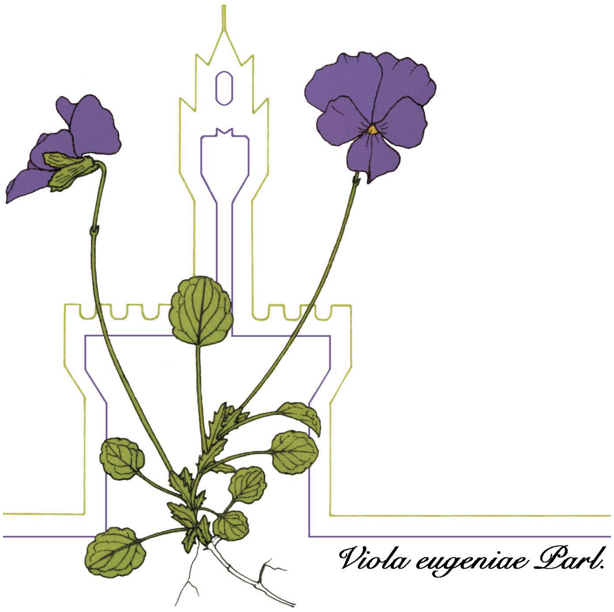
113° Congresso della Società Botanica Italiana

V INTERNATIONAL PLANT SCIENCE CONFERENCE (IPSC)

Fisciano (SA), 12 - 15 September 2018



ABSTRACTS

KEYNOTE LECTURES, COMMUNICATIONS, POSTERS

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DI SALERNO

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113° Congresso della Società Botanica Italiana onlus

V INTERNATIONAL PLANT SCIENCE CONFERENCE (IPSC)
Fisciano (SA), University Campus, 12 - 15 September 2018

Programme

Wednesday 12 September 2018

9:00-12:00 Registration and poster installation

12:00-13:00 Welcome lunch

13:30-14:00 Opening ceremony

Symposium 1

NEW FRONTIERS IN PLANT DEVELOPMENT AND ADAPTATION TO ADVERSE ENVIRONMENTAL CONDITIONS

(Chairpersons: M.M. Altamura and S. Castiglione)

Key words: plant development, stress responses, plant hormones, genome editing, biotechnology

14:00-14:30 • **Bettina Hause**, Martin-Luther-Universität Halle-Wittenberg, Germany (30 + 5 min)
"The plant hormone jasmonic acid - functions in flower development of tomato"

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

- **Camilla Betti**, Gustavo Gudesblat, Eugenia Russinova (12 + 3 min)
  "Brassinosteroid regulation of stomata development via BIN2-SPCH interaction"

- **Alfredo Ambrosone**, Antonello Costa, Stefania Grillo, Antonietta Leone (12 + 3 min)
  "Survive or grow: plant acclimation versus shock response to osmo-stress"

- **Chiara Mizzotti**, Carolina Cozzi, Sara Forlani, Simona Masiero (12 + 3 min)
  "A Fruitalk"

- **Enrico Cortese**, Roberto Moscatiello, Luca Carraretto, Marisa Brini, Lorella Navazio, (12 + 3 min)
  "Measurements of calcium dynamics in the plant endoplasmic reticulum: an aequorin-based approach"

15:30-16:00 Coffee break

16:00-16:30 • **Jochen Kumlehn**, Leibniz Institute of Plant Genetics and Crop Plant Research (IPK), Gatersleben, Germany (30 + 5 min)
"Site-directed genome modification in cereals using RNA-guided Cas endonucleases"

16:30-18:00 **Communications**

- **Martina Cerri,** Francesco Ferranti, Andrea Coppi, Bruno Foggi, Daniela Gigante, Lorenzo Lastrucci, Andrea Onofri, Roberto Venanzoni, Daniele Viciani, Lara Reale (12 + 3 min)
  "Influence of die-back syndrome on reproductive strategies within *Phragmites australis* populations"

- **Maria Cristina Sorrentino**, Fiore Capozzi, Alessia Matzutzi, Simonetta Giordano, Valeria Spagnuolo (12 + 3 min)
  "Evaluation of morpho-physiological responses in three cultivars of *Cynara cardunculus* under metal stress: counting of stomata, leaf ultrastructure and protein content"

- **Gennaro Carotenuto**, Veronica Volpe, Giulia Russo, Janice de Almeida Engler, Andrea Genre (12 + 3 min)
  "Investigating the role of endoreduplication in arbuscular mycorrhizas"

- **Vincenzo D'Amelia**, Riccardo Aversano, Ingo Appelhagen, Cathie Martin, Domenico Carputo (12 + 3 min)
  "New genetic and metabolic insights into *Solanum commersonii* adaptation to environmental stress conditions"
- **Andrea Fasolo**, Alessandra Tondello, Stefania Marcato, Tiziano Bonato, Werner Zanardi, Elisabetta Barizza, Michela Zottini, Giuseppe Concheri, Andrea Squartini, Barbara Baldan (12 + 3 min)
  "Compost, micro-organisms and plants: stimulating a natural dialogue for sustainable future"
- **Matteo Chialva**, Stefania Stelluti, Mara Novero, Paola Bonfante, Luisa Lanfranco (12 + 3 min)
  "Tomato wild relatives and their responsiveness to arbuscular mycorrhizal symbiosis"

18:00-19:30 Poster session and Working Group meetings

Thursday 13 September 2018

Symposium 2

SECONDARY METABOLITES IN PLANT-ORGANISM INTERACTIONS
(Chairpersons: S. Piacente and A. Bisio)

Key words: secondary metabolites, plant-organism interactions, phytochemistry

9:00-9:30 • **John Pickett**, Cardiff University, United Kingdom (30 + 5 min)
"Plant-insect signalling regulates secondary metabolites for defence"

9:30-11:00 **Communications**

- **Daniela Russo**, Hanene Chercar, Massimiliano D'Ambola, Luigi Milella, Zahia Kabouche, Nunziatina De Tommasi (12 + 3 min)
  "Antioxidant activity and chemical composition of *Phagnalon sordidum* L."
- **Fabio Sciubba**, Alberta Tomassini, Maria Enrica Di Cocco, Giorgio Giorgi, Walter Aureli, Gabriella Pasqua, Alfredo Miccheli (12 + 3 min)
  "Maturation of purple and orange carrot roots assessed by Nuclear Magnetic Resonance"
- **Carmen Formisano**, Daniela Rigano, Annalisa Lopatriello, Carmina Sirignano, Lolita Arnoldi, Nicola Sardone, Orazio Taglialatela-Scafati (12 + 3 min)
  "NMR-based phytochemical analysis of bergamot (*Citrus bergamia* Risso) juice"
- **Giulia Potente**, Roberto Mandrioli, Stefano Tartarini, Stefano Lugli, Michela Freschi, Cristina Angeloni, Silvana Hrelia, Fabiana Antognoni (12 + 3 min)
  "A two-year evaluation of bioactive compound profiles of new "UNIBO" sweet cherry (*Prunus avium* L.) cultivars"
- **Stefano Acquadro**, Silvia Appleton, Arianna Marengo, Carlo Bicchi, Barbara Sgorbini, Francesco Gai, Pier Giorgio Peiretti, Cecilia Cagliero, Patrizia Rubiolo (12 + 3 min)
  "Characterization of the phenolic content of leaves and green pruning residues of 16 cultivars of *Vitis vinifera* L."
- **Massimiliano D'Ambola**, Lorenzo Fiengo, Nunziatina De Tommasi, Fabrizio Dal Piaz,, Ziad Omran, Majed Halawani, Ammar Bader (12 + 3 min)
  "Phytochemical study of *Calotropis Procera* (Ait.) R.Br. leaves, fruits, stems and latex extracts and evaluation of their cytotoxic activity"

11:00-11:30 Coffee break

11:30-12:00 • **Leandros Skaltsounis**, National and Kapodistrian University of Athens, Greece (30 + 5 min)
"Olive bioactive compounds: Chemistry and Biology"

12:00-13:00 **Communications**

- **Marinella De Leo**, Fabiano Camangi, Beatrice Muscatello, Anna Maria Iannuzzi, Chiara Giacomelli, Filippo Graziani, Maria Letizia Trincavelli, Claudia Martini, Alessandra Braca (12 + 3 min)
  "Phytochemical analysis and antisenescence activity of *Sorbus torminalis* (L.) Crantz and *Elaeagnus umbellata* Thunb fruits"
- **Natalizia Miceli**, Emilia Cavò, Salvatore Ragusa, Paola Dugo, Francesco Cacciola, Maria Fernanda Taviano (12 + 3 min)
  "Phenolic profile, antioxidant properties and brine shrimp toxicity of a hydroalcoholic extract obtained from the aerial parts of *Matthiola incana* (L.) R. Br. (Brassicaceae)"
- **Massimo Tacchini, Karina Yanza,** Alessandra Guerrini, Alessandro Grandini, Gianni Sacchetti, Matteo Chiurato, Diego Vinueza (12 + 3 min)
  "Leaves and flowers of *Oreocallis grandiflora* (Lam.) R.Br. from Ecuador: a new herbal remedy?"
- **Milena Masullo**, Hamzeh Hossaini, Antonietta Cerulli, Stefania Martucciello, Mahdi Ayyari, Sonia Piacente (12 + 3 min)
  "Antiproliferative cardenolides from the aerial parts of *Pergularia tomentosa*"

13:00-14:00 Lunch

Symposium 3

PLANT LIFE IN EXTREME ENVIRONMENTS: A CHALLENGE FOR ECOLOGICAL RESEARCH

(Chairpersons: C. Wellstein and S. Martellos)

Key words: plant-habitat interactions, species interactions, community assembly, environmental stress

14:00-14:30
- **José María Fernández-Palacios**, University of La Laguna, Tenerife, Spain (30+5 min)
  "Oceanic island summits, an extreme insular environment"

14:30-16:00 **Communications**

- **Giovanna Aronne,** Luigi G. Izzo, Veronica De Micco (12 + 3 min)
  "Plant growth in space: struggling with environmental factors and physical constraints"
- **Silvia Ongaro,** Lorenzo Morosini, Stefano Martellos, Mauro Tretiach (12 + 3 min)
  "Preliminary climatic distribution model for *Pterygiopsis affinis* as a means for field survey planning"
- **Martina Pollastrini**, Filippo Bussotti, Giovanni Iacopetti, Nicola Puletti, Walter Mattioli, Federico Selvi (12 + 3 min)
  "Extreme drought and heat wave effects on Tuscan forests: forest dieback and tree mortality in summer 2017"
- **Veronica De Micco**, Chiara Amitrano, Ermenegilda Vitale, Giovanna Aronne, Carmen Arena (12 + 3 min)
  "Morpho-functional plant traits conferring radioresistance: living in extreme conditions by transforming constraints in opportunities"
- **Mauro Mariotti**, Sara Romeo, Enrica Roccotiello (12 + 3 min)
  "Ecophysiological response in some *taxa* of the urban ecosystem of Genova"
- **Francesco Boscutti**, Enrico Braidot, Valentino Casolo, Antonio Filippi, Elisa Petrussa, Marco Zancani (12 + 3 min)
  "Plant traits and plant-plant interactions shape the effect of elevation on *Vaccinium myrtillus* communities in Alpine tundra"

16:00-16:30 Coffee break

16:30-17:00
- **Martin Grube**, Universität Graz, Austria (30 + 5 min)
  "Lichen symbioses as hubs of microbial complexity"

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

- **Elisabetta Bianchi**, Andrea Coppi, Ilaria Colzi, Cristina Gonnelli, Lorenzo Lazzaro, Stefano Loppi, Luca Paoli, Alessio Papini, Andrea Vannini, Renato Benesperi (12 + 3 min)
  "Adaptations to environmental stresses: the case of *Seirophora villosa* (Ach.) Frödén"

- **Juri Nascimbene**, Renato Benesperi, Elisabetta Bianchi, Gabriele Casazza, Alessandro Chiarucci, Matteo Dainese, Lorenzo Lazzaro, Paola Malaspina, Lorenzo Marini, Pier Luigi Nimis, Daniel Spitale, Paolo Giordani (12 + 3 min)
  "Unraveling how global change and habitat modification are threatening lichen diversity"

- **Angela Cicatelli**, Francesco Guarino, Berthold Heinze, Stefano Castiglione (12 + 3 min)
  "Phytoremediation capacity and rhizosphere microbiome of a black poplar in response to zinc and compost amendment"

- **Adriano Stinca**, Giuseppe D'Auria, Nunzio Fiorentino, Lucia Ottaiano, Assunta Esposito (12 + 3 min)
  "Evidence of changes in reproductive strategies in promoting the invasiveness of *Pistia stratiotes* L.

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

19:00-19:45 Presentation of new botanical books

20:30-23:30 Congress social dinner

Friday 14 September 2018

Symposium 4

EVOLUTION AND DIVERSITY IN EXTREME ENVIRONMENTS
(Chairpersons: L. Peruzzi and G. Domina)

Key words: adaptation, environmental drivers, evolution, floristics, phylogeny, systematics

9:00-9:30

- **Elvira Hörandl**, University of Göttingen, Germany (30 + 5 min)
  "Plant evolution in alpine ecosystems: direct and indirect influence of cold stress"

9:30-11:00 **Communications**

- **Nicoletta Cannone**, Isabella Vanetti, Omar Rota-Stabelli, Peter Convey, Francesco Malfasi, Paolo Gerola, Alberto Vianelli, Mauro Guglielmin, Serena Zaccara (12 + 3 min)
  "Diversity trends of bryophytes in Antarctica are the result of adaptation, evolution and survival (including cryptobiosis): the case study of the phylogeny of *Bryum argenteum*"

- **Federico Selvi**, Aida Bani, Isabella Bettarini, Lorenzo Cecchi, Ilaria Colzi, Andrea Coppi, Guillaume Echevarria, Cristina Gonnelli (12 + 3 min)
  "Diversity, evolution and nickel accumulation in a critical group of metallophytes from a major serpentine hotspot: the genus *Odontarrhena* (Brassicaceae) in Albania"

- **Grazia Cecchi**, Simone Di Piazza, Giuseppe Greco, Ester Rosa, Mauro Mariotti, Mirca Zotti i (12 + 3 min)
  "Mycological characterization of extreme environments and substrates as first step for sustainable remediation technologies"

- **Emanuele Fanfarillo**, Giulio Ferrante, Giovanna Abbate (12 + 3 min)
  "Shifts of maize crop weed flora over 50 years: a case study in central Italy"

- **Salvatore Tomasello**, Kevin Karbstein, Ladislav Hodac, Mareike Daubert, Claudia Pätzold, Elvira Hörandl (12 + 3 min)
  "Diversity and species concept in the polyploid apomictic *Ranunculus auricomus*"

- **Marco D'Antraccoli**, Giovanni Bacaro, Enrico Tordoni, Gianni Bedini, Lorenzo Peruzzi (12 + 3 min)
  "More species, less effort: designing and comparing sampling strategies to draft optimised floristic inventories"

11:00-11:30 Coffee break

11:30-12:00 • **Silvia Matesanz**, King Juan Carlos University, Spain (30+5 min)
"Evolutionary ecology of gypsum specialists: insights into climate change responses"

12:00-13:00 **Communications**

- **Gianniantonio Domina**, Sami Ben Haj, Ridha El Mokni (12 + 3 min)
  "The florula of hypersaline habitats in the central Mediterranean and its biological traits"
- **Stefano Vitti**, Francesco Boscutti, Elisa Pellegrini, Valentino Casolo (12 + 3 min)
  "Study of plant traits, soil features and plant invasion in sandy beach of Grado and Marano lagoon (Northern Adriatic Sea)"
- **Lorenzo Lazzaro**, Daniele Ciampi, Ilaria Colzi, Cristina Gonnelli, Lorenzo Lastrucci, Daniele Viciani, Andrea Coppi (12 + 3 min)
  "An integrated analysis of functional and genetic diversity in depicting the adaptive strategies of plants. The case study of *Silene paradoxa* L."
- **Piero Zannini,** Salvatore Pasta, Riccardo Guarino, Alessandro Chiarucci (12 + 3 min)
  "The Aeolian archipelago as a model system for Island Biogeography Theory in the Mediterranean"

13:00-13:30 Congress closure

13:30-14:30 Lunch

Post-congress event at the University Campus of Fisciano

15:00-16:30 Workshop on the Coordinamento della Rete Italiana dei Musei Botanici (CORIMBO) project

Saturday 15 September 2018

8:00-15:00 Post-conference excursion to the WWF protected area "Oasi di Morigerati" at the Cilento, Vallo di Diano e Alburni National Park

The timetable may be modified

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Symposium 1. New frontiers in plant development and adaptation to adverse environmental conditions

1. *Bettina Hause* - The plant hormone jasmonic acid – function in flower development of tomato
2. *Jochen Kumlehn* - Site-directed genome modification in cereals using RNA-guided Cas endonucleases

Symposium 2. Secondary metabolites in plant-organism interactions

3. *John A. Pickett* - Plant-Insect signalling regulates secondary metabolites for defence
4. *Leandros A. Skaltsounis* - Olive bioactive compounds: chemistry and biology

Symposium 3. Plant life in extreme environments: a challenge for ecological research

5. *José María Fernández-Palacios* - Oceanic island summits, an extreme insular environment
6. *Martin Grub* - Lichen symbioses as hubs of microbial complexity

Symposium 4. Evolution and diversity in extreme environments

7. *Elvira Hörandl, Simone Klatt, Eleni Syngelaki, Christoph Schinkel, Bernhard Kirchheimer, Stefan Dullinger* - Plant evolution in alpine ecosystems: influence of cold stress on polyploidy, mode of reproduction and niche dynamics
8. *Silvia Matesanz* - Evolutionary ecology of gypsum specialists: insights into climate change responses

COMMUNICATIONS

Symposium 1. New frontiers in plant development and adaptation to adverse environmental conditions

Symposium 2. Secondary metabolites in plant-organism interactions

Symposium 3. Plant life in extreme environments: a challenge for ecological research

Symposium 4. Evolution and diversity in extreme environments

POSTER

1. New frontiers in plant development and adaptation to adverse environmental conditions

1.1 Plant development

1. *Leonardo Bruno, Santina Rizzo, Antonella Muto, Marianna Pacenza, Innocenzo Muzzalupo, Adriana Chiappetta, Maria Beatrice Bitonti* - Evaluation of compost effects on soil fertility and plant development: a preliminary study
2. *Laura Carotti, Giuseppina Pennisi, Francesco Orsini, Sonia Blasioli, Ilaria Braschi, Fabiana Antognoni, Andrea Crepaldi, Karina B. Ruiz, Giorgio Gianquinto, Stefania Biondi* - Effects of red: blue ratios in LED lighting on nutritional and nutraceutical properties of hydroponically grown Lactuca sativa L.
3. *Pierluigi Cortis, Claudia Caltagirone, Alberto Caredda, Annalena Cogoni, Antonio De Agostini, Domenica Farci, Alessandra Garau, Francesco Guarino, Michele Lussu, Andrea Vacca, Dario Piano* - A population of Epipactis helleborine subsp. tremolsii (Orchidaceae) growing on mine tailings: a case study in Sardinia (Italy)

1.2 Stress response

4. *Angela Balzano, Katarina Čufar, Giovanna Battipaglia, Veronica De Micco* - Coupling xylogenesis and dendroecology to understand the relations between environmental factors and tree-ring formation in Mediterranean hardwood and softwood species
5. *Stefania Caporale, Marco Marchetti, Silvana Munzi, Sonia Ravera* - Variation of physiological parameters in the lichen Evernia prunastri (L.) Ach. exposed to different environmental stressors in Molise (central Italy)
6. *Barbara Conte, Viviana Maresca, Sergio Sorbo, Milad Heydari, Vincenzo Carginale, Clemente Capassso, Adriana Basile* - Response to heavy metal stress in Leptodictyum riparium Hedw
7. *Michele Ferrari, Anna Torelli, Matteo Marieschi, Radiana Cozza* - Involvement of DNA methylation in Cr(VI) tolerance and in response to sulfur deprivation in the microalga Scenedesmus acutus M. (Chlorophyceae)
8. *Marianna Pacenza, Leonardo Bruno, Antonella Muto, Fabrizio Araniti, Maria Rosa Abenavoli, Adriana Chiappetta, Maria Greco, Maria Beatrice Bitonti* - Modulation of stress response pathways by DNA methyltransferases in A. thaliana
9. *Anna Maria Palermo, Maria Emanuela Mascaro, Giuseppe Pellegrino* - The application of leaf pigment evaluation in forensic science
10. *Stefania Stelluti, Matteo Chialva, Mara Novero, Paola Bonfante, Luisa Lanfranco* - Performances of mycorrhizal tomatoes under water and nutrient stress conditions

1.5 Plant biotechnology

11. *Veronica Volpe, Gennaro Carotenuto, Ludovica Oddi, Mara Politi, Giusto Giovannetti, Elena Barni, Alessandra Salvioli, Luisa Lanfranco, Paola Bonfante, Sergio Capaldo, Marco Bergese, Consolata Siniscalco, Andrea Genre* - AM for Quality - Use of chitin oligomers to enhance forage plant mycorrhization and crop quality

2. Secondary metabolites in plant-organism interactions

2.1 Secondary metabolites

12. *Fabiana Antognoni, Rosaria Arena, Giulia Potente, Giuseppe Gabellini, Elena Lombardini, Karina B. Ruiz Carrasco, Roberto Mandrioli* - Characterisation of bioactive compound composition and antioxidant activities in seeds of a Chilean Chenopodium quinoa (Willd.) cultivar grown in Emilia-Romagna (Italy)
13. *Fabrizio Araniti, Maria Rosa Abenavoli* - Rosmarinic acid induces programmed cell death in Arabidopsis seedlings through reactive oxygen species and mitochondrial dysfunction

14. *Miriam Bazzicalupo, Marcella Denaro, Antonella Smeriglio, Domenico Trombetta, Jacopo Calevo, Annalisa Giovannini, Laura Cornara* - Antioxidant activities of extracts from various portions of giant orchid, Himantoglossum robertianum (Loisel.) P. Delforge
15. *Alfredo Bottone, Marina Creydt, David Schütz, Markus Fischer, Cosimo Pizza, Sonia Piacente* - Metabolite profiling of sweet almond lipids in two Italian cultivars, Toritto and Avola
16. *Lucia Caputo, Marina Daiana Reguilón, Marta Rodrìguez-Arias, Josè Miňarro, Vincenzo De Feo* - CNS activity of Lavandula angustifolia essential oil and its main constituent, linalool
17. *Antonietta Cerulli, Milena Masullo, Paola Montoro, Jan Hošek, Cosimo Pizza, Sonia Piacente* - Metabolite profiling of "green" extracts of Corylus avellana L. leaves by 1H NMR spectroscopy and multivariate statistical analysis
18. *Ilaria Chiocchio, Manuela Mandrone, Mariacaterina Lianza, Giovanna Picone, Lorenzo Barbanti, Ferruccio Poli* - 1H NMR-based metabolomic approach to study Sorghum bicolor
19. *Marcella Denaro, Antonella Smeriglio, Domenico Trombetta, Gianpaolo Grassi and Clara Circosta* - Phytochemical analysis and antioxidant properties of Cannabis sativa L. essential oil from a Chinese accession
20. *Luigi Alessandro Di Iorio, Giustino Orlando, Marcello Locatelli, Nicola Macchione, Annalisa Chiavaroli, Lucia Recinella, Simone Carradori, Gokhan Zengin, Stefania Cesa, Fiorella Di Giambattista, Lidia Leporini, Sheila Leone, Luigi Brunetti, Luigi Menghini, Claudio Ferrante* - Bee pollen to manage prostate inflammation: phytochemistry and protective role of standardized extracts in in vitro assays and on rat prostate specimens
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23. *Mariacaterina Lianza, Manuela Mandrone, Ferruccio Poli, Cinzia Sanna, Giovanna Farruggia, Emil Malucelli, Stefano Iotti E Concettina Cappadone* - Antitumor potential of plants from Sardinia (Italy), a hotspot for biodiversity in the Mediterranean basin
24. *Paola Malaspina, Erica Catellani, Bruno Burlando, Daniele Brignole, Laura Cornara, Simona Candiani, Valentina Obino, Vincenzo De Feo, Lucia Caputo, Paolo Giordani* - Effects of lichen extracts on tyrosinase activity
25. *Arianna Marengo, Cecilia Cagliero, Stefano Acquadro, Barbara Sgorbini, Maria Laura Colombo, Patrizia Rubiolo* - Misidentification of wild plants cooked in soup and sold as traditional gastronomy
26. *Luigi Menghini, Claudio Ferrante, Lidia Leporini, De Simone Simonetta, Marcello Locatelli, Simone Carradori, Gokhan Zengin, Lucia Recinella, Annalisa Chiavaroli, Sheila Leone, Luigi Brunetti, Giustino Orlando* - Crocus sativus L. qualitative fingerprint, antioxidant potentials, and enzyme inhibitory activities: giving added value to high-quality by-products
27. *Stefano Negri, Mauro Commisso, Matilde Merlin, Elisa Gecchele, Sara Zenoni, Massimiliano Perduca, Linda Avesani, Flavia Guzzo* - Plant tryptamine and serotonin: in search of their biological role in fruits
28. *Gabriella Pasqua, Camilla Badiali, Elisa Brasili, Giulia De Angelis, Giovanna Simonetti, Eric De Castro Tobaruela, Eduardo Purgatto, Heng Yin, Alessio Valletta* - Water-soluble chitosan oligosaccharides (COS) affect xanthone, and volatile organic compound content and antifungal activity against human pathogenic fungi, of Hypericum perforatum root culture extracts
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2.2 Plant/organism interactions

2.3 Phytochemistry

3. Plant life in extreme environments: a challenge for ecological research

3.1 Plant-habitat interactions

3.2 Species interactions

3.3 Community assembly

3.4 Environmental stress

4. Evolution and diversity in extreme environments

4.2 Environmental drivers

4.3 Evolution

4.4 Floristics

4.5 Phylogeny

4.6 Systematics

Free Topic

Working Group "Allochthonous Species"

Working Group "Applied Botany"

Working Group "Biotechnology and Differentiation"

Working Group "Botanical and Historical Gardens"

Working Group "Cellular and Molecular Biology"

Working Group "Ecology"

Working Group "Floristics, Systematics and Evolution"

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89. *Andrea Ceci, Flavia Pinzari, Veronica Spinelli, Fabiana Russo, Oriana Maggi, Anna Maria Persiani* - Study of tricalcium phosphate solubilization by some soil microfungi and evaluation of their potential as efficient plant growth-promoting organisms for Glycine max

90. *Simone Di Piazza, Federica Turrini, Raffaella Boggia, Gianluca Damonte, Mirko Benvenuti, Mirca Zotti* - "Closing the loop" with Oyster Mushroom to recycle agricultural organic waste

91. *Giorgio Marozzi, Gian Maria Niccolò Benucci, Leonardo Baciarelli Falini, Emidio Albertini, Domizia Donnini* - Ectomycorrhizal synthesis of Tuber mesentericum Vittad. A review of ECM descriptions and DNA characterization

92. *Fabiana Russo, Antonietta Siciliano, Andrea Ceci, Oriana Maggi, Marco Guida, Eligio Malusà, Małgorzata Tartanus, Anna Maria Persiani* - Exploiting saprotrophic soil microfungi in bioremediation of DDT: stress tolerance, oxidative stress and antioxidants

Working Group "Nature Conservation"

93. *Paola Angelini, Robert P. Wagensommer, Giancarlo Bistocchi, Andrea Arcangeli, Claudia Perini, Roberto Venanzoni* - Red list of fungi from Umbria (Italy): the Boletaceae Chevall.

94. *Giovanni Astuti, Michele Adorni, Francesco Carli, Angelino Carta, Marco D'Antraccoli, Luigi Ghillani, Sergio Picollo, Lorenzo Peruzzi, Gianni Bedini* - Guiding the guidelines for monitoring species under Habitats Directive surveillance: the case of Crocus etruscus Parl. (Iridaceae)

95. *Annamaria Giorgi, Luca Giupponi, Valeria Leoni, Alberto Tamburini* - Contribution to the knowledge and valorization value of "copafam", a landrace of Phaseolus coccineus L. from the Brescia pre-Alps (northern Italy)

96. *Valentina Lucia Astrid Laface, Carmelo Maria Musarella, Giovanni Spampinato* - Conservation status of the Aspromontana flora: monitoring and new stations of Salvia ceratophylloides Ard. (Lamiaceae) endemic species in Reggio Calabria (southern Italy)

97. *Anna Scialabba, Francesco Maria Raimondo, Antonino Messina, Maria Carola Fiore* - Development of a Sicilian bean core collection using morphological descriptors

KEYNOTE LECTURES

The plant hormone jasmonic acid – function in flower development of tomato

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Jasmonic acid (JA) and its derivatives, collectively known as jasmonates, are lipid-derived signaling molecules involved in defense against pathogens and in response to wounding by herbivores, in the regulation of mutualistic interactions of plants with microorganisms, but also in plant growth and development. Many of them alter gene expression positively or negatively in a regulatory network. Their function in development of tomato flowers was concluded from a mutant defective in JA perception (*jai1-1*), being known to exhibit defects in female development leading to female sterility, but also showing additional severe effects on stamen and pollen development. To elucidate occurrence and function of JA in tomato flower organs we applied the following complementary approaches: (i) detailed jasmonate profiling of dissected flower organs, (ii) immuno cytochemical detection of jasmonates using a highly specific JA antibody, and (iii) transcript and metabolite profiling of reproductive tissues isolated from cultivar MicroTom (wild type) and *jai1-1*.

Stamens of *jai1-1* exhibit a senescence phenotype leading to the hypothesis that jasmonates might function in regulation of processes known to be mediated by ethylene. Temporal profiling of transcriptome and metabolome of stamens of wild type and *jai1-1* suggested a role of jasmonates in the temporal inhibition of ethylene production to prevent premature desiccation of stamens. This was further tested by crosses of *jai1-1* with *NeverRipe*, an ethylene insensitive mutant. Indeed, double mutants showed a complementation of *jai1-1* phenotype in terms of dehiscence and pollen release.

Using isolated ovules of three different stages of flower development from both wild type and *jai1-1*, we identified putative JA-dependent regulatory components. Among the high number of differentially regulated genes were two genes encoding MYB transcription factors, which orthologues in Arabidopsis are known to have a crucial role in JA-regulated stamen development. To analyze their function in tomato ovule development, a respective TILLING mutant was identified and CRISPR/Cas9 mutants created. The phenotypic characterization of these mutants confirmed a function of the identified MYB transcription factors in ovule development of tomato. Comparative transcriptomics using carpels of a CRISPR/Cas9-mutant line, *jai1-1* and wild type will help to identify putative MYB transcription factor target genes and to verify their role in JA-regulated tomato ovule development.

Site-directed genome modification in cereals using RNA-guided Cas endonucleases

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Site-directed genome modification triggered by customizable CRISPR-associated (Cas) endonucleases offers novel possibilities for the elucidation of gene functions and the improvement of crop performance. Targeted mutagenesis has been exemplified in many model and crop species and is now being routinely used to produce knock-out lines. To readily take advantage of newly emerging elements such as Cas9-derivatives with specific functionality, a modular vector system has been developed. It also allows for the simultaneous expression of multiple guide RNAs. The co-expression of different guide-RNAs was demonstrated to result not only in the modification of multiple genomic target sites at a time, but also to facilitate the precise deletion of fragments between two such sites. Further, we showed that independent mutations carried by the typically chimeric primary mutants can be readily separated and fixed by producing doubled haploid progeny. We have been using targeted mutagenesis in barley and wheat to study domestication traits and to generate plants with improved quality features as well as with enhanced yield potential. Whereas the use of customizable endonucleases for site-directed mutagenesis (i.e. the formation of random sequence modifications at a predefined genomic locus) is well established, the precise editing of plant genomes (i.e. the creation of predefined sequence modifications) using synthetic repair templates implemented by homology-directed DNA repair remains a major challenge. To demonstrate that the principle of homology-directed editing of genomic sequences is feasible in barley, we precisely exchanged a single nucleotide thereby endowing a previously integrated target (trans)gene with other functionality. For the time being however, this was achieved only at the cell level, because homology-directed repair events are comparatively rarely occurring and therefore not sufficiently associated with the effect of a co-expressed selectable marker gene. Consequently, quite some further research efforts are required to render truely precise genome editing a viable tool for plant research and breeding.

Plant-Insect Signalling Regulates Secondary Metabolites for Defence

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Plant science can deliver sustainable management of pests and other constraints on food production, and can now be developed via biotechnology. Plant secondary metabolites present the main opportunity particularly those involved in stress signalling, for example, associated with insect attack. Approaches involving ecosystem management, including use of companion plants, exploiting secondary metabolism is now, with new molecular tools, readily exploited. The metabolites involved include the tetranorterpenes (homoterpenes), produced by oxidation of higher terpenoid tertiary alcohols and acting as plant defence signals particularly in the powerful enhancement of biological control of insect pests. In addition, external plant defence elicitors, often related to plant hormone systems, are targeted by selecting plants, from species biodiversity studies, for releasing these types of metabolites. Evidence of field effectiveness comes from development in regions relying on resource poor farming and where less sustainable seasonally applied chemical toxicants, still the main intervention in industrialised agriculture, may not be available. Although natural biosynthetic pathways are utilised in selecting or breeding the smart companion plants, GM approaches offer evidenced opportunities particularly with advances in genome editing. There are also examples of synthetic biology now being explored for developing new pathways based on novel synthetic genes for improved plant signalling molecules.

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Olive bioactive compounds: Chemistry and Biology

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The olive tree, closely connected to the Mediterranean region has provided a wealth of goods. Research on the olive has started early but it has proven inexhaustible revealing mainly a vast array of nutritional and health properties. Apart from olive oil and table olives, the by-products coming from olive processing industry have been proven attractive materials for research. The aim of this communication is to present a holistic research strategy towards the multifaceted exploitation of the olive tree including activities such as extraction, fractionation, isolation, analysis of olive tree products as well as investigation of processes related to olive industry and valorization of by-products.

The main products of the olive tree, olive oil and table olives as well as by-products such as leaves, paste, mill wastes and table olive wastewater have been used as sources for the recovery of valuable secondary metabolites. This has been performed with conventional techniques and also by adsorptive resin technology [1, 2]. In addition standardized enriched fractions have been prepared with various techniques, such as MPLC, HPLC, and CCC. Isolation of promising lead compounds with emphasis to olive polyphenols [3] oleuropein (leaves), hydroxytyrosol & tyrosol (olive oil, by-products), oleacein & oleocanthal (olive oil) and lactones (by-products), has been achieved. Additionally advanced analytical techniques and methodologies (UPLC/HPLC-DAD, HPLC-DAD-HR/MSn, and HPTLC) have been developed and applied for the qualitative and quantitative determination of secondary metabolites in all the above mentioned materials [4]. The lab scale processes have been also adapted to pilot scale systems. The biological profile and the therapeutic potential of olive extracts and compounds is explored and supported by several in vitro and in vivo studies while their possible application as nutraceuticals, dietary supplements, cosmetics as well as agrochemicals is also investigated.

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Oceanic island summits, an extreme insular environment

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Summit ecosystems of tropical and subtropical oceanic islands constitute one of the most ephemeral and isolated ecosystems existing, harbouring thus specific characteristics that confer their biota an outstanding singularity.

Summits are ephemeral in the sense that they are the last ecosystems to be constructed during the growth of the new oceanic island, as well as the first to vanished due either to island subsidence, island erosion, or both of them. Whereas their geological emergence/disappearance is controlled by the volcanic/erosion activity, the Pleistocene glaciations, forcing in the last million years the altitudinal shift of the timberline, have also created or destroyed summit ecosystems, enabling in glaciation maxima the appearance of alpine ecosystems where they were not present in interglacial periods and vice versa.

On the other hand, summit ecosystems constitute islands within islands, in the sense that they are more isolated from similar ecosystems than the coastal lines of the islands containing them. Thus summit biota, frequently displaying a very high endemicity, may originate either through dispersal from other close summit ecosystems during peak periods (multiple summit endemics), when a dispersal window remains open for pre-adapted summit biota, or more usually from the colonization of the summits and later evolution to the new conditions from low or mid-altitude generalistic species of the same island (single summit endemics). Conversely, if peak periods are absent, the disappearance of summit ecosystems implies the extinction or extirpation of their constitutive species.

Keywords: climatic emergence, double isolation, endemicity, ephemeral ecosystems, geologic emergence, peak period, summit, timberline,

Lichen symbioses as hubs of microbial complexity

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Lichens represent a self-sustaining relationship between algae and fungi that gave rise to unique and macroscopically distinct phenotypes, the lichen thalli. After compatible partners find together to form thalli, these structures can become highly persistent under natural conditions. While the photosynthesizing symbionts are sheltered underneath peripheric fungal layers, recent microscopic and molecular data reveal that other fungi and bacteria specifically colonize lichen thalli as well. Analyses of the metagenome, -transcriptome and -proteome demonstrate a functional involvement of bacteria in the lichen symbiosis, including also their differential responses to variations of hydration. These results suggest a revised model of this symbiosis as a functional network of multiple participants. Fractions of the lichen microbiome include strains that are closely related to those of importance in other ecological niches, such as plants. Certain lichen-associated bacteria actually display plant-beneficial effects. Lichens therefore also act as environmental reservoir of microbes, which might hint to a microbial buffering system in the environment.

Plant evolution in alpine ecosystems: influence of cold stress on polyploidy, mode of reproduction and niche dynamics

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Plant life in alpine habitats is constrained by extreme abiotic conditions, mainly by cold stress, freezing periods, short vegetation periods and poor soil conditions. Evolution of alpine plants is characterized by polyploidization, shift in modes of reproduction and adaptations to harsh environments. Here we focus on direct and indirect consequences of low temperatures and frost periods in alpine environments in the alpine species Ranunuclus kuepferi. The species occurs with diploid, mostly sexual cytotypes in the southwestern Alps, while tetraploids with faculative apomixis occur in the northern parts of the Alps at higher altitudes, in Corsica and the Apennines. This reflects a classical pattern of "geographical parthenogenesis". Cold stress influences mode of reproduction and appears to trigger unreduced gamete formation and polyploidization. Large scale screening in natural populations revealed that in diploid populations, spontaneous asexual seed formation and polyploidization via female unreduced gametes takes place (Schinkel et al. 2016, 2017). Experiments in climate growth chambers confirmed that actually cold stress can trigger both unreduced female gamete development and apomictic seed formation (Klatt et al. 2018). In the Alps autotetraploids conducted a niche shift towards colder climatic conditions (Kirchheimer et al. 2016). Simulation of range expansions and modelling of the postglacial re-colonization process confirmed our hypothesis that a combination of polyploidy and uniparental reproduction enabled tetraploid apomicts to colonize previously glaciated areas faster than diploids (Kirchheimer et al. 2018).

The genetic background of the shift of mode of reproduction and cold acclimation is still unclear. Population genetic studies revealed no significant differences in genetic diversity measures, but just less geographic structure in tetraploids compared to diploids (Cosendai et al. 2013). In current projects we test the hypothesis that epigenetic changes are the putative background of apomictic seed formation and of cold adaptation. Correlations to apomictic and growth form are being tested both in natural populations and in experimental conditions. Results of methylation sensitive AFLP studies revealed significant differences of DNA methylation profiles between diploid and tetraploid cytotypes, both in wild and experimental conditions. Furthermore, tetraploids differed regarding their reproduction mode, with facultative sexual tetraploids exhibiting greater variation among methylation patterns and differing significantly from obligate apomictic tetraploids. Additionally, patterns varied between non, hemi- and holomethylated epiloci among cytotypes as well as among reproduction modes. Locus-by-locus AMOVA of samples under experimental stress conditions revealed a great amount of significantly differentiated unmethylated epiloci among cytotypes. We conclude that polyploidization and DNA methylation are putative consequences of cold stress conditions in the higher zone of the Alps. In turn, these processes allow plants a quick response to rapid evolution of cold-adapted lineages, and the colonization of high altitudes.

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Evolutionary ecology of gypsum specialists: insights into climate change responses

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Understanding how plant populations will respond to climate change is one of the greatest challenges of our time. One of the potential mechanisms to escape from climate change is migration to more favorable habitats. However, migration may be limited in plant species with specific edaphic requirements, such as plants that only grow on gypsum (gypsophiles). Dispersion in these species may be limited by the non-continuous distribution of gypsum soils, which present both natural and anthropogenic fragmentation, and by their generally-poor dispersal ability. It is thus likely that in situ microevolutionary processes (phenotypic plasticity and natural selection) will be particularly important for gypsophiles populations to face the novel selection pressures imposed by climate change. Despite the extensive knowledge on many aspects of plant life on gypsum soils, we are lacking experimental evidence on the evolutionary ecology of these species. The main goals of our research are: i) to assess the effect of natural selection on ecophysiological traits related to drought adaptation under contrasting natural conditions; ii) to evaluate the evolutionary potential of key functional traits of adaptive value; iii) to assess the importance of neutral and adaptive processes in phenotypic differentiation of populations along climatic gradients, and iv) to evaluate plasticity patterns across populations. Through a multidisciplinary approach combining field, common garden experiments, and molecular analyses, we will gain understanding on how adaptive evolution and phenotypic plasticity can mitigate the effects of climate change on gypsophile populations. Preliminary results show that populations of these gypsum plants show substantial genetic variation for growth, morphology and physiology traits, and that these traits are plastic. We discuss the potential role of these aspects of variation in population responses to climate change and how other drivers of global change such as habitat fragmentation may interact to compromise future adaptation.

COMMUNICATIONS

Brassinosteroid regulation of stomata development via BIN2-SPCH interaction

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Stomata are microscopic pores on the leaf surface that control carbon dioxide and water vapour exchange between plants and their environment. Regulation of stomatal aperture and density ensures optimal photosynthesis and transpiration rates. Stomatal formation is regulated and controlled by multiple developmental and environmental signals that are integrated in a complex manner not yet completely elucidated (1, 2). The stomatal lineage is initiated from an undifferentiated protodermal cell through multiple asymmetric cell divisions and cell fate decisions. Within this cell lineage, the stomatal fate is limited by positional signalling events that ensure that stomata are separated from each other by at least one non-stomatal epidermal cell. In *Arabidopsis thaliana*, development of stomata is regulated by a mitogen-activated protein kinase (MAPK) signalling cascade including the MAPK kinase kinase (MAPKKK) YODA (YDA) that eventually phosphorylates and inactivates the basic helix–loop–helix (bHLH) transcription factor SPEECHLESS (SPCH) required for stomata lineage initiation and progression. SPCH activity is involved in the entry, amplifying and spacing divisions that occur during stomatal lineage development (3). We have shown that in addition to MAPKs SPCH activity is also modulated by brassinosteroid (BR) signalling.

The plant BR hormones control diverse aspects of plant development and are recognised by the LRR RLK BR-INSENSITIVE 1 (BRI1). Upon BR recognition, BRI1 is activated and a signal is relayed through a phosphorylation cascade that inhibits BR-INSENSITIVE 2 (BIN2), a GSK3/SHAGGY-like kinase whose function is to phosphorylate and inactivate two transcription factors belonging to the BRASSINAZOLE RESISTANT (BZR) family (4).

We demonstrated that BIN2 phosphorylates SPCH residues overlapping those targeted by the MAPKs, as well as four residues in the amino-terminal region of the protein outside the MAPK target domain. These phosphorylation events antagonize SPCH activity and limit epidermal cell proliferation. Conversely, inhibition of BIN2 activity *in vivo* stabilizes SPCH and triggers excessive stomatal and non-stomatal cell formation (5). In summary, we have proven that SPCH serves as an integration node for stomata and BR signalling pathways to control stomatal development in Arabidopsis through phosphorylation inputs from both MAPKs and BIN2.

Yet it still remains unclear how BIN2 mutually exclusive phosphorylation of MAPK and its downstream target SPCH is modulated to precisely regulate asymmetric cell divisions in stomatal cell lineage. Current experiments are focused on understanding this mechanism by identifying novel BIN2 biochemical interactors that may alter its subcellular localization and ultimately stomatal production.

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Surviveal or growth: plant acclimation versus shock response to osmo-stress

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The plant response to osmotic stresses, intensively investigated thus far, appears to be driven by complex multicomponent signalling pathways, as revealed by studying plant molecular response to short and intense stress conditions, which are, however, irremediably associated with growth impairment and a consequent yield loss. From an ecological and breeding perspective, more informative are efforts aimed to uncovering the mechanisms underlying the plant response to mild and prolonged exposure to osmotic stress.
To address this point, potato cells (Solanum tuberosum) were acclimated gradually to osmo-stress, and we demonstrated, by a transcriptomic analysis, that distinct regulatory gene networks from those induced upon acute stress were activated. In particular, we provided evidences that acclimation requires a major effort in terms of transcriptional regulation and that distinct transcription factors promote the differential response to short- or long-term water limiting conditions. Modulation of ethylene signalling network appear to be a distinctive trait of gradual acclimation to osmo-stress and may be part of a vast environmentally-driven epigenetic program, which needs further investigations. Gene expression patterns and functional analyses in potato plants confirmed that valuable information on primary acclimation response are achieved from using cell cultures (1, 2). Finally, we provide evidence that AtRGGA gene, coding for a glycine-rich RNA-binding protein, ortholog of StRGGA selected from the catalogue of genes specifically induced in cells acclimated to water limiting conditions, confers tolerance to osmotic stress when overexpressed in the model species Arabidopsis thaliana (3).

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A Fruitalk

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Given the fundamental nature of both the dietary and biological significance of fruit, molecular dissection of fruit growth and maturation has considerable interest. The yield and quality factors associated with fruits are of key importance to agricultural production, future improvements of fruit characteristics will rely on the comprehension of the mechanisms controlling fruit development and maturation.

Here we report our efforts to shed light into the molecular networks controlling fruit development in the model plant Arabidopsis thaliana. In order to identify genes whose products control Arabidopsis fruit development and maturation, a transcriptome analysis by RNA-deep-sequencing has been performed, comparing wild-type (WT) siliques, devoid of seeds, at 3, 6, 9 and 12 DPA, thus covering all the phases of silique development and maturation. The bioinformatics and statistical analysis of the data led to the identification of about thousand genes differentially expressed between early and late stage of silique development. Among all the different clusters, we are currently exploring the role of the NAC transcription factors. Our data strongly indicate that NAC proteins participate to the homeostasis of gibberellins and other hormones. Auxins, gibberellins, cytokinins, abscisic acid, and ethylene have been implicated at various stages of fruit development.

Measurements of calcium dynamics in the plant endoplasmic reticulum: an aequorin-based approach

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Calcium is a fundamental intracellular messenger involved in a wide range of different signalling pathways in all eukaryotes. In plants, Ca^{2+} has been shown to participate in the transduction of a large plethora of environmental stimuli, of both abiotic and biotic nature. A complex Ca^{2+} homeostatic and signalling toolkit allows for a tight regulation of the intracellular concentration of the ion ($[Ca^{2+}]$) and its variations during signal transduction. Recent evidence shows that different intracellular compartments of the plant cell are involved in the fine modulation of these cytosolic Ca^{2+} transients (Costa et al., 2018). The increasing availability of specifically targeted Ca^{2+} reporters is rapidly expanding the possibility of accurately monitoring Ca^{2+} dynamics in different subcellular localizations. In contrast to animal cells, the involvement of the endoplasmic reticulum (ER) in Ca^{2+} handling has long been overlooked, possibly clouded by the prominent role commonly ascribed to the vacuole, and because of the lack of direct measurements of ER luminal Ca^{2+}. The only genetically encoded Ca^{2+} indicator targeted to the ER lumen so far is the FRET-based Ca^{2+} probe cameleon (Iwano et al., 2009: Bonza et al., 2013; Corso et al., 2018). Although GFP-based Ca^{2+} sensors are well-suited to Ca^{2+} imaging, the Ca^{2+}-sensitive bioluminescent protein aequorin still remains the most suitable tool to accurately quantify Ca^{2+} concentrations (Ottolini et al., 2014).

In this work a construct encoding a new ER-targeted aequorin-based probe was designed, by fusing the cDNA for a mutated version of aequorin, characterized by a reduced Ca^{2+} affinity, to the nucleotide sequence encoding a defective, uncleavable N-terminal ER signal peptide. In this chimera, retention in the ER does not depend on the classical C-terminal ER retention sequence K/HDEL (which has been found to alter the bioluminescent properties of the photoprotein) but relies on the predicted anchoring of aequorin to the ER membrane. After *Agrobacterium tumefaciens*-mediated transformation via the floral dip method, transgenic *Arabidopsis thaliana* seedlings were identified by kanamycin resistance screening. The expression, correct localization and functioning of recombinant aequorin were evaluated by RT-PCR analysis of gene expression, Western blot and confocal microscopy analyses. Aequorin-based Ca^{2+} measurements assays were then set up in both seedlings and cell suspension cultures, obtained from the transgenic plants (Sello et al., 2017). Monitoring ER Ca^{2+} dynamics *in vivo* requires separate steps of ER Ca^{2+} emptying (during reconstitution of the holoprotein with the prosthetic group coelenterazine) and subsequent Ca^{2+} refilling (before challenge with the different stimuli). The use of this novel ER-targeted aequorin chimera provided the first actual measurements of $[Ca^{2+}]$ in the plant ER, highlighting significant differences with the animal counterpart. Moreover, the observed stimulus-specific ER $[Ca^{2+}]$ increases suggest that the plant ER acts more as a Ca^{2+} sink than as a Ca^{2+} source, in contrast to the well-recognized role of this compartment as a major stimulus-releasable Ca^{2+} store in animal cells.

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Influence of die-back syndrome on reproductive strategies within *Phragmites australis* populations

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Sexual plant reproduction is a strategy that allows plant populations to increase genetic variability, and consequently to be more efficient in adapting to new environments and to overcome stress conditions. Here, we focus on the reproductive mode of Phragmites australis, an important sub-cosmopolite species that can spread both by clonal propagation and sexual reproduction. In Europe, P. australis is affected by severe decline (known as Reed Die-Back Syndrome or RDBS); this study aims to understand if in RDBS condition the reproductive strategy of P. australis was altered, based on the hypothesis that if stress occurs, plants are likely to use their energy to increase genetic variability to overcome the stress.

Inflorescences in five Italian wetlands were sampled from healthy and RDBS-affected stands of P. australis. Seed production, seed viability and seed germination were evaluated, with germination monitored under two different water conditions: moist and submerged.

Stress induced by RDBS appears to influence the reproductive strategy of P. australis. In RDBS-affected stands, seed production was significantly higher than healthy stands for four of five sites. Seed viability and germination were also higher in RDBS-affected stands, but these differences were lower and site dependent.

Evaluation of morpho-physiological responses in three cultivars of *Cynara cardunculus* under metal stress: stomatal density, leaf ultrastructure and protein content

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Heavy-metal pollution is one of the most pressing issues affecting the quality of water, air and soil. Nowadays, several and expensive chemical and physical methods are applied to clean up soils, often causing serious alterations of its natural structure and biological properties (1). Phytoremediation is a *green* emerging technology, which takes advantage from the uptake capability of tolerant plants, known as hyperaccumulators, to remove or stabilize soil pollutants (2). In previous experiments, we tested three cultivars (cvs.) of *Cynara cardunculus* L. var. altilis (i.e., Sardo, Siciliano and Spagnolo) for their potential use in phytoremediation of metal polluted substrates; we found that all the tested cvs. were able to uptake metals, although providing different responses at morpho-physiological levels (3, 4). In this work, we analyzed leaf morphology, estimated the number of stomata and their total surface (Fig. 1) in the same cultivars grown for 4 weeks in hydroponic culture, under Cd and Pb stress. We observed a different behavior of the three cultivars; particularly, a significant decrease of the percentage of stomata (Fig. 2) and their surface occurred in Sardo and Siciliano cvs. grown with Cd and Pb salts. By contrast, Spagnolo cardoon was able to counteract metal stress, keeping the number of stomata and their surface under Pb stress, or even enhancing these parameters when cultured with Cd (Fig. 2). This compensative response observed under Cd stress is consistent with the preserved leaf morphology and ultrastructure, and the increase of photosynthetic proteins previously found in *C. cardunculus* Spagnolo (3, 4), in comparison with the other tested cultivars.

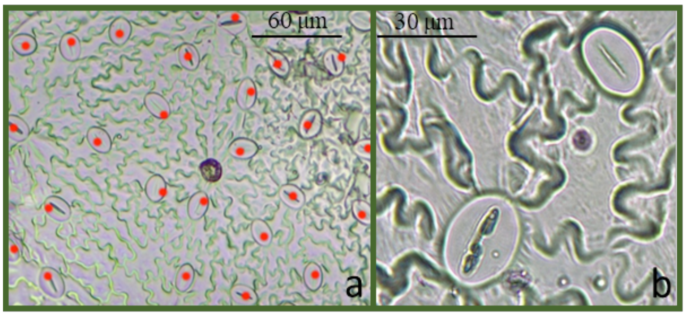

Fig. 1. Light microscope image of a Sardo cardoon control sample (a) and Spagnolo cardoon treated with Pb.

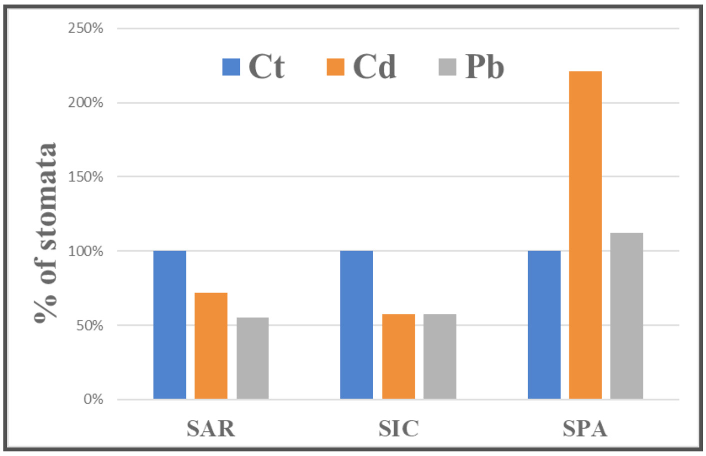

Fig. 2. Percentage of stomata in control and treated plants in the three cvs. of *C. cardunculus*; the counting refers to 4 epidermal peelings x 2 leaves x 2 plants (n=16)

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Investigating the role of endoreduplication in arbuscular mycorrhizas

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More than 80% of all terrestrial plants, including crop species, engaged a beneficial symbiotic association with arbuscular mycorrhizal (AM) fungi. AM fungi penetrate and colonize plant roots, where they differentiate into highly branched structures known as arbuscules, which are the principal sites of nutrient exchange between the two organisms. Plant cells dynamically remodel their architecture and molecular composition during symbiosis establishment, a process referred to as host cell reprogramming (Dörmann et al., 2014). In fact, root epidermal cells dramatically reorganize to accommodate the fungal symbiont, orchestrating precise nuclear movements, cytoplasm aggregation and cytoskeleton remodeling that assemble the pre-penetration apparatus (PPA) (Genre et al., 2005). Furthermore, high levels of metabolic activity have also been observed during the AM colonization and related to a global increase in nuclear DNA content in the root system (Berta et al., 2001). Detailed observation of pre-penetration responses revealed that root cells undergo dramatic changes in advance of fungal colonization, including nuclear repositioning and enlargement (Genre et al., 2008), suggesting that endoreduplication can be part of the pre-penetration response. However, the precise location of such endoreduplication events has not been defined.

On this basis we hypothesized that endoreduplication events can be part of the pre-penetration response. To test this hypothesis, we investigated ploidy changes in wild-type Medicago truncatula roots upon inoculation with the AM fungus Gigaspora margarita. Furthermore, we have extended our studies to M. truncatula dmi2-2 and dmi3-1 mutants, where pre-penetration responses are absent. Our experimental approaches were based on the targeted analysis of endoreduplication in the colonized area of the root. Cell ploidy was analyzed by combining flow cytometry and confocal microscopy imaging. Parallel gene expression and cellular localization analyses were used to highlight the localized activity of endocycle initiation markers. Compared to the controls, 3-day-old sub-hyphopodial root segments showed a marked increase in the percentage of endoreduplicated nuclei and the ploidy level was significantly higher in colonized area when compared to the adjacent uncolonized area of the roots. No such changes were observed in the controls or mutants. Besides, the nuclear size and volume increased all along the intraradical hyphae in colonized wild type roots. In addition, gene expression analysis of endocycle and cell division markers confirmed a significant upregulation in colonized wild type roots compared to uncolonized and mutant roots. Importantly, such expression increases correlated with the expression of early symbiotic markers.

Our results clearly indicate that endocycle activation occurs in the colonized area of the root and is limited to those cells that host fungal structures and a few of the surrounding cells, suggesting that ploidy increase is indeed related to pre-penetration responses.

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COMMUNICATION N. 8

New genetic and metabolic insights into *Solanum commersonii* adaptation to environmental stress conditions

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Solanum commersonii Dun. is a tuber-bearing wild potato species which separated from the common ancestor with *S. tuberosum* about millions of years ago. *S. commersonii* has been reported to be the Solanum species with the highest tolerance to low temperatures, whereas the domesticated *S. tuberosum* is considered cold-sensitive (Fig. 1A, B) (1). However, the mechanism that allows *S. commersonii* to adapt to cold exposure remains unclear. Recently, its genome has been released allowing researchers to find out novel genetic differences with respect to *S. tuberosum* (2). These differences are fundamental to understand the divergent ability of the two species to face environmental stresses. We decided to focus on genes controlling the biosynthesis of secondary metabolites (3). We paid particular attention on molecular regulation of phenylpropanoid pathway, because phenolic compounds are largely known to be involved in plant-environmental interaction (4). The differences we observed comparing *S. commersonii vs* cultivated varieties at molecular and metabolic level, allowed us to obtain intriguing outcomes. With respect to *S. tuberosum*, the wild potato has the ability to transcriptionally activate a gene named *ScAN2* after cold stress (5). The overexpression of *ScAN2* on heterologous system (*Nicotiana benthamiana* Dom.) showed its capacity to activate the biosynthesis of phenolic compounds, and in particular hydroxycinnamic acid derivatives. These compounds localized on the cell wall and plasma membrane when observed with confocal laser scanning microscopy (Fig. 2B). Intriguingly, also vesicle structures, potentially rich of phenolic compounds, were observed in the same cellular area (Fig. 2C). Considering that hydroxycinnamic acid derivatives are precursors of known compounds to face environmental stresses, these results are a first important step to understand the evolutionary differences that allowed *S. commersonii* to gain/retain tolerance ability against cold stress.

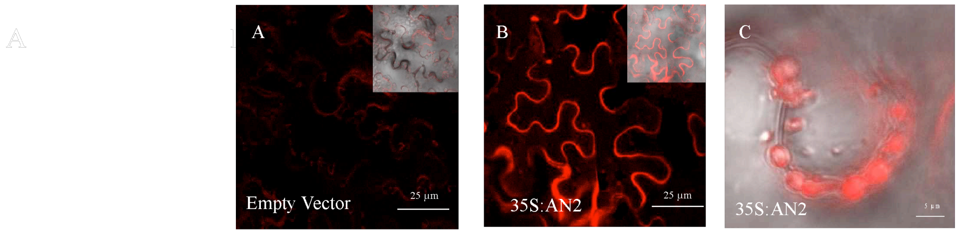

Figure 1 *Solanum commersonii* Dun. (A) and *Solanum tuberosum* L. after cold stress.

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Compost, micro-organisms and plants: stimulating a natural dialogue for a sustainable future

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The world population increase and urbanization lead to an increment of urban biowaste production, hence society must have a proper economic waste management which allows to recycle properly urban biowaste, obtaining end-products like compost. Composting is defined as the biological decomposition of organic matter under controlled aerobic conditions to form a stable, humus-like end-product (1). On the other hand, increasing world population means also a higher demand both for food and sustainable production, while soils are degrading their fertility mainly due to a loss of soil organic carbon (SOC). Compost application, like many other agronomical practices, can restore soil fertility but can be also seen as a source of microorganisms or molecules with Plant Growth Promoting (PGP) activities. This project involves different approaches to study compost obtained from domestic waste, starting with a microbiological characterization of the sample and going to evaluate the effect of compost application on plants and soil. The characterization of the microbiological features involved the isolation from compost of culturable bacterial strains at different temperatures. The obtained strains were tested for their PGP activities using biochemical assays and then identified using 16s rDNA sequencing (2).
The effects of compost application were monitored on sterile Glera plants, on grafted cuttings and in vineyard under field conditions. To assess the abilities of microorganisms contained in compost to colonize plant tissue, sterile Glera plants were transferred on substrate enriched with it, and were grown for a month in a growth chamber.

Plant physiological parameters like photosynthetic efficiency, transpiration rates and stomatal conductance were evaluated after compost application on grafted cuttings grown in greenhouse. To assess its effects under a more complex situation like field conditions, compost has also been applied in two vineyards in Veneto region: enzymatic activities, microbial DNA content, biological fertility changes were analyzed on soil. Due to the homeostasis of plants and soil of a vineyard, several years of study are envisaged as necessary to gather robust results. A multidisciplinary approach gives us the possibility to evaluate the interaction between Glera plants and bacteria living in compost derived from urban biowaste. Compost addition tends to affect soil physical properties by increasing aggregate stability (3), but can also be used as a carrier to deliver useful microorganisms to the soil.

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COMMUNICATION N. 10

Tomato wild relatives and their responsiveness to arbuscular mycorrhizal symbiosis

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Arbuscular mycorrhizal (AM) symbiosis is one of the most widespread beneficial interactions established between the majority of land plants and a group of soil fungi belonging to Glomeromycotina (1). Among crop plants, tomato (*Solanum lycopersicum*) is a valuable model species in plant biology and in the field of plant-microbe interactions, including the AM symbiosis. Indeed, a large body of genetic resources, such as monogenic mutant~~s~~ lines, introgression and breeding lines and wild genotypes, are publicly available. As an example, tomato wild relatives (*Solanum* sect. Lycopersicon) have been used as effective genetic reservoir for cultivated tomato, focusing on the introgression of desirable genes and alleles from wild species (2,3). So far, in tomato, all these resources have been extensively used to unravel many aspects of tomato biology, including fruit ripening or resistance to biotic/abiotic stresses, but rarely used to investigate tomato responsiveness to AM fungi (AMF) (4). Particularly, AMF responsiveness in tomato wild relatives has never been assessed neither at root colonization level nor at plant systemic responses.

Our hypothesis is that the ‘domestication syndrome’, occurred during the domestication process which seriously narrowed genetic variation of modern tomato varieties, also influenced plant responses to beneficial microbes such as AMF. Moreover, at the best of our knowledge breeders have rarely considered responsiveness to AMF as a feature to be selected.

In this work we analyzed the responses of two tomato wild relatives, *Solanum pennellii and Solanum neorickii* in parallel to a reference cultivated tomato accession (M82) to inoculation of a monospecific inoculum of *Funelliformis mosseae,* an AMF very common in natural and agricultural systems. Sterilized seeds were sown in small volume pots with a quartz sand-vermiculite substrate containing or not *F. mosseae* inoculum, and grown for 60 days under controlled climate conditions watered with a modified Long-Ashton solution containing 3.2 μM phosphate to favor mycorrhization. At the sampling time, biometric parameters and SPAD index were measured. Finally, the mycorrhizal status was monitored: the extent of AMF colonization was quantified on methyl-blue stained roots and the morphology of fungal intraradical structures was observed using confocal microscopy after Wheat Germ Agglutinin (WGA) staining.

Preliminary results indicate that, under these experimental conditions, in all tested genotypes, including the tomato reference genotype M82, the AM symbiosis induces a negative response in terms of biomass and growth, as sometimes documented in the literature (5). Morphological analysis of mycorrhizal roots reveals that the fungal intraradical structures (arbuscules and hyphae) are similar among the genotypes with an ‘Arum-type’ colonization model. However, and in contrast to our expectation, mycorrhization is strongly reduced in both wild relatives compared to the cultivated tomato.

Further insights on the symbiosis functionality, based on the expression profiles of selected marker genes and measurements of phosphorus concentration, are required to validate or not the working hypothesis that domestication has had an impact on AM responsiveness.

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Antioxidant activity and chemical composition of *Phagnalon sordidum* L.

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Phagnalon sordidum L. belongs to the Asteraceae family. This genus includes about 36 perennial species widespread throughout Africa, Mediterranean, Irano-Turanian and Saharo-Arabian region (1). Some *Phagnalon* species are used as spice in Italy (2) or herbal medicine in some folkloric systems such as Spain (3), Palestine (4), and Jordan (5). A limited number of phytochemical studies performed in some *Phagnalon* species revealed existence of phenolic acid conjugates, flavonoids, and terpenes derivatives (6). The aim of the present study was to investigate the phytochemical composition and *in vitro* biological activities of the extracts and isolated compounds from *Phagnalon sordidum.* The *P. sordidum* aerial parts, collected in May 2016 at Guelma (North Esatern Algerian), were extracted using chloroform, ethyl acetate (PsEA) and *n*-butanol (PsB).

All the extracts were analyzed for their antioxidant activity by 4 different *in vitro* assays: radical-scavenging activity against synthetic radicals (ABTS and DPPH assays) and biological radical nitric oxide (nitric oxide assay) and lipid peroxidation inhibition (*β*-Carotene Bleaching, BCB). Moreover, the Relative Antioxidant Capacity Index (RACI) was calculated to compare the obtained antioxidant activity (7, 8).

Results showed that the PsEA reported the highest values (2201.45±102.15 mgTE/g in ABTS test, 1442.79±0.49 mgTE/g in DPPH test, 36.06±2.47 %AA at 2 mg/mL and IC_{50} of 0.78±0.04 mg/mL in Nitric oxide test). In according to previous assays, PsEA showed the highest relative antioxidant capacity index (0.71). PsB showed a slightly lower activity in comparison with PsEA, demonstrating to be also an interesting source of bioactive compounds. Successively, the bioactive extracts were separated on Sephadex LH-20 column and HPLC. The compound structures were elucidated by spectroscopic and spectrometric analysis (NMR, UV, MS). A total of 18 compounds were isolated, two out of which have been isolated for the first time, and their structures have been determined as follows: 3, 4 dihydroxy-4(2-hydroxy, 5-acetyl phenyl)-3[(β-*O*-glucopyranosyl)]-2-methyl-but-1-en and 5-*O*-β-D-glucopyranosyl-2,10-dihydroxy-p-cymene. Isolate antioxidant activity was investigated by ABTS, DPPH and BCB assays. The 3,5 dicaffeoylquinic acid presented the highest Relative Antioxidant Capacity Index (1.93), followed by 7-methyl eriodictyol (1.25) and 7-*O*-β-glucopyranosyl luteolin (0.99). In conclusion, this study provide phytochemical investigations on *Phagnalon sordidum* aerial parts and suggest that its antioxidant activity may account for its use in inflammatory diseases and other ROS related pathological conditions.

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Maturation of purple and orange carrot roots assessed by Nuclear Magnetic Resonance

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The carrot (*Daucus carota* L.) is a root of *Apiaceae* family. It is one of the most important and cheapest vegetable crops in the world. It can be consumed as it is, in the form of juices or be the basis of more elaborate products. From a nutritional point of view, carrots do not bring a significant amount of calories (43 kcal per 100g), but contain numerous chemical species with documented anti-inflammatory, antioxidant, antimicrobial, antiviral and anti-tumor properties. Historically, different varieties of carrots have been grown, which generally differ according to color (1), and it is important to underline that this is not a purely aesthetic parameter but has a direct correlation with the nutritional properties of the food. In fact, while orange carrots contain mainly carotenoids as coloring agents, black carrots (*Daucus carota* ssp. *sativus* var. *atrorubens*) contain anthocyanins and other biologically active compounds, about 9 times more abundant than in orange (2). This is very important since food with a high content of flavonoids and antioxidants has been accepted by the scientific community as a good starting point for diets able to prevent the onset of various diseases, and this has made the carrots subject to spectroscopic studies about their chemical composition (3,4).

Root ripening is a process that, as observed in the case of fruits, can have a significant influence on the phytochemical composition of the food. Studies on fruit ripening have shown that harvesting time can be adjusted to consider the potential health effect of consumers of components that increase or decrease during development time (5). Moreover, in other studies it was also highlighted that cultivars of the same plant species develop at different rates, even when the overall maturation time is comparable (6).

While the ripening process is well documented for fruit, root development has not been investigated in the same way, and as such a detailed investigation of changes in chemical composition is required.

In the present study, NMR spectroscopy (coupled with PLS multivariate static analysis) was used to monitor the development of two carrot cultivars, black and orange, from 1 to 3 months after sowing. Moreover, since it is known that the excessive development of carrots, especially the black variety, can give rise to a bitter taste in the crop, the development of this cultivar was extended to 5 months to better analyze the chemical basis of this phenomenon.

It was possible to determinate that, during the ripening process, an increase of free aminoacids and of organic acid, belonging to the Krebs cycle, was observed in both cultivars while only in the black cultivar there was an increase of metabolites belonging to the phenylpropanoids pathway. Concerning the over ripening of black carrots, at the 5th month an increase of chlorogenic acid and polyacetylene was detected, and both molecules were associated to a bitter taste.

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NMR-based phytochemical analysis of bergamot (*Citrus bergamia* Risso) juice

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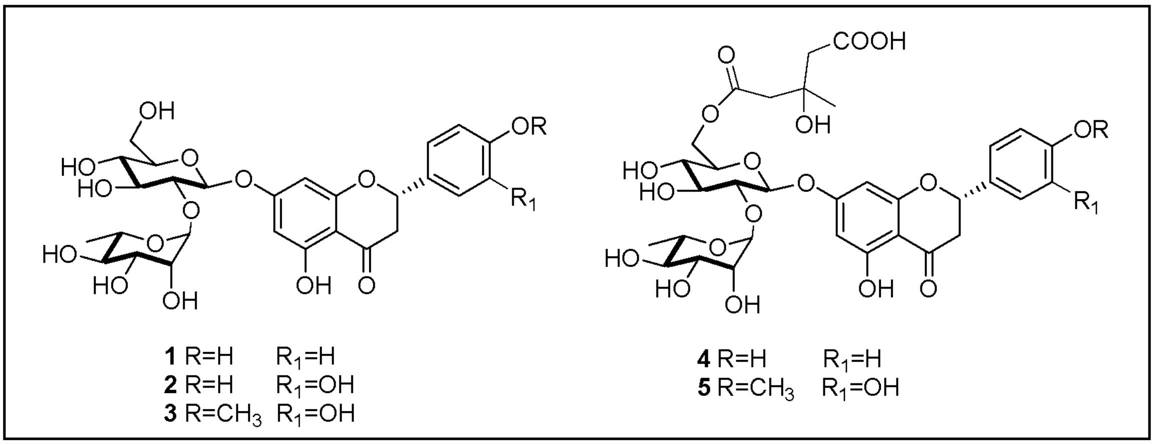
Citrus bergamia Risso, a small tree belonging to the Rutaceae family, is defined as a hybrid between bitter orange (*Citrus aurantium* L.) and lemon (*Citrus limon* (L.) Burm. f.) or a mutation of the latter (1). The plant presents big leaves similar to those of lemon, white flowers and round yellow fruits. It has been known in the Mediterranean area for several centuries and was described as early as 1708. The Italian production is limited to the Calabria region (Southern Italy) and, more precisely, in a restricted area of the province of Reggio Calabria and contributes over 95% of bergamot world production of essential oil, the most precious citrus fruit essential oils. The lack of interest by the Italian citrus fruit industry for bergamot juices is the main reason for the scarcity of studies on its composition. Indeed, among the 800 papers/patents present in the scientific literature about *Citrus bergamia* (2-3), the vast majority describes the antioxidant potential, anti-inflammatory and anti-proliferative activities, improvement of cognitive functions (2) exerted by essential oils, while the chemical composition of bergamot juice is probably the least studied among citrus juices. Almost all these studies based their conclusions on HPLC and/or Mass Spectrometry (MS) techniques that, while allowing highly sensitive and rapid analyses, often miss new, even abundant, metabolites contained in the vegetal matrices (4). In this communication, we will present the results of the first NMR-based comprehensive characterization of bergamot juice.

The chemical composition of the juice has been preliminarily analyzed by UPLC-DAD-HRMS and then, after chromatography with two different and complementary procedures (semipreparative RP-18 column and Sephadex LH-20), through NMR spectroscopy. This combined approach provided the most comprehensive description of bergamot juice available to date. This is dominated by the five flavanone glycosides naringin (**1**), neoeriocitrin (**2**), neohesperidin (**3**), metilidin (**4**) and brutieridin (**5**), also contained nine additional flavanones, five flavone C-glucosides, two flavone-O-glucosides, three limonoids and three compounds belonging to other biogenetic/structural classes.

Some of these compounds had never been reported before for bergamot, and one of them was unprecedented in the scientific literature.



1 R=H R_1=H
2 R=H R_1=OH
3 R=CH_3 R_1=OH

4 R=H R_1=H
5 R=CH_3 R_1=OH

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COMMUNICATION N. 14

A two-year evaluation of bioactive compound profiles of new "UNIBO" sweet cherry (*Prunus avium* L.) cultivars

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Cherry is the common name for several species of the genus *Prunus*, Rosaceae family, Prunoideae subfamily. One of these is *Prunus avium* L., also known as sweet cherry, which is geographically distributed around the world, especially in temperate climate areas, like Mediterranean Europe, North Africa, South Australia, New Zealand, and parts of America (1). Sweet cherry is one of the most popular temperate fruits, being studied by the scientific community both for its taste, colour, sweetness and for its nutritional and health-promoting properties (2). Sweet cherry fruits are rich in phenolic compounds, including hydroxycinnamic acids, antocyanins, flavonols and flavan-3-ols (2), which have been recognized to play a clear role in the prevention of several diseases (3).

In this work, the levels of bioactive compounds, namely phenolic acids, flavonoids and antocyanidins, as well as the antioxidant properties, have been investigated in seven new cultivars of sweet cherry obtained through a natural breeding program carried out at the University of Bologna, and thus called "UNIBO" cultivars. The aim of the study was to investigate if the fruits of these cultivars, which have excellent physicochemical traits, as well as nutritional and organoleptic features, are also characterized by improved health-promoting features. For comparison, three reference cultivars, that have long been present on the market, were utilised. The study was conducted for two consecutive years, with the aim of understanding how environmental factors may influence the drupes' phytochemical profile.

Phenolic compounds were analysed by HPLC-DAD and HPLC-FL. Antioxidant properties of new sweet cherry cultivar extracts were evaluated by both in vitro cell-free assays (ORAC, DPPH), and using oxidative-stressed animal cells. The neuroprotective potential of the novel cultivars assays was also investigated using neuron-like SH-SY5Y cells.

Results clearly show that some of the new UNIBO cultivar have a significantly higher anthocyanin and hydroxycinnamic acids content, compared to reference ones, and the antioxidant capacity did correlate with the anthocyanin profile. These results allow defining the new UNIBO cherry cultivars as a new prospective functional food, both for their high content of bioactive substances, and for their potential neuroprotective activity.

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Characterization of the phenolic content of leaves and green pruning residues of 16 cultivars of *Vitis vinifera* L.

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Vitis vinifera L., commonly known as grapevine, is a woody perennial plant belonging to *Vitaceae* family. Mankind has benefited from vine since ancient times. Initially, wild species were used and, subsequently, with the domestication of the plant, men cultivated vine to obtain grapes that could be eaten fresh or dried (raisins), or used to produce wine. Nowadays, grape crops are one of the main and most widespread agro-economic activities in the world. A large proportion of the grape production is used in vinification processes. Unfortunately, viticulture and wine production generate huge amounts of residues, which are destined mainly to composting or discarded in open areas, potentially causing environmental issues. Hence, the need to find solutions in order to reduce the amount of waste on landfill sites, such as reuse, recycling and recovery of resources (1).

Although numerous studies have been conducted on the chemical composition of several grapevine by-products (i.e. grape seeds and skins) (2, 3), little information is available on that of the leaves and even less on the green pruning residues (GPR) generated by the annual pruning of vineyards (Fig. 1) (4, 5). The aim of the present study is therefore to determine the phenolic content of leaves and GPR from *V. vinifera*, to establish whether they could be a valid source of antioxidants with nutritional properties and biological potential, thus increasing their economic value and, at the same time, limiting their waste and impact on the environment.

For this purpose extracts of leaves and GPR samples from 16 red and white cultivars of *V. vinifera*, harvested in Piedmont and used to produce some of the most prestigious wines, were evaluated.

The phenolic compounds were extracted using ultrasound-assisted extraction method, a simpler, faster, more efficient and cost-effective system than the conventional extraction techniques that entailed a greater consumption of time, energy and polluting solvents. The extraction conditions were optimized using an experimental design that ensures that the highest yield is obtained reliably, quickly and efficiently.

The extracts were then subjected to High Performance Liquid Chromatography (HPLC) analysis combined with UV and triple quadrupole MS detection for the qualitative and quantitative analysis.

On the basis of the data obtained, GPR and leaf phenolic contents of the various cultivars were compared and the similarities and differences were evaluated using unsupervised chemometrics techniques (Fig. 2).

Results show that, even though leaves and GPR can be discriminated using statistical tools, the qualitative and quantitative results of the two by-products are comparable and they both may be further investigated in order to evaluate their effective antioxidant activity.

Fig. 1. Experimental field and GPR of *V. vinifera*

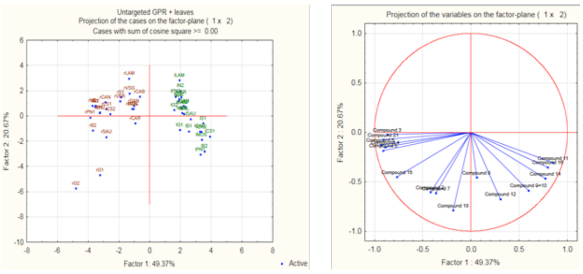

Fig. 2. Score and loading plot obtained by PCA of analysis results

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COMMUNICATION N. 16

Phytochemical study of *Calotropis Procera* (Ait.) R.Br. leaves, fruits, stems and latex extracts and evaluation of their cytotoxic activity

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Calotropis procera (Ait.) R.Br. is a wild growing plant belonging to the family of Asclepiadaceae famous in several traditional medicine systems of Saudi Arabia, Sudan and India. *C. procera* is also used by various tribes of the world as a curative agent for the treatment of leprosy, ulcers, tumors, piles and diseases of spleen and liver (1). Different parts of the plant have been reported to possess several biological properties as proteolytic, antimicrobial, larvicidal, nematocidal, anticancer, anti-inflammatory and antiparasitic (2). *Calotropis procera* has been deeply studied for its specialized metabolites content revealing the presence of a wide variety of secondary metabolites such as cardiac glycosides, triterpenoids, flavonoids, lignans, tannins, sterol, and cardenolides (3). Due to the variety in secondary metabolites and their bioactivities, the phytochemical studies on this species are currently in the limelight. The scope of this work was to verify if the environment of Saudi Arabia may affect the secondary metabolites pattern as evidenced in several other cases, and to study the antitumoral activity of isolates in the frame of a project searching for new anti-proliferative or cytotoxic agents.

The organs of an accession of *Calotropis procera* growing in Saudi Arabia were investigated. Various techniques including ultrasound-assisted extraction, microwave-assisted extraction, maceration under gentle agitation have been used for the extraction of *Calotropi procera* organs (1 g) in order to increase the extraction yield target compounds, and enhance the quality of extracts. The ultrasound-assisted extraction was selected as the best extraction method. The extracts were analyzed for their anti-proliferative activity in Jurkat, and HeLa cancer cell lines, results showed that the chloroform and n-buthanol extract were the most active (IC_{50} 10±0.3 and 8.5±0.6μg/ml, respectively). The phytochemical study was conducted on the active chloroform and n-buthanol extracts obtained from the leaves. The components were separated by classical column chromatography and the identification was performed by means of extensive spectroscopic (1D and 2D-NMR) and spectrometric (ESI-MS) techniques. The secondary metabolites pattern showed a composition on line with those reported in other accessions of *Calotropis procera* being mainly composed by Uscharidin and Calotropine, 15*β*-Calactine, Calactine and Calotropagenin (3,4).

The antiproliferative activity of isolates was evaluated in Jurkat, and HeLa cancer cell lines. Cells were exposed to increased concentrations of compounds and cell viability was evaluated at 48 h by MTT assay. Half maximal inhibitory concentration (IC50) values, obtained from dose-response curves, showed that Uscharidin has an interesting antiproliferative activity with similar potency in all cell lines (12.3±0.8 μM).

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Phytochemical analysis and antisenescence activity of *Sorbus torminalis* (L.) Crantz and *Elaeagnus umbellata* Thunb fruits

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In Italy, many spontaneous plants are used as food in folk traditions and are now being re-evaluated as healthy products with high nutritional value. In the ethnobotanical field, we selected a fruit tree that modern gastronomy has forgotten: the "Ciavardello" (*Sorbus torminalis* (L.) Crantz). The Italian phyto-alimentary tradition (1) uses its fruits to make jams, jellies, syrups, fresh snacks and, less often, alcoholic beverages.

The "Ciavardello" is a species of *Sorbus* native to Europe, North Africa, and Asia Minor and it is a member of the Rosaceae family. It is a deciduous tree or shrub which grows 1-7 m tall (sometimes reaching 20 m) and it is a slow-growing and long-lived species found in forests of broadleaf trees. The trunk is straight and the crown is expanded, globose and dense, while the bark is smooth and greyish. The leaves are alternate, simple, glabrous, petiole, ovate (4–10 cm long and 2–8 cm wide), with five to nine acute lobes, serrate and dark green colored on both sides. The flowers are hermaphrodite, 5-merous, with white petals and they are produced in corymbs. It blooms in spring (April-May) and bears fruit in autumn (September-October). The fruit is a globose to ovoid pome 10–15 mm in diameter and it is greenish to russet or brown and patterned with small and pale lenticel spots when ripe, with a pleasantly acidulous taste (2).

Another interesting plant is 'Albero dei coralli' (*Elaeagnus umbellata* Thunb.), an allochthonous species belonging to the Elaeagnaceae family. It is cultivated in Italy for ornamental purposes, while the fruits are eaten fresh, a custom that was imported from tropical and temperate Asia, its native region. The "Albero dei coralli" is a deciduous shrub or tree, more or less spiny, which grows 3-5 m tall. The leaves are alternate, lanceolate (4-10 cm x 2-4 cm), with wavy margins, green colored above and covered with silvery scales below. The flowers are hermaphrodite, fragrant, whitish, tubular and 4-lobed and they are found in the leaf axils in clusters of 1-7 elements. The fruits are small roundish drupes (3-9 mm diameter), reddish to pink, dotted with scales and they are pulpy, juicy and sweet. It blooms in the spring and its fruits ripen in the fall (2).

Both fresh fruits, collected in the Tuscany region (Italy), were extracted at room temperature with 80% EtOH (three times, every 24 h) and the obtained residues were partitioned between *n*-BuOH and H_2O. The *n*-butanolic extracts were finally analyzed by HPLC-PDA/UV-ESI-MS/MS techniques. The chemical profile of *S. torminalis* revealed the presence of phenolic acids and flavonol glycosides (3), while *E. umbellata* extract was rich especially in quercetin and kaempferol derivatives.

In the scenario of regenerative medicine, the gingival mesenchymal stem cells (GMSCs) have arisen as a promising tool to repair damaged tissues. Herein, the GMSCs were used to investigate the beneficial effects of *n*-butanolic extract of investigated fruits. Both extracts were able to increase the GMSC proliferation and decrease the intracellular accumulation of ROS. Furthermore, the extracts were able to counteract the senescence phenomena in GMSC with different extent. In particular, they contrast the ROS production mediated by hydroxyurea and hydrogen peroxide and reduced the age-related phenotypic changes (SA-β-gal staining).

In conclusion, these results highlight *S. torminalis* and *E. umbellata* fruit extracts as novel sources of antioxidant phytocomplexes able to decrease the senescence process in mesenchymal cells. The ability of both extracts per se to ameliorate the GMSC well-being and decrease cellular senescence shed light on their possible use in regenerative medicine and in particular in all the GMSC *in vitro* application.

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Phenolic profile, antioxidant properties and brine shrimp toxicity of a hydroalcoholic extract obtained from the aerial parts of *Matthiola incana* (L.) R. Br. (Brassicaceae)

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The spontaneous flora of Sicily (Italy) includes several species belonging to the Brassicaceae family, potential source of bioactive compounds. *Matthiola incana* (L.) R. Br. is a plant native to the Mediterranean region in Southern and Western Europe, North Africa and the Middle East. It is a biennial, short-lived perennial or annual herb (10-80 cm), often rather shrubby, almost hairless to densely hairy; the leaves (1-3 x 5-12 cm) are linear to oblong-lanceolate or obovate, entire or sometimes slightly sinuous-pinnatifid, obtuse or almost acute. The cruciform flowers, single or double, are gathered in terminal racemes with violet, purple, red, pink or white petals (1,2). *M. incana* is commonly used for ornamental purposes, due to its colourful flowers, and has become an economically important floral crop. Literature data report the use of this species in the traditional medicine of different countries for the treatment of various ailments, including inflammations and cancer; nonetheless, very few researches have been performed to investigate the phytochemistry and the biological activities of *M. incana* aerial parts. Thus, in continuation of our studies on species of the Brassicaceae family growing in Sicily, the present work was undertaken to characterize the phenolic profile and to evaluate the antioxidant properties and the toxicity of a hydroalcoholic extract (80% methanol) obtained from the aerial parts (leaves and flower buds) of *M. incana* grown wild around Capo d'Orlando (Messina).

The quali-quantitative characterization of the phenolic compounds contained in *M. incana* extract was attained by HPLC-PDA-ESI-MS. The analysis led to the identification of 12 compounds, 2 out of them belonging to the group of phenolic acids (5.46 mg/g extract) and 10 to flavonoids (155.85 mg/g extract). Among the detected compounds luteolin-glucoside turned out to be the most abundant one (57.07 mg/g ± 0.87% RSD), followed by dihydrokaempferol (40.46 mg/g ± 1.01% RSD) and naringenin-glucoside (30.92 mg/g ± 0.98% RSD).

Antioxidant activity, especially of phytocomplexes, cannot be evaluated satisfactorily by a simple antioxidant test, but it is strongly suggested the use of various methods in order to acquire a more complete antioxidant profile. In order to extensively characterise the antioxidant potential of *M. incana* extract, three "in vitro" systems based on fundamentally different approaches and mechanisms were utilized: the primary antioxidant activity was examined by DPPH and reducing power assays; the secondary antioxidant properties were determined by ferrous ions chelating activity assay (3,4). Butylated hydroxytoluene (BHT) and ethylenediaminetetraacetic acid (EDTA) were used as reference standards. *M. incana* extract exhibited mild activity both in the DPPH test (IC_{50} = 2.32 ± 0.24 mg/mL) and in the reducing power assay (ASE/mL = 12.29 ± 0.42). By contrast, the extract exhibited good chelating properties, reaching nearly 80% activity at the dose of 1 mg/mL.

Finally, the extract resulted non-toxic in the *Artemia salina* Leach (brine shrimp) lethality bioassay (LC_{50} > 1000 μg/mL) (3,4).

Our findings contribute to an increase in knowledge about *Matthiola incana*, also supporting the traditional use of this species.

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COMMUNICATION N. 19

Leaves and flowers of *Oreocallis grandiflora* (Lam.) R.Br. from Ecuador: a new herbal remedy?

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O. grandiflora (Lam.) is an Ecuadorian species belonging to the Proteaceae family, commonly known as *cucharillo* (Loja and Zamora provinces), *cucharilla* (Sierra region), *gañal* (Bolívar province) and *algil* (Chimborazo province) (Fig. 1). Its leaves and flowers, collected during blooming, are traditionally used for oral administration to treat liver diseases, vaginal bleeding, ovary/uterus inflammation and as digestive, diuretic, hypoglycemic remedy (1-3). To the best of our knowledge, the related literature does not report any scientific papers regarding the chemical composition of the used parts of this species (leaves and flowers), while few indications are reported about the healthy properties of their preparations as crude drugs. Based on these premises, the present research was performed with the objectives to fill the gaps of the chemical and biological knowledge about this species, enriching the knowledge related to the plant biodiversity of Amazonian Ecuador and to the ethno-botanical tradition of Andean communities.

In fact, the chemical and biological investigation (*in vitro* antioxidant, anti-inflammatory and cytotoxic properties) of the flowers and leaves hydroalcoholic extracts, shed a light on the functional metabolites putatively involved in healthy properties of the *O. grandiflora* traditional preparations. The chemical fingerprinting achieved by HPTLC (Fig. 2) and ^{1}H NMR analyses showed the presence of phenols, flavonoids in particular, subsequently quantitatively estimated by $AlCl_3$ complexation assay. Silica-gel chromatography allowed the isolation of quercetin 3-*O*-β-glucuronide and myricetin 3-*O*-β-glucuronide, the two main compounds detected in the flowers extract. HPLC-MS analyses showed instead the presence of quercetin 3-*O*-rutinoside and isorhamnetin 3-*O*-rutinoside in the leaves extract (4, 5). Regarding the antioxidant and the anti-inflammatory activities performed by DPPH test and WST-1 assay respectively, leaves extract showed the most promising IC_{50} values (6.69±1.39 µg/mL and 4.08±0.07 µg/mL, respectively). Finally, starting from the concentration of 50 µg/mL, the cytotoxicity (cell viability assay) of the leaves extract against human isolated neutrophils was approximately 4-fold higher than that showed by the flowers extract. However, despite this result, the very pronounced anti-inflammatory activity of leaves extract encourage further research to strengthen its possible as a new herbal health remedy (6).

Fig. 1. *Oreoacallis grandifora*

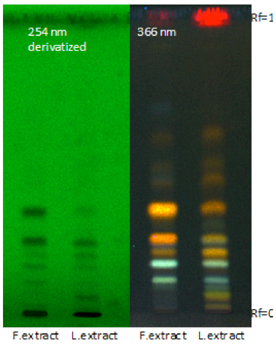

Fig.2 HPTLC of extracts

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Antiproliferative cardenolides from the aerial parts of *Pergularia tomentosa*

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Pergularia tomentosa is a milkweed tropical plant belonging to the family Asclepiadaceae, with dimensions close to 50 cm high. It is a native species of the Middle East and North of Africa (1). Different part of *P. tomentosa*, including aerial parts and roots are used in traditional medicine for the treatment of several diseases such as bronchitis, tuberculosis, molluscidal activity, persistent hypoglyceaemic effects, constipation and skin diseases (2). Previous investigations on *P. tomentosa* highlighted the occurrence of cardiac glycosides namely desglucouzarin, coroglaucigenin, and uzarigenin in the leaves (1,3), and uzarigenin, ghalakinoside, calactin, 6'-hydroxycalactin, 6'-hydroxy-16α-acetoxycalactin, 16α-hydroxycalactin, 12'-dehydroxyghalakinoside, 3-*O*-*β*-glucopyranosylcalactin, 6'-dehydroxyghalakinoside in the roots (4, 5).

Chemically, cardiac glycosides are compounds showing a steroidal nucleus with a lactone moiety at position C-17, containing a sugar moiety at C-3. The nature of the lactone ring at C-17 defines the compound class: cardenolides (with an unsaturated butyrolactone ring) and bufadienolides (with an α-pyrone ring) (6, 7). In cardiac glycosides produced by plants from the milkweed family Asclepiadaceae (such as calactin, ghalakinoside) the A/B rings are trans fused, resulting in rather flat structures with a markedly more potent binding to Na^+/K^+-ATPase pump (particularly to Na^+/K^+-ATPase α1 subunits) (5).

As a part of our ongoing research on new bioactive compounds from *P. tomentosa* and on the basis of the interesting activity shown by cardenolides isolated from the roots, our attention was focused on the aerial parts of this plant with the aim to highlight the occurrence of cardiac glycosides with potential activity.

Therefore, the LC-MS profile of the MeOH extract of the aerial parts of *P. tomentosa* was performed to guide the isolation of 23 compounds, of which structures were unambiguously elucidated by NMR analysis. Four new double-linked cardenolides (4, 7, 13, 14), along with known cardenolides (1-3, 5, 8, 15-23) and flavone glycosides (6, 9-12) were identified. With the aim to observe differences in the qualitative profile of the aerial parts and roots of *P. tomentosa*, known for the occurrence of double-linked cardenolides, a LC-ESI/HR/MS analysis, in negative ionization mode, of the methanol extract of the roots was also performed.

On the basis of the activity reported for cardenolides, the isolated compounds were tested for the ability to decrease the cell viability on five different human cancer cell lines (PC3, prostate carcinoma), Hela (cervical carcinoma), Calu-1 (epithelial lung cancer), MCF-7 (breast cancer), and U251MG (human glioma). All tested compounds were able to reduce *in vitro* cell viability with IC_{50} ranging from 0.2 to 8.9 μM. Moreover, a S-phase entry assay was performed to investigate if the compounds could affect cell cycle progression of PC3 cells. Our findings highlight the double-linked cardenolides isolated from the aerial parts of *P. tomentosa* as potential antiproliferative agents.

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Plant growth in space: struggling with environmental factors and physical constraints

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Long-duration missions to deep-space destinations, such as Mars, rely on the possibilities to grow food crops in controlled environments, while exposing them to extra-terrestrial factors, such as altered gravity and radiation.

Expected scenarios for a mission to Mars are 6 to 8 months of travel (one way) and about 500 days on the surface of the planet. For such missions, the option of supplying all the biological needs from Earth, as it is currently done for the International Space Station (ISS), is not feasible. The solution is thus to recycle consumables, and use *in situ* resources.

The development of a bioregenerative life support system (BLSS), supplying astronauts with all the oxygen, water and food they require, is the goal of the Micro-Ecological Life Support System Alternative programme (MELiSSA), a long lasting European Space Agency (ESA) initiative based on a terrestrial lake ecosystem concept.

In the BLSSs higher plants play a crucial role: they purify water, organicate CO_2, and produce O_2 and food. Difficulties to set up an artificial self-sustaining life support system in space are increased by the interactions with specific environmental factors, and physical constraints (e.g. confinement). Although plants on Earth have evolved strategies to adapt to a wide range of environmental parameters, and also to extreme environments, no species has naturally evolved to deal with reduced gravity, such as on the Moon or Mars, or the microgravity (weightlessness) conditions occurring in orbital stations, such as the ISS.

We have studied the effect of microgravity on plant growth, in the framework of several projects funded by the European or Italian Space Agencies (ESA and ASI). In this contribution, we focus on the effects of the interaction between weightlessness and other environmental factors on root development.

On Earth, gravity is considered the main factor directing root growth into the soil (gravitropism). However, other external stimuli have been identified to affect plant root growth. The most extensively studied include hydrotropism, phototropism, chemotropism, halotropism and thigmotropism. These stimuli are generally studied in a reductionistic approach, without other tropic influences. However, in any natural or artificial environment, the final growth strategy relies on the integration of proportional influences of all tropic signals.

We have recently investigated the role of gravitropism, hydrotropism and chemotropism as external stimuli for root tip orientation in order to clarify the interactions between different attractive factors. The experiments were performed on Earth in simulated microgravity conditions and in space on the ISS. In our laboratory, we used a uniaxial clinostat to alter the gravity conditions by constantly changing the gravity vector. In Space, we executed the MULTITROP experiment, the winner of a call by ASI (Youth ISS Science) for activities to be performed by the astronaut Paolo Nespoli during the VITA Mission (Expedition 53/54). The study aimed at disentangling the role of gravity from other stimuli causing root orientation, namely hydrotropism and chemotropism.

The experiment was implemented in a hardware previously developed for yeast culture in Space, and refurbished for seed germination by Kayser Italia. Several months of activities were aimed at selecting the suitable plant species. Seeds had to be best adapted for the limited volume of the growth chamber, the expected temperature range for launch and flight operations, and the NASA timeline from sample integration to the arrival in microgravity conditions. At the end of the pre-flight phase, 32 carrot seeds (*Daucus carota* L.) were chosen for the experiment. Implementation in the hardware occurred at the launch site (NASA, Kennedy Space Centre, FL, USA). Seed imbibition began during the implementation, but seed germination had been planned to take place in microgravity. After germination, root growth was interrupted on board the ISS by the injection of a chemical fixative.

At the end of the MULTITROP mission samples were retrieved, analyzed and compared to those obtained in the 1-g ground control, and in simulated microgravity, allowing us to evaluate the contribute of the different tropisms to direct root tip development.

COMMUNICATION N. 22

Preliminary climatic distribution model for *Pterygiopsis affinis* as a means for field surveys planning

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Pterygiopsis affinis (1,2) is a rare, crustose cyanolichen with Mediterranean distribution. While this species is known to reproduce sexually, several specimens bearing also soredia (asexual reproductive structures) have been collected. In lichen taxonomy, reproductive strategy is a character normally used for delimiting different species. In this study, we aimed at understanding whether the sorediate forms of *P. affinis* deserve to be recognized taxonomically (3). The morphology and anatomy of both sorediate and non-sorediate specimens were analyzed, to understand whether the production of soredia correlates with other features. Preliminary results do not evidence a clear clustering, but a range of variability among sorediate and non- sorediate samples, mainly in spores, algal mucilage, and pseudoparenchymatous cells size. A distribution modeling approach was then applied to identify suitable areas for *P. affinis* in the country, and hence to collect new specimens. An ensemble forecasting approach (4) was applied, creating models with 3 different algorithms (GLM, RandomForest and MaxEnt), and then averaging the results in one final model, weighed on the performance of individual models, which was assessed by True Skill Statistic (TSS) (5), setting the threshold at 0,7. Bioclimatic variables obtained from Chelsa have been used to train a number of preliminary models and tested for correlation, identifying most influencing factors, hence excluding meaningless variables. Preliminary results show that the most influencing climatic variables for the species are Precipitation Seasonality and Temperature Annual Range. *P. affinis* has a higher suitability in areas with less than 20% of variation in monthly precipitation, and with a temperature annual variation lower that 15°C. These areas are mostly the Adriatic side of Italy, with another hotspot in Friuli Venezia Giulia and Istria, and some other scattered locations occur in Western Maritime Alps and southern Tuscany.


Fig.1. *Pterygiopsis affinis*, typical form.

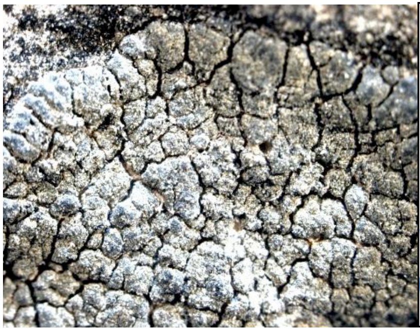
Fig.2. *Pterygiopsis affinis*, sorediate.

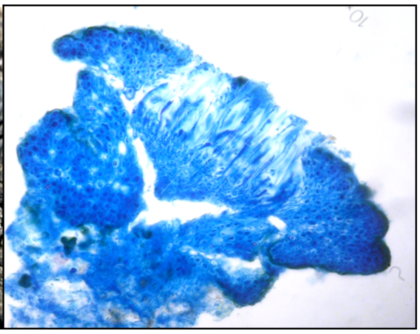
Fig.3. Apothecia thin section (10 μ).

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Extreme drought and heat waves effects on Tuscan forests: forest dieback and tree mortality in summer 2017

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The reports of forest die-off events triggered by dry spells have increased significantly in the last decades. During summer 2017, central Italy was hit by intense drought and heat waves, with temperature peaks over 40°C. Starting from mid July, impacts, such as leaf discolouration, desiccation and, in August, early foliar shedding in deciduous broadleaf tree species, and diffuse desiccation of leaves and branches in evergreen broadleaf species, were observed.

This study describes the results of a preliminary analyses of these impacts in Tuscany (central Italy), based on field observations and remote sensing surveys. To this purpose, we adopted three different approaches: (i) mapping of drought-induced forest damage, by means of the analysis of canopy reflectance; the Normalized Difference Vegetation index (NDVI) and Sentinel 2 spectral bands (NIR, RedEdge 3, RedEdge 4) were evaluated for drought-affected and unaffected forest stands for each forest type studied; (ii) analysis of non-structural carbohydrates content in woody tissues (small branches and twigs) in drought-damaged and non-damaged trees for the most relevant species; (iii) evaluation of the resilience of evergreen sclerophyllous species, by analyzing their capacity to produce new shoots and leaves from the damaged crown.

Beech (*Fagus sylvatica* L.), downy oak (*Quercus pubescens* Willd.), Turkey oak (*Quercus cerris* L.) and holm oak (*Quercus ilex* L.) forest stands, located respectively in the Apennines, hilly and Mediterranean areas of the region, were the forest ecosystems which suffered the most relevant impacts. The strongest impacts were observed in the sites at higher altitudes, south exposed and/or on poor substrata, especially on calcareous and serpentine soils. Remarkably, deciduous trees were affected by strong crown defoliation but, apparently, no mortality, whereas evergreen species showed foliar desiccation and large crown dieback. Crown defoliation, desiccation and tree mortality affected also drought-tolerant sclerophyllous shrubs, like *Arbutus unedo* L., *Phillyrea latifolia* L., *Erica arborea* L., and perennials herbs of the Mediterranean shrubland.

The studied tree species, which mostly suffered of the severe dry spell in 2017, and their distribution in Tuscany, suggest a relevant role of the site conditions (slope aspect, bedrock, soil properties as depth and capacity of water retention) in the occurrence and diffusion of forest dieback. The analysis of these factors allows to map the sensitivity and vulnerability of forests to extreme climate events. This is the premise for the development and application of new management strategies aimed at decreasing climate-induced risk and promoting forest resistance.

COMMUNICATION N. 24

Morpho-functional plant traits conferring radioresistance: living in extreme conditions by transforming constraints in opportunities

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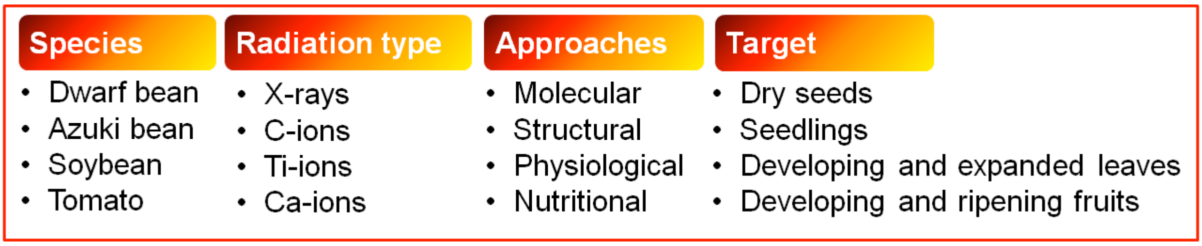
The effect of ionizing radiation on plants has been studied with several purposes. Ionizing radiation has been used to generate stable mutants in breeding programs, or as a substitute for conventional decontamination methods. However, the larget amount of data on the effects of ionizing radiation on plants was obtained from radioecology studies, as well as from Space exploration applications.

Events as Chernobyl or Fukushima disasters created "natural experimental fields" where to study radiation effects in contaminated ecosystems. The direct effects of radionuclides on plants and indirect long-term effects due to the contamination of other components of the ecosystem have been widely explored. However, Space biology is currently one of the most active fields in which radiation effects on plants are studied (1). In the sight of long duration exploratory-class manned missions, like the one towards Mars or the deep space, the protection of organisms from cosmic radiation is considered one of the main challenges (2). In such missions, plant cultivation is mandatory, since plants not only have an important role in mitigating psychological stress due to isolation, but are also key organisms for regeneration in artificial ecosystem (e.g. Bioregenerative Life Support Systems). Plants regenerate oxigen through photosynthesis, allow water recovery through transpiration, participate in waste recycling, and produce fresh food (3). However, the Space is an extreme environment where ionizing radiation is known to influence deeply organisms' growth at molecular, morpho-structural, physiological and biochemical levels, thus affecting the efficiency of plants as regenerators.

Herein, we summarize the key results of many experiments performed on crop species (e.g. soybean, *Glycine max* (L.) Merr.; azuki bean, *Vigna radiata* (L.) R. Wilczek; dwarf bean, *Phaseolus vulgaris* L.; tomato, *Solanum lycopersicum* L.), where plants, during several phenological phases; have been exposed to ionizing radiation. Seeds and adult plants were exposed to different types of radiation (e.g. Low- and High-LET, Linear Energy Transfer), and at different doses. The combined effect of radiation and other environmental factors, such as light quality, has also been investigated. The consequence of irradiation was assessed through the analysis of morphological development, some molecular traits, anatomical features of leaves and fruits, photosynthesis, flowering phenology, fruit development, and nutritional traits.

Depending on several factors, including species, cultivar, plant phenological stage and traits of the target tissue/organ, the exposure to ionizing radiation produces several effects, which range from detrimental outcomes at very high doses, harmful consequences at intermediate levels, and stimulatory effects at very low doses. A severe interaction with other factors has also been evidenced.

The interaction between factors and the different radiosensitivity of plants should be taken into account in ecosystem recovery in case of radiation contamination, as well as in the development of artificial ecosystems in extreme environments like Space.



Species	Radiation type	Approaches	Target
Dwarf bean	X-rays	Molecular	Dry seeds
Azuki bean	C-ions	Structural	Seedlings
Soybean	Ti-ions	Physiological	Developing and expanded leaves
Tomato	Ca-ions	Nutritional	Developing and ripening fruits

Fig. 1. Plant material, radiation type, and analyses conducted to explore plants' response to ionizing radiation

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Ecophysiological response in some *taxa* of the urban ecosystem of Genova

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Urban air pollution is one relevant environmental issue. All plants are able to remove pollutants from air, but some of them are more efficient, depending on their morpho-functional, and species-specific characteristics, such as: structure of the leaves (thickness, shape, density and morphology of the stomata) and seasonal persistence (1).
The present study assesses the ecophysiological response in relation to air pollutants of 4 plant *taxa* commonly used for greening urban areas in the city of Genoa. This research aimed at: a) identifying the most performing of the 4 *taxa*; b) understanding the possible influence of leaf exposure time to air pollutants in relation to ecophysiological parameters; c) verifying the possible difference in each *taxon* within and among the sampling areas with different levels of air pollutants.
The 4 *taxa* were: *Photinia* x *fraseri* Dress, *Pittosporum tobira* (Thunb.) W.T. Aiton, *Hedera helix* L. and *Cedrus libani* A. Rich. The different plant disposition in space when exposed to the pollutants, plant size and heights of exposition respect to the ground level (from 0.5 to 3 m), and their *habitus*, have allowed to study the effect of air pollution on the ecophysiology of each *taxon*.
The study area includes two urban green sites in the metropolitan city of Genoa, one with high level of car traffic in the district of San Martino (A) and one with 'background' level of air pollutants in the residential district of Quinto (B). Both sites have similar climatic conditions, and are close to "Liguria Region Environmental Protection Agency (ARPAL, Agenzia Regionale per la Protezione dell'Ambiente Ligure) station" for air quality monitoring.
The samplings were carried out on the leaves at different exposure time to air pollutants (i.e., "young", 0-3 months or "old", > 6 months), following the protocol described in a previous study (2). All the samples were measured with Handy PEA (Photosynthesis Efficiency Analyzer) direct fluorimeter to measure the polyphasic curve of Kautsky O-J-I-P (which describes the passage of electrons between the two photosystems of the photosynthetic apparatus), the photosynthetic efficiency (Fv/Fm) (3), and the Performance Index (P.I., an overall indicator of the vitality of the plant).
Data analysis was performed with PEA Plus Application and Minitab 15 Statistical software. The t-test for the comparison between the non-paired samples mean (two sample test) was used. Differences were considered significant at $p < 0.05$ using one way ANOVA.
The ecophysiological parameters revealed that the 4 *taxa* perform differently as far as air pollutants are concerned, in the order: *P. tobira* > *P.* x *fraseri* > *H. helix* > *C. Libani* (A site), and *P. tobira* > *C. libani* > *H. helix* (B site). The ecophysiological response in both sites of 'old' and 'young' leaves revealed that longer exposures does not affect the photosynthetic apparatus, with the exception of *H. helix.* The comparison of all parameters between the two sites highlighted a significant difference in the performance of all *taxa*, with those of sites B subjected to lower stress, due to lower concentration of air pollutants, as described in the monitoring carried out by ARPAL (4). The behaviour of *H. helix*, albeit in contrast to the other *taxa*, is consistent in the response to environmental stresses in B site; this may indicate that this species is more sensitive to other air pollutants limited to B site (e.g., O_3).

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COMMUNICATION N. 26

Plant traits and plant-plant interactions shape the effect of elevation on *Vaccinium myrtillus* communities in Alpine tundra

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A relevant evidence of vegetation change due to climate warming in both arctic and alpine regions is shrub expansion (1,2). Hence, understanding the response of dwarf shrubs to climate warming can be of outmost importance for the understanding of the forthcoming changes in high altitude ecosystems. Climate changes induce plants to develop both morphological and physiological strategies in order to achieve acclimatization. Along an elevation gradient (as a proxy for temperature decrease), we demonstrated that changes of non-structural carbohydrates (NSC) in alpine stands of *Vaccinium myrtillus* were affected not only by altitude - which, in some cases, acts as secondary player - but also by some crucial plant and population traits. In particular, plant traits (age and stem tissue pattern) of the populations and plant–plant interactions (shrub density) can shape the effect of elevation. Glucose content was positively correlated with altitude, but negatively correlated with shrub density, and percentage of phloem tissue. Instead, sucrose decreased at high altitude, and in older populations, but increased in individuals with higher percentage of vascular tissues (i.e. xylem and phloem). Starch content increased along elevation, and with high shrub density, whereas age of *V. myrtillus* stands showed a weak negative relationship. NSC exhibit different patterns with respect to elevation and plant traits. In conclusion, we suggest that plant traits could represent modulators of species response to temperature stress, which act specifically on each different NSC, showing that they cannot be analyzed altogether for understanding species acclimation processes. In light of shrub expansion due to global warming, we showed that species acclimation to temperature relies not only on the environmental stress on each individual, but also on community structure. This highlights the importance of intra- and interspecific effects on plant metabolism, in order to evaluate the outcome of plant–plant interactions as a function of abiotic conditions (3). Our results open also new perspective in the light of the Stress Gradient Hypothesis (SGH) (4), which postulates that facilitative interactions would be dominant in harsh environments, shifting to competitive ones as abiotic conditions ameliorate. These results might reverberate on the whole plant community, affecting the overall diversity, by changes in species richness and species composition (5).

Fig. 1a- 1b - 1c.Study plant community, species (*Vaccinium mirtyllus*) and trait (ramet coss-section)

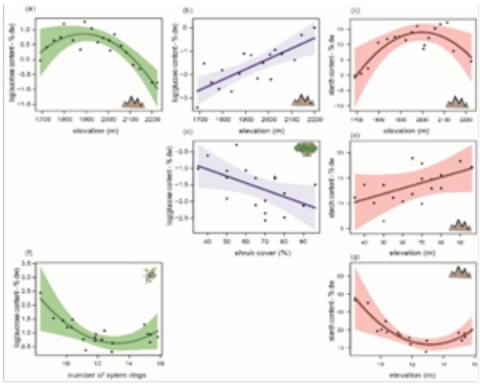

Fig. 2. Graphical abstract of main results

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Adaptations to environmental stresses: the case of *Seirophora villosa* (Ach.) Frödén

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In the Mediterranean basin, coastal dune systems are a priority habitat for nature conservation. *Seirophora villosa* is strictly associated with undisturbed dune juniper formations and could be used as an indicator of the status of conservation (1). Light regime, water availability and high salt concentrations are generally the main ecological factors affected by habitat fragmentation, which could be detrimental to the survival and establishment of *S. villosa* populations. This study aims at investigating how light regime and water availability affect individual specimens of *S. villosa*, by studying the relationship between photosynthetic activity and water content in thallus areas of different sizes. Furthermore, we investigated the role of the thin hairiness on thallus surface characterizing *S. villosa* to withstand the effects of seawater by continuous exposure to marine aerosol. Thalli of *S.villosa* were repeatedly sprayed on the upper surface with mineral water, until fully hydrated. Each thallus was weighed at the beginning of the dehydration cycle, and thereafter the rate of water loss and the photosynthetic activity were assessed every 40 minutes. Light exposure was carried out in wet and dry thalli in a climatic chamber at 20°C and 60% RH using a photon flux intensity of 2000 μmol $m^{-2}s^{-1}$. The hydration status of each sample was maintained for the entire exposure time and once photoinhibited, samples were left to recover (2). The impact of salinity stress was detected exposing thalli of *S. villosa* with and without microscopic hair (in the latter case they were removed mechanically by using a razor), to 0, 0.5, and 1 M NaCl. Thalli were repeatedly sprayed on the upper surface with each solution until fully hydrated and once dehydrated, sodium concentrations were determined by atomic absorption spectrophotometry. Therefore, we investigated its effect on membrane permeability and on the photosynthetic apparatus. *S. villosa* thalli are susceptible to sudden increases in light exposure, especially in the case of small and dry specimens, which after photoinhibition exhibited a reduced ability to recover. The presence of highly branched laciniae covered by thin hairs improves the lichen's surface/volume ratio, thus increasing the absorption surface, and keep out part of the salt depositions sprayed on the thallus. High salt concentration caused dehydration and the inhibition of photosynthesis as in other lichen species. However, in *S.villosa* the morphological trait of hairiness plays a main role slowing down thallus dehydration, hence allowing prolonged photosynthetic activity even with low water content (25%). Several studies have shown that after logging or habitat fragmentation, epiphytic lichens are exposed to both sudden increases in PAR and to dry conditions which, when exceeding the normal ecological range, can negatively impact on their photosynthetic performance (3). As a consequence, incorrect management of dune systems can cause vegetation loss and a resulting sudden increase in light exposure and greater exposure to salt spray. Therefore, habitat fragmentation is a significant threat to lichen species, particularly in more vulnerable phases such as dispersal and establishment.

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Unraveling how global change and habitat modification are threatening lichen diversity

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Rapid global change is threatening biodiversity globally (1), and climatic changes related to the increase in CO_2 concentrations in particular are expected to become the main driver of biodiversity loss (2). Both direct and indirect effects may affect species distribution and community assembly, e.g. causing latitudinal and altitudinal range, compositional and functional shifts, phenology anomalies, and mismatches among species, which could imperil the effectiveness and stability of biological networks. Global change may also exacerbate the impacts of other relevant drivers of biodiversity loss, as in the case of habitat modification associated with agriculture, forestry, and urbanization. This scenario fully applies to lichens, whose poikilohydric nature determines a close relationship between eco-physiology and climatic conditions and a strong sensitivity to anthropogenic disturbance (e.g. air pollution, eutrophication, forest management). Results of our research clearly indicate that global change is rapidly affecting lichen diversity in many ways, also interacting with local processes that involve habitat modification. In a number of studies across Italy, spanning climatic and anthropogenic gradients, we detected the lichen response in terms of richness loss, compositional and functional shifts, floristic homogenization, habitat suitability reduction, decoupling between epiphytes and substrate, indicating that many species may soon experience local extinction. While lichens greatly contribute to ecosystem biodiversity and functioning, being also associated to relevant ecosystem services (3, 4), they are still receiving little consideration in conservation policies. Hopefully, our results could contribute to increase public awareness on their future.

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Phytoremediation capacity and rhizosphere microbiome of a black poplar in response to zinc and compost amendment

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Phytoremediation is an economically and environmentally effective *in situ* method for cleaning heavy metal (HM) polluted soils (1). The environmental behavior of HMs in soils, and the potential risks they constitute for plants and animals depend by the interaction among them and organic and inorganic components of the soil. These interactions influence mobility, bioavailability and toxicity of HMs. A promising strategy for the treatment of HM polluted soils is their amendment with compost (CMP; 2). CMP amendment affects physical-chemical and biological characteristics of soil, and can significantly modify the mobility and bioavailability of HMs. At the same time, it introduces new organic matter, nutrients and microbial organisms, which can considerably improve plant productivity and soil quality (3), and provide environmental and nutritional conditions effective in selecting microorganisms which are useful for reclamation of multi-polluted soils.

The aims of the study were to evaluate the phytoremediation capacity (in terms of biomass production and metal uptake) of a metal tolerant (4) poplar clone (NG12), grown on zinc (Zn) artificially polluted soil, even in presence of CMP, and to characterize poplar rhizosphere microbiome, and its modifications in response to treatments (metal contamination and/or CMP amendment). Cuttings of NG12 clone were singularly put into plastic pots, containing CMP amended (20% of the pot volume) or not amended soil; some pots were added with Zn solution up to a final concentration of 450 mg kg^{-1} of soil dry weight (DW). Therefore, the experimental design was as follow (3 cuttings per treatment): control (plant on unpolluted soil); Zn treatment (plant on Zn polluted soil); CMP Control (plant on unpolluted and CMP amended soil); CMP+Zn treatment (plant on Zn polluted and CMP amended soil). At the end of experimentation, root, stem and leaf samples were collected and stored separately for biomass DW measurements and for determination of Zn content. Therefore, root rhizosphere soils were harvested at the end of the trial from each pot and microbiomes underwent Next Generation Sequencing (NGS) procedure in order to analyze the hypervariable region of 16S rDNA and of ITS (Internal Transcribed Spacer), and characterize the bacterial and fungal communities.

The addition of Zn to the soil did not significantly influence the growth of poplar clone, while the CMP amendment significantly improved growth (mainly of leaves) and biomass production. Poplar clones accumulated Zn mainly in the roots, then in the stems and leaves; however, CMP amendment of soil did not significantly influence Zn accumulation in plant organs. The NGS analysis revealed a significant variability of the microbiomes associated with the rhizospheres of NG12 clone depending on the treatment (addition of the Zn and/or CMP amendment). The most abundant bacterial classes in both CNT and treated rhizosphere samples were Acidobacteria, Alpha-, Beta- and Gamma-proteobacteria. However, compared to CNT rhizosphere Acidobacteria and Beta-proteobacteria were reduced mainly in CMP amended rhizosphere; while Gamma-proteobacteria were enriched in the CMP amended rhizosphere. Actinobacteria were identified in Zn and CMP rhizospheres. Pezizomycetes and Sordariomycetes were the most abundant fungal classes associated to NG12 rhizospheres, and their abudances were modulated mainly in the presence of CMP alone. At the same time the Dothideomycetes were strongly reduced in the rhizosphere of clone amended with CMP; while the abundance of Agaricomycetes class was increased in the presence of Zn and CMP. This research provides essential information about the variation and adaptation of rhizosphere microbiome of tolerant poplar clone, in relation to HM stress and CMP amendment.

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Evidence of changes in reproductive strategies in promoting the invasiveness of *Pistia stratiotes* L.

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Pistia stratiotes L. (Araceae) is a free-floating freshwater macrophyte native to tropical and sub-tropical regions. In invaded areas, this alien plant can produce extensive mats with a high environmental impact on aquatic systems: reduction of dissolved oxygen concentrations, increase of evaporation rates in comparison with open water areas, increase of malaria vectors, and competition with native biodiversity. Water lettuce is considered one of the most emerging invasive alien hydrophytes in Mediterranean Basin and it is included in the Alert List by European and Mediterranean Plant Protection Organization (EPPO). However, few studies deal with the ecological parameters which affect the biology and invasion strategies of this weed.

In this study, combined effect of conspecific density (3, 6, 12, 24 plant/0.175 m^2) and water salinity (0, 0.01, 0.05, 0.1% NaCl) on growth and reproductive strategies of *P. stratiotes* were analyzed by means of greenhouse experiments.

The growth rate of *P. stratiotes* is negatively affected by population density and salt concentration. Similar trend was evidenced in vegetative reproduction (number and length of stolons) that dramatically decreased in treatments with the higher plants density. As a consequence N content in plant shoots showed a linear increasing trend associated to plant density with higher values recorded for higher plant densities. On the contrary, the production of inflorescences increases in the treatments with higher plants density.

Data highlighted the effects of environmental conditions on reprodutive strategies of *P. stratiotes* suggesting an adaptative capability in modulating its high and very fast spread and invasiveness.

Diversity trends of bryophytes in Antarctica are the result of adaptation, evolution and survival (including cryptobiosis): the case study of the phylogeny of *Bryum argenteum*

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Terrestrial vegetation communities in Antarctica are predominantly composed by bryophytes and lichens, as the extreme climatic conditions of this remote continent limit the colonization of vascular plants to 2 species in the Antarctic Peninsula.

A decline in bryophyte species richness occurs with latitude across the sub-Antarctic islands, Antarctic Peninsula and Antarctic continent, with a clear continent-Antarctic Peninsula separation, consistent with the Gressitt Line, a boundary identified for the invertebrate fauna, and now demonstrated also for bryophytes, suggesting a common feature in the evolutionary history of the vegetation and invertebrate fauna. Within continental Antarctica, the highest bryophyte diversity occurs in the Ross sector, despite its location is the least favorable for species migrating from the southern Hemisphere continents.

Moreover, the occurrence in Antarctica of cosmopolitan species, such as *Bryum argenteum*, further highlights the need to understand the origin and evolution of the Antarctic flora and to investigate the role of Antarctica in explaining the dispersal routes of taxa with transoceanic or transcontinental disjunctions, with *B. argenteum* being an ideal target species.

We reconstructed the phylogeny and divergence times of *B. argenteum*, both at global scale and focusing within Antarctica and Southern Hemisphere, using the nrITS region from more than 200 samples applying a molecular clock based on previously estimated substitution rates for mosses. Our analyses indicate that the distribution patterns of *B. argenteum,* at global scale, are the result of major paleoclimatic events occurring across the planet, and involving differentiation and migration/colonization during periods warmer than today since the Mid Miocene Climate Optimum (MMCO), as well as fragmentation during severe cooling, such as during the extensive Antarctic glaciations in the mid Miocene 14 Ma.

Bryum argenteum first extensively colonized Antarctica around 9.77 Ma during the warm Tortonian (Miocene), using an intra-Antarctic dispersal route after reaching Antarctica from South America, moving the length of the Antarctic Peninsula, and reaching continental Antarctica along the coast or through inland mountains as far as Victoria Land (Ross sector), where it survived glaciations in nunataks regions that have remained ice-free for ≥ 5 My. One potential survival mechanisms during glaciations involved cryptobiosis under cold-based glaciers. Cryptobiosis is a reversible ametabolic state of life characterized by the ceasing of all metabolic processes, allowing survival of periods of intense adverse conditions. In the Antarctic Peninsula we demonstrated that entire moss individuals, dated by ^{14}C, survived through cryptobiosis during six centuries of cold-based glacier burial in Antarctica, and that after re-exposure due to glacier retreat these mosses were able to return to a metabolically active state and remain alive.

The case study of *B. argenteum* allows to demonstrate that Antarctica was colonized several times and that migrations occurred globally during periods of climatic optima in the Miocene and Pliocene, thus providing an analogue for biotic responses in a future warming world, in particular demonstrating the potential for future biological invasions in Antarctica.

Diversity, evolution and nickel accumulation in a critical group of metallophytes from a major serpentine hotspot: the genus *Odontarrhena* (Brassicaceae) in Albania

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Serpentine soils are extreme environments for plant life due to their physical and chemical anomalies, especially the high levels of heavy metals such as Ni, Cr and Co. Only a few plants groups are adapted to live on these soils and these are termed "metallophytes" (1). A special group of metallophytes is that of Ni-accumulator plants, which are able to accumulate more than 1000 µg Ni g^{-1} leaf dry weight without any toxicity symptom. In the Euro-Mediterranean region, the most diverse and widely distributed of these plant groups is *Odontarrhena* C.A.Mey. Several experimental studies on the physiological and molecular mechanisms of Ni-hyperaccumulation have been performed on model taxa of this genus, and many of these are currently used or considered for phytoremediation or phytomining practices (2). Despite the importance of such plants, however, our knowledge about the diversity, evolutionary dynamics and metal accumulation capacity in *Odontarrhena* is still incomplete. Taxonomic interpretation of several species groups remains problematic due to the often large phenotypic plasticity of populations, weak correspondence between morphological, ecological or geographical variation, low phylogenetic divergence and relatively homogeneous karyotype features (3). Past authors proposed complex treatments based on a narrow morphological species concept. More recent views mostly recognize a lower number of taxa, that, however, are considerably different from author to author. Nomenclatural inconsistencies caused by the divergent taxonomic opinions hamper further studies of ecological and physiological type, as well the use of these plants for practical applications.

Because of the large occurrence of serpentine soils and its geomorphological complexity, Albania is a major center of diversity of *Odontharrhena*. The country is botanically still poorly known and, to date, no consensus has existed either about the number and the distribution of native taxa of this genus or about the names to be adopted for them (4). Patterns of genetic variation and diversity within and between such taxa/populations were also mostly unknown, despite a few previous phylogenetic studies (5, 6).

To fill these gaps, we started a series of investigations mainly based on material collected from over thirty natural populations from the entire Albanian territory during numerous field trips started in 2006, on serpentine and non-serpentine soils. After in-depth studies of literature and (scarce) herbarium collections, including type materials, we especially focused on the *loci-classici* of the 18 taxa described from the country. The study of this material with morphological, karyological and molecular tools allowed us to provide answers to the following questions: 1) how many and which taxa do exist in Albania? 2) Which are their phylogenetic relationships and patterns of population genetic structure and diversity? 3) Is there a relationship between the levels of heavy metals (especially Ni) in their native soil and those in their below- and above-ground tissues?

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Mycological characterization of extreme environments and substrates as first step for sustainable remediation technologies

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Fungi are pioneer microorganisms able to survive in extreme conditions and to colonize every kind of habitat. Many studies have demonstrated how they have developed specific strategies and tolerance mechanisms against limiting factors (e.g. salinity, pH, T, nutrients), and toxic organic and/or inorganic compounds (1, 2). These adaptation strategies generally involve fungal metabolism. Thanks to their enzymes, pigments, organic acids, and secondary metabolites, microfungi can change the microenvironment parameters, degrade or inactivate contaminants, induce chemical reactions, and solubilise insoluble compounds for their advantage (3). The study of extremophile fungal communities allows not only the selection of fungal strains potentially employable in biotechnological processes of remediation, but also the selection of important fungal metabolites usable in a number of sectors, such as medicine, natural science, engineering, etc. In bioremediation context, native fungi can represent a promising answer to the metals and hydrocarbons remediation. Many researchers have shown that fungi isolated from extremely compromised sites are the best to be employed in the restoration processes of the same sites (4, 5, 6). Mycological characterization consists of the isolation of vital fungal strains from various matrices (both solid and liquid), in their cultivation, identification, and analysis in order to select a pool of stronger fungal species exploitable in mycoremediation protocols of contaminated soils and waters.

In this context, our studies have faced the mycological characterization of numerous extreme environments, such as soils contaminated by Zn, Ni, Cd, and PAHs, or extreme substrates, such as organic waste, composting waste, diesel fuel, port sediments, port seawaters, and industrial sewages. Until now we have isolated about 800 fungal strains belonging to 70 morphotypes from 40 samples. This allowed selecting several strains that appear promising in some mycoremediation tests and fruitfully exploitable in biotechnologies.

Shifts of maize crop weed flora over 50 years: a case study in central Italy

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During the second half of the twentieth century, agricultural practices in Italy underwent a fast transition from extensive and traditional to intensive and specialized. Weed assemblages colonizing cultivated fields were thus subjected to major shifts, mainly driven by the increased use of herbicides and chemical fertilizers. Moved by the early intuition that the fight against weeds could deeply alter the balances of arable agroecosystems, already in the 1960s' several phytosociological surveys were carried out on maize weed communities of many Italian regions (1, 2, 3, 4, 5). Based on the study carried out in 1964 (4) on 21 maize fields across Latium (central Italy), we re-surveyed weed communities in as many fields, in the same localities, and in the same period of the year, to highlight the changes that agricultural intensification induced in their features. The agricultural practices lately introduced are irrigation, chemical weeding, and chemical fertilization.

Floristic diversity underwent a relevant decrease from 1964 to 2017, as the number of detected species dropped from 99 to 69, consistently with the current common use of herbicides. Besides, a major species turnover was highlighted: by 2017, 71 taxa had disappeared from fields and new 41 had appeared. Out of a total amount of 130 species, only 28 were present both in old and new relevés; the latter are essentially represented by generalist taxa as *Convolvulus arvensis* L., *Cynodon dactylon* (L.) Pers., and *Solanum nigrum* L. The most represented families are Poaceae, Asteraceae, and Fabaceae in both the surveys; a decrease of Asteraceae and Brassicaceae and an increase of Euphorbiaceae and Solanaceae was detected.

Many alien taxa, mostly American, appeared in 2017, suggesting how intensive agriculture can enhance biological invasions. Particularly relevant are the rates of occurrence of the invasive neophytes *Paspalum distichum* L. and *Datura stramonium* L. (36.4% and 31.8% of the relevés, respectively), followed by those of the archaeophyte *Abutilon theophrasti* Medik. (27.3%) and of the neophyte *Xanthium italicum* Moretti (18.2%). Other newly appearing invasive neophytes are *Euphorbia maculata* L., *E. prostrata* Aiton, and *Amaranthus blitoides* S.Watson. There was also a casual presence of several cultivated species deriving from previous or surrounding cultivations. Many taxa typically related to winter arable land were common in 1964, but had disappeared by 2017. Between these, there are *Lolium temulentum* L., *Lysimachia arvensis* (L.) U.Manns & Anderb., *Anthemis arvensis* L., *Phalaris brachystachys* Link, *Legousia speculum-veneris* (L.) Chaix, and *Centaurea cyanus* L.; their disappearance is to be related to the current absence of crop rotations and to their sensitivity to fertilized soils. Very relevant was the increase in frequency of *Sorghum halepense* (L.) Pers. (from 4.8 to 54.5%) and *Cyperus rotundus* L. (from 14.3 to 50%); these monocots are often resistant to herbicides (6) and, as rhizomatous geophytes, their propagation is favoured by ploughing.

The comparison of chorological spectra shows how Eurimediterranean species are the predominant ones in both the years, though a slight decrease in their occurrence is detectable in 2017. Cosmopolitan taxa highly increased, becoming the second most important chorotype instead of alien taxa, which became the third. The disappearance of Stenomediterranean taxa is linkable to the spread of irrigation practices and the consequent artificial elimination of drought conditions.

The similarity between the two life forms spectra points out how species were replaced by others with similar life strategies, as already detected in Latium for wheat weeds (7). The slight decrease in Therophytes is consistent with the disappearance of Stenomediterranean taxa, while the higher occurrence of Geophytes can be related to an increased intensity of ploughing.

The increase in C4 plants (such as Panicoideae, *Sorghum* sp. pl., *Cyperus rotundus*, *Amaranthus* sp. pl., *Euphorbia* sect. *Chamaesyce* - from 18.1 to 39.1%, frequency-weighted values), as maize itself is, suggests that more specialized agricultural practices favour weeds being physiologically closer to the cultivated species.

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Diversity and species concept in the polyploid apomictic *Ranunculus auricomus*

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Polyploidization is an important evolutionary process particularly prominent in plants. If combined with hybridization (allopolyploidization) is capable to create rapidly novel biotypes with novel genomic composition and morphological or physiological features. Formation of hybridogenic polyploid complexes is often coupled with the capability of those of reproducing asexually. Apomixis (i.e., the asexual reproduction via seed) is relatively common in species-rich, widespread families and genera (1), and chiefly observed in polyploid complexes (2). Apomictic polyploid complexes comprehend normally a few sexual parental species, and several apomictic hybrid derivatives. The substantial lack of recombination and cross-fertilization results in the formation of numerous clonal hybrid lineages, in which certain morphological and ecological traits can be rapidly fixed. Occasional facultative sexual reproduction and crossings among lineages can produce then several, locally distributed morphological phenotypes (agamospecies).

Classification of highly diverse agamic polyploid complexes has been notoriously difficult (3). The biological species concept, based on sexual reproduction and reproductive isolation, is by definition inappropriate for asexual and/or hybridogenic taxa (4). For asexual lineages, species definition has often been solely based on morphological characters, and the different attempts of defining agamospecies usually applied to few specific organismic groups. A generally applicable species concept for classification of apomictic plant complexes is still missing (5).

The *Ranunculus auricomus* complex comprises a handful of diploid to tetraploid sexual species and numerous polyploid agamospecies (833 names in Euro+Med database; 6). It is, therefore, a suitable group to investigate diversification processes and species concept in facultative apomictic polyploid complexes. Molecular markers have helped to reconstruct reticulate relationships and population genetic structure of apomictic plants (e.g., 7, 8). However, traditional markers have failed in properly reconstructing phylogenetic relationships (8). Therefore, we applied Target Enrichment to produce sequence information for hundreds of single-copy nuclear genes. Using coalescent-based species tree/network reconstruction methods, we aimed at resolving the gross phylogenomic patterns within the *Ranunculus auricomus* complex (ie. interspecific relationships of sexual taxa, monophyly vs. polyphyly of "morphogroups", hybrid origin and parentage of derivative agamic lineages). Restriction site-associated DNA sequencing (RAD-seq), was then used to infer relationships among agamic lineages and post-origin evolution.

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More species, less effort: designing and comparing sampling strategies to draft optimised floristic inventories

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Floristic inventories are of indispensable support to basic and applied research, conservation management, and monitoring activities. However, obtaining exhaustive lists of species, especially in large areas is very difficult, when not impossible. Floristic inventories are generally drafted according to opportunistic sampling, without standardised quantitative approaches, following the so-called 'botanical internal algorithm' (1), which consists of a highly subjective combination of ability, experience, expertise and intuition. While this approach may help in maximising the number of species recorded in field excursions (1), inventories drafted in this way are likely to differ widely in the amount and the quality of the data provided, depending on the abilities and expertise of the botanists as well as on the time invested (2). A switch to probabilistic - instead of opportunistic - sampling is required to achieve the following goals: (i) to apply rigorous statistical analyses, (ii) to compute unbiased estimates of species richness (1), (iii) to perform reliable comparisons among floras in different regions and times (2), and (iv) to facilitate the reproducibility of methods. The aim of this study is to investigate and measure the benefits of probabilistic approaches in floristic research, and to contribute to answer questions which are still open in this field: (i) what is the best sampling strategy to optimise species detection against sampling effort? (ii) Which are reliable estimators of the completeness of a floristic inventory?

We selected three sites as study cases within the Migliarino-San Rossore-Massaciuccoli Regional Park (Tuscany, Italy) showing different vegetation complexity: site A (1.28 km^2, psammophilous vegetation), site B (2.39 km^2, prevalence of thermophilous forests and maquis), and site C (2.07 km^2, patches of open spaces, thermophilous and hygrophilous forests). Even if species lists for these sites are not available in literature, reliable floristic inventories can be obtained through the geodatabase Wikiplantbase #Toscana (3). These inventories are used as *a posteriori* references.

We planned, carried out and then compared two different sampling strategies: (i) a stratified random sampling design based solely on a spatial optimization algorithm, and (ii) a sampling design based on the maximisation of the spectral heterogeneity among sampling units (hereafter, plots), quantified in terms of Normalized Difference Vegetation Index values (NDVI). The former design simulates a context in which no prior information is available to arrange plots. The second one integrates environmental information based on remote sensing techniques according to the 'Spectral Variation Hypothesis' (4), which states that sites with the highest spectral variation are expected to have the highest number of species. Plots (15 per site per sampling strategy) were represented by permanent quadrats of 10 m^2, surveyed from March to November once every two months, in order to obtain exhaustive floristic inventories. Times to reach the plot and to perform the sampling were also recorded.

Spatially Explicit Rarefactions curves (5), which take into account the spatial autocorrelation among sampling units, were used to perform analyses between strategies and across sites. The optimal sampling strategy was then defined based on a combination of quantitative statistics, such as the steepness of the rarefaction curves, the partition of diversity elements across spatial and temporal scales and the completeness of the collected floristic inventory.

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The florula of hypersaline habitats in Central Mediterranean and its biological traits

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Salinity is one of the most widespread soil degradation processes. In fact in saline soils uneven temporal and spatial water distribution and localized high concentration of salts occur, characterizing restricted habitats where most of the present organisms are halophilic or salt tolerant.

Soils could be classified hypersaline when salt concentration exceeds certain thresholds (1, 2). For this study were analysed 10 hypersaline soil localities from Sicily (Birgi, Isola Lunga, Salinelle Marsala, Capo Feto, Piana del Signore, Vendicari) and Tunisia (Qurba, Khniss, Ras Dimas, Chebba).

These areas are temporarily flooded in autumn and winter, with longer permanence of water in some zones. Salt crusts with thickness of 1–3mm appear above the soil surface during the dry season, from June to September. Climate of these areas belongs to semiarid Mediterranean, characterized by an intense summer drought and a mean annual rainfall that ranges from 361 to 383mm. Mean daily air temperature ranges from a maximum of 26.6 °C in August to a minimum of 4.9 °C in Januarywhereas for Monastir area it ranges from a maximum of 27.8 °C in August to a minimum of 12.2 °C in January. The vegetation belongs to the *Thero-Salicornietea* A. & O. Bolòs, 1950 class and is structured as a scattered mosaic of patches whose distribution is related to the flooding pattern and duration. Vegetation, in which most plants. The physiognizing elements of these communities are halophilous pioneer swards typical of salt marshes: *Salicornia fruticosa* (L.) L., *Suaeda vera* J.F.Gmel., and *Atriplex halimus* L. The field investigations were done between spring 2013 and spring 2018. Floristic surveys and biological traits observations and measurements were performed during repeated visits. On the whole, 20 traits from those available in the TRY Plant Trait Database (https://www.try-db.org) were selected and were measured in site. These concerns vegetative, reproductive and dispersal traits as well: Plant growth form; Plant height vegetative; Stem diameter; Crown (canopy) length: diameter along the longest axis; Crown (canopy) width; Leaf angle (inclination, orientation); Leaf compoundness; Leaf distribution along the shoot axis (arrangement type); Leaf length; Leaf width; Leaf thickness; Flower color; Flower sex; Diaspore Typology; Dispersal unit appendages; Dispersal unit length; Dispersal unit shape; Seeds per Diaspore; Fruit type; Plant reproductive phenology timing; Species occurrence range: native vs invasive; Plant chromosome number. Chromosome data were retrieved from Chrobase, Chromosome numbers for the Italian flora, database (http://bot.biologia.unipi.it/chrobase/index.php) or from the Index to Plant Chromosome Numbers (IPCN) (http://www.tropicos.org/Project/IPCN). Nomenclature of taxa follows the updated checklists of native and alien flora of Italy (3, 4) and Euro+Med (http://www.emplantbase.org/) for Tunisia.

The recorded florula was compared by means of statistical analysis to that of neighboring areas with lower content of salt. The main differences concern the number of taxa that is 4 or more times lower in hypersaline soils. Saline habitats are highly selective but host species with large distribution range. A limited number of local endemic species (*Limonium* cf. *oblanceolatum* Brullo & Erben, *Linaria aegyptiaca* (L.) Dumort.) was found only in the studied areas with lower content of salt in the soil.

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Study of plant traits, soil features and plant invasion in sandy beach of Grado and Marano lagoon (northern Adriatic Sea)

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Biotic invasions are altering the world's natural communities and their ecological characters at an unprecedented rate. If we fail to implement effective strategies to curb the most damaging impacts of invaders, we risk impoverishing and homogenizing the ecosystems on which we rely to sustain ecosystem services given by biodiversity with irreplaceable natural services (1). As other extreme environments, sandy beaches are undergoing severe changes due to global changes. Climate changes are drastically shifting the most important ecological drivers affecting their plant communities (e.g., sand salinity, sand deposition, wind, marine aerosol and water table). These modifications can trigger rapid changes in species composition and abundance of a naturally highly dynamic ecosystem, often favoring invasive alien plants. The aim of this study is to evaluate which are the ecological drivers that cause alien species plant invasion, analyzing plant response from cellular to community level, through a laboratory-field approach, measuring plants traits and evaluating the species composition of the whole plant community. The study sites are barrier islands of the Marano and Grado lagoon (45°40’40” N 13°03’50” E to 45°46’30” N 13°27’20” E). It is located in the north part of the Adriatic Sea (2). The lagoon is morphologically classified as a leaky lagoon (3), with strong tidal influence. The experimental design includes a gradient study (from water edge to dune’s back side) of alien species on native species occurring in diverse plant communities evaluating plant traits and species composition in relation to the most important soil features (e.g. soil salinity, grain size). Plants traits can be divided in physiological (e. g. Non-Structural Carbohydrates, osmolites, secondary metabolites) and morphological (e. g. Specific Leaf Area, biomass, height, number and dimension of leaves) while plant communities can be evaluated with taxonomical and functional diversity and plant – plant interactions. Particular attention is given to *Ambrosia psilostachya* DC., *Cyperus esculentus* L., and *Sporobolus pumilus* (Roth) P.M. Peterson & Saarela (4, 5). We expect to clarify the role of soil proprieties on biological invasion, focusing on salt tolerance strategies, which might be a key driver for future scenarios of rainfall reduction and consequent increase of soil sand content.

COMMUNICATION N. 39

An integrated analysis of functional and genetic diversity in depicting the adaptive strategies of plants. The case study of *Silene paradoxa*

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According to Grime's CSR theory (1) the selective pressures to which plants are subjected induce adaptive responses that follow three main directional lines. C-selected plants have developed highly competitive abilities, S-selected plants possess physiological peculiarities necessary to tolerate environmental stress and R-selected plants display characteristics necessary for the colonization of disturbed habitats. Such strategies reflect in a number of different plant traits, whose measurement can be used to place the plant in the CSR framework. Recently, Pierce et al. (2) showed that the position of individuals in the CSR framework can be reasonably deduced *via* the measurement of only three specific functional traits: leaf area (LA), leaf dry matter content (LDMC) and specific leaf area (SLA), representing interspecific variation in plant size and conservative vs. acquisitive resource economics, respectively. It is conceivable that individuals of the same species, grown in different environments, present different adaptive strategies and consequently differences in functional traits (3). Such adaptations to environmental changes may be the response of the combination of two main mechanisms: phenotypic plasticity and adaptive genetic variation.

The present study aimed to verify whether there are detectable differences in the CSR coordinates, identified through the measurement of LA, SLA and LDMC of individuals of *Silene paradoxa* L. (Caryophyllaceae) from environments with contrasting stress levels. The two selected environments were serpentine soils, characterized by high levels of heavy metals, and non-contaminated soils. We sampled 120 individuals from 12 populations, 6 growing on serpentine sites and 6 on non-serpentine sites. Moreover, the present study aimed to investigate the possible relationship between phenotypic plasticity and genetic diversity, through DNA-fingerprinting techniques. The analysis of the functional traits showed a marked phenotypic plasticity of *S. paradoxa*, with the populations of serpentine sites significantly polarized towards the S component of the CSR triangle. The ecological constraints responsible for the development of functional plasticity could have also influenced the genetic structure of populations. In fact, even if the values of genetic diversity at the intra-population level did not differ between populations of serpentine and non-serpentine sites, we recorded a shift in gene pool selection. It is therefore conceivable that the stress due to the presence of heavy metals acted as a filter of the populations' gene pool. The effect due to the particular edaphic conditions seems to have also acted on the selection of some regions of the genome of the species, with various portions of the genome that are exclusive, or clearly prevalent, of the serpentine and non-serpentine populations.

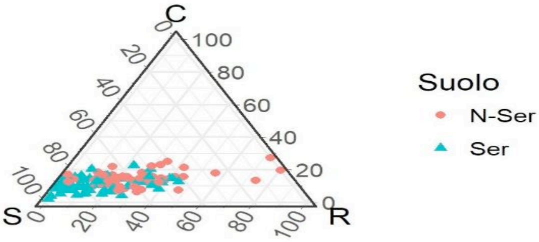

Fig. 1 *Silene paradoxa* on serpentine soil. Fig. 2. CSR tri-plot for the 120 individuals of *S. paradoxa* growing on serpentine (Ser) and non-serpentine (N-Ser) sites.

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The Aeolian archipelago as a model system for Island Biogeography Theory in the Mediterranean

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Islands have long played a crucial role in biological studies, especially volcanic archipelagos. The Aeolian Archipelago (Sicily, Italy) is a relatively young volcanic system located in the Central Mediterranean with a long history of human settlement. This archipelago has been largely studied by ecologists, who collected huge amount of data, but it has been the object of only few large scale biogeographical investigations. To fill this gap, we investigated the biogeographical patterns of the Aeolian Islands, using both published and unpublished floristic data, describing the flora of the 7 major islands and 23 minor islets. In these analyses, we also included data on age of island emersion, elevation, distance from the nearest most probable source of propagules, average acclivity and shape-index. We calculated species-area relationships (SARs) by the Arrhenius power model both for the entire dataset, for the 7 major islands and for the 23 islets as distinct groups. We then searched for correlations between the residuals from the SARs and the other features of the islands. As expected, the Aeolian Archipelago as a whole stands out as to biogeographical features, while the single islands of Vulcano and Stromboli are placed far below the SAR curve probably because of their active volcanism, which reduces the species pool and the realized niches. This study opens the way to other investigations of the Aeolian islands to make it a perfect model system for studying biogeographical and ecological processes in the Mediterranean area, similarly to the Hawaian, Canarian or Azorean archipelagos in oceanic context.

POSTERS

1.1 = Evaluation of compost effects on soil fertility and plant development: a preliminary study

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Global warming is strongly depleting land currently under agricultural use whilst world population is increasing (1). On the other hand, climate change and human pressure, including intensive agriculture, are intertwined and strongly contribute to deleterious environmental changes. Therefore, sustainable agricultural practices are needed to do not further aggravate soil erosion, loss of soil organic matter and leaching of nutrients. At this respect, an increasing attention is currently paid to the use of organic fertilizers such as compost, produced through aerobic digestion of organic fraction of solid waste (SW). Namely, the use of SW compost as fertilizer, beside contribute to waste disposal, could add organic matter, reintegrate essential elements and retain water thus improving the soil chemical-physic and biological characteristics. Due to the potential of compost use in agronomic field, and in order to improve its efficiency and safety, it appears of great relevance to add information on both the effect of SW compost on plant growth and crop's productivity but also on its impact on soil's biological community, mainly after prolonged use of this fertilizer. To meet such target, as part of an integrated research project, we planned to evaluate the effects of SW compost on growth and productivity of horticultural plants commonly used in the south of Italy, by estimating a panel of morphological, physiological and molecular parameters and performing toxicity tests. To this aim, an experimental field area, of about 1000 m^2, located in San Marco Argentano (CS) Italy (39,6280070, 16,2180550) has been selected and divided into three sectors characterized by: i) no fertilization; ii) fertilization with compost (8 Mg ha^{-1}, 16 Mg ha^{-1}); iii) mineral fertilization (11-22-16 NPK from YaraMila®, 500 Kg ha^{-1}). Here we report the preliminary results so far obtained using the species *Cucumis melo* var. *cantalupensis.* Seedlings of this species were implanted on the different sectors. Soil fertility was estimated through chemical and biochemical analysis, carried out on soil samples before plant implantation and at the end of the crop cycle (2). In the early stages of development, a faster growth rate was observed by measured the area of leaf series in plants implanted in the sector under compost fertilization compared to plants growing both without fertilization and under mineral fertilization. Namely, after 2 weeks from the implant, it was possible to notice an increase of size of the plants of about 20%, estimated considering the superficial area occupied by the plants. Plant productivity and fruit size and quality including total soluble solids in juice (°Brix), nutritional elements and heavy metals content are under investigation.

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POSTER N. 2

1.1. = Effects of red: blue ratios in led lighting on nutritional and nutraceutical properties of hydroponically grown *Lactuca sativa* L.

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Plants respond to light intensity, photoperiod, and spectral quality in terms of growth and development as well as at physiological and metabolic (primary and secondary) levels. For crops, this means that light impacts dramatically on phenology, yield, and nutritional quality. Artificial lighting is gaining relevance in agriculture, since it enables intensification of production, improves quality, and allows cultivation wherever natural light is not sufficient (e.g., indoor cultivation). Light-Emitting Diodes (LEDs) are more efficient than other commonly used light sources due to their long functional life, low operating temperatures, low energy consumption, and selective spectral output [1]. Light may also influence product quality in nutraceutical terms. In fact, consumer interest in functional foods containing flavonoids has been increasing and cultural methods, such as lighting, that enhance the content of these compounds are being explored [2]. The antioxidant potential of phenolic compounds contributes to the health benefits of many fruits and vegetables, including lettuce [3]. In order to gain insight into the nutrient uptake capacity and phytochemical profiles in response to different spectral components, lettuce (*Lactuca sativa* L., var. Rebelina) plants were hydroponically grown under a LED lighting system with different red:blue light ratios. A cool fluorescent light control was also used and in all treatments a constant photosynthetic photon flux (200 mmol $m^{-2}s^{-1}$) and a long-day photoperiod (14/10 h day/night) was provided. Mineral assimilation from the nutrient solution and leaf tissue accumulation were measured during growth and at final harvest (18 DAT) by using Kjeldahl, and ICP-OES techniques. Total leaf phenolic and flavonoid concentrations were determined by spectrophotometric methods; concentrations of single phenolic compounds were obtained by HPLC-DAD. The antioxidant capacity of leaf methanolic extracts were evaluated using several *in-vitro* methods (DPPH, ORAC, FRAP-FZ). The expression levels of genes encoding nitrate, ammonium, and phosphate transporters and of those for several enzymes in the phenylpropanoid and flavonoid pathways (phenylalanine ammonia-lyase, chalcone synthase, and flavonol synthase) were evaluated in roots and/or leaves by real-time quantitative Reverse Transcription-Polymerase Chain Reaction (RT-qPCR). Results show that specific red:blue light ratios significantly affected plant biomass, nutrient uptake, and ion transporter transcript levels. The phytochemical profiles and antioxidant activity of leaf methanolic extracts were also modulated by the different lighting regimes.

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1.1 = A population of *Epipactis helleborine* subsp. *tremolsii* (Orchidaceae) growing on mine tailings: a case study in Sardinia (Italy)

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This study investigates ecological features of a large population of *Epipactis helleborine* subsp. *tremolsii* (Orchidaceae) growing on mine tailings. The studied site was subjected to intense Zinc, Copper, and Argentiferous Lead mining. Soils are Spolic Technosols directly formed from the mine tailings. This is the growing context of ~100 individuals of *E. helleborine* subsp. *tremolsii* for which we aim at understanding:
i) How this orchid could colonize this extreme ecological context; ii) Whether the *E. helleborine* subsp. *tremolsii* accumulates heavy metals and in which tissues; iii) The possible protection role against heavy metals of the mycorrhizal symbiosis – typical of the Orchidaceae family (1); iv) Possible advantages that this symbiosis may offer to a sciaphilous species in order to contrast adverse conditions such as high sunlight exposure. Different approaches were used to address these questions. Chlorophylls fluorescence and the related photosynthetic activity were measured to evaluate the stress levels. Results were compared with those from individuals growing in non-contaminated areas. Morphometric features were measured and compared between plants growing on contaminated and non-contaminated areas. Ecological parameters were used to study and characterize the mycorrhizal symbiosis. In particular, the specific mycorrhizal fungus of this symbiosis was isolated from plants found in contaminated soils and grown on a specific culture medium (SDA and PDA added with CHL) supplied or not with heavy metals characteristic for this soil. These experiments will allow to estimate plants tolerance to heavy metals toxicity thanks to the symbiosis with this specific fungus ecotype (2).
Three selected topsoil samples (0-25 cm), representing different areas of the studied site, were collected for physical and chemical analyses. The bulk soil samples were air dried and sieved to < 2 mm. Sand (2.00–0.05 mm), silt (0.050–0.002 mm), and clay (<0.002 mm) fractions were separated after the removal of organic matter through an oxidative treatment with H_2O_2 and dispersion aided by Na-hexametaphosphate. Soil pH was measured by potentiometry in soil/solution suspensions in water with a 1:2.5 ratio. The organic carbon content was determined by C elementary analyser (Leco, USA). The total content of Fe, Al, As, Cd, Cu, Cr, Pb, Zn, Ni, and Mn was determined from the sieved samples after digestion in conc. HNO_3 according to the EPA 3050-B method. Bioavailable fractions were estimated by the BCR extraction method (acetic acid 0.11 M). The extracted were analysed, in both cases, by an Inductively Coupled Plasma-Atomic Emission Spectroscopy (ICP-AES).
Chlorophylls fluorescence and related photosynthetic activity were measured with Plant Photosynthesis Meter (EARS miniPPM-100). Morphometric parameters have been collected on 20 individuals growing on the contaminated soil: plant total height; inflorescence height (when not completely developed); length and width of the bigger leaf. Measured individuals were explanted and root, leaves, and stem separated. These parts were dehydrated in oven at 45° C for 48 hours. Dehydrated samples were pulverized with liquid N_2 in a mortar or reduced to ash by baking at 550 °C for 2 h, and finally used to quantify heavy metals in plants' tissues. For each part, three replicates have been analysed. All samples (soils and plants' parts) will be digested with an acid mixture (HNO3 65%/ HF, 50% = 2:1 v/v) in a microwave oven (Ethos, Milestone). The concentration of each element will be determined by an Inductively Coupled Plasma (ICP-OES - Optima 7000DV, PerkinElmer). Standard reference material (1575a Pine Needles; NIST, 2004) will be also analysed in order to verify the accuracy of the obtained data.

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1.2 = Coupling xylogenesis and dendroecology to understand the relations between environmental factors and tree-ring formation in Mediterranean hardwood and softwood species

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In Mediterranean environments, the increasing risk for drought stress and heat waves due to the ongoing climate change may impact wood formation in trees. Therefore, negative consequences on forest productivity and changes in ecosystem vegetation dynamics are expected. However, Mediterranean woody species evolved to cope with seasonal environmental fluctuations, also adjusting their wood anatomy to optimize the efficiency and safety of the hydraulic system depending on water availability. Indeed, Mediterranean species show peculiar patterns of cambial activity recorded in wood anatomical functional traits, often resulting in changes in wood density within the tree ring, called Intra-annual Density Fluctuations (IADFs). IADFs are unexpected and abrupt changes in wood anatomical traits along the tree ring and appear either as latewood-like cells within earlywood or earlywood-like cells within latewood. Tree rings with IADFs are able to modulate water flow to maintain high conductivity when water is available while preventing embolism phenomena during water shortage. Therefore, IADF analysis may provide accurate information on plant's growth responses to climate at the intra-seasonal level.

We monitored xylogenesis in *Pinus halepensis* Mill., *Pinus pinea* L. and *Arbutus unedo* L. at sites characterised by Mediterranean climate in Southern Italy to link IADFs with climate variables triggering their formation. Moreover, we analysed interspecific differences in cambial production and functional wood traits in long tree-ring series in Mediterranean species with intra-annual resolution. The relations between series of functional anatomical traits and climatic factors were also analysed.

We were able to detect the periods of IADF formation and environmental factors triggering them and highlighted the different sensitivity to environmental fluctuations recorded in wood anatomy of the various species that should be taken into account in forest management.

In conclusion, the occurrence of IADFs in tree rings suggests the plasticity of the investigated species to respond to frequent intra-seasonal variations of water availability, which is typical for Mediterranean sites. Such plasticity can be considered a competitive advantage compared to those species which are less prone to form IADFs.

1.2 = Variation of physiological parameters in the lichen *Evernia prunastri* (L.) ach. exposed to different environmental stressors in molise (central italy)

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The Molise region is, among the regions of central Italy, the least industrialized both for precise political choices and because of its geomorphological characteristics that make difficult the construction of infrastructures and the transportation of people and goods. The industrial areas, as well as the main urban areas, are confined to the flat part of the territory that offers better conditions for the development of human activities. On the other side, the region largely preserves the typical characters of traditional agriculture, like small farms and scarce use of chemical fertilizers.

Despite this relatively low anthropogenic impact, the recent urban and industrial development occurred in the region (1) set up the bases for a survey to monitor the environmental impacts of these activities.

In this study, some physiological parameters of the lichen *Evernia. prunastri* (chlorophyll *a* fluorescence, degradation of chlorophyll to pheophytin, water-soluble protein content and ammonium content) were used to evaluate the impacts of different pollution sources in Molise. *Evernia prunastri* is a sensitive lichen species widely used as a biomonitor of environmental stress (2), both in studies of bioaccumulation of trace elements and to evaluate physiological alterations in response to nitrogen pollution (3), currently acknowledged as a major threat to ecosystem and human health.

The impact sources considered in the sampling design reflect the three most characteristic of the territory: small villages, small and medium farms and complex (industrial plus urban) areas.

In order to place the transplants in an environment with microhabitat conditions that are as similar as possible between the sampling stations and the collecting area (represented by a remote wooded area of Alto Molise), sampling stations were selected among the Trees Outside Forest (TOF) of the region (4).

The land use analysis carried out within a radius of 1 km in the vicinity of the sampling stations showed a clear prevalence of agricultural use that can be considered as the common trait to all plots.

Starting from these data, this study aims to establish whether there are differences due to the other sources of impact present in the vicinity of the sampling stations.

For each type of impact (villages, farms, complex areas), 5 sampling stations were selected among the TOFs closest to the source of impact. In each sampling station transplants of *E. prunastri* , were exposed for 90 days (from July to November 2017).

The lichens exposed to the impact source show a photosynthetic capacity similar to the control values which demonstrates that the transplanted thalli have remained viable despite the displacement from the area of origin to that of use.

The content of total pigments seems to show a general increase compared to the control values probably due to the increased availability of nutrients in transplant areas.

Preliminary results show no significant differences near complex areas and only slight differences in the vicinity of villages and farms. This seems to indicate the widespread agricultural use as the main source of impact.

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1.2 =Response to heavy metal stress in *Leptodictyum riparium* Hedw

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Aquatic bryophytes are considered excellent biomonitors of metals and other contaminants (1) because of their unique morphology and physiology. This study aims to evaluate the biological effects of heavy metals using the aquatic moss *Leptodictyum riparium* (Hedw.), known as pollution-tolerant species. *L. riparium* protonema and gametophytes samples cultured *in vitro* in Mohr medium were exposed to the heavy metals concentrations (Cd, Pb at 10^{-4} -10^{-5} M) choices among the highest ones measured in the most polluted fluvial sites in Italy.

The biological response to heavy metals was investigated considering metals bioaccumulation, ultrastructural damage, and oxidative stress: ROS production and enzymatic activity (GST, SOD, CAT and Peroxidase), and putative phytochelatin presence.

The results showed severe ultrastructural damage both in protonema and gametophyte samples, an increase of ROS production and of antioxidant activity, and different expression levels of HSP in samples exposed to high representative heavy metals concentrations.

These results suggest that *L. riparium* can be used as a low cost model organism in biomonitoring projects. In fact, it can be considered a natural "green filter" both thanks to their antioxidant enzymes activity and as promising biomarker of heavy metals toxicity.

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1.2 = Involvement of DNA methylation in Cr(VI) tolerance and in response to sulfur deprivation in the microalga *Scenedesmus acutus* M. (Chlorophyceae)

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Epigenetic, referred to heritable changes in gene expression without mutating DNA sequences, can play a role in organism response both to biotic and abiotic stress factors. DNA methylation is a conserved epigenetic mark important for development and stress responses in plants and many animals.

In plants in particular, environmental stressor (including heavy metals, drought, and cold) can trigger DNA methylation changes at a genome-wide scale (1). So, the single genome in a plant cell could give rise to multiple epigenomic changes in response to developmental and environmental cues. In fact, stresses can induce changes in gene expression through hypomethylation or hypermethylation of DNA (2) but may also involve genome regions which have an adaptive significance during stress responses and can direct genome evolution (3). The acclimation of plants to adverse environmental conditions requires reversible, as well as persistent, epigenetic modulation of gene expression. Stress-induced epigenetic modifications are not only persisting during exposure to stress, but can also be transmitted hereditarily, thus ensuring the transfer of "stress memory" to progeny (4).

Despite the studies on stress-responsive epigenomes and transcriptomes are important to understand mechanisms related to stress adaptation, very few data have been reported in algae.

To gain additional information on the involvement of epigenetic mechanisms in heavy metal tolerance, we analysed the global DNA methylation in two strains, namely wild type (wt) and chromium-tolerant (Cr-tolerant), of the freshwater green alga *Scenedesmus acutus* with different Cr(VI) sensitivity. A transient increase in Cr(VI) tolerance has been already observed following a 3-day sulfur (S) starvation in both wild type and Cr-tolerant strain. However, this was lost after 2-day recovery in standard medium (5). Therefore, the analysis of methylation was conducted after Cr(VI) exposure following 3-day culture in standard or S-deprived medium. Both the level and the labeling pattern of the DNA cytosine-rich zones showed significant differences between the two strains. In addition, hypomethylation was observed in both strains upon S-starvation condition. These results suggest that an epigenetic mechanism could be at the basis of the Cr tolerance in *S. acutus*, as supported by our previous data reporting that the acquired tolerance was inherited and maintained through the progeny.

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1.2 = Modulation of stress response pathways by DNA methyltransferases in *A. thaliana*

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Because of their sessile status, plants are constantly exposed to a variety of abiotic stresses. The ability of plants to cope with stressful conditions relies on their promptly conversion of stress signal perception into appropriate responses and adaptive modifications, through a rapid modulation of existing genetic information[1]. At this respect, epigenetic mechanisms can play a relevant role in regulating the expression of stress-related genes at both transcriptional and post-transcriptional level. Among these mechanisms, DNA methylation is especially interesting to study, since it's partially controlled by hormonal fluxes, which are in turn influenced by abiotic and biotic factors[2]. For this reason, understanding DNA methylation involvement in response to stress is important to decipher plant adaptation mechanisms.

For some time, we are using in our studies the *drm1 drm2 cmt3* (*ddc)* triple mutant of *Arabidopsis thaliana,* defective for three methyltransferases involved in *de novo* and maintenance activity, as a suitable tool to understand the DNA methylation involvement in plant response to cadmium (Cd) stress. Cd was chosen because of its large diffusion, high solubility in water and high toxicity for both plants and animals.

In particular, in plants Cd exposure affects mineral nutrition and several physiological and metabolic processes, that lead to a general inhibition of plant growth and development[3,4] depending on several Cd-induced effects such as genotoxicity, free radicals generation, cell membrane damage, enzyme inhibition, and alteration at transcriptional, post-translational and epigenetic levels[1,3,5]. In this context, we planned to perform a functional phenotyping of *ddc* mutant *vs* wild type (WT) plants of *Arabidopsis thaliana* exposed to Cd (25 μM and 50 μM), through a transcriptomic approach.

In a first step, clear differences in hormone-related genetic pathways emerged from transcriptomic analysis[6]. In the present work, attention has been focused on genetic pathways related to stress response. As expected, following Cd exposure, in both *ddc* mutant and WT we observed a global up-regulation of genes involved in MAPK signalling pathways which work in the activation of stress response. However, we also observed that, in presence of Cd 25 μM, all these pathways were strongly up-regulated in *ddc vs* WT. Interestingly, these pathways were activated by both ROS and hormones which, as above mentioned, were differentially regulated in *ddc vs* WT.

A different behaviour was observed for pathways related to the oxidative stress response. Indeed, also in this case, gene expression related to these pathways was up-regulated both in WT and *ddc* mutant. No differential expression was found when comparing *ddc* mutant vs WT. The only exception was observed for GSH metabolic pathway, which in presence of Cd was up-regulated in *ddc* mutant *vs* WT, especially under Cd 25 μM treatment. Assays of the activity of principal enzymes involved in antioxidant response, such as SOD, CAT and GPX, and the quantification of principal antioxidant compounds, like ascorbate and GSH, were also performed. Globally, the obtained results confirmed that *ddc* was less affected by Cd exposure than WT. In fact, a decrease of both enzymatic activities and antioxidants amount was observed following Cd exposure, but at a lower level in *ddc* mutant than in WT plants. In addition, although for most of the analysed pathways a good correlation with transcriptomic data was observed, some differences in SOD and CAT data strongly suggested that epigenetic status can act at post-transcriptional or post-translational level to modulate stress-related pathways, that need to be further explored.

In summary, these results suggest that hypomethylation status of *ddc* triple mutant could directly or indirectly provide the opportunity for the plant to activate more readily the defence responses, thus limiting Cd-induced damage, consistently with the differential growth response of mutant and WT to Cd exposure.

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1.2 = The application of leaf pigment evaluation in forensic science

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In the last few years, the Forensic Botany is getting more and more interesting due to the contribution it can make during the discussion in law court. Indeed, the analysis of plant material found on the scene of a crime can reveal essential information in the forensic field such as the chronological location of the crime, the location of the victim's discovery, the place of the crime but also the mode of death and the location of the site where it is the burial (1). It's important to remember that all plants at a crime scene may be valuable evidence. Often under the victim's body or in his hands, but also inside cars and wheel wells, it's possible to find, fragments of plant remains such as a leaf. These plant fragments torn from their natural location undergo a series of morphological and physiological modifications whose accurate evaluation can provide useful information. For this reason, an experiment was conducted to evaluate the decrease in photosynthetic activity, in terms of the quantity of pigments present, in leaves belonging to species of different families of Italian flora. The preparatory procedures, in order to simulate the finding of a leaf in the victim's hand in a hypothetical crime scene, provided for the removal of some leaves and the covering of the lower half with aluminum paper. So half the leaf remained in natural light conditions while the other half was in the dark. From every half leaf every two days the concentration of chlorophyll a, b and carotenoids was evaluated with the aid of a spectrophotometer extracting the pigments in acetone (2). The results showed a progressive decrease in the concentration of pigments, which is already halved after 10 days, reducing further in the following days. The main decline mainly concerns the chlorophylls, while the carotenoids have a less evident decrease (3). Furthermore, there is a different variation in the concentration of pigments extracted from the half leaf left in the light and that of the half left in the dark. It should be noted that even if the decrease in chlorophyll occurred in all the species examined, these showed differences in the trend. This shows how each species has a different response to stresses (4).

This preliminary data highlights how knowledge of the concentration of pigments linked to photosynthetic activity allows to acquire useful information on the time elapsed since a leaf has been detached from its natural place or how many days it has been in the dark. This information can be fundamental especially when the finding of a corpse occurs many days after the criminal event, in cases where the human parameters used to date the death of an individual, tanatological parameters, are no longer usable. The application of these results are, in particular, more effective the more distant the time is the criminal event.

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1.2 = Performances of mycorrhizal tomatoes under water and nutrient stress conditions

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Tomato (*Solanum lycopersicum*) has been one of the first non-legume models to be used in Arbuscular Mycorrhiza (AM) studies, since it responds to mycorrhizal colonization (1) and native microbiota (2) providing a useful tool to investigate metabolic processes and gene expression regulation in plant-microbe interaction. Tomato avoids the overlapping signal transduction pathways, since it does not establish the Rhizobium - AM tripartite symbiosis that occurs in legumes (3), thus permitting to investigate additional clues concerning the non-common symbiotic signaling pathway.

Our work is developed in the frame of the TOMRES European Project, whose purpose is to enhance resilience to combined water and nutrient stress in tomato, as a model crop, in the context of global climate change evoking a scenario of frequent drought events and with the aim to reduce the environmental impact of agricultural activities. Our working hypothesis is that mycorrhization is a beneficial driver for tolerating adverse environmental conditions.

The aim of the poster is to illustrate the performances of the tomato reference genotype M82 when grown in a miniature industrial culture system, i.e. tomatoes are grown in little volume plastic-pots (100 ml), using a quartz sand-vermiculite substrate with and without a monospecific inoculum of *Funneliformis mosseae*, an AM fungus which is widespread in nature.

The well-watered and water stress conditions, with about 100% and 40% soil water content, respectively, were identified in a preliminary experiment measuring the stem water potential by using a Sholander pressure chamber. Seedlings were maintained for 16 h light at 24°C and 8 h dark at 21°C. The low nutrient solution was a modified Long-Ashton solution containing 3.2 µM phosphate to favor mycorrhization. After 70 days from the start of the experiment, at the sampling time biometric parameters and SPAD index were measured. The mycorrhizal status was also monitored with morphological observations.

The results indicate that under nutrient deprivation and in a small-substrate volume the growth of M82 is in general stunted and that water stress reduces root and shoot biomass, regardless of the AM fungus presence. Even though plants in both well-watered and water stress conditions show a good level of mycorrhization, the AM symbiosis leads to a growth reduction: non mycorrhizal plants show higher biomass in both watering conditions compared to mycorrhizal plants. However, the AM symbiosis seems to improve the nutritional status of water-stressed plants over time: after 22 days from the start of the experiment the SPAD index was lower in mycorrhizal plants, but after 70 days the values were reversed.

In conclusion, these results suggest that the beneficial effects usually provided by AM fungi to tomato are more limited when the M82 cultivar is maintained in small-plastic pots, as the ones used in the industrial cultivation, but some parameters, as the general nutritional status, seem to be positively affected.

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1.5 = AM for Quality - Use of chitin oligomers to enhance forage plant mycorrhization and crop quality

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Ecosystem welfare and world food request urge XXI century farmers to send an SOS message to soil microrganisms such as arbuscular mycorrhizal (AM) fungi, ancient symbionts providing mineral nutrients and water to most crop plants. One possibility is to use signal molecules that trigger symbiosis. A few chemical signals are known to be exchanged between symbiotic fungi and their host plants during the establishment of arbuscular mycorrhiza (Catoira *et al.*, 2000; Bonfante and Genre, 2015). Among the AM fungus-released molecules (Myc factors), short-chain chitin oligomers (Myc-COs) have been shown to be active in a wide range of host plants (Maillet *et al.*, 2011; Genre *et al.*, 2013), including legumes and cereals. The discovery of the key role played by Myc-COs in the early stages of AM establishment paved the way to possible applications in sustainable agriculture.

The *AM for Quality* project (funded by Fondazione Cassa di Risparmio di Cuneo - Ricerca Scientifica 2015) aims to investigate the feasibility of field applications of Myc-COs to enhance mycorrhizal intensity in forage plants, eventually increasing yield and nutritional properties of crops. During this four-year project two research lines are being carried out:

Line 1 - in the lab: the model legume *Medicago truncatula* is grown in the presence or absence of AM inoculation; with and without CO treatment. Here we investigated the impact of exogenous Myc-CO application on pot-grown *M. truncatula* inoculated with the AM fungus *Funneliformis mosseae*. We evaluated the colonization level by morphological and molecular approaches over 4 weeks.

Line 2 - in the field: four experimental meadows (CTRL; CTRL+CO MYC; MYC + CO) specifically prepared for the project with a mixture of native species, are regularly monitored to record AM colonization intensity and plant productivity.

The Myc-CO treatment enhanced AM colonization in *M. truncatula* with an extensive development of arbuscules in several layers of root cortical cells, compared to untreated mycorrhizal plants. We also observed an increase in biomass production and leaf photosynthetic surface in treated and untreated mycorrhizal plants. Furthermore, these preliminary results suggest that the Myc-CO treatment anticipated the whole process of AM development, encouraging the use of Myc-COs as a promising tool to promote AM establishment in sustainable agricultural practices.

The results from the first year of studies showed a significant increase in the intensity of AM colonization and biomass in Myc-CO treated-mycorrhizal fields when compared to control, fully supporting our laboratory results. Moreover, Myc-CO treatment improved plant species equitability, by generating a better balance among forage species. We are currently monitoring the experimental meadows for the second growing season, and our preliminary results confirm the positive effect of Myc-COs on mycorrhization.

Concerning the nutritional and organoleptic properties of forage, we will relate the symbiotic status of the forage plants with their content in crude protein, neutral detergent fibre (NDF) and acid detergent fibre (ADF).

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2.1 = Characterisation of bioactive compound composition and antioxidant activities in seeds of a Chilean *Chenopodium quinoa* (Willd.) cultivar grown in Emilia-Romagna (Italy)

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Quinoa (*Chenopodium quinoa* Willd.) is a native food plant of the Amaranthaceae family, traditionally cultivated in the Andean region of South America, which has recently received much attention because of its exceptional nutritional value and potential health benefits. Due to their content in several classes of plant secondary metabolites behaving as bioactive compounds, quinoa seeds are currently considered an excellent example of "functional food". Among the compounds of interest, natural antioxidants such as polyphenols and tocopherols have been recognized to play a major role in maintaining health, by virtue of their capacity to reduce the risk of various degenerative diseases associated with oxidative stress, such as cancer, cardiovascular disease and osteoporosis (1).

Despite its geographical origin, quinoa is characterised by a huge genetic variability, which makes possible to adapt cultivars to a wide range of environmental conditions (2). Currently, quinoa is commercially grown outside South America, and the number of producer countries is rapidly increasing, especially in Southern Europe, where the climatic conditions are closer to those of its genetic origin (2).

In this work, we characterised the bioactive compound composition and antioxidant activities of seeds of the Chilean cultivar Regalona (registered variety of BAER, Chile) grown near Ravenna (Emilia-Romagna), and we compared them with those of another cultivar (Titicaca) adapted to grow in Europe. The effect of the seed desaponification process on the profile of functional compounds was also evaluated. Total polyphenol content (TPC) and total flavonoid content (TFC) were determined by spectrophotometric assays, and specific phenolic acids and flavonoids were detected by HPLC-DAD in both free and conjugated fractions. Genistein and daidzein levels were also analysed in seed extracts. Antioxidant activities of quinoa seed extracts were assayed through in vitro tests based on different mechanisms (DPPH, ABTS, ORAC), and possible correlations between the functional compound profile and antioxidant properties were investigated.

Results indicate that TPC in the free phenolic fraction in seeds of locally-grown Regalona was very similar to that in Titicaca. In both cultivars, TPC levels were significantly higher in the acid-hydrolysable phenolic fraction compared to the base-hydrolysable one. HPLC-DAD analysis of phenolic acids and flavonoids indicate that major compounds were simple phenolic acids, hydroxycinnamic acids and flavonol glycosides. Antioxidant activity, tested through different tests, shows a good correlation with polyphenol profiles. Based on these results, it can be concluded that the agro-ecological conditions in the cultivation area near Ravenna allowed to produce seeds with good nutraceutical traits.

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2.1 = Rosmarinic acid induces programmed cell death in Arabidopsis seedlings through reactive oxygen species and mitochondrial dysfunction

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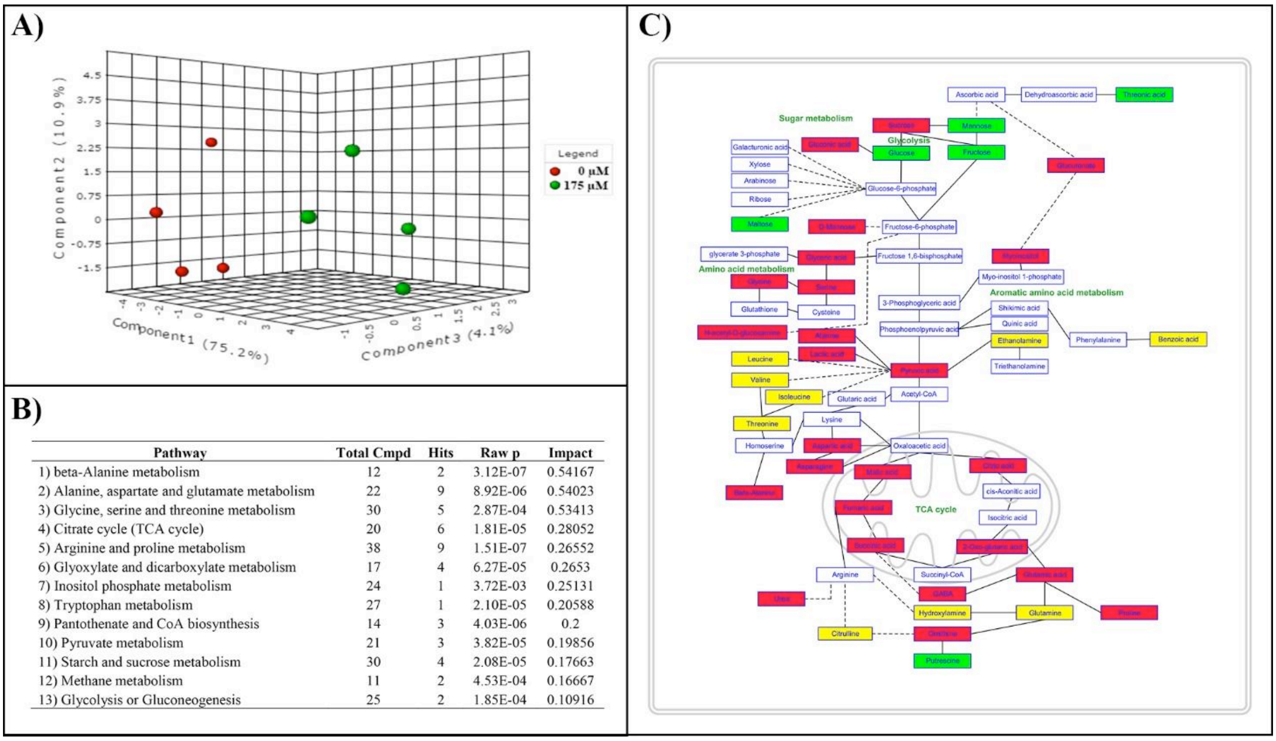
The phytotoxic potential of rosmarinic acid (RA), a caffeic acid ester largely found in aromatic species (1,2), was evaluated on *Arabidopsis* through metabolomic (GC-MS), microscopic (TEM and confocal microscopy) approaches. *In-vitro* bioassays pointed out that RA affected root growth and morphology, causing ROS burst (H_2O_2 and O_2^-), ROS scavengers activity inhibition (SOD and CAT) and consequently, an alteration on cells organization and ultrastructure. In particular, a strong vacuolization and an alteration of mitochondria structure and function were observed. The RA-treatment (175 µM) caused an increased number of mitochondria and a consistent ROS-induced reduction of mitochondrial transmembrane potential ($\Delta\Psi_m$). These data suggested a cell energy deficit also confirmed by the metabolomic analysis through Principal Component Analysis (PCA) (Fig. 1) that evidenced a clear separation between control and treated plants (Fig. 1-A). Further, both TCA cycle and amino acids metabolism were significantly altered by the RA-treatment (Fig. 1-B, C). Moreover, the increase in H_2O_2 and O_2^- contents suggested that RA-treated meristems underwent an oxidative stress, resulting in apoptotic bodies and necrotic cells. Taken together, the results suggested that RA inhibited two of the main ROS scavengers causing high ROS accumulation, responsible of the alterations on mitochondrial ultrastructure and activity through $\Delta\Psi_m$ dissipation, TCA-cycle alteration, cell starvation and consequently cell death on *Arabidopsis* seedlings. All these effects resulted in a strong inhibition on root growth and development, which converted RA in a promising molecule to be explored for further use in weed management.



B)

Pathway	Total Cmpd	Hits	Raw p	Impact
1) beta-Alanine metabolism	12	2	3.12E-07	0.54167
2) Alanine, aspartate and glutamate metabolism	22	9	8.92E-06	0.54023
3) Glycine, serine and threonine metabolism	30	5	2.87E-04	0.53413
4) Citrate cycle (TCA cycle)	20	6	1.81E-05	0.28052
5) Arginine and proline metabolism	38	9	1.51E-07	0.26552
6) Glyoxylate and dicarboxylate metabolism	17	4	6.27E-05	0.2653
7) Inositol phosphate metabolism	24	1	3.72E-03	0.25131
8) Tryptophan metabolism	27	1	2.10E-05	0.20588
9) Pantothenate and CoA biosynthesis	14	3	4.03E-06	0.2
10) Pyruvate metabolism	21	3	3.82E-05	0.19856
11) Starch and sucrose metabolism	30	4	2.08E-05	0.17663
12) Methane metabolism	11	2	4.53E-04	0.16667
13) Glycolysis or Gluconeogenesis	25	2	1.85E-04	0.10916

Fig. 1: A) PCA 3D-Score plot of metabolite profile of control (0 µM) and treated (175 µM) plants exposed to RA; B) Result from "*Pathway Analysis*" carried on the concentrations of metabolite identified in *Arabidopsis* roots treated with RA (175 µM). Total Cmpd: is the total number of compounds in the pathway; Hits: is the matched number from the uploaded data; P value: is the original p value calculated from the enrichment analysis; Impact: is the pathway impact value calculated from pathway topology analysis; C) Schematic representation of the qualitative changes of metabolite abundance mapped onto the metabolic network. Green filled boxes were used for increased metabolites; red for decreased metabolites; yellow for not significantly affected metabolites and empty boxes for unmeasured metabolites. Statistical differences were evaluated through Student's test with $P \leq 0.05$. N=4.

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2.1 = Antioxidant activities of extracts from various portions of giant orchid, *Himantoglossum robertianum* (Loisel.) P. Delforge

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Himantoglossum robertianum is a terrestrial, vegetatively robust orchid with long racemes of large flowers characterized by violet-colored and spotted, elaborate labella. The species is widespread throughout the Mediterranean region and is actually extending its range (1). The plant grows up to 1 m in height showing a size similar to those of cultivated orchids, and has tubers that can reach very considerable dimensions. Such tubers have been traditionally collected for *Salep* (2) or other alimentary preparations: e.g. in Sicily, they are cut in slices and roasted on the barbecue (3). Historically, the genus *Himantoglossum* has been used for the treatment of gastrointestinal problems, skin disorders, and wounds (4). Previous studies (5) have reported that the flower extract shows a high anthocyanin content, with orchicyanin II and serapianin as main components. However, there is limited information about the chemical composition of the plant's different portions. Therefore, the aim of our study was to characterize from morphological and phytochemical points of view the flowers, stem, leaves and tubers of this orchid and to evaluate the antioxidant activity of their extracts. Furthermore, in order to ensure the conservation of natural populations, micropropagation was carried out by aseptic culture from seeds, according to Calevo et al (2017) (6). Plants were collected from Taggia (Imperia, Western Liguria) according to the Regione Liguria's legislation and authorization (L.R. 9/84; D.G.R. 363 1/18). To investigate the polyphenol content as well as the antioxidant properties of extracts, we carried out an ultra-sound assisted extraction using methanol/water mixture (7) in order to obtain polyphenol rich extracts. A preliminary phytochemical screening revealed a high variability of total phenols (93.52-243.76 mg gallic acid equivalent/100g fresh weight) and flavonoid content (38.71-548.46 mg quercetin equivalent/100 g of fresh weight) among the examined extracts. The flower extract showed the highest total phenol and flavonoid contents, probably due to high anthocyanins, as previously reported (5). All extracts showed remarkable antioxidant and free-radical scavenging activities towards all assays performed, with the following order of potency: ORAC > FRAP > TEAC > β-carotene bleaching > Iron-chelating activity > DPPH. Flowers showed the best antioxidant power followed by leaves, bulb, floral axis and stem. Furthermore, the comparison of the stem with the floral axis extracts demonstrated that the second one showed the highest antioxidant power despite a similar phenolic content, probably due to higher flavonoid content. This was well correlated with the strong iron-chelating activity found in the floral axis extract (IC50 90.42 μg/ml), significantly higher with respect to the other ones (IC_{50} 285.54-571.72 μg/ml). Data suggest that *H. robertianum* is a rich source of phenols and flavonoids although a high variability among the different portions of the plant was observed. Further and more detailed phytochemical studies are needed to better characterize the polyphenolic profile of these extracts and to evaluate their biological activities as potential sources of antioxidants for medicinal use.

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2.1 = Metabolite profiling of sweet almond lipids in two Italian cultivars, Toritto and Avola

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Prunus dulcis Mill. (Rosaceae) is a tree native of Minor Asia, mainly cultivated for the production of its edible seeds, known as sweet almonds. Even if they are usually consumed as they are, fresh, dried or roasted, almonds are widely employed in confectionery, cookery and pastry, for the manufacturing of typical dishes. Much is known about the chemical composition of the oily fraction, rich of mono- and polyunsaturated fatty acids (1), reported for their capacity to reduce the hematic levels of Low Density Lypoproteins (LDL) and to enhance those of High Density Lypoproteins (HDL), preventing the onset of heart diseases (2). On the other hand, there is still a lack of informations about the polar lipids. In addition, there is a low number of reports about the metabolite profile of Italian almond varieties. Aiming at achieving a deeper knowledge on the chemical composition of the lipids of Italian almond cvs., as well as at evaluating if different growing conditions may affect the metabolome of a plant species, the most appreciated Italian cvs, Toritto (Apulia) and Avola (Sicily), were submitted to phytochemical investigation.

The analysis of the polar lipids was carried out by extracting the almonds of the selected cvs. by employing the method described by Bligh and Dyer (3), with slight modifications; the obtained extracts were submitted to LC-ESI-(Q-ToF)-MS/MS experiments, allowing the identification of the major polar lipids as phospholipids, by comparing their accurate masses and typical fragmentation patterns with those reported in literature and in online databases.

The analysis of the oily fraction was performed by extracting the almonds of the selected varieties with *n*-hexane, and by evaluating the content of free fatty acids of the obtained oils by GC-FID experiments, previously converted in their related Fatty Acids Methyl Esthers (FAME), and identified by comparing their retention times with those a standard FAME mixture.

Moreover, the raw data obtained from the LC-ESI-MS experiments were processed by a Multivariate Data Analysis approach. In particular, Principal Component Analysis (PCA) showed not only significant differences among the Apulian and the Sicilian cvs., but also among the three Avola cvs (Fascionello, Pizzuta and Romana). Partial Least Square (PLS) analysis further confirmed the results obtained from PCA.

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2.1 = CNS activity of *Lavandula angustifolia* essential oil and its main constituent, linalool

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Animals and humans are continually exposed to various anxiety-promoting situations in their surrounding environment and the management of anxiety and social relationships has become salient in contemporary urban life (1). The administration of natural substances in aromatherapy, folk and complementary medicine has been known for a long time, and its relevance is steadily growing. Different studies have been carried out to unravel the effects of essential oils and aromatic species on the central nervous system (CNS) (2). *Lavandula angustifolia* Mill. (lavender, Lamiaceae) is an aromatic plant recommended in folk medicine for relief of convulsion, anxiety and insomnia and in treatment of several neurological disorders (3). In this research, we studied the effects of *L. angustifolia* essential oil and of its main constituent, linalool, *in vitro* on proteins involved in the transmission of the signal (pERK and PKA) and *in vivo* in mice followed up in a series of experimental procedures. Elisabetsky and coworkers, in a psychopharmacological *in vivo* evaluation of linalool, showed that this compound possesses dose-dependent sedative effects on the Central Nervous System (4, 5). Our experiments showed that linalool inhibited pERK and PKA expression whereas *L. angustifolia* essential oil showed no significant effects on protein expression. Moreover, an injection intraperitoneal of linalool (100 mg/Kg) and of *L. angustifolia* essential oil (200 mg/Kg) had sedative effect, reduced motor activity and increased significantly social interaction. Mice experiencing aggression develop an aversion to social contact (6). In this study, we compared the effect of acute and chronic administration of linalool and *L. angustifolia* essential oil and the results demonstrated that these natural substances are capable to reverse social aversion acting as antidepressant substances.

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2.1 = Metabolite profiling of “green” extracts of *Corylus avellana* L. leaves by ^{1}H NMR spectroscopy and multivariate statistical analysis

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The common hazel plant (*Corylus avellana* L.), a tree native to Europe and Asia belonging to Betulaceae family, grows in temperate climates like Turkey, Spain and Italy. Turkey is the leading producer and exporter of hazelnuts, accounting for more than 70% of the world crop, Italy is the second producer with over 13% (1).
The Italian “Nocciola di Giffoni”, also known as “Tonda di Giffoni”, is a labeled PGI (Protected Geographical Indication) product of the Campania region, representing an important economic resource (2). The leaves of *C. avellana* are used, as infusion, in folk medicine for the treatment of hemorrhoids, varicose veins, phlebitis, and edema, as a consequence of their astringent, vasoprotective, and antiedema properties and also for their mild antimicrobial effects (3). They are claimed for their beneficial properties and are widely distributed in on-line market.
Our previous investigations on the leaves of *C. avellana* led to the isolation of cyclic diarylheptanoids and diaryletherheptanoids, some of which highly hydroxylated, named giffonins A-P, along with flavonoid derivatives, which displayed the capacity to prevent oxidative damages of human plasma lipids (4, 5). With the aim to give an interesting and economically feasible opportunity to *C. avellana* leaves as source of functional ingredients for pharmaceutical and cosmetic formulations, “green” extracts were prepared by employing “eco-friendly” extraction protocols as maceration, infusion and SLDE-Naviglio extraction.
Metabolite profiles of the extracts were obtained by ^{1}H NMR experiments and data were processed by multivariate statistical analysis to highlight differences in the extracts and to evidence the extracts with the highest concentrations of bioactive metabolites. Based on the NMR data, a total of 31 compounds were identified. The metabolite variation among the extracts was evaluated using Principle Component Analysis (PCA) and Partial Least Squares-Discriminant Analysis (PLS-DA).
Furthermore, the total phenolic content of the extracts was measured by Folin-Ciocalteu colorimetric assay and the antioxidant activity of extracts was assayed by the spectrophotometric tests DPPH$^\bullet$ and ABTS and by an *in vitro* test based on the evaluation of cellular reactive oxygen species production stimulated by pyocyanin. In particular, at 5 µg/ml, the extract obtained by maceration and by SLDE-Naviglio reduced intracellular ROS levels of about 30.92 % and 42.41 %, respectively; this activity was comparable to that exerted by quercetin, used as reference compound, confirming the antioxidant potential of *C. avellana* leaves.

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2.1 = ^{1}H NMR-based metabolomic approach to study *Sorghum bicolor*

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Sorghum bicolor (L.) Moench, belonging to Poaceae family, is the fifth most important cereal and is mainly cultivated in Africa, Asia and south-west USA for food, feed, fiber and fuel (1). The cultivation of sorghum for food is recently increasing in Mediterranean countries, due to its lack of gluten (2). Although this cereal is characterized by high nutritional value (3), it also produces a cyanogenic glycoside (dhurrin), whose content varies depending on plant age and growth conditions (4), and must be kept extremely low at harvest. The relevant role played by sorghum in global agriculture makes it important to investigate whether specific agricultural practices or environmental features could positively affect sorghum in terms of both grain yield and quality. Thus, the present study was aimed at evaluating different *S. bicolor* crops by metabolomic analysis of different plant organs at different stages: thus, the relationships between the metabolome and agronomic parameters were investigated. Sorghum samples were collected in seven different fields of Emilia-Romagna region (Italy). The ^{1}H NMR-based metabolomic approach combined with the Multivariate Data Analysis provided interesting information. The PCA, carried out on the spectral data of seedlings, adult leaves and grains, showed the inter-field differences in term of metabolites variations, while the OPLS-DAmodel used to compare seedlings and adult leaves highlighted the differences in the metabolome related to the plant growth. Figure 1 shows the results of the final PCA carried out on all the investigated traits (content of the most variable metabolites, geographical position, agronomic parameters, meteorological conditions, soil physical and chemical features and so on). It revealed that some soil features are associated with high crop yield, despite most unfavorable meteorological course. For example, the field called BT showed high yield despite severe drought (lowest precipitation). This field was subjected to intensive organic fertilization and deep soil tillage, and it had high percentage of clay and associated humidity, compared to the others. Moreover, according to metabolomic analysis, BT soil revealed the highest content of threalose, probably linked to intense microbiological activity. Low yield crops (mostly on the left side of the graph) showed worst quality in term of metabolome, with highest concentration of dhurrin in the grains. The field called SG represents an interesting case of study since it shared common features with MT and BT but with a lower yield; further analysis are ongoing to explain this data. In conclusion, ^{1}H NMR-based metabolomics,combined with agronomic study, turned out to be a valid tool for smart agriculture, allowing the evaluation of crops in different growth conditions and analyzing their relationships with the environmental factors.

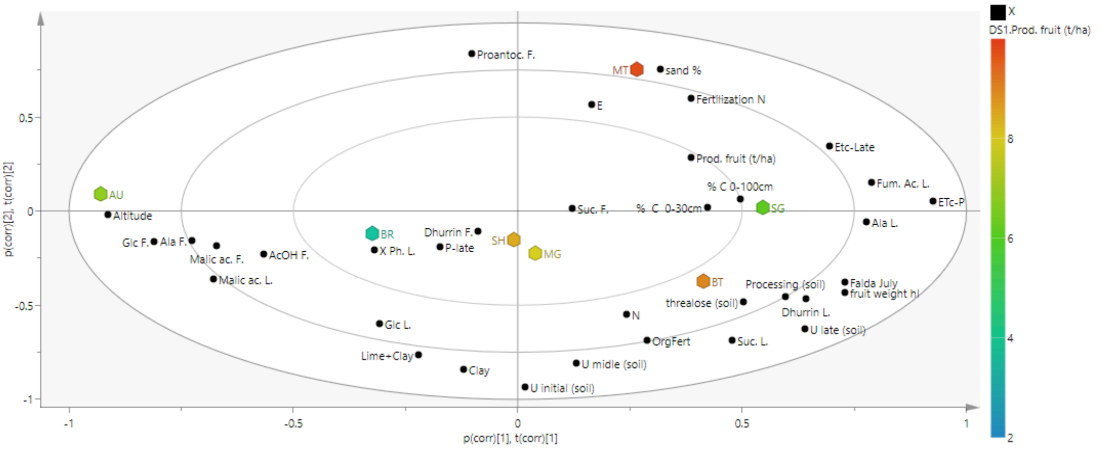

Figure 1. Biplot of PCA build for 7 Sorghum bicolor crops. Each hexagon represents a field, and the reddish ones showed highest grain yield. Black dots are the considered x variables of the model

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POSTER N. 19

2.1 = Phytochemical analysis and antioxidant properties of *Cannabis sativa* L. essential oil from a Chinese accession

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Cannabis sativa L. (Cannabaceae) has been cultivated in China for millennia as one of their main crops, and utilized as source of fiber, oil, food and medicine. Most botanists consider the plant monotypic, while some regard it as consisting of more than one species and numerous varieties. In Meijer's opinion (2014) *Cannabis* genus should be considered as monospecific, comprising only the single species *C. sativa* L. (1). The long and close association of *Cannabis* plant with man has caused high variations. Today, in China the ancient cultivation of *Cannabis* results in genetically distinct regional varieties of fiber-rich hemp that are adapted to local environmental conditions throughout the country (2, 3).

The essential oil of *Cannabis* is a complex mixture composed primarily of mono- and sesquiterpene hydrocarbons and their derivatives with simple substitutions such as alcohols or ketones, a number of other compounds such as alkanes and some cannabinoids like those having propyl side chains common only in a specific cannabis biotype (4-6). Phytocannabinoids, in particular, comprise more than 60 terpenophenolic compounds uniquely belonging to *Cannabis* genus recognized as extremely important chemotaxonomic markers (6).

It seems likely that differences in terpenoids composition that characterize *Cannabis* biotype essential oils are naturally occurring in its wild progenitor and are not primarily a result of human selection. However, the significant differences in terpenoid composition could be responsible of distinctive medicinal properties of different *Cannabis* biotypes (7).

In the present study, the essential oil of dried flowering tops of a new Chinese accession (G-309; Fig. 1) of *Cannabis sativa* L. with low Δ^9-THC content (<0.2%) was extracted by hydro-distillation with a Clevenger-type apparatus according to the European Pharmacopoeia. After that, the volatile fraction was characterized by GC-FID and GC-MS analysis and its antioxidant properties investigated by several *in vitro* assay.

The quali-quantitative GC analysis identified 61 constituents of which sesquiterpenes represent the most abundant class (58.06 %) followed by sesquiterpene oxygenated (23.48 %), phytocannabinoids (8.57 %), monoterpenes (8.52 %), monoterpene oxygenated (0.17 %) and other compounds (1.19 %) like ketones and esters. Major compounds include Caryophyllene (20.98 %), α-Bisabolol (15.41 %), Caryophyllene oxide (6.98 %), γ-Catinene (6.23 %) and Δ^9-Tetrahydrocannabivarin (6 %). Moreover, GC analysis revealed a significant amount of non-psychotropic phytocannabinoids, of which Δ^9-Tetrahydrocannabivarin (THCV, Fig.1) is the most representative (6 %).

Cannabis essential oil from Chinese accession (G-309) showed a remarkable antioxidant and free-radical scavenging activity with the following order of potency: ORAC (IC_{50} 2.11 μg/ml) > TEAC (IC_{50} 16.09 μg/ml) > Folin-Ciocalteu (IC_{50} 155.98 μg/ml) > β-carotene bleaching (IC_{50} 256.82 μg/ml) > DPPH (IC_{50} 453.90 μg/ml) > FRAP (IC_{50} 629.67 μg/ml).Most probably, the high antioxidant properties of the essential oil, obtained from Chinese accession G-309, are mainly due to the synergy of terpenoids and phytocannabinoids, which make it hypothesize a potential use to prevent the onset of several oxidative stress-related diseases.

Fig. 1. *C. sativa* L. Chinese accession

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2.1 = Bee pollen to manage prostate inflammation: phytochemistry and protective role of standardized extract in *in vitro* assays and on rat prostate specimens

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Acute and chronic prostate inflammation are common diseases that are often related to the presence of infectious agents of bacterial or non-bacterial origin (1). Plants extracts are largely investigated as potential candidates to manage clinical symptoms of prostatitis because of their multiple synergistic actions on the modulation of oxidative stress and inflammation pathways (2). Different extracts of bee pollen were already tested as medicinal agent for the same purpose. In the present work, *Graminex* (Graminex, Deshler, OH, USA), a commercially available standardized pollen extract, is investigated as a protective agent, *ex vivo*, on rat prostate specimens (3). We assessed the possible mechanism of action of the reconstituted powder on multiple pathways significantly increased by pro-inflammatory stimuli, including reduction of prostaglandin E2 (PGE2), nuclear factor kappa-light-chain-enhancer of activated B cells (NFκB) and malondialdehyde (MDA). Phytochemical characterization was defined by means of chromatographic and colorimetric studies on pollen extract to provide a better correlation of its biological activity on immortalized prostate cells (PC3) and rat prostate specimens challenged with *Escherichia coli* lipopolysaccharide (LPS). As a result, a reduced production of radical oxygen species (ROS) from PC3 cells (Fig. 1) and MDA, NFκB mRNA, and PGE2 levels on rat prostate specimens were obtained. According to our experimental evidences, the efficacy of *Graminex* pollen in the modulation of inflammatory pathways is confirmed and it appears to be a promising naturally derived product to prevent and manage symptoms related to inflammatory mechanisms of prostatitis.

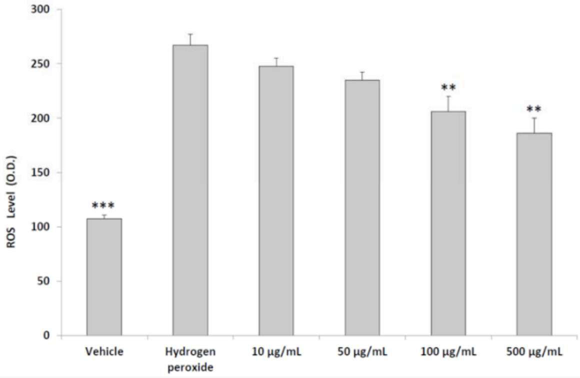

Fig.1. Effects of pollen extract (10–500 µg/mL) on ROS production from PC3 cell line

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2.1 = Roasted hazelnut (*Corylus avellana* L.) skins: from a by-product to an active ingredient for food packaging films

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Hazelnut (*Corylus avellana* L., Betulaceae family) is one of the most consumed nut in the world, not only as whole fruit (raw or roasted) but also as an ingredient of manufactured bakery and confectionery products. A lot of by-products, including hard shells and skins, are produced during the processing of the edible part (1). The skins are usually removed by blanching or roasting to improve the kernel flavour, colour and crunchy and their disposal represents both an economic and environmental problem. In the last years, great attention has been paid to the recovery and upgrading of agro-industrial residues as potential sources of bioactive compounds with beneficial effects on human health, and some papers have already showed the potential use of Roasted Hazelnut Skin (RHS) derivatives as natural antioxidants (1). In recent years, the development of edible films and coatings is considered a potential tool to improve safety of food and to protect it from the influence of external environmental and biotic factors, thus increasing its shelf life (2). Natural compounds, such as flavonoids and phenolic acids, have been identified as food preservatives safe for human consumption (3). Therefore, the aim of this work was to develop an antioxidant and antimicrobial food packaging film by incorporating the hydroalcholic extract obtained from RHS (RHS-H) into a polysaccharide film and to elucidate the extract impact on physico-mechanical properties, and antimicrobial and antioxidant activities of the obtained bioactive films. The proanthocyanidins-rich RHS-H was produced by an optimized Accelerated Solvent Extraction (ASE) technique. Pullulan, a water-soluble polysaccharide, edible, biodegradable, biocompatible to human and environment was used as polymeric matrix, with the addition of a plasticizer (4). The films were produced by casting technique and RHS-H was incorporated at concentrations of 1, 5 and 10% (w/w). Thermal stability, structure, optical properties, mechanical performances of films were investigated. Moreover, the antioxidant and antibacterial activities against Gram-positive and Gram-negative foodborne pathogens, were evaluated. The results showed that the addition of RHS-H reduced the light transmittance of films in the visible and UV ranges, which can contribute to the reduction of food deterioration. The addition of RHS-H had a poor effect on mechanical properties of the films, but improved their antioxidant and antimicrobial activities. All the results suggest that RHS-H can be use as a natural antioxidant and antimicrobial agent in flexible bioactive films promoting the reuse of a food by-product to increase the eco-sustainability of the food processing industry.

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2.1 = Testing the antiproliferative activity of lichen extracts

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Lichen secondary metabolites are produced by the mycobiont and are activated as a consequence of biological processes characteristics of the symbiotic association with the algae. These metabolites mostly derive from different chemical pathways and it has demonstrated that they have relevant potential pharmacological applications, due to their biological activities, which include, among others, antitumoral effects (1, 2).

Preliminary studies of our research group explored the effects of crude methanolic extracts of four different lichen species on the proliferation of human tumor cell lines. However, comparison with the corresponding chloroform-based extracts revealed that the former were more efficient in arresting cell proliferation.

Based on these evidences, in this work we aimed at testing the effects of chloroformic extracts of two lichen species, *Cetraria islandica* and *Flavopamelia caperata*, on human osteosarcoma (MG-63) and small lung carcinoma (A-549) cells. Both species produce several secondary metabolites among their major compounds, including fumarprotocetraric acid.

For both cell lines, the Alamar Blue® proliferation assay was used to assess the IC_{50}. The assay was performed by reading the culture supernatants at 570-600nm, and determined after 6 days of culturing as of 50 µg/mL and 25 µg/mL, for *C. islandica* and *F. caperata*, respectively.

Results revealed that the antiproliferative effect was not detectable in control cultures exposed to the extract adjuvant alone (dimethylsulfoxide), whereas it paralleled the growth inhibition caused, in positive control cell cultures, by treatment with usnic acid (4 µg/mL).

Moreover, the effect was not transient for any of the extracts, since removal of exposed cells and their transfer to a standard medium did not support reprisal of cell growth and proliferation.

Furthermore, we used control and extract-treated cell aliquots to isolate total mRNA and assess the transcript levels of specific genes involved in proliferation (ki67), apoptosis/anti-apoptosis (p53 and BCL-2, respectively). As a result of the gene expression analysis, we observed a drastic reduction of Ki67 transcript level, in accordance with an impaired proliferation, both in MG-63 and in A-549 cells, as well as with clear cell morphological changes (*i.e.* cell rounding), emerging soon upon extract exposure. In the attempt to grant a regular cell cycling, at the same time extract-exposed cells enhanced their BLC-2 expression levels.

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2.1 = Antitumor potential of plants from Sardinia (Italy), a hotspot for biodiversity in the Mediterranean basin

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Valorization of territory and endogenous resources are nodal points for sustainability. Sardinia (Italy), due to its geographical isolation and high geological diversification, is a hotspot for biodiversity within the Mediterranean basin, which makes Sardinian plants an interesting source of new bioactive chemical scaffolds (1). Moreover, plant-based traditional medicine is still widely practiced in Sardinia, providing valuable information about safety and uses of the different plants growing on the Island. This work was aimed at draw attention on Sardinian plants, in terms of their significance for Mediterranean cultural heritage, biodiversity conservation and valorization, sustainable rural development strategies and vitality of traditional medicine and food systems. In particular, thirty-seven samples from Sardinian plants, including three endemic and exclusive species of Sardinia and nine endemics of Sardinia and Corse, were investigated for their *in vitro* anti-proliferative activity. Coping with cancer prevention and treatment, the contribution of natural drugs has been historically remarkable and still remains of great importance (2). In addition, epidemiological studies established associations between certain dietary patterns and reduced cancer risk and these findings were also widely supported by laboratory researches (3). In this context, significant results were obtained in experimental and clinical trials on different secondary metabolites produced by edible plants, such as: carotenoids, phenolics, and organosulfur compounds. Hydroalcoholic extracts were prepared for all the selected plants, and MTT test was performed using U2OS human osteosarcoma. Five out of the thirty-seven tested plants, namely: *Arbutus unedo* L., *Centaurea calcitrapa* L., *Cynara cardunculus* L., *Smilax aspera* L. and *Tanacetum audibertii* Req, significantly reduced cell proliferation. The EC_{50} calculated for these plants were: 63.14 μg/mL for *C. calcitrapa*, 39.85 μg/mL for *C. cardunculus*, 125 μg/mL for *T. audibertii,* 94.79 μg/mL for *Arbutus unedo* and 127.9 μg/mL for *Smilax aspera.* Hence, the mechanism of action of these plants was deepened by performing a cytofluorimetric analysis of DNA profiles. All the five extracts were able to induce a significant cell cycle arrest in G2/M phase. A preliminary phytochemical analysis, carried out by ^{1}H-NMR, highlighted a similar aromatic profile of *T. audibertii* and *C. cardunculus.* These aromatic compounds might be responsible for the common mechanism of action shown by these two plants. *C. cardunculus* was enriched in cynaropicrin, a guaianolide sesquiterpenoid known to possess antitumor activity (4). This compound was also quantified by q-NMR reveling a concentration of 76 μM/mg of extract. Signals typical of guaianolides were also evident in *C. calcitrapa* ^{1}H-NMR spectrum, while *A. unedo* was enriched in flavonoids and arbutin, a hydroquinone glycoside endowed with cytotoxic potential (5). In conclusion, this work allowed the selection of five plants endowed with antitumor potential and which are interestingly used in form of decoction in the Sardinian traditional medicine, suggesting a safe oral administration and a potential use to develop chemopreventive food supplements.

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2.1 = Effects of lichen extracts on tyrosinase activity

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Tyrosinase is a key enzyme responsible of melanin synthesis in animals and browning in plants. It catalyses the hydroxylation of monophenols to *o*-diphenols and their subsequent oxidation to *o*-quinones using molecular oxygen. These reactive *o*-quinones then undergo non-enzymatical polymerization to form melanin. Although melanin in human skin is widely accepted as an adaptation to UV radiation, the overproduction and accumulation of melanin can result in hyperpigmentation disorders. Therefore, the discovery of new natural inhibitors is becoming of great importance for the pharmaceutical and cosmetic industry to prevent and treat different types of damage related to anomaly in skin pigmentation. Lichens are symbiotic associations between a fungus and a population of unicellular algae, widely distributed around the world. Lichens produce various unique extracellular secondary metabolites, known as lichen substances, which have several biological activities. For this reason, they are used in traditional medicines by cultures across the world and recently they have been taken up for many researches concerning phytochemical and pharmaceutical applications. The aim of this work was to test the potential inhibition effect of 4 lichen extracts on tyrosinase activity and to verify if different solvent systems may affect or not this process. The extracts that showed the highest inhibition rate were tested *in vivo* on the melanization process of zebrafish embryo (*Danio rerio*). For achieving our goal, we selected 4 lichens, *Cetraria islandica*, *Letharia vulpina*, *Flavoparmelia caperata*, and *Parmotrema perlatum*, characterized by different pools of lichen substances. Each lichen was extracted in 4 solvents with increasing polarity: chloroform, a mixture of chloroform-methanol (9:1), methanol, and water. The results of the *in vitro* tests showed that the methanol extract of *L. vulpina* and the chloroform-methanol extract of *C. islandica* have the highest inhibition rate on the enzyme tyrosinase. The tests *in vivo* confirmed these data by showing that lichen extracts can also inhibit the melanization process in zebrafish embryos. Finally, the autobiography technique was used to characterize the lichen substances and to verify their inhibitory activity against tyrosinase.

2.1 = Misidentification of wild plants cooked in soup and sold as traditional gastronomy

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In the North eastern Piedmont region, in Ivrea town and surroundings, a soup made with wild spring herbs is very much appreciated. Many people gather wild plants and in particular ajucca *Phyteuma spicatum* L. (Campanulaceae family), to prepare a soup, whose recipe is linked to popular tradition (1).

In this context, a case of a misidentification of *Phyteuma spicatum* young leaves with poisonous plants was responsible for the access of a female subject, who ingested the soup, to the hospital Emergency Department, at Ivrea Hospital (Torino province).

A complication, from a legal point of view, was due to the fact that the soup was sold in a store of prepared and cooked foods.

The Istituto Zooprofilattico of Torino, responsible for food emergency and intoxication for the Piedmont region, provided the sample of the soup for the botanical and phytochemical identification of the poisonous plant material.

Considering the reduced size of the plant material in the soup, it was not possible to identify the poisonous plant, while the presence of some leaves of ajucca was confirmed; therefore a chemical evaluation of the constituents potentially responsible for the intoxication was mandatory. The phytochemical analyses revealed the presence of tropane alkaloids in particular atropine (+/- Hyoscyamine) in the soup, confirming the possibility of a contamination with species from Solanaceae family since species belonging to *Phyteuma* genus don't biosynthetize tropane alkaloids (2, 3). The presence of atropine supported also the clinical outcomes on the hospitalized woman (i.e. marked pupillary dilation). The erroneous collection can be related to the morphological similarity between *Phyteuma spicatum* L. young leaves in spring time and those of other poisonous plants, in particular Solanaceae species growing in the same environment.

The case here described can be considered as an emblematic example of the lack of cultural knowledge of many people collecting wild plants for human consumption and sale. It especially draw the attention to the importance of the identification of wild plants, since not all the natural products are necessarily safe.

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2.1 = *Crocus sativus* L.: qualitative fingerprint, antioxidant potentials and enzyme inhibitory activities: giving added value to high-quality byproducts

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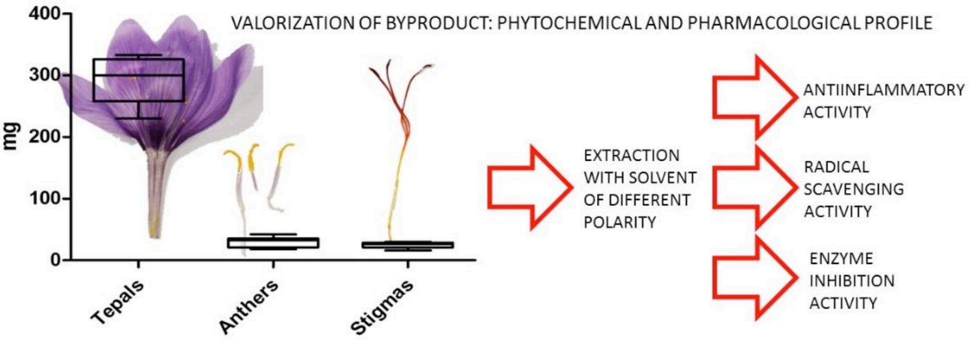
Saffron (*Crocus sativus* L.) has been previously reported to be active as a protective agent in multiple experimental models of oxidative stress, inflammation and cancer (1,2). These findings refer to the protective effects of stigmas, not byproducts such as tepals and anthers. In this context, the aims of the present work were to characterize the phytochemical profile of saffron stigmas (CST) and high quality byproducts (tepals + anthers - CTA) extracts. Additionally, we studied the antioxidant and chelating effects of CST and CTA extracts by preliminary in vitro assay. The antioxidant activity was further investigated through the evaluation of reactive oxygen species (ROS) levels and lactate dehydrogenase (LDH) activity on mouse myoblast (C2C12) and human colon cancer (HCT116) cell lines. Additionally, we evaluated CST and CTA extract treatment on cholinesterases, α-glucosidase and α-amylase activity, *in vitro*. Finally, we studied the effects of CST extract on malondialdehyde (MDA) level in rat colon specimens challenged with *E. coli* lipopolysaccharide (LPS).

We observed that water CST extracts are rich in phenolic content, whereas for CTA the olive oil was the elective extraction solvent. As expected, water CST extracts were the most effective in reducing hydrogen peroxide-induced oxidative stress in both cell lines and *in vitro* assays. Furthermore, both CST and CTA water extracts reduced the LDH activity in HCT116 cells challenged with hydrogen peroxide and LPS-induced MDA levels in rat colon specimens.

Concluding, the present findings showed protective effects exerted by CST and CTA extracts in *in vitro* and *ex vivo* models of inflammation and oxidative stress.



VALORIZATION OF BYPRODUCT: PHYTOCHEMICAL AND PHARMACOLOGICAL PROFILE

EXTRACTION WITH SOLVENT OF DIFFERENT POLARITY

ANTIINFLAMMATORY ACTIVITY

RADICAL SCAVENGING ACTIVITY

ENZYME INHIBITION ACTIVITY

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2.1 = Plant tryptamine and serotonin: in search of their biological role in the fruit

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About a hundred of plant species, that include some common fruits and vegetables, are able to produce and accumulate the two neuroactive indolamines tryptamine and serotonin (1).
Although these molecules have been well studied in animal systems in which they regulate various physiological functions (2), still more information is required to clear their biological role in plants. In some species, tryptamine - beyond being the precursor of serotonin - is also an intermediate in the biosynthesis of IAA (3) and indole alkaloids (4), whereas serotonin can be converted to melatonin (5) and various phenolic derivatives (6). Recent works, that suggest the involvement of serotonin in many plant physiological processes (1) (flowering, senescence (7), plant architecture, adaptation to environment, stress response (8), did not focus on its production in reproductive organs such as the fruit and the seeds where the highest levels of this substance have been reported (9) (Fig.1).
This project aims to unravel the role of tryptamine and serotonin in the model fruit of tomato (*Solanum lycopersicum* cv. Microtom), a natural producer of these indolamines, by using a metabolic engineering approach. The strategy is to induce the depletion and a further accumulation of tryptamine and serotonin in the fruit by targeting a fruit-specific tryptophan decarboxylase (*TDC*), responsible for the conversion of tryptophan into tryptamine. Three tomato genes have been identified and characterized as *TDCs* through *in-vivo* functional assay in *Nicotiana benthamiana* . *SlTDC1*, whose expression pattern is fruit specific, has been chosen as the target to generate knock-out and overexpressing mutants that will be subjected to phenotypical analysis along the whole ripening process and tested for their ability to respond to abiotic and biotic stress. Moreover, the analysis of the accumulation pattern of tryptamine and serotonin by HPLC-ESI-MS in different organs and developmental stages of wild-type tomato plants, added further details to the comprehension of their functions in plants.

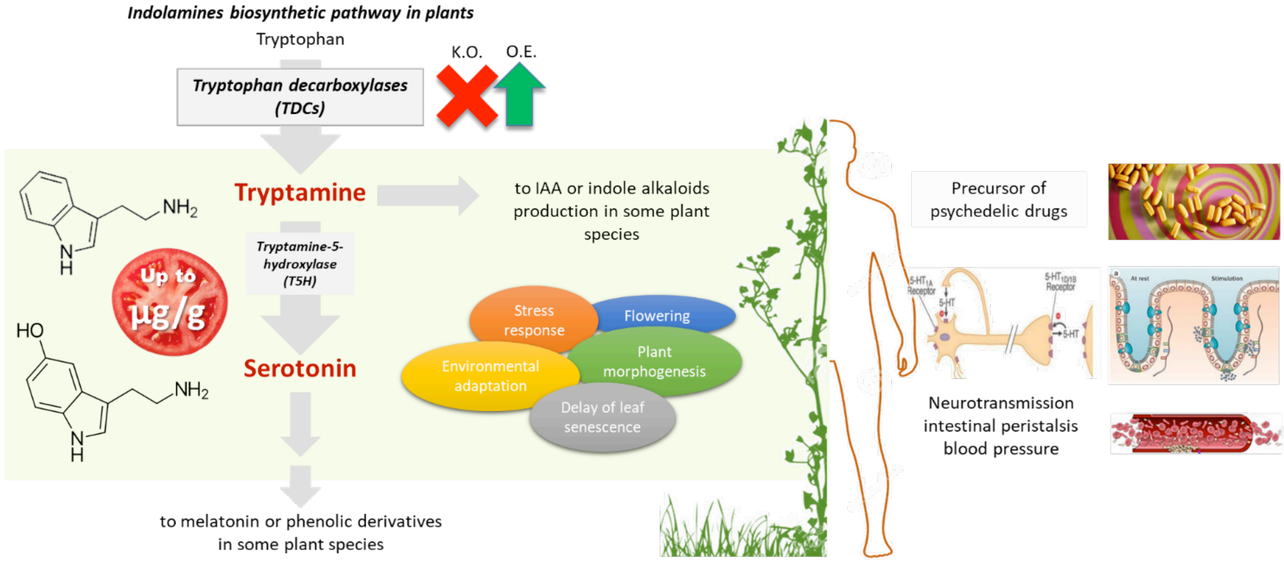

Fig. 1. Tryptamine and serotonin: what do we know?

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2.1 = Water-soluble chitosan oligosaccharides (COS) affect xanthone, and volatile organic compound content and antifungal activity against human pathogenic fungi, of *Hypericum perforatum* root culture extracts

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Chitosan (CHIT) is a natural non-toxic biopolymer produced by the partial deacetylation of chitin, the main component of fungal cell wall. Among elicitors, CHIT is one of the most used to increase the biosynthesis of plant secondary metabolites of pharmacological interest. However, CHIT is poorly soluble in neutral water as well as in most organic solvents, therefore it is commonly dissolved in water acidified with acetic acid, which greatly limits its application. Recently, we found that short-chain monocarboxylic acids, as acetic acid, could alter the xanthonic profile in *Hypericum perforatum* root cultures, masking the effect of CHIT [1]. This problem could be overcome by using water-soluble CHIT derivatives, also known as chitosan oligosaccharides (COS), which can be obtained by enzymatic and/or chemical hydrolysis of CHIT. To date, few studies are available on the use of COS as elicitors to enhance the production of phytochemicals in plant *in vitro* cultures. At present, *H. perforatum* is one of the most prominent and best investigated medicinal plants due to a plethora of biological activities including antidepressant, antibacterial and antitumoral activities. *In vitro* root cultures of *H. perforatum* have been recognized as a valuable source of xanthones, a class of non-flavonoid polyphenols with numerous pharmacological properties. Xanthones are synthetized only in traces in the root of *H. perforatum* plant. Differently, in root cultures a higher xanthone production was observed, greatly enhanced by CHIT. *H. perforatum* is also an interesting source of numerous volatile organic compounds (VOCs) such as monoterpenes and sesquiterpenes with antibacterial and antifungal activities, that have been detected in the shoot organs of wild plants and in *in vitro* shoot cultures, but not yet in *in vitro* root cultures. Several studies have demonstrated the elicitor power of chitosan on xanthone biosynthesis in root cultures of *H. perforatum*. One of the major limitations to the use of chitosan, both for basic and applied research, is the need to use acidified water for solubilization. To overcome this problem, the elicitor effect of water-soluble chitosan oligosaccharides (COS) on the biosynthesis of both xanthones and volatile organic compounds (VOCs) was evaluated in the present study. The analysis of xanthones and VOCs was performed by HPLC and GC-MS headspace analysis. The obtained results showed that COS are very effective in enhancing xanthone biosynthesis. With 400 mg L^{-1}COS, a xanthone content of about 30 mg g^{-1} DW was obtained. The antifungal activity of extracts obtained with 400 mg L^{-1}COS was the highest, with MIC_{50} of 32 μg mL^{-1} against *Candida albicans* and 32-64 μg mL^{-1} against dermatophytes, depending on the microorganism. Histochemical investigations suggested the accumulation of isoprenoids in the secretory ducts of *H. perforatum* roots. The presence of monoterpenes and sesquiterpenes was confirmed by the headspace analysis. Other volatile hydrocarbons have been identified. The biosynthesis of most VOCs showed significant changes in response to COS, suggesting their involvement in plant-fungus interactions.

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2.1 = Metabolomic analysis of *Arbutus unedo*: relationship between phytochemical profile and anti-tyrosinase activity

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Arbutus unedo L. (Ericaceae family) is a fruit tree species widely distributed in the Mediterranean-Atlantic area where is also used in traditional medicine. The leaves of this plant showed different biological activities such as anti-inflammatory, antifungal, antibacterial, antioxidant, and skin-whitening action (1). Among its secondary metabolites, *A. unedo* produces also arbutin, a glycosylated hydroquinone used in cosmetics as skin-whitening ingredient due to its activity against tyrosinase. Tyrosinase is a copper-containing enzyme that catalyzes two distinct reactions of melanin synthesis: the hydroxylation of tyrosine by monophenolase action and the oxidation of 3,4-dihydroxyphenylalanine (L-DOPA) to o-dopaquinone by diphenolase action (2). Nevertheless, arbutin is able to contrast only the monophenolase activity of this enzyme. Moreover, arbutin is effective only at a specific dosage, while at high dosage (0.5-8 mM) it determines iperpigmentation and cytotoxic effects (3). On this basis, this work was focused on *A. unedo* leaves and their potential as skin-whitening ingredients, aimed at verify if metabolites, other than arbutin, may contribute to skin-whitening activity inhibiting also tyrosinase diphenolase function and allowing to use extracts with low concentration of arbutin and high biological activity. Hence, *A. unedo* leaves were collected in different areas of Sardinia Island and at different time points and their inhibitory activity of diphenolase function of tyrosinase was correlated to their metabolomic profiles measured by ^{1}H NMR. This is an emerging technique in natural products-based drug discovery, used to facilitate the identification of the active principle(s) of a crude plants extract (4, 5). The IC_{50} values of enzyme inhibition, measured for the hydroalcoholic extracts of the collected samples, showed a wide range of activities (from 15.01 to 89.39 µg/mL), proving that *A. unedo* leaves, differing for harvesting areas and/or season of the year, are not comparable in term of bioactivity. This observation was supported also by the metabolomic analysis, which highlighted several differences among the samples, also in respect of arbutin content (varying from 9.69 to 1074 µM/mg of extract). By performing OPLS (Ortogonal Partial Least Squares) model it was possible to establish a correlation among ^{1}H NMR signals possibly due to flavonoids and the increasing biological activity. Thus, the most active extract was fractionated by flash chromatography obtaining four fractions, and confirming that the two fractions containing flavonoids were the active ones.

In conclusion, *A. unedo* proved to contain active principles, other than arbutin, endowed with tyrosinase inhibitory activity, and the metabolomic approach was useful to shorten and guide the identification of these active principles. Moreover, ^{1}H NMR fingerprinting and multivariate data analysis resulted as an interesting tool for a broad-spectrum quality control of *A. unedo* extracts, allowing selecting the autumn as the best season to obtain the extracts with the highest effectiveness against tyrosinase.

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2.1 = Phytochemical screening and antioxidant activity of *Euphorbia dendroides* L. leaf extracts

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Euphorbia is the largest genus of the Euphorbiaceae family with more than 2000 species of flowering plants[1,2]. *Euphorbia dendroides* L. is a typical Mediterranean plant and prefers rocky places along the coasts, up to an altitude of about 700 m above sea level, mainly in Spain (Balearic Islands), France (including Corsica) and Italy (including Sardinia and Sicily). It is a perennial plant and takes the form of a rounded shrub or small tree up to 3 m tall (Fig. 1). The leaves are small, oblong-lanceolate, and fall in early summer as an adaptation to the semiarid Mediterranean climate. As all *Euphorbia* species, it produces a white latex that exudes when cut, and it is rich in secondary metabolites that contribute to the pharmacological properties of many *Euphorbia* species [3, 4]. Our previous study on the latex from *E. dendroides,* which grows naturally in Sicily, highlighted also high amounts of phenolic compounds mainly as phenolic acids and flavanones as most abundant flavonoids. Moreover, *E. dendroides* latex showed significant antioxidant activity (unpublished data). In this study, an ultra-sound assisted extraction of leaves of *E. dendroides*, using methanol/water mixture (80:20, v/v) was carried out. A phytochemical screening of total phenols and flavonoids on *E. dendroides* leaves extract as well as tannins, glycosides, alkaloids, saponins and anthraquinones on powdered dried leaves has been carried out using standard procedures. The antioxidant and free radical scavenging properties, by several *in vitro* assays such as DPPH, TEAC, ORAC, FRAP and β-carotene bleaching have been evaluated. Moreover, iron-chelating activity was estimated.

The preliminary phytochemical analyses showed that *E. dendroides* leaves extract contains a high total phenols content (1,294.97 mg GAE/100 g FW) of which 8.57 mg QUE/100 g FW of flavonoids. Furthermore, the extract was found highly positive to condensed tannins test while cardiac glycosides, saponins and antrachinones were not detected.

A significant and concentration-dependent antioxidant and free radical scavenging properties was found with the following order of potency: ORAC (IC_{50} 6.34 μg/ml) > TEAC (IC_{50} 16.77 μg/ml) > FRAP (IC_{50} 26.25 μg/ml) > DPPH IC_{50} 62.50 μg/ml) > β-carotene bleaching (IC_{50} 174.34 μg/ml). In addition, the extract showed a strong iron-chelating activity (IC_{50} 19.35 μg/ml).

Most likely, there is a direct relation between the content of active compounds in extract, mainly phenols and flavonoids, and antioxidant activity.

Fig. 1. *Euphorbia dendroides* L.

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2.1 = Using *Cynara cardunculus* L. allelopathic activity for weed control

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In the last decades, the increasing search in eco-sustainable strategies for weed management, has led the scientific community to explore the utilization of plant secondary metabolites, commonly called allelochemicals, in agriculture. Recently, Cynara cardunculus L., a perennial herbaceous plant belonging to Asteraceae family, has been studied for its allelopathic activity caused by sesquiterpene lactones such as cynaropicrin, aguerin B and grosheimin (1), and by polyphenols such as chlorogenic acid, luteolin- and apigenin derivatives, both presented in high quantities in the leaves.

In a first step the phytotoxic activity of aqueous leaf extracts (40 and 80% concentrations) of globe artichoke [var. scolymus (L.) Fiori], cultivated cardoon (var. altilis DC.), and wild cardoon [var. sylvestris (Lamk) Fiori] (Fig. 1) was evaluated on seed germination and mean germination time of six weed species (Amaranthus retroflexus L., Diplotaxis erucoides (L.) DC., Portulaca oleracea L., Lavatera arborea L., Brassica campestris L. and Solanum nigrum L.) widespread in Mediterranean Basin, along with the autoallelopathic activity of wild cardoon (2). Effects varied with the weed species and the extract concentrations. The results showed a mean reduction of about 41% in germination of all test species in comparison with the controls and the best result was obtained with the leaf extracts (80%) of cultivated cardoon, which resulted the most efficient also in comparison with wild cardoon, allowing only 9% of seed germination.

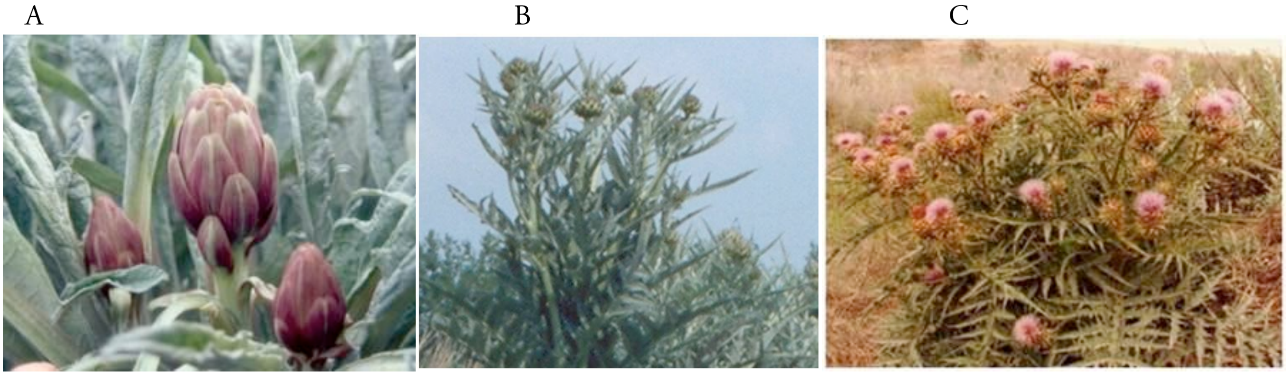

Fig. 1. Cynara cardunculus three botanical varieties: globe artichoke (A), cultivated cardoon (B) and wild cardoon (C)

Since cultivated cardoon extract resulted the most phytotoxic among the three botanical varieties, it was further investigated. In particular, the inhibitory activity of leaves (fresh, dried and lyophilized) of cultivated cardoon, treated with three different solvents (bidistilled water, 70% methanol and 80% ethanol) in presence/absence of citric acid (20%), on four common Mediterranean weed species (A. retroflexus, P. oleracea, Stellaria media (L.) Vill., and Anagallis arvensis L.) was compared (3). On the average of the four weed species under study, all leaf extracts showed inhibitory effects. Regarding plant material, lyophilized leaves permitted only 3.2% of seed germination as compared to 73% of control, followed by dried leaves (10%) and fresh leaves (16%). For what concern the solvents, the ethanolic extracts completely inhibited weed seed germination, while bidistilled water was the least effective (34% vs 73% of control) compared to the other solvents. Moreover, the polyphenolic profile of dried leaves aqueous, methanolic and ethanolic extracts of cultivated cardoon were analysed by HPLC. Results showed that the highest total measured polyphenols was found in the MeOH extract (14727 mg kg-1 of DM), followed by EtOH one (10561 mg kg-1 of DM) and aqueous extract (2777 mg kg-1 of DM), with caffeoylquinic acids representing more than 50% of the total measured polyphenols.

These results suggest that C. cardunculus extracts can be used for the potential production of a bioherbicide.

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2.1 = Sardinian plant biodiversity: a reservoir for the discovery of new anti-HIV-1 agents

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Plant products represent over one-quarter of all approved new molecular entities (1) and they continue to serve as a reservoir for the discovery of new bioactive compounds, including anti-HIV agents. However, despite the intensive investigation of plant kingdom, it is estimated that only 6% of the approximately 300,000 species of higher plants have been pharmacologically investigated, and only 15% phytochemically studied (2). A number of attempts have been made in the fight against the HIV-1 infection and several natural compounds, able to inhibit the viral enzymes, have been reported (3). However, so far all anti HIV-1 approved drugs were obtained only by chemical synthesis. Therefore, plants should be further investigated because new compounds, with original structures and novel modes of action, are continuously required for the management of HIV-1. An innovative approach in searching for new anti HIV-1 agents is aimed at identifying dual inhibitors, small molecules that can inhibit two viral functions (4), so giving the possibility of reducing the number of administered drugs and their chronic toxicity.

In our ongoing research of new natural compounds for developing innovative dual HIV-1 inhibitors, we focused on Sardinian endemic flora, in which geographical isolation and peculiar geological and geomorphological features have selected original metabolic patterns (5, 6). Interestingly, even if the scientific evidences show that endemic plants are interesting for their phytochemical and biological characteristics (5-8), the majority of them have not yet been investigated.

In the present study, crude extracts of eighteen endemic plants collected in Sardinia (Italy) have been tested for their ability to inhibit the HIV-1 Reverse Transcriptase-associated Ribonuclease H (RNase H) activity in biochemical assays and the HIV-1 integrase (IN) activity in presence of LEDGF/p75 cellular cofactor in Homogeneous Time Resolved Fluorescence assay (9).

Among them, thirteen extracts displayed strong inhibition against both key viral enzymes, two were able to inhibit only the HIV-1 IN activities in presence of LEDGF/p75, showing a selectivity for this viral enzyme, and three were found inactive on both enzyme activities.

Although deeper investigations are still required, our results suggest that Sardinian endemic plants have the potential for preventing HIV-1 infection. Noteworthy, Helichrysum saxatile Moris, Limonium morisianum Arrigoni, Plagius flosculosus (L.) S. Alavi & V.H. Heywood and Scrophularia trifoliata L. exhibited IC50 values ranging from 2.29 to 11.46 µg/mL (anti RNase H activity) and from 2.5 to 3.4 µg/mL (anti IN activity), becoming candidates as sources of potential multi-targeted HIV-1 inhibitors.

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2.1 = VOCs composition of *Salvia ceratophylloides* Ard. (Lamiaceae), a Calabrian endangered species, through targeted and untargeted metabolomic analyses

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The biotic and abiotic interactions between plants and their environment are mainly mediated by secondary metabolites (1). Among these metabolites, the volatile organic compounds (VOCs) are involved in a wide class of ecological functions for plants such as defense against herbivores and pathogens, attractors of pollinators, beneficial animals and microorganisms (2). Moreover, they play a pivotal role as plant stress signals (2). Environmental conditions, organs, genotypes, species, populations etc are several factors affecting VOCs composition, production and release .(3).

Salvia ceratophylloides Ard. is an endemic scapose hemicryptophytes, which was declared extinct in 1997 and recently rediscovered in around 100 individuals in the hills of Reggio Calabria. Therefore, according to IUCN criteria, it is considered as a critically endangered species which should be deeply studied through different approaches in order to enhance its conservation (4).

As first step, we have focused our attention on the chemical characterization of the VOCs produced by the different plant organs and on the evaluation of potential differences in chemical profiles among the three rediscovered populations. In particular, the aerial parts of *S. ceratophylloides* were sampled on three different locations at Reggio Calabria (Southern Italy), characterized by different disturbance levels: high (HD) and low disturbance (LD) (by anthropogenic nature) and natural habitat (NH). The volatiles produced by the different organs [young (YL) and old leaves (OL), flowers (FL), thin roots (TR), fine roots (FR) and woody roots (WR)] of *S. ceratophylloides* were chemically characterized using the HS/GC-MS method. As second step, an untargeted metabolomic analysis was carried out on the full aerial parts of the three different locations in order to highlight potential chemical differences among them.

The 40 compounds identified were differentially distributed among the different organs ranging from 35 compounds in FL to 6 volatiles in WR. Among the VOCs identified, the most abundant were monoterpenes (18 monoterpenes and 3 monoterpenic alcohol) and sesquiterpenes (7 chemicals). Moreover, four aldehydes, one thioether, three ketones, two alcohols and one acetate were also isolated. Concerning the aboveground organs, the flowers showed more abundance of monoterpenes than leaves: *β*-pinene was the most abundant compound (three folds higher than in leaves). On the contrary, the leaves produced more sesquiterpenes than flowers with predominance of *β*-caryophyllene. Concerning the different root types, isovaleraldehyde and α-methyl-n-butanal were the most abundant volatiles more released in FR. Moreover, volatile dimethyl sulfide was highly released by GR but its presence was not detectable in the other roots. Among the monoterpenes, eucalyptol was the most abundant and its abundance was higher in FR. Finally, the PCA, carried out on the metabolomic data obtained from the individuals of three locations, pointed out a clear separation among them: the individuals of HD and LD grouped together and were clearly separated from the NH individuals. These results highlighted differences of VOCs emission between above- and below-ground plants, different root and shoot organs and individuals of *S. ceratophylloides* in disturbed and natural habitats.

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2.2 = Anti-biofilm and anti-quorum sensing activities of *Melaleuca alternifolia* essential oil and its major component, terpinen-4-ol, against *Staphylococcus aureus* methicillin-resistant strains

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In the last decades, the incidence of human pathogens resistant to several antimicrobials has increased worldwide. The lack of effectiveness of traditional antibiotics has created serious problems for the treatment of infectious diseases (1). In general, due to their chemical variety, essential oils represent a distinctive group of possible novel antimicrobial agents that have attracted special attention (2). In particular, the tea tree oil (TTO), an essential oil obtained from *Melaleuca alternifolia* (Maiden & Betche) Cheel (Myrtaceae), has been known for its antimicrobial and anti-inflammatory effects. TTO consists of about 100 different components, including terpinen-4-ol, which is one of its main antibacterial components (3).

The aim of this study was to evaluate the anti-quorum sensing (anti-QS) and anti-biofilm potential of *Melaleuca alternifolia* essential oil and of its main constituent, terpinen-4-ol, to prevent the infections due to meticillin resistant *Staphylococcus aureus* strains as an alternate to antibiotics. The tea tree oil (TTO) was assessed for its activity in inhibiting QS-dependent phenomenon such as violacein pigment production in *Chromobacterium violaceum*, swarming motility of *Pseudomonas aeruginosa* PAO1 and biofilm formation in MRSA strains on glass. Terpinen-4-ol is able to inhibit by 73.70% the biofilm formation on the glass strips of the MRSA strains. TTO inhibits by 69.3% the violacein production (Fig.1, at a MIC value of 0.048 mg mL^{-1}). At 100 µg mL^{-1} TTO and terpinen-4-ol exhibited inhibition in swarming motility of PAO1 by 33.33% and 25%, respectively. Because TTO demonstrated anti-QS and anti-biofilm activities at very low concentrations, it could be further exploited for novel molecules useful in treatment of infections due to MRSA strains.

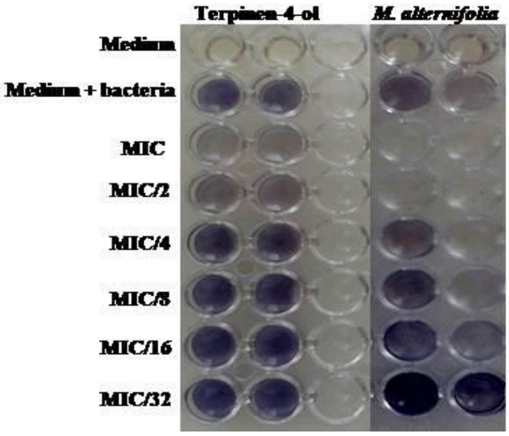

Figure *1*. Effects of different MIC values of *M. alternifolia* and terpinen-4-ol on violacein inhibition (qualitative method with *C. violaceum* ATCC 12472).

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2.2 = Chemical composition and phytotoxic activity of *Rosmarinus officinalis* essential oil

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Rosmarinus officinalis L. is an aromatic plant belonging to the Lamiaceae family used for culinary and medicinal proposes, thanks to its aromatic properties and health benefits (1). The biological activities of this plant are related to its phenolic and volatile constituents (2-4). The use of natural substances as biocontrol agents is receiving increased attention (5), due to the potential human and environmental toxicity of synthetic pesticides (6). Different studies have indicated the possible use of natural compounds as safe phytotoxic agents (5). In this study we determined the chemical composition of *R. officinalis* essential oil and evaluated its possible phytotoxic activity. The chemical composition of essential oil of *R. officinalis* from Salerno was studied by GC and GC-MS analyses. A total of 57 compounds were identified and the main components were α-pinene (24.9%), verbenol (8.5%), verbenone (8.5%), 1,8-cineole (8.2%) and isoborneol (8.1%). Moreover, the essential oil was evaluated for its possible *in vitro* phytotoxic activity against germination and initial radicle growth of radish (*Raphanus sativus* L.) - a species frequently utilized in biological assays - rue (*Ruta graveolens* L.), lettuce (*Lactuca sativa* L.) and tomato (*Solanum lycopersicum* L.). The results showed that both germination and radical elongation were influenced by this oil. In particular, the oil affects in different ways the radicle elongation of *R. sativus*, *R. graveolens*, and *L. sativa* and the germination of *S. lycopersicum*.

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2.2 = Comparative study of cardamom species

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The commercial cardamom (also known as “small cardamom”) is the “true cardamom”, and is obtained from the plant botanically known as Elettaria cardamomum. Many other plants belonging to the genera Amomum and Aframomum, belonging to the cardamom family, produce aromatic seeds. Among them, Amomum subulatum is the most used plant. The others are clubbed together as “false cardamoms”. They do not have commercially importance except in the case of Aframomum corrorima. Most of these “false cardamoms” are important locally as spices and flavoring materials and as remedies for various ailments (1).

Elettaria cardamomum (L.) Maton is a tall, perennial, reed-like herb growing wild in rainforests of South India, Sri Lanka and other tropical countries (2). The plant, common name “green” or “true cardamom”, is a very ancient and expensive spice and it is known as "the queen of spices" (3). Amomum subulatum (A. Braun) P.C.M. Jensen, “black cardamom” or “large cardamom” (4), native to Sikkim, is a perennial herbaceous plant with subterranean rhizomes which produces several leafy shoots and panicles. Korarima (Aframomum corrorima (Braun) P.C.M. Jansen), is a perennial, aromatic herb, also called “Ethiopian cardamom” or “false cardamom”, is native to Ethiopia. It is mainly grown in southern, south-western and western Ethiopia, and it is cultivated on a small scale in some West African countries. Korarima is one of the aromatic medicinal plants used in traditional medicine by the people of southern Ethiopia (5).

In this study, we compared the phytochemical profiles and biological activities of three essential oils from Elettaria cardamomum, Aframomum corrorima and Amomum subulatum. The oils were analyzed using GC and GC/MS techniques and were mainly constituted by oxygenated monoterpenes, especially 1-8 cineole. The essential oils showed significant antimicrobial activity against Gram-positive and Gram-negative microorganisms. The three tested essential oils and their main component (1,8-cineole) increased significantly elastase and protease production, and motility in Pseudomonas aeruginosa PAO1 in a dose dependent manner. Moreover, they also inhibited the violacein production in Chromobacterium violaceum.

The common use of spices and aromatic plants in food preparation might be another factor contributing to the health-promoting effects of the Mediterranean diet. A thorough knowledge of the biological and safety profiles of essential oils can produce applications of economic importance.

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2.2 = Secondary metabolites of floral nectar affect survival and locomotion of pollinators

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Floral nectar is an important food for animals engaged frequently in a mutualistic relationship for pollination. These animals range from arthropods to birds and mammals (1). Nectar has a high content of simple sugars that are easily absorbed and utilized by animals as a source of carbon and energy (2). It also contains all 20 proteinogenic amino acids (2). Several secondary metabolites were recently found in nectar, some of which affect the foraging behaviour of animals and potentially increase the benefits to the plant (3, 4). Very little is known about these substances or their real effects on insects and ultimately on plant fitness. One class of such substances is the non-proteinogenic (non-protein) amino acids that are not used to build proteins. GABA (γ-aminobutyric acid) and β-alanine are two of the more abundant and frequent non-protein amino acids found in floral nectar and they are recognized as important neurotransmitters in the insect nervous system (5). In this study we tested the effect of these secondary metabolites on honey bees (*Apis mellifera*), bumble bees (*Bombus terrestris*) and mason bees (*Osmia bicornis*). The insects were reared in cages and an fed artificial diet enriched with the two non-protein amino acids at low (naturally occurring in nectar) and high concentration. Their survival and behaviour (flying, walking, feeding, staying still) were assessed by scan sampling. The GABA diet had a positive (bumble bees) or neutral effect on insect survival (mason bees and honey bees) whereas the β-alanine diet had a negative (bumble bees and mason bees) or neutral effect (honey bees). The enriched diets affected behavioural parameters differently according to species and concentration. Bumble bees showed increased walking activity when fed the high-concentration β-alanine diet and increased flying activity with the same solution at low concentration. Mason bees were sensitive to the high-concentration GABA diet, showing increased walking activity. Behavioural parameters of honey bees were less significantly affected by the two diets.

The fact that plants affect the survival and locomotion of pollinators through nectar composition may have important ecological consequences for the plants, as it increases the possibility of pollinator movements between flowers, plants and populations, thus promoting higher reproductive outputs.

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2.2 = *In silico* evidence of trans-kingdom RNA silencing in the arbuscular mycorrhizal symbiosis

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Small RNAs (sRNAs) are short non-coding RNA molecules (20-30 nt) that regulate gene expression at transcriptional or post-transcriptional levels in many eukaryotic organisms, through a mechanism known as RNA silencing. Beside the well-established roles in development and stress responses, recent investigations have highlighted that sRNAs are also involved in trans-kingdom communication. In particular, concerning the interactions between plants and fungal pathogens or parasitic plants, recent data suggest that sRNAs can move across the contact surfaces, from one organism to the other. Once in the host cells, sRNAs can target the expression of specific mRNAs, sometimes triggering secondary sRNAs production and thus leading to a modulation of host metabolic pathways and defenses responses (1-3). Almost nothing is known about RNA silencing mechanism and sRNAs occurrence in Arbuscular Mycorrhizal (AM) fungi, a key component of the plant root microbiota, that can provide several benefits to host plants, such as improved mineral nutrition and tolerance to biotic and abiotic stresses (4).

Focusing on the symbiosis between the AM fungus *Rhizophagus irregularis* and the model plant *Medicago truncatula*, we exploited a NGS (Next Generation Sequencing) approach to characterize the fungal sRNAs population. *In silico* analysis revealed that several sRNAs produced by *R. irregularis* have the ability to target specific *M. truncatula* genes. Taking advantage of published PARE (parallel analysis of RNA ends) sequencing data (5), collected in similar experimental conditions, we detected cleavage activity at 60 hypothetical fungal sRNAs - plant mRNAs binding sites. We did not find any plant sRNAs able to target the mRNAs at the same sites. Remarkably, microarray expression analysis (6) revealed that about half of the 60 potential target genes are down-regulated in arbuscule-containing cells compared to adjacent non-colonized cells. Overall, these data may represent first evidence of the occurrence of trans-kingdom RNA silencing in the AM symbiosis. Further experiments are on going to validate these results.

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2.2 = *Alnus glutinosa* (L.) Gaertn. and *Alnus cordata* (Loisel) Duby as new sources of safe cosmetic and pharmacological anti-melanogenic agents

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The genus *Alnus* (Betulaceae) comprises many species with a long history in traditional medicines. The crude extracts and isolated compounds from *Alnus* species exhibit a wide spectrum of *in vitro* and *in vivo* pharmacological activities (1). Phytochemical investigations revealed the presence of diarylheptanoids, a class of natural products typically found in *Alnus* genus with two aryl groups joined by a heptane chain in the main skeleton that have drawn attention due to their multiple biological properties and their therapeutic potential (2). A previous study reported that oregonin and other structurally analogous diarylheptanoids isolated from the bark of *A. hirsuta* showed inhibitory effects on melanogenesis in B16 melanoma cells (3). Nowadays the discovery of new whitening agents from natural sources is increasing, due to the weak effectiveness and unwanted side effects of currently available compounds.

In this context, the aim of this study was to evaluate the skin whitening capabilities of crude extracts (80% aqueous MeOH) obtained from the fresh bark of *Alnus glutinosa* (L.) Gaertn. and *Alnus cordata* (Loisel) Duby, an endemic species in the Mediterranean areas (4). As tyrosinase is the rate-limiting enzyme in melanin biosynthesis, the inhibitory effects of *A. glutinosa* and *A. cordata* extracts (AGE and ACE, respectively) on mushroom tyrosinase activity were preliminary evaluated. In addition, the anti-melanogenic ability of AGE and ACE was further investigated on the pigmentation of early stage zebrafish at 72 hours post fertilization (hpf) to find new skin whitening agents without cytotoxic concerns.

Results of the enzymatic assay showed that ACE was capable to inhibit dose dependently L-DOPA oxidation catalyzed by tyrosinase (IC_{50} = 77.44 ± 0.54 µg/mL) as compared to the reference inhibitor kojic acid (2.24 ± 0.18 µg/mL). Unlike, AGE exhibited a lower anti-tyrosinase activity (100 µg/mL reached 28% of inhibition while higher doses showed pro-oxidative effects). Moreover, the zebrafish *in vivo* assay revealed that ACE (50 µg/mL) has equivalent inhibitory effects on the pigmentation (76.57%) to that of phenylthiourea (PTU, 30 µg/mL), used as the reference inhibitor (77.80%), as compared to control, while they did not affect the embryos development and survival. Conversely, the depigmenting effects of AGE were about 10 fold less than ACE (45.28% at 500 µg/mL). A mild anti-melanogenic activity was also evidenced for the diarylheptanoid oregonin (10% of inhibition at 20 µg/mL).

A preliminary phytochemical screening evidenced that ACE and AGE have a high phenolic content (399.27 ± 14.30 and 534.17 ± 20.60 mg GAE/g of extract, respectively). However, despite AGE showed the highest phenolic content, the quali-quantitative RP-HPLC-DAD analysis highlighted as it is predominantly composed by oregonin (418.45 µg/mg of AGE *vs* 1.23 µg/mg of ACE) that exhibited a mild anti-melanogenic activity both *in vitro* and *in vivo* assays. Further phytochemical investigations are still in progress to identify the bioactive compounds of ACE as to be considered a potential candidate for the treatment of skin disorders due to its bleaching properties and favorable safety profiles.

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2.2 = Evaluation of antimicrobial activity of *Fuscoporia torulosa* (Pers.) T. Wagner & M. Fisch. against human pathogenic fungi and bacteria

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Fuscoporia torulosa (Pers.) T. Wagner & M. Fisch. (*Hymenochaetaceae*) is the causal agent of white rot that particularly infects the roots and the collar of old trees and shrubs of many species.

As the antimicrobial activity of this fungal species has been little investigated, the aim of the present study was to determine the activity of the methanolic extract of the fruiting body of *Fuscoporia torulosa* against various pathogenic microrganisms.

The methanolic extract was investigated *in vitro* against a wide range of species such as *Candida* spp., *Aspergillus tubingensis*, *A. calidoustus*, *Fusarium oxysporum*, *Penicillium chrysogenum*, *Bacillus subtilis*, *Streptococcus pyogenes*, *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Xantomonas maltophila* (1, 2). The methanolic extract of *Fuscoporia torulosa* showed antimicrobial activity against several fungi and Gram positive and Gram negative bacteria tested at a variety of degrees.

The positive results of this screening of the fruiting bodies of *Fuscoporia torulosa* for antimicrobial activity constitute a primary platform for further phytochemical studies and the development of new drugs for the therapy of different infections.

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2.3 = *Calluna vulgaris* (L.) Hull: chemical investigations and immunomodulatory effect on human mononuclear cells

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Calluna vulgaris (L.) Hull. (Ericaceae) is a perennial shrub common in Italy and in many European countries, found in plains, hilly and mountainous areas. In ethnobotany and in traditional medicine, *C. vulgaris* aerial parts decoction is used for urinary complaints and as antimicrobial (1); furthermore *C. vulgaris* is also one of the 38 Bach Flowers and it is used to treat selfish and oppressive people.

More recently, studies on the photo-protective activity of water and ethanolic extracts were published (2-3).

Preliminary data obtained in our lab lead us to better investigate a new interesting characteristic of *C. vulgaris*, namely its immunomodulatory effect. The aim of this work was to chemically characterize a soft water extract (DER 4:1) of Italian *C. vulgaris* aerial parts and, for the first time, to investigate its immunomodulatory effect.

Phytochemical analyses were carried out combining enzymatic, colorimetric and HPLC-DAD-MS methods. Immunomodulatory effects of the *C. vulgaris* extract were evaluated using an *in vitro ex vivo* model with human peripheral blood mononuclear cells incubated with the extract for 24 hours. IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-17, TGF-b and TNF- a levels was evaluated by ELISA and NF-kB activation by immunofluorescence after 30, 60 and 120 mins of incubation. Bacterial lypopolysaccharide was used as control.

The extract was found to contain mainly polysaccharides, over 7.50% m/m. Also polyphenols were found to be abundant, over 5.5% m/m, phenolic acids, tannins, chromen-4-one derivatives, hydroxycinnamic acids and flavonoids the most representative subclasses and chlorogenic acid and hyperoside the most abundant single constituents.

C. vulgaris water extract exhibited a strong immunomodulatory effect from 5 mg/ml: IL-6, IL-2, IL-10, TGF-b and TNF- a were significantly increased after 24 hours of incubation, compared to basal levels, and IL-6 resulted the most upregulated cytokine. Interestingly, NF-kB nuclear translocation was not affected by the extract after 30, 60 and 120 minutes of incubation, while LPS produced a marked NF-kB activation after 60 minutes.

C. vulgaris water extract was found to be a novel immunumodulatory agent, being able to regulate innate and adaptive immunity, by targeting cytokines released by monocytes and macrophages such as IL-6, TGF-b and TNF- a and activating lymphocytes subpopulation by IL-2 upregulation.

Finally, the lack of NF-kB activation suggest that the immunostimulant effect of *C. vulgaris* is not related to a LPS-like effect and could involve other molecular pathways, such as MAP kinases.

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2.3 = Antimicrobial and ATP synthase modulating activity of the surface constituents of *Salvia tingitana* Etl. (Lamiaceae)

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During our research on plant compounds from *Salvia* species able to face the constantly increasing resistance to antibiotics (1), the antimicrobial properties of the dichloromethane extract obtained from the surface of the aerial parts of *S. tingitana* were investigated following a bioassay-oriented fractionation approach. The surface extract showed moderate antimicrobial activity against Gram positive multi-resistant bacterial strains; no activity was observed against the Gram-negative strains or *Candida albicans*.

The hexane insoluble and soluble fractions of the crude extract, as well as the semi purified fractions obtained by silica gel column chromatography (I_a-VI_a and I_b-V_{IIb}) were evaluated at doses corresponding to that of the crude extract. Among the tested fractions, fraction I_a, IV_a, V_a and VI_a were active against *Staphylococcus aureus* and *S. epidermidis*. Fractions III_b, IV_b and were active against *S. aureus, S. epidermidis, Enterococcus faecium and E. faecalis,* while V_b was active against the two *Staphylococcus* species.

The separation of the fractions 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, identified by IR, 1D and 2D NMR, HR-MS analysis. The antimicrobial activity of the isolates was analysed on several Gram positive multi-resistant 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*, *E. gallolyticus*). The compounds showed antimicrobial activities against Gram positive bacteria with MIC values ranging from 32 to 128 μg/mL, with the exception of manool and sclareol, which exhibited MIC values ranging from 4 to 64 μg/mL.

Moreover, taking into account that the modulation of ATP synthase has been described as the base of the activity of several compounds against multidrug-resistant mycobacteria and Gram-positive pathogens and that the overall structure and energy transduction mechanism of the ATP synthase are well conserved from bacteria to mammalians (2), the inhibitory action of the more active compounds on the ectopic F_1F_o-ATP synthase (ATP synthase) of purified rod outer segments (OS), as a subcellular system naturally expressing extra-vesicularly the molecular machinery for the oxidative phosphorylation, was also evaluated (3, 4). The compounds that displayed the best antibacterial activities *i.e.* salvileucolide methylester, sclareol and manool showed ATP synthase inhibition of 60, 70 and 80%, respectively. Interestingly, their time killing curves, performed against the susceptible bacterial species, showed bacteriostatic activity.

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2.3 = Alimurgic plants from Calabria (Italy): bioactive components and therapeutic potentials in the treatment of inflammatory disorders and obesity

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Different studies demonstrated the interesting health benefits of various species belonging to traditional Mediterranean cuisine (1, 2). In Italy, several wild edible plants have been traditionally used for culinary purposes. Actually, a rising attention towards a healthy diet and the need to restore a link with nature and our old traditions have determined a new interest towards alimurgic plants (3, 4).

The aim of this study was to realize a screening of four wild edible species from Calabria: *Asparagus officinalis* L., *Bellis perennis* L., *Daucus carota* L. and *Sambucus nigra* L. as anti-inflammatory, anti-obesity and antioxidant agents.

Samples were extracted through maceration using methanol. Apolar constituents were identified by means of GC-MS while HPTLC analyses allowed the identification and quantification of phenolic constituents: the presence of chlorogenic acid and the flavonoid glycoside rutin was verified in various samples. Total phenolic and flavonoid contents were also determined. The antioxidant activity of raw extracts was assessed by means of different tests: DPPH, ABTS, FRAP-Ferrozine and β-carotene bleaching test. The best ability to scavenge DPPH radical was observed for *S. nigra*, with an IC_{50} value of 43.65 ± 0.48 μg/mL. This species showed also the highest total antioxidant capacity in both the ABTS assay and FRAP-Ferrozine assay (TAC values 0.11 and 0.26, respectively). Moreover, *S. nigra* extract resulted the best inhibitor of lipid peroxidation.

All investigated species have been traditionally used as antirheumatic and anti-arthritic remedies (5). The anti-inflammatory potential was verified through the evaluation of their capacity to inhibit lipopolysaccharides (LPS)-induced production of nitric oxide (NO) in murine macrophage RAW 264.7 cell line. The crude extract of *D. carota* showed the best activity, with an IC_{50} value of 45.1 ± 1.0 μg/mL. A lower but still interesting effectiveness was observed for *B. perennis* and *A. officinalis* crude extracts. Finally, to assess the potential health benefits of different alimurgic plants in the treatment of obesity, samples were investigated for their ability to inhibit pancreatic lipase, a key enzyme in dietary fat absorption. *D. carota* raw extract showed the best inhibitory activity, with an IC_{50} value of 1.63 ± 0.07 mg/mL.

According to obtained results, investigated dietary plants, particularly *S. nigra* L. and *D. carota* L., could be interesting sources of bioactive principles useful for the development of new pharmaceutical products against inflammatory conditions and obesity.

Fig. 1. *Sambucus nigra* L.

Fig. 2. *Daucus carota* L.

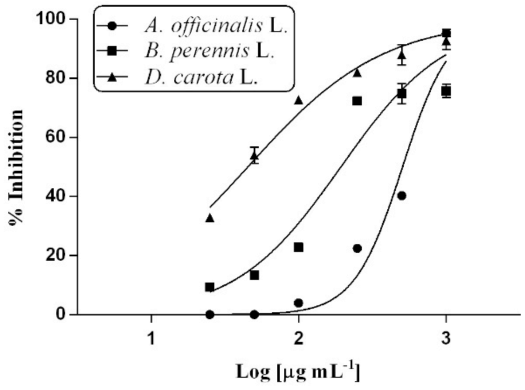

Fig. 3. NO inhibition induced by plants extracts

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2.3 = Metabolite and lipid profiles of *Abelmoschus esculentus* L. Moench by LC-ESI-Orbitrap-MS and NMR analysis

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Okra (*Abelmoschus esculentus* L. Moench.) (Fig. 1), belonging to Malvaceae family, is a very popular vegetable crop in North-eastern African countries where its fruits are commonly used in the local cuisine; it is also an important medicinal plant in the Ayurveda, Siddha and Unani medicines (1). Nowadays, this species is widespread and it can be easily found in European local markets. Okra seed oil is rich in unsaturated fatty acids such as linoleic acid. Along with the edible use, extracts from okra fruit have been used in food and pharmaceutical industry as emulsifiers, drug tablet formulations or blood plasma replacement, due to their high content in biopolymers, such as polysaccharides (mainly pectins), and bioactive compounds such as ascorbic acid and beta-carotene (2). Recently, bioactive compounds, with antiproliferative activity, have been isolated from okra (3). Furthermore, its mature fruit and stems contain crude fiber, which is used in the paper industry. Although the extensive and spread use of this vegetable, there is no comprehensive literature information on the polar lipids occurring in the oil of okra seeds and on the secondary metabolites occurring in the fruit. In this study, the analysis of the hydroalcoholic extract of okra fruits, by high-performance liquid chromatography coupled to multiple-stage linear ion-trap and orbitrap high-resolution mass spectrometry using negative electrospray ionization mode (LC-ESI/LTQOrbitrap/MS/MS^n), was carried out. Polar metabolites, mainly belonging to flavonoid class, and to polar lipid classes, as oxylipins, phospholipids, glycolipids, and cerebrosides, were tentatively identified by this analytical approach. Flavonoid structures were confirmed by NMR analysis.

Fig 1: Okra fruit

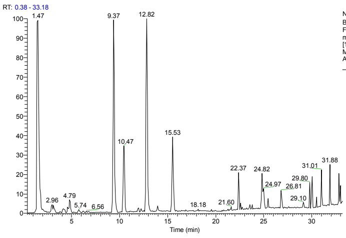

Fig 2: LC-MS profile of polar metabolites

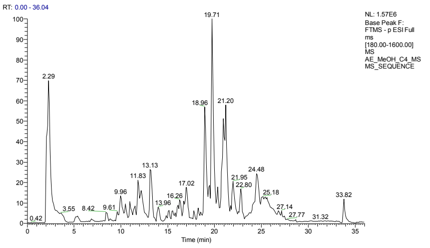

Fig 3 LC-MS profile of polar lipids

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2.3 = Optimization of non-psychotropic *Cannabis sativa* L. extract and evaluation of the *in vitro* anti-inflammatory activity on microglial cells

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Cannabis sativa L. (Cannabaceae) is an annual flowering plant. The first appearance of *Cannabis* was believed to be in central Asia about 5000 BC, whereas it introduction in Central Europe was around 500 BC. For millennia, the plant has been used for fiber and oil production, as well as for other traditional uses, such as for feeding animals (1). After the discovery of cannabinoids and of the endocannabinoid system, *C. sativa* is attracting the interest of the scientific community for its potential therapeutic use, even if this was limited by the presence of the psychoactive compound Δ^9-tetrahydrocannabinol (THC) (2).

More recently, a vast amount of literature has been published regarding the biological effects of the non-psychotropic constituents of cannabis, in particular cannabidiol (CBD), for the treatment of central and peripheral inflammation, gastrointestinal upset, epilepsy and neurodegenerative diseases (3).

Currently, *C. sativa* is approved in many countries for medical purposes; in Italy, the national cultivation FM2 as cut dried female inflorescences and the Dutch varieties Bedrocan as dried entire drug, containing different THC:CBD ratios, are approved. Nevertheless, patients are mostly required to prepare for themselves a decoction, or to inhale vaporized drug. Clinicians and pharmacists would significantly benefit from the standardization of an easy to apply drug extraction method.

In this work, starting from the female inflorescences of *C. sativa* L. var. *carmagnola*, kindly provided by Doctor Gianpaolo Grassi (CRA, Rovigo), we tested different extraction methods by using 96% EtOH and olive oil as solvents, and by varying the time of extraction and the heat-decarboxylation of the herbal material. We applied two methods obtained from the Italian Pharmacopoeia FUI XII (maceration and percolation), an automatic extraction method by means of Naviglio Estrattore®, and a published method (4), which is used as a reference method for the preparation of cannabis based drugs in Italian pharmacies.

Each extract was compared for the content in cannabinoids, total phenolic compounds and volatile constituents by means of colorimetric (Folin-Ciocalteu) and chromatographic/spectrometric (TLC/HPTL, HPCL-DAD, GC-MS) methods. The total flavonoid content was quantified by direct spectrophotometric revelation (5) and by applying the method reported in the European Pharmacopoeia 9.

The antiradical capacity of each extract was assessed through the 2,2-diphenyl-1-picrylhydrazyl (DPPH) assay.

The extract possessing the best phytochemical profile and antiradical capacity was then compared to CBD for its *in vitro* activity on neuro-inflammation by dosing the production of TNF-α in LPS-stimulated microglial cells (BV2), through non-competitive sandwich ELISA.

The ethanolic extract obtained by maceration of the decarboxylated herbal material, was found to contain cannabidiol 466 mg/l, total flavonoids 90 mg/l (of which vitexin 12 mg/l), sesquiterpenes (being b-caryophyllene the most representative) and very few amount of monoterpenes (<0.1 mg/l).

The better phytochemical profile correlated well with the antiradical capacity, with the IC_{50} of the ethanolic extract being 18.7 mg/ml.

Finally, contrary to cannabidiol alone (1 μg/ml), the selected extract, used at the same concentration, was able to significantly reduce by 28.9 % the LPS-induced production of TNF in BV2 microglial cells.

C. sativa should be not considered just a fashion or a psychoactive drug, but it can be actually considered one of the most promising medicinal plant for painkillers non-responsive patients and for neuro-inflammatory diseases. Nevertheless, the actual potential of cannabis phytocomplex has yet to be studied, being not only represented by cannabinoids. It is crucial to define and optimize the extraction method in order to preserve the phytocomplex and this study represents our first effort on the topic.

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2.3 = *Hyssopus officinalis* subsp. *aristatus* (Godr.) Nyman from Abruzzo region: morphological features, chemical and biological characterization of essential oil and hydroalcoholic extract

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Aerial parts of hyssop wildly grown in natural habitat of the Abruzzo region (Italy) were classified as *Hyssopus officinalis* subsp. *aristatus* (Godr.) Nyman (synonym *H. officinalis* subsp. *pilifer* (Pant.) Murb.) and then characterized on the basis of its essential oil (EO) composition and hydroalcoholic extract (HE). *Hyssopus officinalis* L. (Lamiaceae) is a polymorphous species, well known for its pleasant aromatic scent, as an ornamental and bee-attracting plant, and in food industry as a condiment and spice or as a minty flavor (1). In Italy, the subspecies *aristatus* is found in Lombardia, Trentino-Alto Adige, Veneto, Friuli-Venezia Giulia, Umbria, Lazio, Abruzzo, Molise, Campania, Basilicata and Calabria. The literature (2,3) reports the peculiar features of the subsp. *aristatus* as floral leaves ending with an arista of 1-3 mm, a calyx divided into teeth of 2–3 mm with lanceolate or ovate-lanceolate shape and a 7-9 mm blue-violet corolla. We confirmed these morphological aspects (Fig.1) and observed microscopic features.
The EO, characterized with GC-MS and NMR analyses, revealed an uncommon composition, never described in literature: limonen-10-yl acetate (67.9%) was the main compound, followed by 1,8-cineole (15.5%) and limonene (5.8%) (4,5). The fingerprinting (HPLC DAD, HPTLC) of hydroalcoholic extract showed chlorogenic and rosmarinic acid among the main secondary metabolites.
Regarding the antioxidant activity, performed by DPPH test, HE evidenced interesting IC_{50} value (38.9±2.12 µg/mL), if compared to Trolox (4.28±0.48 µg/mL), due to chlorogenic and rosmarinic acid (IC_{50} of 7.29±0.17 µg/mL, 4.31±0.34 µg/mL, respectively) as confirmed by DPPH bioautographic assay (Fig.2). The EO did not show radical scavenging capacity. The antimicrobial bioautographic and microdilution tests against *Staphylococcus aureus* are currently in progress.
HE was assayed for biological activities employing two cell lines. The antiproliferative effects and the modulation of oxidative stress were investigated on C2C12 and HCT116 cell lines by MTT test and ROS production. In range concentration 50-300 µg/ml, HE resulted well borne by both cell lines.
On HCT116 cell line, at concentrations higher than 150 µg/mL, HE is able to completely revert the induced ROS production.

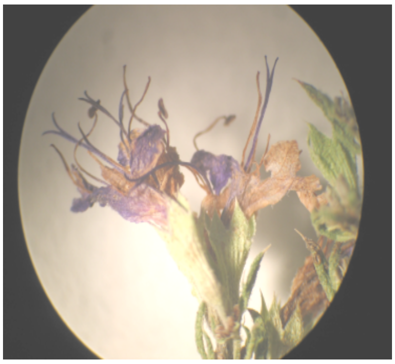

Fig. 1. *H. officinalis* subsp. *aristatus* crude drug

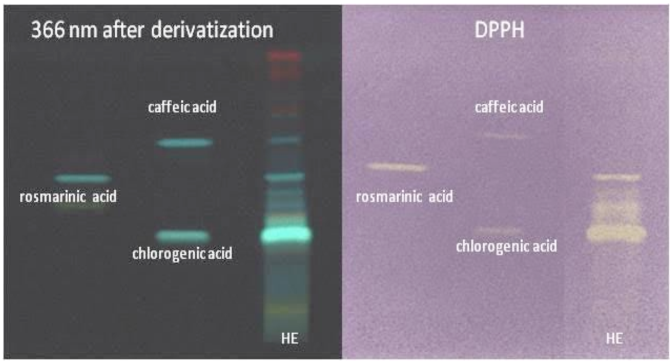

Fig. 2. DPPH bioautographic assay of HE

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POSTER N. 47

2.3 = *Moricandia arvensis* (L.) DC.: phytochemical profile and potential anti-obesity activity

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Natural compounds have recently attracted much interest as novel therapeutic agents in the treatment of obesity. Different classes of phytochemicals have been recently proved effective, such as polyphenols, terpenes, phytosterols and saponins. Herbal products play a role in body weight control by different mechanisms of action, such as lipid absorption inhibition, increase of energy expenditure, decrease of pre-adipocyte differentiation and proliferation (1). The inhibition of pancreatic lipase, a key enzyme for lipid absorption, is one of the most important strategy to prevent obesity (2).

The aim of this study was to investigate the potential health benefits of *Moricandia arvensis* (L.) DC. (Brassicaceae). This species is present in northern Africa, southern Europe and western Asia (3). The leaves have been traditionally used both in medicine and as food (4).

Aerial parts of *M. arvensis* were extracted through maceration using methanol. Raw extract was then fractionated using solvents with increasing polarity: *n*-hexane, dichloromethane and ethyl acetate.

Apolar constituents were identified by means of GC-MS. Compositional profiling by HPLC and LC-MS revealed that flavonoid glycosides were the main specialized metabolites present in the methanolic extract with kaempferol and quercetin representing the two *O*-glycosylated aglycones. Kaempferol-3-*O*-(glucosyl)-rutinoside and kaempferol-3-*O*-arabinosyl-7-*O*-rhamnoside were the most abundant flavonols. Total phenolic and flavonoid contents were also determined.

The methanolic extract and fractions from *M. arvensis* were investigated for their ability to inhibit pancreatic lipase, which was verified through the *in vitro* evaluation of the prevention of *p*-nitrophenyl caprylate hydrolysis. The raw extract and the dichloromethane and ethyl acetate fractions showed a strong lipase inhibitory activity, with IC_{50} values of 2.06 ± 0.02, 1.52 ± 0.02 and 1.31 ± 0.02 mg/ml, respectively. The antioxidant activity was also verified by means of DPPH and β-carotene bleaching test.

Obtained results support the hypothesis that *M. arvensis* (L.) DC. could be a source of active principles for the pharmacological inhibition of dietary lipids absorption.

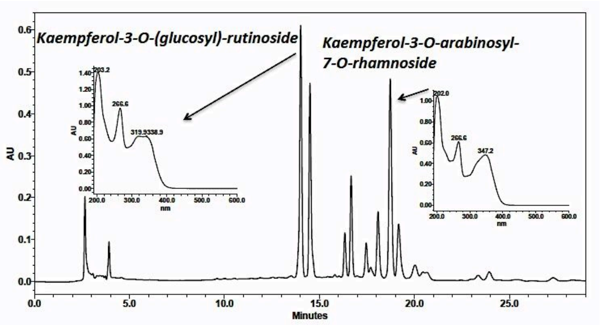

Fig. 1. HPLC trace of flavonoids from *M. arvensis* (L.) DC. fractions.

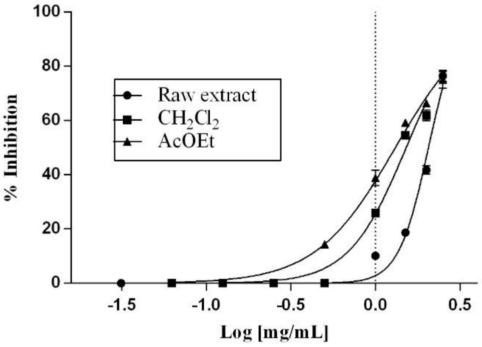

Fig. 2. Lipase inhibitory activity of *M. arvensis* (L) DC. extract and fractions.

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2.3 = Multi-trait approach to characterise fruit quality in Calabrian peach cultivars for the international export market

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Fruit quality has become an important issue for the consumer: therefore, the fruit industry needs to ensure and optimize product quality throughout the supply chain. Peach fruit production plays a key role in the agricultural sector of several Italian regions, both in Northern and Southern regions (such as Calabria and Campania). In Calabria there is an increasing interest in innovations in peach cultivation to increase their commercialization for national and international markets (according to the technical document for the 2014-2020 Calabrian PSR programme). Calabrian producers directed particular attention to the cultivation of local varieties, which are characterized by intense skin colour and high flavour, characteristics particularly prized by consumers. Low temperature storage is the most common current method for delaying ripening after harvest and extending peaches' commercial life (1). However, chilling can affect production of flavour- and health-related metabolites in peach (2, 3). The FRUITY project aims to characterise Calabrian peach quality and its resilience to chilling, introducing innovative technologies. The main objective is to provide tools to enable the fruit supply chain to apply objective quality tests to optimize internal and sensorial quality of fresh fruits throughout the whole supply chain, especially following long distance transport and storage involving chilling. We used six peach cultivars with different ripening periods through the summer, sampled at two time points – immediately after commercial harvest (D0) and after storage at low temperatures (1°C) for 7 days (D7). We performed a multi-trait approach that included sensory analysis, phytochemical profiling, monitoring of the volatile organic compounds (VOCs) and gene expression profiling - with the overall goal of exploring the metabolic and genetic changes occurring in fruit post-harvest and providing a suite of simple diagnostic checks to monitor fruit quality. A total of 117 VOCs were identified for the six peach cultivars studied through identification using the NIST-2017 database. Canonical Analysis of Principal coordinates (or CAP) on VOCs profiles showed a discrimination between cultivars and between ripening periods. A combination of sensory evaluation and VOC profile shows the same trend reported by CAP analysis. Furthermore, correlation between the expression profile of flavour-related genes and related VOCs was shown. Overall the combination of sensory evaluation, VOC profiles and gene expression could help the producer to understand which traits/aroma are more relevant in consumer perception and perhaps the export of locally produced cultivars in an unexplored market.

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3.1 = Morpho-anatomical and physiological responses of *Robinia pseudoacacia* L. plants to anthropogenic dust deposition in the Vesuvius National Park

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Anthropogenic impacts affect plants also in several protected areas of the Mediterranean basin. These impacts can also produce changes in vegetation dynamics. In Southern Italy, some areas of the Vesuvius National Park are particularly exposed to the impact of seasonal tourism. The frequent and intense presence of tourists, both hiking and moving by means of vehicles, is responsible for the formation of dust clouds which are mainly deposited on the vegetation close to the trails (Fig.1). Dust deposition on leaf lamina may negatively affect gas exchanges, compromising the plant health status.

In this study, we analysed the effects of dust deposition on the leaf surface in *Robinia pseudoacacia* L., an invasive tree, native to north America, widespread in many areas of the Vesuvius National Park. Samplings were conducted in different sub-areas either located a) along the side of the roads, where a high deposition of dust (HD) was reported, or b) at least five meters far from the roads/trails, where plants receive a lower amount of dust (LD). For each site, the amount of dust on leaves was quantified in different periods of the year (spring-summer-autumn), which have a different touristic pressure. On both HD and LD leaves, the effects of the dust deposition on anatomical and physiological traits were evaluated by performing eco-physiological measurements (e.g. chlorophyll fluorescence emission, photosynthetic pigment extraction and leaf functional traits) and light microscopy analysis (e.g. thickness of spongy and palisade tissues, incidence of intercellular spaces, stomatal size and density) (Fig. 2). Our results showed dust-induced changes in some of the anatomical and physiological traits, suggesting that dust deposition acted as a natural screen to protect the leaf surface especially in HD plantsIn HD plants, photochemistry was not affected by the presence of dust, being comparable to LD plants thus showing no sign of photoinhibition. HD plants also presented a higher amount of photosynthetic pigments, which could be an adaptation to the shielding phenomenon by which the plant increment chlorophylls and carotenoids in order to capture more light for photosynthesis especially during the summer, when the photosynthetic tissues are exposed to the excess of sunlight. These responses can be considered additional adaptive traits when the leaf heliotropic movements are not sufficient to avoid photo-inhibition. The leaf morphological and physiological plasticity of *R. pseudoacacia* in response to dust deposition is another critical trait which allows this species to be particularly competitive, and to spread in natural areas of the Vesuvius National Park subjected to anthropogenic impact.

This research activity has been realised in collaboration with the Vesuvius National Park (Ente Parco Nazionale del Vesuvio) within the "Azione di Sistema - Impatto antropico da pressione turistica nelle aree protette: interferenze su territorio e biodiversità"funded by "Ministero dell'Ambiente e della Tutela del Territorio e del Mare", Direttiva Conservazione della Biodiversità.

Fig. 1. Dust clouds in the area of Vesuvius National Park

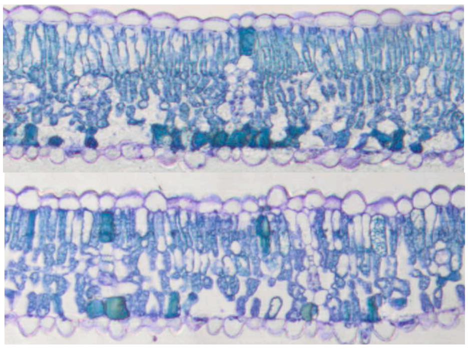

Fig. 2. Light-microscopy views of cross sections of the leaf lamina in *R. pseudoacacia* HD (a) and LD (b) plants

3.2 = Interaction between *Santolina pinnata* Viv. and *Orobanche apuana* Domina & Soldano, two species endemic to the Apuan Alps (Tuscany, Italy)

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Santolina pinnata Viv. (Asteraceae) is a perennial species endemic to the Apuan Alps (NW Tuscany) and it is the only Italian *Santolina* for which a parasitic relationship with another plant species – *Orobanche apuana* Domina & Soldano (Orobanchaceae) – is reported (2, 3, 4). The latter is the only holoparasite *Orobanche* with a specific requirement for *S. pinnata* (5). In this work, we aim to investigate the parasite effects on the host and to analyze phenology and biology of both species.

We studied the interaction between the two species by examining both vegetative (6) (shoot length, capitulum size) and reproductive (7) traits (fruit-set and seed-set).

To investigate the effects of *O. apuana* on its host we studied two populations of *S. pinnata* at the same altitude and aspect, one with and the other without the parasite. In each population we randomly chose ten individuals and collected three fruiting heads from each one of them, then we counted and weighed the seeds in the laboratory. The fitness of *O. apuana* was investigated in two populations at the same altitude and aspect, one in the central-northern, the other in the central-southern sector of the mountain range. In each population we counted the *O. apuana* individuals (ramets) growing in 20 randomly selected host plants, collected 20 fruiting stems, counted the number of the capsules and weighed their contents. We performed the non parametric Kruskal-Wallis test; the statistical significance was assessed for a p-value < 0.05.

Parasitized *S. pinnata* individuals showed a reduction of vegetative shoots length (p-value = 0.020), flower heads diameter (p-value = 4.834e-09), number of flowers per flower head (p-value = 1.272e-09), and number of fertile seeds (p-value = 5.217e-06). Seed mass was higher in non-parasitized individuals (p-value = 5.217e-06). The number of shoots and capsules of *O. apuana* was not significantly related to qualitative and quantitative traits of *S. pinnata*; in contrast, seed mass was significantly different between the two sampled populations (p-value = 0.025).

Our results confirm previous reports of negative effects of *Orobanche* species on the host's vegetative and reproductive fitness (8). We enhanced the knowledge about two endemic species of the Italian flora and about their relationship, with important implications both on their conservation and on their evolutive history. Indeed, studies concerning other species of *Orobanche* showed that the effects of infestation are much reduced or contrasted in perennial species compared to their magnitude in annual crop species (8). Accordingly, *S. pinnata* might have evolved a similar strategy to cope with its parasitic symbiont.

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3.3 = Plant community, ecology and functional strategy of *Linaria tonzigii* Lona, a steno-endemic species of the Italian pre-Alps

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Linaria tonzigii is a rare steno-endemic species of Community interest that grows on some limestone screes in the Orobie Bergamasche Regional Park (Italian Alps) (Fig. 1). Currently, its presence is certain in just a few sites of the Orobic pre-Alps. Many of these sites were refuge areas during the glaciations of the Quaternary Age: Mt. Arera/Mt. Corna Quadra group (locus classicus), Mt. Menna, Mt. Secco, Mt. Ferrante/Mt. Presolana group and Mt. Cavallo/Mt. Pegherolo group. According to the IUCN Red List of threatened species, *Linaria tonzigii* belongs to the category 'Endangered D', and it is therefore subject to strict protection under law LR10/2008 in Lombardy. Information on its ecology (and synecology), and on its Grime's CSR functional strategy are scarce. This study, other than analyzing the floristic composition and ecology of the communities hosting *Linaria tonzigii* by means of traditional methods (ecological indices), also evaluated its Grime's CSR strategy using the latest methods and tools.

Analysis of 24 phytosociological relevés conducted in five different areas revealed that this species is part of a single plant community (*Linaria tonzigii-Hornungia alpina* community), consisting of basophile and xerophile species, mostly typical of limestone screes. The analysis of the CSR strategy revealed that the main strategy of *Linaria tonzigii* is R/CSR (Fig. 2), although the species adopts slightly different strategies in different sampling areas. The analysis of plant height showed that the isolated population at the northern limit of the distribution range has significantly taller, and less stress-tolerant individuals, than those in other areas, suggesting that this population may be a different ecotype (1). This study aims at stimulating researchers to study poorly known endemic species, in order to preserve and valorize the biodiversity of protected areas, and hence that of the planet.

Fig. 1. *Linaria tonzigii* Lona

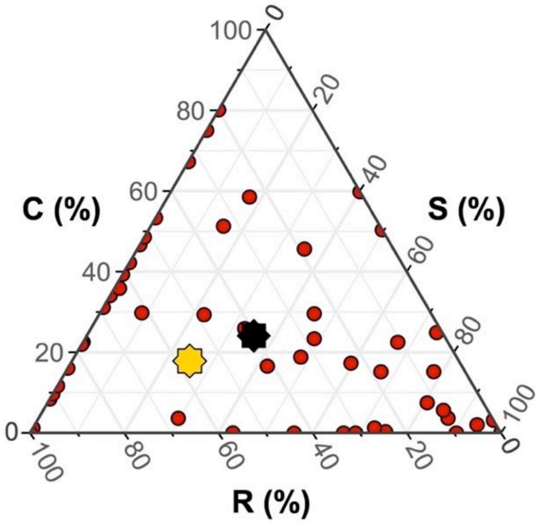

Fig. 2. CSR strategy of the species of the *Linaria tonzigii-Hornungia alpina* community (red dots), mean strategy of the community (black star) and strategy of *Linaria tonzigii* (yellow star).

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3.4 = Rhizosphere microbiota responses to nickel stress

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The serpentine soils, characterized by high level of metals like Ni, Cr, Co, Mn (1,2), and low levels of N, P, K, Ca (3), provide inhospitable habitat for many plant species (4,5), except for hyperaccumulators, able to store metals such as nickel (Ni) in aboveground biomass (6). Despite the high number of research on plants growing on serpentine substratum, the interest on the root system of hyperaccumulators, and in its interactions with the other components of the rhizosphere is quite recent (7). The rhizosphere plays a crucial role in hyperaccumulation, since plant root-associated bacteria and fungi provide beneficial effects on their host, improving the efficiency of phytoremediation processes (7).

This study aims at characterizing the microbiota associated with the rhizosphere of the facultative Ni-hyperaccumulator *Alyssoides utriculata* (L.) Medik. from serpentine and non-serpentine sites, and at obtaining a screening of bacterial and fungal strains which are capable to promote metal uptake, and hence allow plant development. Culturable bacteria and fungal strains were isolated on agar by a dilution plate technique from the rhizosphere of *A. utriculata*, as well as from bare soil samples. Microbiota isolated from serpentine soil were selected on the basis of their Plant Growth-Promoting Rhizobacteria (PGPR) properties, and Ni tolerance.

Isolated strains from the rhizosphere of plants that grow on serpentine soils were evaluated for their ACC deaminase activity, production of phytohormone IAA, synthesis of siderophores, phosphate solubilizing capacity, and Ni tolerance, up to 20 mM of nickel sulphate hexahydrate ($NiSO_4*6H_2O$) on agar.

Eight tested bacterial isolates were positive for more than one plant growth-promoting character. The rhizobacteria *Pantoea* exhibits all PGP activities, showing high production of IAA and siderophores, such as *Pseudomonas*. The solubilization of phosphates is mainly observed in *Pantoea* and *Erwinia*, while *Streptomyces* grows better on ACC as the sole source of N. Only two strains (*Pseudomonas* and *Streptomyces*) are able to tolerate up to 15 mM $NiSO_4*6H_2O$.

Among fungal strains, *Trichoderma harzianum* Rifai group exhibits Ni tolerance (up to 500 mg l^{-1} of $NiSO_4*6H_2O$), and high bioextraction capability (more than 10000 mg kg^{-1}) (8).

Bacteria and fungal communities associated with root system could be useful to alleviate metal stress, and to promote plant growth and Ni uptake, through the development of an integrated plant-microbiota system.

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3.4 = The effects of cold stress on Cypress pollen intine permeability

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Background: Cold injuries include both chilling and freezing damages, which can impact on several biological functions of plants. Freezing can induce the formation of extracellular ice, which produces mechanical stress on both cell wall and membrane, inducing cell rupture (1). Cold, as other environmental stressors, is sensed by receptor proteins on plasma membrane. These receptor proteins generate downstream signaling networks, which trigger appropriate plant reaction. The Ca++ balance is often involved as second messenger in those events (2). In this study we analysed the effects on airborne cypress pollens of a sudden shift to extreme low temperature occurred in Perugia (Central Italy) at the end of February 2018.
Methods: Standard protocols for aerobiological monitoring were used; a fluorescent probe (FURA) was applied to measure intracellular calcium concentration.
Results: Under light microscopy observation the presence of abnormal pink coloration of the cytoplasm was highlighted in a large amount of aerobiological pollen samples. In these samples, as often happen, exine was broken, but the thick layer of intine was intact. Intine, under normal conditions, does not react with the sporopollenin stain (basic fuxine in glycerine), and prevent it to pass through and reach the cytoplasm. As in the case of cold tolerance, the signaling network which operates in pollen are also poorly understood. We hypothesized a possible involvement of Ca++ balance, since in plant tissues the Ca++ signaling is involved in susceptibility to different environmental stress. Thermal shock can induce an inhibition of Ca++ signalling in cypress pollen, which was responsible of leads to a modification of cell membrane permeability causing an increase of cold sensitivity. Conclusions. In consequence of climatic change, seasonality of temperatures is biased, and sudden freezing stresses can suddenly occur (3). These stresses could induce, even in winter airborne pollens, a physiologic stress, which damages pollen wall, causing an increased permeability of intine. As a consequence, the alteration of the pollen performance occurred as reduction of pollen germination, and also a possible release of new allergens. These effects could occur in other winter allergenic pollens (as Corylus spp.), with possible relevant economic consequences (4).

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4.2 = Forest diversity and tree health relationships in Italian forests depend on environmental context

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Forest tree diversity plays a relevant role in promoting forest productivity and overall capacity to deliver ecosystem services. Tree health is positively influenced by forest diversity, especially in relation to insect and pathogen attacks. However, there is no clear general evidence about the possible relationships between stand-scale tree diversity and tree health (estimated as degree of crown defoliation), as assessed in the current European forest monitoring programme (the International Co-operative Programme on Assessment and Monitoring of Air Pollution Effects on Forests, ICP Forests).

It is recognized that the influence of stand-level tree diversity on forest health may be variable in relation to the environmental context, and it can be expected that different patterns of crown defoliation occur on different tree species in different eco-regions. Italy is an excellent case of study because of the ecological heterogeneity of the forest areas, that includes alpine, mountain and Mediterranean climatic regions as well as a broad range of soil types. Aim of this study was to investigate the relationships between forest diversity and tree health conditions of the main forest types in Italy, using the current forest monitoring network.

In this study, 250 plots belonging to the Italian Level I ICP Forests network were grouped in four groups of plots by means of a multivariate cluster analysis, considering ecological and structural parameters of the stands (geographical, climatic and soil characteristics, taxonomic (Shannon) and structural (mingling) diversity indices), obtained from various datasets. Relationships between crown defoliation and diversity (at tree- and stand-level) were analysed on the whole dataset and in homogeneous clusters for the main forest tree species (*Fagus sylvatica* L., *Picea abies* (L.) H.Karst., *Quercus cerris* L., *Q. pubescens* Willd., *Castanea sativa* Mill.).

The main results show that at country level, tree species richness, diversity and composition of the stands reflect different and often contrasting ecological conditions. The responses of trees (crown defoliation) seems driven more by the environmental context rather than diversity. In homogenous ecological conditions, low levels of stand diversity were more effective to improve the crown conditions (i.e. minor defoliation) than either monospecific or highly-diverse stands. This aspect should be considered to promote and apply forest management options aimed at enhancing stand diversity in monospecific stands in areas where the main tree species are in their ecological optimum.

4.3 = On the taxonomy, origin and diversification of *Euphorbia gasparrinii*

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Euphorbia gasparrinii Boiss. (Euphorbiaceae) is an Italian endemic species distributed in two main disjunct areas with two different subspecies: *E. gasparrinii* subsp. *samnitica* (Fiori) Pignatti in Central Apennines (Abruzzo, Marche, Molise) and *E. gasparrinii* subsp. *gasparrinii* in S Italy (Calabria and Sicily) (1). During the last years, *E. gasparrini* has not been found again in Calabria, while in Sicily it is considered as Critically Endangered according to IUCN categories and criteria (2, 3).
The phylogenetic relationships of *E. gasparrinii* are unclear. This species was historically considered as related to *E. fragifera* Jan and to *E. verrucosa* L. by Boissier (4), but later it was considered as belonging to *E. epithymoides* L. group (5, 6). Whereas *E. verrucosa* and the closely related *E. flavicoma* DC. and *E. montenegrina* (Bald.) K.Maly (7) are distributed in southern and central Europe from Spain to Hungary, the *E. epithymoides* group (including *E. fragifera* and *E. polychroma* A.Kern.) is distributed in central and south-eastern Europe (8, 9). From a biogeographical point of view, there are suggestions to investigate the relationship between *E. gasparrinii* and *E. verrucosa* (and closely related species), considering the occurrence of this taxon also in northern Italy. On the other hand, the chromosome number supports a possible connection between *E. gasparrinii* ($2n = 16$) and *E. epithymoides* ($2n = 14, 16$), while *E. verrucosa* and *E. flavicoma* mostly show only $2n = 14$ chromosomes (with some cases of tetraploid cytotypes with $2n = 28$ chromosomes in *E. flavicoma*) and *E. fragifera* has $2n = 26$ chromosomes (10). In order to elucidate the unclear phylogenetic position of *E. gasparrinii* and its morphological differentiation from related taxa, an integrative approach based on morphometric, karyological and molecular techniques was used in this study.
Results of molecular analysis (AFLP fingerprinting and nuclear ITS sequences) highlight a close relationship between *E. gasparrinii* and *E. verrucosa*/*E. flavicoma*, but not with the *E. epithymoides* group. In most of the characters used for morphometry, there is a strong overlap among taxa, suggesting a limited morphological differentiation, in particular concerning *E. gasparrinii* and *E. verrucosa*. Also the morphological differences between the two subspecies of *E. gasparrinii* are unclear. On the other hand, concerning karyology, while Relative Genome Size measurements show no difference in DNA amount between *E. gasparrinii* and *E. verrucosa*, *E. flavicoma* is clearly separated. In addition, *E. gasparrinii* subsp. *gasparrinii* from Sicily shows a completely different DNA-plody level (putatively tetraploid) with respect to *E. gasparrinii* subsp. *samnitica* from central Apennines, albeit these subspecies are extremely close genetically. New chromosome counts further confirm the dysploid relationship between *E. gasparrinii* and *E. verrucosa*. Accordingly, we hypothesise a simultaneous separation from an ancient, more widely distributed, taxon in 3-4 different areas. Our results in particular suggest the differentiation of *E. flavicoma* in Iberian Peninsula, of *E. gasparrinii* in Italian Peninsula (and Sicily) and of *E. verrucosa* in Balkan Peninsula, during Pleistocene glaciations.

1) S. Pignatti (2017) Flora d'Italia. Edagricole-New Business Media
2) F. Bartolucci, L. Peruzzi, G. Galasso et al. (2018) Plant Biosyst., 152(2), 179-303
3) S. Orsenigo, C. Montagnani, G. Fenu et al. (2018) Biol. Cons., 224, 213-222
4) A. Candolle de (ed.) (1862) Prodromus Systemais Naturalis Regni Vegetabilis, 15(2.1). Paris
5) A. Fiori, A. Beguinot (1902) Flora analitica d'Italia, 2
6) T.G. Tutin, V.H. Heywood, D.M. Moore et al. (eds.) (1968) Flora Europaea, 2
7) R. Riina, J.A. Peirson, D.V. Geltman, et al. (2013) Taxon, 62(2), 316-342
8) Meusel H, Jäger E, Rauschert S, Weinert E, eds. (1978) Vergleichende Chorologie der Zentraleuropäischen Flora *2*. Jena, Gustav Fischer.
9) R. Govaerts, D.G. Frodin, A. Radcliffe-Smith, S. Carter (2000) World checklist and bibliography of Euphorbiaceae (with Pandaceae). Royal Botanic Gardens, Kew
10) A. Rice, L. Glick, S. Abadi, et al. (2015) New Phytol., 206(1), 19-26

Poster n. 56

4.4 = Occurrence of *Chara* spp. in the Bussento and Calore rivers of the "Cilento, Vallo di Diano e Alburni" National Park (Salerno, southern Italy)

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Charophyte are freshwater algae constituting the monophyletic clade in which land plants evolved (1). The Italian flora, according to the most recent revision (2), includes 33 species belonging to 6 genera: *Chara*, *Lamprothamnium*, *Lycnothamnus*, *Nitella*, *Nitellopsis* and *Tolypella*. Species distributional data, however, are often incomplete and outdated, especially for the Campania region, where only 3 species (*Chara intermedia* A.Braun, *Ch. braunii* C.C.Gmel., *Lamprothamnium papulosum* J.Groves) were historically reported (2).

With the aim to contribute to the understanding of the Charophyte flora of the "Cilento, Vallo di Diano e Alburni" National Park, the largest protected area in Campania and one of the largest in Italy, extensive field surveys were carried out during the years 2016-2018 on the two main river systems of the area: the Bussento and the Calore Salernitano. Overall, 8 populations belonging to 4 taxa were observed (Figure 1), encompassing 3 species new for the Campania region (*Ch. vulgaris* L., *Ch. gymnophylla* A. Braun, *Ch. globularis* Thuill.) and one new infraspecific taxon for the Italian flora (*Ch. vulgaris* var. *papillata* K.Wallroth). Detailed analyses of each population, involving morphological (number of branches and branch segments, cortication, size of internodes, branch segments, spines, stipuloides, oogonia), biochemical (photosynthetic pigments, nitrogen balance index), and physiological (fluorescence parameters, carbonate encrustation) traits were performed in order to evaluate population variability and differentiation.

The occurrence of most of the observed taxa in rivers, instead of the lentic systems usually colonized, further expands our understanding of the ecology of this peculiar and evolutionary pivotal group of algae.

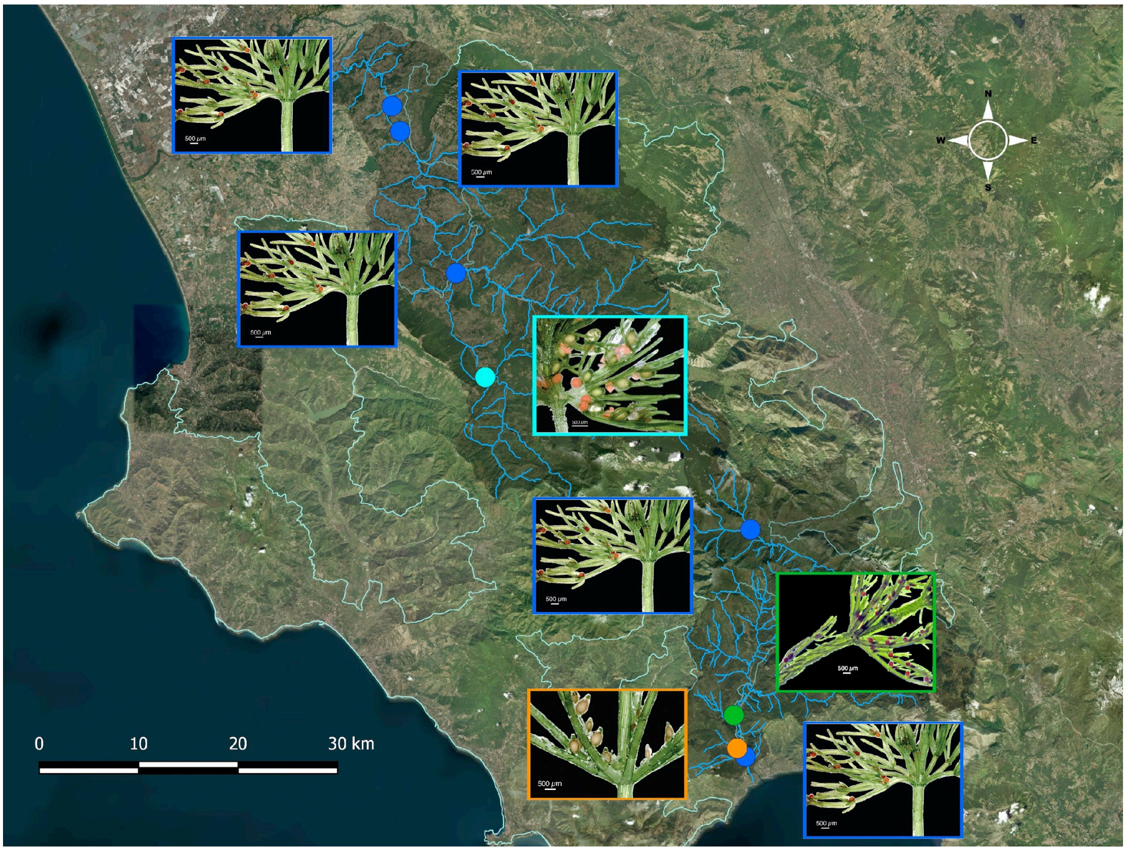

Fig. 1. Distribution of *Ch. gymnophylla* (blue), *Ch. vulgaris* (cyan), *Ch. globularis* (orange) and *Ch. vulgaris* var. *papillata* (green) in the Bussento and Calore Salernitano rivers. Park boundaries and river courses are indicated by cyan and blue lines, respectively. All the photographs from Bazzichelli and Abdelahad (2), with the exception of *Ch. vulgaris* var. *papillata* from www.algaebase.com.

1) J. De Vries, J.M. Archibald (2018) New Phytol., 217, 1428-1434
2) G. Bazzichelli, N. Abdelahad (2008) Ministero dell'Ambiente e della Tutela del Territorio e del Mare

POSTER N. 57

4.4 = The vascular flora of Montarbu of Seui and Perda ‘e Liana: an annotated checklist

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The areas of Montarbu and Perda ‘e Liana (C-E Sardinia) consist of a group of isolated limestone mountains which are commonly called "Tonneri" or "Tacchi" (1) with a total surface area of 10,730 ha. The geographical position, the elevation, the isolation and the complex geology of this area determined an extraordinary richness of plant species, as well as a variety of ecosystems and unique landscapes.

This area is characterised by mountainous peaks above 1,200 m a.s.l. and other particular environments, such as rocky cliffs and gorges, small wetlands and vast scrublands and woodlands represent other important floristic territories. Also, there are peculiar vegetation coenosis of biogeographic and conservation interests, such as the relict forest formations of *Taxus baccata* L., *Ilex aquifolium* L., *Acer monspessulanum* L. subsp. *monspessulanum*, and *Ostrya carpinifolia* Scop. Even if several researchers studied this area in the past (e.g. 1, 2), further endemic plant species were more recently described or found, such as *Senecio morisii* J.Calvo & Bacch. and *Pinguicula sehuensis* Bacch., Cannas & Peruzzi (3, 4).

We present a checklist of the vascular flora of Montarbu of Seui and Perda ‘e Liana in order to update the knowledge about the vascular plant species diversity and distribution, with particular regard to its endemic component, in order to better assess the conservation priorities. This work was based on bibliographic and herbarium studies, integrated by several field surveys carried out from 2012 to 2017. A total of 763 taxa, belonging to 88 families had been found. As regards the endemic component, 92 taxa with 36 exclusive for Sardinian territories and additional 26 taxa with phytogeographical interest, have been identified. The results shows that this area is one of the most important micro hotspot of the island, mainly driven by its isolated conditions and degree of wilderness.

Due to the relatively high number exclusive endemics, and the geologic and geomorphologic peculiarities, and despite the already implemented initiatives, further activities supporting the conservation of this area are suggested and encouraged.

Fig. 1. (a) Chorological spectrum of endemism sub-elements, (b) Typical lanscape of the "Tacchi" region, (c) *Senecio morisii*, (d) *Pinguicula sehuensis*

1) M.C. Loi, A. Lai (2001) Flora Medit., 11, 385-418
2) M.C. Loi, G. Marras, A. Maxia (2004) Flora Medit., 14,153-172
3) J.Calvo, C. Aedo (2015) Syst. Bot., 40, 900-913
4) G. Bacchetta, M. Cannas, L. Peruzzi (2014) Phytotaxa, 186, 279-286

4.4 = Forest flora of the "Dossone della Melia" (Aspromonte National Park – Calabria, Italy)

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The forest resources of Calabria are a high value heritage not only in productive but also landscape, environmental and social terms. The aim of this study is to analyses the forest plant biodiversity and in particular of nemoral flora of "Dossone della Melia" located in the northern part Aspromonte National Park. This territory is the central part of the long peninsular ridge, which connects the Aspromonte massif with the Serre Calabre mountain system with altitudes between 800 and 1000 m (1, 2). The flora survey was carried out through collection of flora in different forest types. The collected species are conserved at the Herbarium of the Mediterranean University of Reggio Calabria (REGGIO). For each species we report the binomial updated according to Bartolucci et al. (3), the biological form and the chorological type according to Pignatti (4) and the habitat in accordance with the classification of the CEE directive 43/92. In order to give a synthetic framework of the flora, were elaborated the biological, ecological and chorological spectra. The taxa belong to 21 families and 35 genera. The most represented families are *Poaceae, Fagaceae*, *Aceraceae* and *Lamiaceae*. The Italian endemic taxa reach 5% of the total flora, while no alien unit has been found, with the exception of some specie used for reforestation (*Chamaecyparis lawsoniana* (A.Murray) Parl., *Quercus rubra* L.). The biological spectrum shows a prevalence of Phanerophytes while less represented are the other biological forms Nanofanerophytes, Geophytes, and Hemicryptophytes. The chorological analysis shows a dominance of taxa with Mediterranean distribution, followed by the Euri-Mediterranean ones. The ecological spectra agree with the bioclimatic and geo-pedological characteristics of the area investigated and show the main variations related to the forest type. The surveys highlight that the forest with the highest number of taxa are the beech woods of *Anemono apenninae-Fagetum sylvaticae* attributable to the directive habitat "9210* Apennine beech forests with Taxus and Ilex" (Fig. 1), widespread in the supratemperate bioclimate belt. A lower number of taxa is hosted by holm oak woods of the *Teucrio siculi-Quercetum ilicis* of habitat directive "9340 *Quercus ilex* and *Quercus rotundifolia* forests"(Fig. 2) located in the supra-Mediterranean belt. This study made it possible to evaluate the floristic richness in homogeneous portions of an ecological and vegetational point of view. Biodiversity is one of the most important environmental assets to ensure stability and resilience to forest ecosystem, as well as a fundamental component for the environmental assessment of a territory.

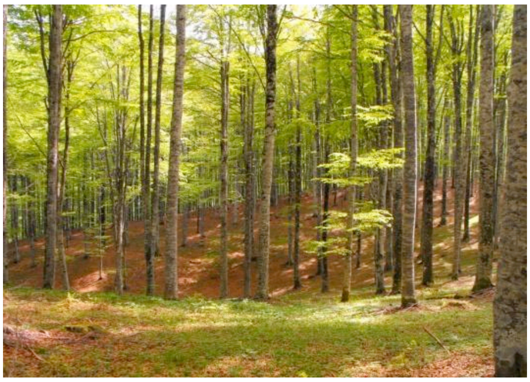

Fig. 1. *Anemomo apenninae-Fagetum sylvaticae.*

Fig. 2. *Tecrio siculi-Quercetum ilicis.*

1) S. Brullo, F. Scelsi, G. Spampinato (2001) La vegetazione dell'Aspromonte. Laruffa Editore, Reggio Calabria
2) G. Spampinato, P. Cameriere, A. Crisafulli, D. Caridi (2008) Quad. Bot. Amb. Appl., 19, 3-36
3) F. Bartolucci, L. Peruzzi, G. Galasso et al. (2018) Plant Biosyst., 152(2), 179-303
4) S. Pignatti (1982) Flora d'Italia, 1-3. Edagricole, Bologna

4.5 = The phylogenetic resolution of four different markers to study closely related-taxa within the genus *Fritillaria* (Liliaceae)

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Phylogenetic analysis with the outcome of biotechnology has allowed us to better understand the relationship among the different taxa within different lineages. The use of RFLP, followed later by the routine Sanger Sequencing Method and recently with the spread of Next Generation Sequencing, molecular data have become available to establish phylogenetic relationships between closely related species and populations. The latest technologies provide insights even in the exome and in the whole nuclear genome of individuals. However, the processing power required by current methods using genome-scale data (phylogenomics) (ML, BY and PP) makes difficult if not impossible to complete phylogenetic analyses from alignments involving several thousand bases in acceptable computational time. The use of a set of markers may help to solve this problem nonetheless the congruence between the different markers, the availability of an open-access share resource of DNA sequences and of effective computing resources are among the limiting factors which can influence the discriminatory power of this approach.
Within the plant kingdom, the choice of *matK* as a core barcode is based on the discriminatory power of the *matK* region when used for species identification (BOLD SYSTEM). *MatK* is one of the most rapidly evolving coding regions of the plastid genome, unfortunately, in some species or species groups *this region* can be difficult to PCR amplify using routine primers sets. In the present work, the resolution power of *mat*K, *rpl*16, *rpo*C1 and *pet*A regions has been tested by analysing the interspecific genetic distances observed and the efficacy of the different marker combinations to form well supported clades.
Twenty species (15 *Fritillaria* from GenBank, three *Fritillaria* in this work and two *Lillium* as outgroups) were considered. Sequences were analysed both separately and in different combinations in order to test the phylogenetic resolution in terms of the number of well supported clades. The sequences were aligned using MAFFT v 7.017 with default conditions for gap openings and gap extension penalties. Phylogenies were inferred with RAxML v.7.2.8 and the GTR+G model was used. A total of 1000 bootstrap replicates were performed to assess the relative robustness of the branches of maximum likelihood in RAxML. Only maximum likelihood bootstrap (MLB) values ≥ 70% were regarded as statistically significant. When considering a single region, best results were obtained with *mat*K (eight clades with MLB ≥ 70; two clades with MLB = 100). The combinations of the *Mat*K-*rpl*16 regions returned the best resolution (16 clades MLB ≥ 70; two clades MLB = 100). *Mat*K-*rpl*16-*pet*A and *mat*K-*rpoc*1-*pet*A combinations returned the same results (14 clades, MLB ≥ 70; three clades, MLB = 100). Combination of the *mat*K-*rpl*16-*pet*A-*rpoc*1 regions returned 15 clades with MLB ≥ 70 and three clades with MLB = 100. When *mat*K was omitted from the analysis, the *pet*A-*rpl*16-*rpoc*1 combination gave the best resolution (12 clades, MLB ≥ 70; one clade, MLB = 100).

4.6 = Bio- and geodiversity of vermiculations from Pertosa-Auletta Cave (southern Italy)

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Caves are largely unknown environments, hosting in their peculiar ecological niches a wide diversity of extremophile microorganisms, highly specialized and adapted to the prohibitive conditions of caves (1). The role of cave biota in the formation of enigmatic structures occurring in underground environments, like vermiculations, is still largely unexplored (2). Vermiculations are thin, irregular and discontinuous deposits of incoherent particles, commonly found on the walls and ceilings of natural or artificial caves, worldwide (3). They show several kinds of morphology (dots, dendritic, hieroglyphic…), color (red, brown, grey, white) and size (4), and are considered "life hotspots", representing a valuable focus for scientists and researchers in the field of cave microbiology.

The aim of this study, funded by MIdA Foundation (Italy), was to investigate the microbiological and physico-chemical properties of vermiculations from Pertosa-Auletta Cave (Campania, southern Italy), in order to clarify the mechanisms involved in their formation and to define the factors affecting their diversification. Indeed, the understanding of biodiversity and genesis of vermiculations is a key step toward the protection and conservation of these peculiar biosignatures.

Vermiculations from Pertosa-Auletta Cave, abundant in all the galleries, showed a considerable variety of morphologies (Fig. 1). Overall, X-ray diffraction analysis indicated calcite and quartz as the dominant minerals of the 15 vermiculations studied, whereas clay minerals were almost absent. Elemental analysis showed wide variations in C, Ca, Co, Cr, Cu, Fe, K, Mg, Mn, N, Na, Ni, P, S, Ti, V, and Zn concentrations among vermiculations. Applying the most innovative techniques in molecular biology, only *Prokaryotes* were observed. In particular, 16S rRNA gene-specific next generation sequencing analysis showed that *Proteobacteria* (48.0%) was the most abundant phylum in the *Bacteria* domain, followed by *Acidobacteria* (11.6%), *Actinobacteria* (7.1%), *Nitrospirae* (5.8%), *Firmicutes* (4.3%), *Planctomycetes* (3.2%), *Chloroflexi* (1.9%), and *Gemmatimonadetes* (1.1%). *Archaea* (0.1%) and a significant percentage of unclassified microorganisms (13.1%) were also observed. Clustering analysis suggested that microbial communities dwelling in the deposits partially influence their morphologies, likely mainly dependent on physico-chemical factors. Confocal laser scanning and field emission scanning electron microscopy confirmed the presence of active microbial mats, suggesting they may have an important role in the formation of vermiculations, throughout a number of processes, like production of organic matter, precipitation of secondary minerals (*e.g.* biogenic calcite), enhancement of sediment trapping and binding, etching or pitting of the host rock.

Fig. 1. Some vermiculations from Pertosa-Auletta Cave. A. Dendritic morphology with evidences of photoautotrophic activity (green color and drops); B. Spot-like morphology; C. Hieroglyphic morphology.

1) K. Tomczyk-Żak, U. Zielenkiewicz (2016) Geomicrobiology J., 33, 1-20
2) A. Bini, M. Cavalli Gori, S. Gori (1978) Int. J. Speleology, 10, 11-33
3) C.A. Hill, P. Forti (1997) Cave Minerals of the World. National Speleological Society, Ed. 2, 463 pp.
4) P. Parenzan (1961) Memoria Rassegna Speleologica Italiana, 5(2), 120-125

4.6 = The rediscovery of an endemic sage species in Calabria (Italy): an assessment of genetic diversity and structure in *Salvia ceratophylloides* Ard. (Lamiaceae)

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Salvia ceratophylloides Ard. is a critically endangered scapose hemicryptophyte, narrow endemic to Calabria. It was declared extinct in 1997 (1), but was recently rediscovered in the hills around Reggio Calabria, where it survives with three populations, about 10 km apart each other, consisting of about 1,000 individuals growing in Mediterranean dry grasslands (2). Considering the economic importance of plant biodiversity in providing food, medicine and fuel but, above all, in the ecosystem functioning and services, we started a project for the assessment of the genetic diversity within and between the three *S. ceratophylloides* populations. The analysis of the genetic structure in endemic and isolated species is essential to evaluate the extent to which stochastic or anthropogenic factors affect the species. The final goal of this study, carried out by SSR markers developed for *S. officinalis* L., is to estimate the degree and distribution of genetic diversity within and among populations of this endangered species, and to infer past demographic processes by means of coalescent-based approximate Bayesian computation (3).

Fig. 1. *Salvia ceratophylloides* individual (on the left) and close-up of the leaf with typical deep sinuses and lobes (on the right).

1) F. Conti, A. Manzi, F. Pedrotti (1997) Liste Rosse Regionali delle Piante d'Italia. WWF Italia, Società Botanica Italiana
2) G. Spampinato, A. Crisafulli, A. Marino, G. Signorino (2011) Inform. Bot. Ital., 43(2), 381-458
3) G. Bertorelle, A. Benazzo, S. Mona (2010) Mol. Ecol., 19, 2609-2625

4.6 = Does morphometric analysis of two sympatric *Pulmonaria* species help their discrimination in spring?

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Pulmonaria (Boraginaceae) is an extremely variable and taxonomically controversial genus. Some puzzling systematic relationships are particularly evident within closely related and morphologically similar species that may naturally hybridize, such as *P. apennina* Cristof. & Puppi and *P. hirta* L., which were even considered as belonging to a single species (1). Shape, maculation and hairs of summer basal leaves are considered as key diagnostic characteristics for these taxa (2). *Pulmonaria hirta*, in peninsular Italy, is confined to the northern-central Apennines, overlapping in that area with the range of *P. apennina*, which is instead spreading all along Apennines. The taxonomic value of spring basal leaves and flowers has been ignored and no studies using geometric morphometrics of leaf shape traits have been carried out in this group so far. Accordingly, the main aim of our study is to provide some preliminary quantitative data to assess the diagnostic value of these characters. 119 plants belonging to 6 populations of *P. hirta* and *P. apennina* (3 populations each) were collected from topotypical localities, or from localities in which authors agree on species identification based on karyological, morphological and/or geographical distribution (2, 3). All these plants are currently cultivated at the Botanic Garden of Pisa. Length of corolla tube and lobe, as well as length of calyx tube and tooth, were measured in one flower, chosen randomly among those open on the inflorescence. Based on the same flower, each individual was assigned to two possible morphs: L-morph (longistylous) or S-morph (brevistylous). For the evaluation of size, shape, maculation and hairs, undamaged, well developed basal leaves were collected randomly and colour images of the adaxial surface were captured with a scanner at a resolution of 300 dpi. Leaf shape was calculated using a geometric morphometrics approach: outlines of whole leaves were automatically recorded on the scanned images and reduced to the coefficients of elliptic Fourier descriptors (EFDs, 4) using SHAPE software (5). To reduce dimensionality, these coefficients were subjected to principal component analysis (PCA), and both a linear discriminant analysis (LDA) and a MANOVA (multivariate analysis of variance) for testing differences in shape among populations and species were also carried out on the effective components using PAST version 3.14 (6). Depigmented areas ("spots"), total leaf area, leaf length and width have been calculated by means of ImageJ v1.47 (7) on leaf pictures. Hair type, length and density have been evaluated under a stereomicroscope, on a 6 mm^2 area, located at ca. 1 cm on the left from the middle vein. Hair types have been categorized as follows: normal, glandular and microglandular. Discriminant Analysis on PCs obtained after PCA on EFDs, followed by Jackknife, gave an 85% of correct *a priori* classification, indicating that spring leaf outline can help discriminating the two species. The possible confusion is mostly due to a population of *P. apennina* from Abruzzo, which shows values that overlap with populations of *P. hirta*. Accordingly, MANOVA returned significant differences ($p < 0.01$) between all pairs of *P. apennina*/*P. hirta* populations, whereas no differences were found among populations of *P. hirta.* However, a significant difference was also found between a population of *P. apennina* (again from Abruzzo) and a co-specific population from Calabria. These results indicate that *P. apennina* is more variable than *P. hirta*. Generally, *P. apennina* shows plumper spring leaves with base and petiole being more distinct than in *P. hirta*, which has instead a more lanceolate spring leaf shape with a winged base running into the petiole. On the contrary, the other evaluated features of spring basal leaves and flowers were not able to discriminate the two species, in accordance with previous authors (2, 3 and literature cited therein). Interestingly, we recovered a balanced morph ratio in all populations, with the exception of the one from Calabria (*P. apennina*), in which longistylous flowers predominate (ca. 90%). In conclusion, a geometric morphometric approach seems suitable for studying such complicated taxa, which show some differences also in spring leaves, at least for their outline. A comprehensive biosystematic study based on morphometric study of summer leaves, karyotype reconstruction, DNA fingerprinting and phylogenetic analyses is ongoing on the same individuals. In addition, these analyses will be extended to other populations including the related *P. vallarsae* A. Kern.

1) C.G. Vosa, G. Pistolesi (2004) Caryologia, 57, 121-126
2) L. Cecchi, F. Selvi (2015) Plant Biosyst., 149, 630-677
3) G. Puppi, G. Cristofolini (1996) Webbia, 51, 1-20
4) F.P. Kuhl, C.R. Giardina (1982) Comput. Graph. Image Process., 18, 236-258
5) H. Iwata, Y. Ukai (2002) J. Heredity, 93, 384-385
6) Ø. Hammer, D.A.T Harper, P.D. Ryan (2001) Palaeontol. Electron., 4, 1-9
7) W.S. Rasband (1997) http://imagej.nih.gov/ij/

4.6 = Critical forms in Sicilian populations of annual *Lysimachia* L. (Primulaceae)

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Based on recent literature, in the Primrose family the genus *Lysimachia* L. absorbed *Anagallis* L. (1). As a result, new name combinations have been introduced for this genus, thus involving the increase of specific and intraspecific taxa (for Italian flora see 2 and 3). A further consequence, focused in this contribution, concerns the binomial combination *Lysimachia arvensis* (L.) U.Manns & Anderb., which has different synonyms and includes several specific and intraspecific taxa. As far as the taxonomic history of the annual taxa of *Anagallis* is concerned, different authors preferred to include some of them in *A. arvensis* and in *A. foemina* at the level of variety (4) or subspecies (2), while others recognized their specific rank (5, 6, 7). Among these taxa, *A. parviflora* Hoffmanns. & Link is frequently reported, even included among the synonyms of *A. arvensis* (5) and then considered as a distinct species (7). The already complex *status* is further complicated by the occurrence of hybrids, as reported by Pignatti (6). The cited case would regard *A. foemina* and *A. parviflora*, but other taxa are also mentioned in literature [cf. *Anagallis* × *intermedia* Giraudias = *Lysimachia* × *intermedia* (Giraudias) B.Bock [(*Lysimachia arvensis* (L.) U.Manns & Anderb. × *Lysimachia foemina* (Mill.) U. Manns & Anderb.]. The analysis of the variability observed in a Sicilian population referable to this group (Fig. 1) revealed a quite critical case, introducing additional variables in the different interpretations of varieties, forms and hybrids of this group of complex species. Plant samples collected in the territory of Palermo particularly showed new colours of the corolla and different dimensions of petals. This recurrence of form and colour observed in the same station for two years, with the presence of congeneric taxa, allows to hypothesize a temporary recurrence of the mentioned features; the authors were induced to interpret this population at variety level or consider it the result of recurring hybridization processes. Field researches were extended on this basis and a second population was found in the territory of Misilmeri, near Palermo. Both populations coexist with the best known and widespread *A. arvensis* (incl. *A. phoenicea* Scop.) and *A. foemina*. The possible hybrid origin of this unit – elsewhere reported by amateurs not just in Italy – led to start genetic investigations which are still in progress.

Fig. 1. Different colours and forms of corolla in annual *Lysimachia* (= *Anagallis*) observed in Sicily.

1) U. Manns, A.A. Anderberg (2009) Willdenowia, 39(1), 49-54
2) L. Peruzzi (2012) Inform. Bot. Ital., 42(1), 383-384
3) F. Bartolucci, L. Peruzzi, G. Galasso et al. (2018) Plant Biosyst., 152(2), 179-303
4) A. Fiori (1926) Nuova Flora Analitica d'Italia, 2, 224-225. Firenze
5) L.F. Ferguson (1972) *Anagallis* L. In: T.G. Tutin, V.H. Heywood, N.A. Burges et al. (Eds.), Flora Europaea, 3, 28-29. Cambridge University Press, Cambridge
6) S. Pignatti (1982) Flora d'Italia, 2, 290-291. Edagricole, Bologna
7) P.V. Arrigoni (2010) Flora dell'Isola di Sardegna, 2, 566-573. Carlo Delfino editore, Sassari

5 = From knowledge to awareness: the LIFE-ASAP project educational path on the invasive alien flora in the Botanical Garden of Cagliari (Italy)

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The project LIFE ASAP aims to stop new introductions and reduce the voluntary and accidental spread of invasive alien species, through education and communication to the general public. At European level, this is the first project that addresses the practical implementation of the Regulation (EU) No. 1143/2014 on invasive alien species and the Italian Legislative Decree No. 230/2017 in force since the 18th February 2018. LIFE-ASAP focuses on the role that Botanic Gardens play in bringing society closer to the different scientific activities and, above all, to the overarching themes of nature conservation and education. Among the activities proposed in the project, an educational trail will be created within the Botanic Garden of Cagliari that will allow visitors to learn about a selected group of alien plants introduced in Italy and Sardinia for ornamental or other purposes. The path will include 13 self-explanatory panels, three of which are introductory and 10 are dedicated to key alien species. Garden visitors will be invited to take the path, and, in addition to a guided tour of the "Invasive alien flora path", a multidisciplinary educational activity will be proposed. Importantly, primary and secondary school students will be involved, through a game, in a real hunt for invasive alien species. Clues and multiple-choice questions will allow student teams to identify invasive alien species within the Botanical Garden and learn the best practices suited for prevention and management.

POSTER N. 65

5 = Assessment of the invasion potential of *Heracleum mantegazzianum* Sommier & Levier in the upper Valle Seriana (Lombardy, Italy)

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The aim of this study is to evaluate the invasion potential of *Heracleum mantegazzianum* (*Apiaceae*) over the course of the River Serio, in the upper Valle Seriana, between Clusone and Ponte Nossa (BG).

H. mantegazzianum is an alien species native to the western Caucasus, introduced in Europe for ornamental proposes in the 19th century and then spontaneously spread. According to the Commission Implementing Regulation (EU) no. 2017/1263 it is included in the list of invasive alien species of Union concern. The EPPO (European and Mediterranean Plant Protection Organization), reports it as an invasive alien species in Denmark, Finland, Norway, France, Germany, Hungary, Slovenia, the United Kingdom and Switzerland. In Italy it is reported as invasive in Valle d'Aosta, naturalized in Lombardy, Alto Adige and Emilia-Romagna, casual in Trentino, Piedmont and Veneto, no longer found in Tuscany (1,2). In these regions the species occurs mainly in mountain areas, along river banks, wet slopes, fallow fields and road edges, but it could potentially spread out very quickly, especially in northern Italy.

This alien species is very dangerous for the human health due to the presence in its lymph of furanocoumarins, organic chemical compounds that cause phytophotodermatitis. However, another problem related with the spread of this species is the impact on the local biodiversity: its high competitive strength could threat native species. In fact, it forms densely populated colonies and has large leafs that shade the underlying species slowing down or inhibiting their growth (1,3).

The Italian scenario has evolved over the years: while its distribution was circumscribed in the past, , nowadays we have observed its gradual expansion. We have investigated a mountain area near Bergamo where *H. mantegazzianum* was reported by Galasso in 2006 (4). The goal was to evaluate its current distribution, its invasion potential using Weber & Gut protocol and Tyler Invasiveness Index.

Importantly, the application of Weber & Gut protocol has attributed *H. mantegazzianum* to the highest risk class, although this species is recorded only as naturalized in Lombardy: this highlights the possibility that it could became a threat for native vegetation. This method represents a reliable risk assessment to evaluate the capacity of an alien species to become invasive and therefore to represent a danger for ecosystem biodiversity. These results, together with a medium value of Tyler Invasiveness Index, support the hypothesis that *H. mantegazzianum* could have a real impact on plant biodiversity and human health. In fact, the species has spread since 2006 (4), when it was only reported along the banks of the river Serio, limited to Ponte Nossa. Nowadays it has spread to the North-East area beyond Clusone, with population density sometimes high.

Our results confirm the need to monitor the population of *H. mantegazzianum* not only in this area, but also throughout the mountain areas in the province of Bergamo.


Fig. 1-3: *H. mantegazzianum* details


Fig. 4: Phytophotodermatitis

1) L. Celesti-Grapow, F. Pretto, E. Carli, C. Blasi (2010) Flora vascolare alloctona e invasiva delle regioni d'Italia, Roma: Casa Editrice Università La Sapienza, 208 pp.
2) E. Banfi, G. Galasso (2010) La flora esotica lombarda, Milano: Museo di Storia Naturale di Milano, 273 pp.
3) P. Pyšek, M.J.W. Cock, W. Nentwig, H.P. Ravn (2007) Ecology and management of giant hogweed (*Heracleum mantegazziannum*), CAB International, 324 pp.
4) G. Galasso, S. Frattini, I. Moreschi (2007) Notulae alla checklist della flora vascolare italiana 3 (1267-1310), Informatore Botanico Italiano, 39, 1, 237-253

5 = Italian street trees: evaluation of the floristic biodiversity in several Italian cities

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Street trees, i.e trees allocated in the border of streets, are too often low considered in the urban ecology assessment and in urban landscaping. On the contrary, they are natural key elements of the cities, playing an important role from an ecological, socio-economic and functional point of view. Then, we carried out a comparative analysis of the street trees of the main Italian cities to assess the species biodiversity and their selection criteria, and to understand their effective functionality at urban ecosystem level. Considering the bioclimatic gradient occurring in Italy, and in relation to the data availability both from the literature and both from the various Garden Services Offices, we collected information related to the following 15 cities, in a latitudinal order: Trento, Turin, Trieste, Milan, Padua, Bologna, Perugia, Florence, Rome, Campobasso, Bari, Naples, Sassari, Cagliari and Palermo.

Our study highlighted that only few scientific studies report floristic data of street trees, and the Gardens Service Offices provided most information. On the national scale, we obtained 275 tree species, for 47 families concerning also Palmae and Asparagaceae, not properly trees but sometimes functionally considered as such in this context. The high biodiversity found had not a homogenous distribution among the cities, and some cities, such as Trento, Cagliari and Padua showed relatively high values considering their size. Few species resulted recurrent, such as *Celtis australis* L. and *Quercus ilex* L. followed by *Cercis siliquastrum* L., *Cupressus sempervirens* L. and *Robinia pseudoacacia* L., whereas the highest number was present only in few cities. The general selection trend of the tree species resulted according to a phytoclimatic gradient of the cities. From a quantitative point of view, the frequency of the genus *Platanus,* followed by *Tilia, Celtis* and *Pinus,* resulted the highest. Moreover, the exotic species resulted more of 50% than the native species, with dominance in the different cities of East-Asiatic and North-American species. From this point of view, Naples was an exception, being highly characterized by Mediterranean species.

This study highlighted the need to develop and implement attention in the selection of species to have guidelines for this important element in urban landscaping.

Fig. 1 Street tree lines of *Pinus* pinea in Rome city

Fig. 2 Street tree lines of *Quercus iles* in Campobasso city

5 = The importance of linking management systems with vegetation surveys: the terraced landscape of the Amalfi Coast (Salerno, Italy)

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The landscape of the Amalfi Coast is typified by a rugged geomorphology, woodlands and cultivated land stolen from the steep mountains thanks to stone terraces. Indeed, terraced vineyards and orchards are identified as key descriptors of the Cultural Heritage site of the Costiera Amalfitana [1]. This unique landscape is threatened by the potential collapse of these terraces once they start deteriorating after the abandonment of agricultural activities [2,3]. The new touristic oriented economy has polarized the local businesses and lured the younger generations who are no longer interested in cultivating the land [3]. Since these cultivated terraces represent social-ecological systems, we wanted to use a new interdisciplinary approach, linking ecological considerations with Traditional Ecological Knowledge [4,5]. Specifically, in this study, we are combining vegetation surveys [6] with semi-structured interviews [7] to understand how the maintenance activities of the terraces affect the vegetation on the walls and hence the stability of the terraces. Although the study is still in progress, we have already performed 25 interviews and 24 vegetation surveys on terrace walls and have already obtained interesting results. All interviewed agriculturists have stressed the importance of removing plants structures emerging from the walls (see also [3]), and indeed the vegetation resulted always herbaceous since woody plants are not allowed to grow or develop. The surveyed plant communities can usually be referred to the *Parietarietea* or *Artemisietea* classes. Another relevant topic, about which informants had conflicting opinions, is the removal of the roots from the fissures among the stones. Some agriculturists believe that the roots contribute to the stability of the structure, acting as a sort of plaster that is not sealing (water needs to percolate to avoid its accumulation and the creation of pressure on the walls). Other people instead prefer to remove them, because in this way the sprout of plants is slower and the time between interventions of vegetation removal is longer. In both cases, the workload is not excessive since in the first case the vegetation removal can be done using a brush cutter, and in the second case, although it has to be done by hand, interventions are fewer. Managing the vegetation on the walls contribute to keeping the walls stable and is less expensive than rebuilding the terraces after a collapse. Moreover, our informants pointed out that if people are working on the terraces the risk of fires is low. An important collateral result of our study is the documentation of the Traditional Ecological Knowledge about the most effective strategies to manage terraces and their stability. Terraces are not only important as a key element of the cultural landscape of the Amalfi Coast but also because their collapse can affect the stability of entire mountain slopes and threaten houses, villages and human lives.

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2) Caneva, G., & Cancellieri, L. (2007). Vegetation landscape of the Amalfi Coast. Gangemi Ed., Rome, Italy (in Italian).
3) Savo, V., Caneva, G., McClatchey, W., Reedy, D., & Salvati, L. (2014). Combining environmental factors and agriculturalists' observations of environmental changes in the traditional terrace system of the Amalfi Coast (Southern Italy). Ambio, 43(3), 297-310.
4) Berkes, F., & Folke, C. (1998). Linking social and ecological systems for resilience and sustainability. Cambridge University Press, Cambridge, UK.
5) Martin, J. F., Roy, E. D., Diemont, S. A., & Ferguson, B. G. (2010). Traditional Ecological Knowledge (TEK): Ideas, inspiration, and designs for ecological engineering. Ecological Engineering, 36(7), 839-849.
6) Braun-Blanquet, J. (1932). Plant sociology. The study of plant communities. McGraw-Hill Book Co., Inc., New York and London, US, UK.
7) Bernard, H. R. 1988. Research Methods in Cultural Anthropology. Sage, Newbury Park, CA, US.

5 = Hierarchisation of biodiversity hotspots from global to local scale: a concrete example with policy and conservation planning implications

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The term, 'biodiversity hotspot', was coined in the late 1980s in order to identify priority conservation regions with a considerable loss of natural habitat and with a large number of endemic species (1). Since its introduction, the concept of hotspots has been used as a key strategy for global conservation actions, and it has become the principal global conservation prioritisation approach, attracting over $1 billion in conservation investment (2). Within the Mediterranean mega-hotspot, the levels of macro-hotspot (e.g. Tyrrhenian islands), meso-hotspot (e.g. Sardinia) and micro- (maximum extent of 500 km^2) and nano-hotspots (maximum extent of 10 km^2) have been applied at regional and local scales for endemic vascular plant species (e.g. 4,5). This approach has the advantage of being simply applicable and understandable through the selection process of areas for conservation, without recurring to extrapolations or automated computations. On the other side, the complete identification of such areas requires a–not always available–vast amount of information about the precise location of species.
This study shows a replicable example of hotspot hierarchisation from the Mediterranean mega-hotspot up the finer level of nano-hotspots of Montarbu area (CE Sardinia), within the intermediate level of micro-hotpots of the Sardinian "Tacchi", a calcareous area in the central part of the Island. Such exercise have several policy implications and could represent a valuable tool for *in situ* conservation planning. Nonetheless, our example showed also gaps in efforts for the protection of endemic plant species.

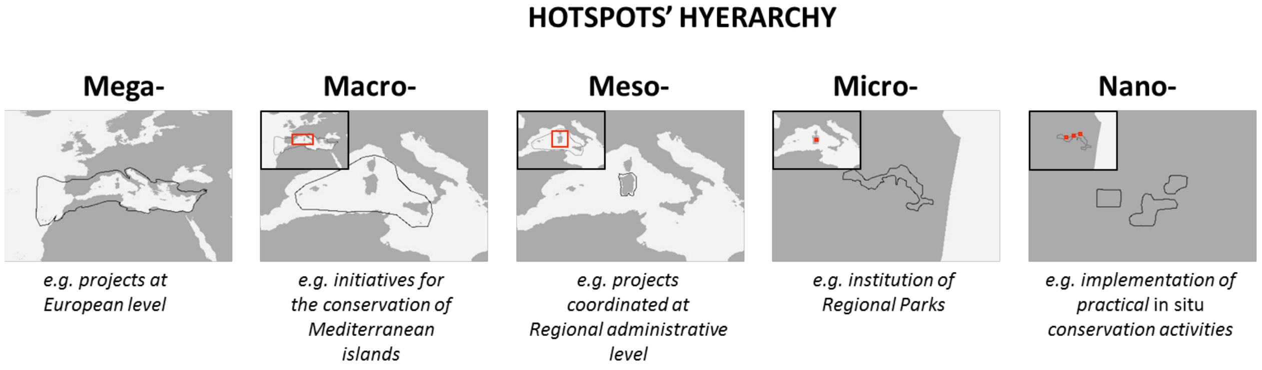

Fig. 1. Hierarchy proposed to organize different levels of hotspots nested in hotspots; examples of different policies to be implemented at different levels.

1) N. Myers (1988) Environmentalist 8, 187-208
2) C. Marchese (2015) Global Ecol. Conserv. 3, 297-309
3) E.M. Cañadas, G. Fenu, J. Peñas, J. Lorite, E. Mattana, G. Bacchetta (2014) Biol. Conserv., 170, 282-291
4) M. Fois, G. Fenu, G. Bacchetta (2018) Plant Ecol. Divers. (in press)

5 = The 18th century herbarium of Giovan Battista Casapini: an example of "hortus siccus" in Parma (Italy)

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Giovan Battista Casapini, a doctor and botanist who lived in the early eighteenth century, practiced medicine in the Duchy of Parma and gathered in the provinces of Parma and Piacenza numerous medicinal plants that he gathered in herbariums, two of which have come down to us. One of them is still conserved in the Modena Botanical Garden and the other entered, in 1837, to be part of the collections of the Palatine Library of Parma and later granted, in 1921, on permanent loan to the Botanical Garden of Parma. There is no information referred to the time, such as the provenance or the state of conservation. The Naturalis Botanicae Icon, known as Erbario Casapini dates back to 1722 and is dedicated to the Marquis Maurizio de Sanctis, advisor to the Duke Francesco Farnese; it consists of five files, in which the plants are classified and annotated in relation to their medicinal virtues. It includes a collection of 199 dried species to a large extent officinal. The document is a precious museum find but also an extraordinary testimony of the medical-therapeutic knowledge of the time. The Erbario Casapini is a work contained in five paper files, of size 22.5 X 44 Cm, which have the value of unity even if they are individually assembled and physically independent from each other. With the exception of the first one, all the sections are wrapped by a double sheet of paper which shows the file number. The files are joined with green silk stitching points. In the right half of the sheet there is a watermark representing a two-headed crowned eagle. The collected botanical samples are glued onto the sheet of paper using a natural adhesive. In some cases, in addition to the glue applied on the plant, the author has reinforced the adhesion with small pieces of paper. Each of the specimens is accompanied by handwritten notes of the doctor: scientific name and therapeutic uses. In some cases the origin of the sample is also indicated. The Herbarium presents a decoration almost certainly made by the same Casapini. The caps and the frames were colored using Gallic metal ink and blue, green, yellow and red pigments. From a first visual examination there are many elements of the Herbarium showing signs of deterioration: in particular the structure of the files, the paper support, the specimens and the writings. Sometimes the observed damage is a testimony of events passed by the manuscript, in other cases they are the result of the conditions of conservation and usury to which the manuscript has been subjected during the centuries. The paper support on which the plants were fixed shows damage of a mechanical, chemical and biological nature. The corrugations of the sheets due to the three-dimensionality and the thickness of the specimens and the fixing system have determined the deformation of the files. These deformations accentuate the fragility of the plants. The pressure, determined by the overlapping of the files and the absence of inter-fragment between the samples, cause fragmentation and detachment of the same. In many cases there are traces of degradation of the inks of the writing and of the decorations as well as erosions of the paper and of the specimens due to insects. The project on the Herbarium, tries to remedy the identified criticalities and secure the botanical species so that they are not further damaged and the fragments are not lost; the removal of particulate matter from the surface of the paper will be fundamental to avoid the development of mocrorganisms. The restoration work was entrusted to the "Laboratorio di restauro Cartantica in Parma" and is still ongoing.

1) F. Lona et al. (1981) Il verde a Parma, Edizioni Artegrafica Silva-Parma
2) F. Fossati (1992) L'Orto Botanico dell'Università di Parma In: Raimondo, F.M. (ed.), Orti Botanici, Giardini Alpini, Arboreti Italiani. Palermo, Edizioni Grifo, pp. 209-218
3) G. Caneva , M.P. Nugari , O. Salvadori (2005) La biologia vegetale per i beni culturali, Vol. I – II
4) L. Bertoli, F. Fossati, L. Sarritzu (2006) Analisi diagnostiche rivolte al restauro e alla conservazione dell'Erbario ottocentesco della contessa Albertina Sanvitale. 101°Congresso SBI, Caserta, p. 37

5 = Analysis of vascular flora on three historical Calabrian churches: preliminary data

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The problem of deterioration of cultural heritage is particularly significant in countries such as Italy that are rich in these assets. Vascular plants are the biodeteriogens causing most damage, such as cracks, collapse, and detachment of materials mainly due to biophysical and biochemical processes. The colonization of a material by an organism depends on specific conditions, such as the bioricepticity of the building material itself, which relies on its chemical-physical composition, porosity, roughness of the surface and its state of conservation, and the physical environmental parameters such as temperature, light, humidity and air movements (1).

In this study, we analyzed the vascular flora biodeteriogens of three Calabrian historical architectures located in three different geographic areas. The aim is to highlight biodiversity and considering its biodeteriogenic potential. The areas of study are the Church of Santa Maria della Serra in Montalto Uffugo (CS), the Church of the Santissima Annunziata in San Fili (CS) and the "gothic church" of Santa Maria della Pietà in Squillace (CZ).

The main climatic characteristics of these three sites are a summer drought and moderate winter cold with an average annual temperature of 15 ° C. Squillace town is proximity of the Ionian sea, while the other two sites are located inland, at higher altitudes.

The plants specimens, collected in late spring, were identified in the field according to Flora d'Italia (2). The vascular flora found comprises 25 plant species, 17 of which were gathered on the walls and 8 in the external perimeter, classified into 24 genera and 13 families among which the most representative are the Asteraceae (54%). The analysis of biological forms highlights the predominance of therophytes and hemicriptophytes (28% each).

The species detected are widespread throughout the Italian territory and therefore endemic species are not in place. Many belong to the ruderal species (*Taraxacum officinale, Picris hieracioides, Diplotaxis tenuifolia*), and *Parietaria judaica* is the only one that is present in all the three sites.

The Hazard Index (IP) (3), used to evaluate the biodeteriogenic potential, show an average value between 5 and 6 in 52% of cases.

The data collected in this work aim to provide a preliminary understanding of the spontaneous vascular flora and its biodiversity. The founding of a different composition in the three sites sheds light on how climatic conditions are an important factor in this kind of analysis. The knowledge of the biological form of the species and their biodeteriogenic potential can help to characterize the plant species detected in historical sites.

1) M. Lisci, M. Monte, E. Pacini (2003) Int. Biodeterior. Biodegradation, 51, 1 – 17.
2) S. Pignatti (1982) Flora d'Italia. Edagricole, Bologna.
3) M.A. Signorini (1996) Inform. Bot. Ital.,28, 7-14.

5 = New update on ethnobotanical uses in southern Calabria (Italy)

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The word “Ethnobotany” comes from the Greek *Ethnos* which means "people", and *Botane* meaning "grass": so, literally the meaning would be "the study of people and herbs", which is usually generalized, as "the study of people and some plants". The term was coined in 1875 by the American botanist John Hashberger (Philadelphia, Pennsylvania-USA, 1869-1929) to indicate the study of the traditional use of plants by man. Subsequently, Schultes defined ethnobotany as "the study of the enhancement by man and the manipulation of plants and their derivatives" (1).

For a better comprension of the traditional knowledge and uses of wild plants, the “Herbarium and Geobotany Laboratory” of “Mediterranea” University since 2011 is studying the Calabrian territory (Southern Italy). By several excursions field, we interviewed several people, recording typical uses of wild and cultivated plants in the local tradition, for food, medicine, religion, magic, aroms, handicraft and more uses.

The investigated territories are located in the provinces of Reggio Calabria, Catanzaro and Vibo Valentia, but we are starting in the other provinces of Cosenza and Crotone.

The methodology used was the same used in the previous investigations carried by us (2,3). This study was conducted by interviewing local people with a semi-structured questionnaire and analysing bibliographic data (4,5,6,7,8). During the interviews realised in the field, the interviewers taken also photos and collected specimens of plants and, when possible, of the derivate products. With the collected specimens were prepared several herbarium samples collected in the herbarium of “Mediterranea” University of Reggio Calabria (REGGIO) and recorded in a specific database containing all the informations proceeding from the interviews.

With the interviews we recorded common and unusual plant uses. At the moment, more than 600 species belonging to more than 100 families having some use in Calabrian tradition are recorded.

In local food tradition, is very common to press capocollo (a typical pork cold cut – “capicollo” in local slang) with thin strips obtained from *Arundo donax* L. and then tied all around of the cut with young stems of several tree species (*Olea europaea* L., *Salix sp.*) (Fig. 1). *Euphorbia characias* L. is an upright, compact evergreen shrub growing in areas of Mediterranean scrub and garrigue, on arid and xeric soils, up to 1000 m of altitude. In several interviews, local people declared that euphorbia seeds were prepared in infusion and used as a laxative. Cladods of *Opuntia ficus-indica* (L.) Mill. were cut out to reproduce the parts of a cart that were then assembled with some small pieces of reed (Fig. 2).

Preserving all these knowledge (not only “information”!), we can contribute to a sustainable development for this southern Italian region. This work can represent a good pattern for similar territories that want grow economically and culturally. Only studying and understanding cultural heritage it will be possible to live good in the future.

Fig. 1. Capocollo ready for maturing

Fig. 2. Artisan car play with Opuntia ficus-indica cladod

1) C R. Evans Schultes, A. Hoffman, C. Ratsch (1979) Plants of the Gods, 210 pp.
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3) G. Spampinato, I. Paglianiti, C. M. Musarella (2017). Book of abstracts, IV IPSC, Parma 20-23/09/17
4) N. G. Passalacqua, P. M. Guarrera, G. De Fine (2007) Fitoterapia,78, 52-68
5) M. L. Leporatti, M. Impieri (2007) Journal of Ethnobiology and Ethnomedicine,3, 34
6) G. Tagarelli, A. Tagarelli, A. Piro (2010) Journal of Ethnobiology e Ethnomedicine,6, 27
7) N. G. Passalacqua, G. De Fine, P. M. Guarrera (2006) Journal of Ethnobiology and Ethnomedicine, 2, 52
8) S. Nebel, B. A. Pieroni, M. Heinricha (2006). Appetite (47): 333–3

5 = Mycofilm development on inorganic substrates: preliminary study

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Many fungi can survive and grow in high concentrations of organic pollutants and some of them are used in mycoremediation. Recently, there was an attempt to exploit fungi in wastewater treatments which, in this sense, can be defined as a type of mycoremediation *in situ* for organic pollutant. In our work, commercial hydrophobic hollow fiber polypropylene membranes were studied with the aim of verifying the adhesion and growth of micro-fungi possibly useful in different bioreactor for the degradation of organic substrates in wastewater. A thin culture medium was added on a membrane before fungal liquid inoculum to favour the mycofilm development. Little it is still known about the adhesion of culture medium on tubular polypropylene membranes, therefore we studied the culture medium at different density, changing temperature and agar percentage.

Fig. 1 MEA starter on membrane, with and without methylene blue as dye

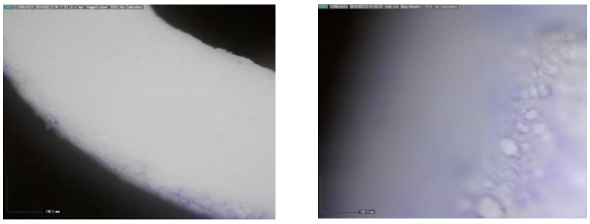

Fig. 2 MEA starter on membrane with methylene blue as a dye under microscope

We chose a culture medium with agar 0,5% in a temperature range of 30-40°C to prove adhesion of two common
micro-fungi: *Penicillium expansum* Link and *Trichoderma harzianum* Rifai group. The membrane was firstly immersed for 30 seconds in MEA (0,5% agar) and after in sterile solution with inoculum of each specie. In figure 3 shows the membrane immersed in sterile water after the fungi growth. The mycofilm on membrane is uniform and the fungi isolated from each membrane do not present contamination.

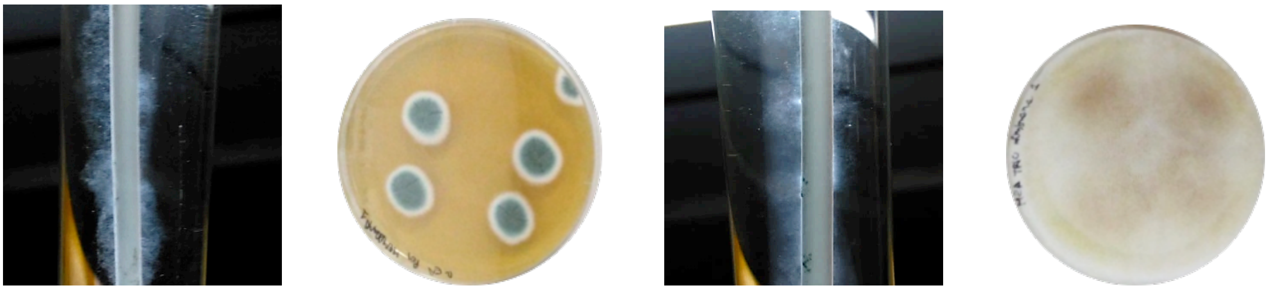

Fig. 3 *P. expansum* and *T. harzianum* on membrane and petridish, respectively

This preliminary study demonstrates that it is possible to develop bioreactors with mycofilm because the fungi can grow on the membrane surface in aquatic medium. The tested fungi show a different adherence on membrane and difference ability of resistance in aquatic medium.

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5 = DNA fingerprinting of extra-virgin olive oils as a tool to protect and enhance the value of typical Italian productions

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Due to the globalization of food markets and the increased variability and availability of food products from other countries, consumers are increasingly interested in knowing the genetic identity of the material from which the transformed products have originated. The European Union (EU) harmonised rules on food labelling, presentation and advertising aim to protect consumers and facilitate trade inside and outside Europe. Recently, an initiative of the European Parliament has identified a number of foods including olive oil, as being the target of fraudulent activities.

Olive oil is the product of the olive tree (*Olea europaea* L.) fruits and differs from most of the other vegetable oils in the method of extraction. It can be consumed in crude form, hence conserving its vitamins and other natural healthy high-value compounds. At international level, olive oil quality is regulated by the International Olive Council (IOC) trade standard, Codex Alimentarius at EU level by Regulation (EEC) n° 2568/91, that establish a list of chemical and organoleptic characteristics, as well as methods for their analysis. Adulteration of extra-virgin olive oil (OEVO) is frequent and occurs not only by accidental contamination during the stages of oil processing, but it is an act of deliberate addition of less expensive olive oils by fraudsters for financial profit.

It is well known that climate, soil, cultivar and time of harvest contribute to determine the different organoleptic properties of olive oils. The authenticity of olive oil, especially the OEVO, has been extensively studied by using several analytical approaches such as chromatography, stable isotope analysis, spectroscopy and nuclear magnetic resonance [1, 2]. Recently, non-conventional methods based on DNA analysis have gained attention due to their high specificity, sensitivity and accuracy to detect the varietal origin of olive oil as well as the botanical origin of plant oils [3-5]. These methods also aimed to trace the chain of oil production and marketing. Rotondi and collaborators (2011) [6] have performed olive oil traceability by the combination of the chemical and sensory analyses with SSR biomolecular profiles.

Starting from these assumptions, the project *Olio extra vergine d'oliva Digital Id maNagement – ODIN*, linking the genotype/s to environment, is aimed to define a model of easy application for the enhancement of the OEVO of high quality, produced by the medium / small size farms of the Calabria region, which have an interest in enhancing their product. The proposed model intends to guarantee the traceability of OEVO, using data and information uniquely ascribable to the farm and to the oil produced. This approach starting with the study of the characteristics of the manufacturers and the cultivars used for the production of the OEVOs, to elaborate a code that will identify the bottles acquired by the final consumer, providing the latter with guarantee of origin and quality. The olive germplasm present in the Calabrian farms involved in the project and the OEVO authentication will be obtained through SSR molecular marker. The molecular profiles obtained by the selected genotypes and the oils produced will be associate. The OEVOs will be also subjected to sensory analyses through the use of a panel of tasters that will certify the most significant organoleptic parameters. Finally, a document traceability method will be put in place, which can also be used for the certification of quality and / or certification of the supply chain. Based on the information acquired in all the phases of the *ODIN* project, a software platform will be developed for the traceability, the monitoring and the identification of the products.

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5 = *In vitro* tissue culture and plant regeneration of the alpine endemic *Tephroseris balbisiana* (Asteraceae)

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The plant: its habitat and range

Tephroseris balbisiana (Dc.) Holub (Asteraceae) is a tall broad-leaved forb –*megaforbie*– that lives along the banks of the mountain streams on soils with a high nutrient status and well supplied with moisture fed by snowmelts. Seeds consist of 3-3.5 mm long achenes with pappus and disperse by wind and water. *T. balbisiana* is an endemic orophyte of the South-western Alps characterized by a disjointed distribution. The main range is centered in the Mercantour-Argentera Massif between the French department of Alpes-Maritimes and the Ligurian and Maritime Alps. Numerous stations are documented for the southern valleys of Piedmont and a few populations are located northernmost in the Po Valley, Cottian Alps.

The species status and the rationale of the project

The species is well protected since most of the populations are located in the Parc national du Mercantour and in the Parco delle Alpi Marittime (CN) in Italy. Nonetheless, the stability of these stations is strictly dependent on the status of conservation of the hydrological network of this region. Recent surveys on the Italian territory by the Staff of the Park have shown that in Italy the population density and number of *T. balbisiana* are declining. This protocol for the *in vitro* plant regeneration of *T. balbisiana* is part of a project of the Ente di gestione delle Aree Protette delle Alpi Marittime aimed at improving the conservation status of the species and habitats within the Natura 2000 Network. The research was financially supported by the Italian Rural Development Program (2014-2020).

Seed germination and in vitro culture preparation

Seeds of *T. balbisiana* were collected in Valle Gesso (CN) at an average altitude of 1730 m a.s.l. (Fig. 1). They were germinated in axenic conditions at 23±0.5°C and 8/16h dark/light photoperiod within 14 days of incubation. Cotyledons, hypocotyls and apical shoots were cut transversally, and cultured on a MS medium supplemented with benzyladenine (BA) and α-naphthalene acetic acid (NAA) at five different levels (hereafter indicated as medium variants). Number of calluses and axillary buds were recorded and expressed as absolute and relative percentages.

Main results of the in vitro cultures

After 37 days of culture, the explants produced both callus and axillary buds. To evaluate significant differences among the medium variants and the types of explant, one/two-way ANOVA analysis were conducted at a 0.05 significant level with the test of Tukey for means comparison. After 60 days of incubation, BAP/NAA 4.4/0.54µM stimulated the production of callus in 54.3±1.1% and 65±1.9% of the hypocotyls and cotyledons, respectively (ANOVA, F-ratio = 12.1, P= 0.0000); much less callus was produced in general by the shoots. This medium variant induced also a significant higher percentage of explants with axillary buds in hypocotyls and cotyledons (48.7±6.0% and 35.0±4.1% respectively; F-ratio = 19.6, P= 0.0000). The % of cultured shoots producing on average one new bud was between 45.9–62.5%, however no significant differences among medium variants were observed in this explant. On average, 36.8-60.7% of the budding explants developed from 5 to 8 new buds with no significant differences among the explants. A significant higher number of axillary buds/explant (9-11 buds) was produced only in the hypocotyls (48.6±3.9% of the explants) (ANOVA, F-ratio = 51.0, P= 0.0000). These results indicate that BAP/NAA 4.4/0.54µM is the best medium variant for the *in vitro* production of axillary buds of *T. balbisiana* only in the case of hypocotyl and cotyledon cultures. Hypocotyls are to be preferred to cotyledons at this purpose.

Fig. 1 (left) A sight of the habitat of *T. balbisiana* in Valle Gesso (CN). (right) 60-day-old plants grown in pot in the greenhouse of the botanical garden of Torino obtained from the *in vitro* cultures.

5 = Drupe development in different species of *Prunus* (L.)

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The genus *Prunus* (L.) is widely cultivated in Europe and comprise species with an important commercial role, used both for food and for rootstock.

Ovaries of these plants consist of a monostratified epidermis, an external hypodermis, a parenchyma, an internal hypodermis and an internal epidermis; the fruit is a typical drupe.

The aim of this work was to investigate if, in *Prunus* species, the fruit size was related to ovary dimension. The mechanisms driving drupe differentiation has been investigated in different species such as *Olea europea*, in which fruit weight was related to ovary size: bigger fruit derived from bigger ovaries [1].

Moreover, previous work about ovary abortion in olive demonstrated a correlation between starch storage and ovary development. So, we analysed different species and cultivar and measured ovary wall dimension, mean cellular area, and numbers of cellular layers. In fruits, we measured the fruit wall dimension, mean cellular area, and numbers of cellular layers. We investigated also starch accumulation to verify its role in *Prunus* fruit development.

Our data highlight no correlation between ovary and fruit dimension: cultivar with bigger ovaries did not produce the biggest fruits. Moreover, the major role in fruit growth seemed to be played by cellular distension rather than cellular division. Even starch deposition seemed to be not correlated with fruit development.

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5 = Biological control at the Florence Botanical Garden: It can be done!

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During the last two years the Botanical Garden of Florence has chosen to stop using chemical products for plant protection, in favour of biological control: the use of natural substances and useful insects to fight pests. The main reason was to protect, besides plants, both gardeners and visitors too. Obviously, the transition from the use of traditional chemical products to biological ones has been a road full of difficulties and today it still represents a daily challenge.

Unfortunately, in such a diverse environment and with several plant species it is practically impossible to standardize the control of individual pests, as often desired by the gardeners. So, a costant monitoring is necessary so that control measures to can be taken promptly to keep pest populations below the damage threshold.

Excellent results were achieved against Aphids, present in various plant collections, through the release of *Chrysoperla carnea*’s larvae. In a few days, this predator managed to eradicate the most numerous colonies of aphids, especially on ferns and *Philodendron*. The activity of *C. carnea* was helped by that of specific parasitoid wasps, particularly useful in small greenhouses.

Mealybugs are currently the most harmful insects to the plants of the Botanical Garden (especially to the several tropical plants present in the greenhouses): mealybugs live in the most shaded and moist parts of the vegetation, thus remaining sheltered and not easily spotted. The biological control of mealybugs begins with treatments with mineral oil and azadiractin (Neem oil), followed by washing of the vegetation with potassic soap. Then, several larvae of *Cryptolaemus montrouzieri*, a predatory coccinellidae, are released. In a few months they have led to a significant reduction of mealybugs populations.

The management of Thrips has also been a success: for years these insects have been a major problem for plants. The remarkable effectiveness of spinosad, combined with the action of natural enemies such as the anthocorid *Orius laevigatus* and the mite *Ambliseius swirskii*, have allowed long-lasting results. Furthermore, a permanent monitoring network has been set up with blue adhesive traps.

In conclusion, the results obtained so far are very promising, excellent in some cases, even though some cases still need improvements. Undoubtedly, the continuation of biological control would most likely restrain the presence of some harmful insects below the damage threshold.

Specific training of personnel will be required, both on botany and plant defense, so as to be ready to act at the right time, thus reducing costs.

Hence, the Botanical Garden of Florence needs an expert able to plan and coordinate biological control activities for the coming years.

Fig. 1A Box with natural enemies

Fig. 2B *Cryptolaemus montrouzieri*

Fig. 1C Parasitoid dispenser

5 = Morphological and physiological responses of *Arabidopsis thaliana* L. mutants to filtered light and N starvation

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Plants are sessile living organisms and to ensure their survival, fitness and productivity, they have developed sophisticated mechanisms to adjust to environmental inputs. For instance, in response to light and nitrogen (N) availability, plants are capable to change their morphological and physiological traits (1). N is one of the main elements for plants, as a key component of many macromolecules, such as proteins, nucleic acids and secondary metabolites. Because in nature the N availability is often not uniform, to satisfy their daily N requirement, plants respond to N variations by altering uptake and recycling mechanisms. Similarly, light is the predominant natural input driving plant growth and development, and plants are able to photosynthesize by perceiving and transducing light signals as well. Among several types of photoreceptors, phytochrome A (phyA) and phytochrome B (phyB) play key roles in the red (R) and red/far-red (R/FR) modulated responses. Although there is certain redundancy between different phytochromes, they also have distinct light sensing properties. In addition, the signalling capacity of the photoreceptors is linked to many transcription factors, among which the bHLH-Phytochrome Interacting Factors (PIFs) and Long Hypocotyl 5 photomorphogenesis-promoting factor (HY5) play key roles. Mechanistically, for several responses PIFs act antagonistically to HY5: HY5 driving photomorphogenic development that is supressed by PIFs that maintain skotomorphogenesis (3). Indeed, the detailed molecular links between phyB, phyAB, PIFs and HY5 and N metabolism are still unknown. However HY5 is known to positively regulate the expression of some genes involved in N transport and metabolism, in response to light (4). Thus, our aim is to investigate the effects of different illumination regimes and N starvation conditions on the morpho-physiological growth of *Arabidopsis thaliana* mutants, and to identify the factors linking the light-induced development with the response to N availability. We studied *Arabidopsis phyB*, *phyAB*, *hy5* and *pifQ* (*pif1-1*; *pif3-3*; *pif4-2*; *pif5-3*) grown in hydroponic condition with and without N starvation treatment, in combination with different light intensities, to investigate morphological and physiological traits related to seedling establishment such as a hypocotyl and root elongation, leaf area, fresh weight and chlorophyll content.

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5 = Effect of stand structure, topography and climate on vascular plants and epiphytes in mountain beech forests of northern Apennines (Italy)

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Forest management, topography, and climate are among the main factors driving biodiversity patterns in forest ecosystems. However, their relative importance may depend on the organisms that are considered, and potentially provide contrasting patterns among taxonomic or functional groups exploiting different resources. In this study, we tested the effects of stand structure (indicative of forest management), topography and climate on species richness and composition of vascular plants and epiphytes (lichens and bryophytes) in mountain beech forests located in the Foreste Casentinesi National Park (Northern Apennines). Fifty-five 20 m x 20 m plots and 220 trees were surveyed in 10 unmanaged (abandoned coppices) and 10 managed (coppices in conversion to high forests) stands with comparable range of topographical, climatic conditions, age and past management history until the ‘60s. Significant differences in species richness and composition between unmanaged and managed stands were found only for vascular plants. These latter showed higher species richness in the managed stands, a result largely due to generalist species. Considering the specific structural, topographic and climatic characteristics of each plot, regardless of the treatment (managed/unmanaged), species composition of vascular plants was mostly influenced by the time since the last intervention, the canopy closure and the topography. The response of bryophytes suggested a main influence of microclimatic conditions (light, temperature and humidity), while lichens mainly responded to stem diameter, stand maturity and climatic factors. The contrasting results among different organism groups support the importance of the multi-taxon approach for improving the conservation effectiveness of forest biodiversity in a climate-change scenario.

5 = Monitoring of shrub and larch encroachment in a subalpine grassland: a new methodological approach

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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 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. 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 (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 the same year (August 2015) and three years before (October 2012) 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 larches distribution followed a patchy pattern with areas of significant higher similarity in density and dimensions. Moreover, the comparison between methods showed that photo-interpretation underestimated the number of larches, but had good performance for shrubs cover estimation.

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 seeds germination. Finally, the efficiency of phot-interpretation of drone images showed that this method might be a good alternative to vegetation survey of wide areas, although an improvement of this method is still needed.

5 = Lack of vegetation-plots in restoration ecology: the case of a rehabilitated site in a dune system

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Vegetation plots are commonly used as fundamental tool for comparison analyses with nearby sitesin the evaluation of the recovery process of a rehabilitated site in restoration ecology. Then,what to do when vegetation plots are not available? Are there equally fruitful techniques that can replace this lack? The intent of this study is to report a study case which examines the integrity at fine-scale of a dune environment, that is among the most threatened habitat in Europe (1), in central Italy (Duna Feniglia Natural Reserve, Fig. 1). The selected study area had to undergo rehabilitation interventions 100-year time ago for side reasons, by means of pine afforestation and dune species plant activities (Fig. 2). Thus, we had a perfect scenario to study fine-scale species composition recovery on a dune system in the Mediterranean context. Moreover, the Feniglia Dune Natural Reserve has already been the subject of a previous study carried out on the same rehabilitated site through the use of vegetation plots, investigating dune habitats with herbaceous or shrub vegetation (2). In the present study, the ecological quality of the restoration was assessedthrough the analysis of the vascular flora (complete species inventory) of the rehabilitated site and species-related attributes (growth form strategy, distribution range, endemics, Ellenberg indicator values). Equal attributes for the whole floristic inventories of nearby coastal sites under similar environmental conditions but with different degrees of disturbance – low, medium, high – were used in a complementary manner. We primarily measured analogies and dissimilarities by means of SDR-simplex analysis (3). In addition, we calculated a parameter for estimating the average species distinctiveness in a community using taxonomic singularity (4). Secondly, we used biological and chorological spectra, Ellenberg indicator values, diagnostic dune species (5) and alien species for the Italian flora according to Galasso et al. (6) with the intention to underline ecological analogies and dissimilarities. All our analyses revealed an overall fine-scale species composition more similar between the site rehabilitated 100 years ago and the most natural one considered, than between all the other pairwise combinations. Differently to alien species, EU diagnostic speciesare present in the rehabilitated site indicating impressive recover in one century. Our results are similar to those of the previous vegetation study (2). Thus, we demonstrated that species-related attributes are valid indicators of the overall ecological status and can be used as a useful surrogate by means of comparisons with nearby sites, showing that the most natural site considered is the most similar to the rehabilitated one, as found by the vegetation plots approach study.

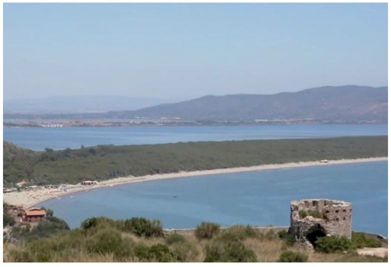
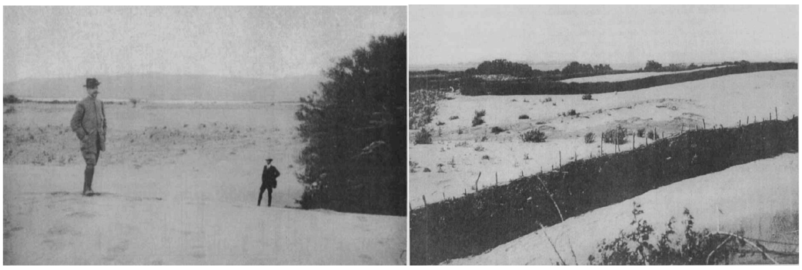


Fig. 1 The Duna Feniglia Natural Reserve



(a) (b)

Fig. 2 State of the Duna Feniglia before the beginning of the rehabilitation Reserve (a) and example of defence works (b)

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5 = A database of alimurgical plants in Italy

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The rediscovery of popular culture has become a scientific investigation, but also a preservation and an enhancement of the local traditions and of the "popular knowledge". This cultural context, arisen in the last decade, has exponentially increased the interest in food consumption of Wild Edible Plants (WEP).

The high consumption of fruit and vegetables, as a characteristic of the Mediterranean diet, has traditionally included wild fruits but especially wild vegetables, as reported by many ethnobotanical studies (1, 2, 3).

These WEP, also known as alimurgical plants, have in the past played an important historical role in integrating and enriching the diet based on basic agricultural food (eg 4, 5), considered an integral part of the Mediterranean basin diet (6, 7).

Although many species considered alimurgic are widespread throughout the Mediterranean basin, only few species (about 30) are currently used in the human food comsuption (2).

The creation of a database of the Italian alimurgical flora has therefore the aim to systematize the baggage of the extensive (and often disordered) knowledge on the WEP acquired in Italy in the last 100 years, creating an easy-to-read tool not only for purely speculative purposes but also for the practical ones (eg cultivation, presence of active compounds, etc.).

The design and population of the database have been performed by means of the discovery and the analysis of bibliographic resources from 121 ethnobotanical and phytoalimurgical works published in Italy since 1918 to these days. For each of the 465 alimurgic species considered and reported in the database, 95 fields related to taxonomic, morphological, geographical and food use characteristics were considered.

The organization of this database and its internal structure will allow the retrieval of information through the online query, an evidence-based information resource, which will be available in the near future and useful for those scientific communities that will want to take advantage of it by having of a shared data set.

The aim of this study is, in short, to provide an interesting tool to be used with the aim of increasing in value the alimurgical species - not commonly or rarely used - but considered of a great nutritional and organoleptic value. The study presumes for the WEP, also the creation of a production chain (from population research to cultivation and marketing).

This research protocol, started with the creation of this database, is an integral part of a currently in progress PhD, which deals specifically with 5 alimurgical species (*Sonchus asper* (L.) Hill, *S. oleraceus* L., *Crepis vesicaria* L., *Tragopogon porrifolius* L. subsp. *porrifolius*, *Blitum bonus-henricus* (L.) Rchb.).

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5 = Revision of *Parmelia saxatilis* group in Italy on the basis of morphological, chemical and molecular evidence

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This work is a preliminary study for the systematic and taxonomic revision of the Italian specimens of the *Parmelia saxatilis* group, by means of morphological, chemical and molecular evidences. Two new species, *Parmelia ernstiae* Feuerer & A. Thell (1) and *Parmelia serrana* A. Crespo, M.C. Molina & D. Hawksw. (2), have been recently described for Europe within the *P. saxatilis* group. Both of them differ from *P. saxatilis* (L.) Ach. (3) only by micro-characters, visible at the microscope only, and by a more in-depth investigation on a chemical and molecular level (2). In the last decades, several new findings of *P. ernstiae* and *P. serrana* were confirmed for Europe. Nevertheless, their presence and distribution in Italy was not confirmed yet. The aims of this study are: i) implementation of a key for the identification of the taxa of the *Parmelia saxatilis* group, and ii) revision of *P. saxatilis* group in Italy, based on morphological, chemical and genetic characters.

Sixty-seven Italian specimens of *P. saxatilis* s.l., present in the Central Italian Herbarium of Florence, and 18 specimens collected in the field were used. The morphological analysis was carried out by means of a dissecting microscope, and an identification key was created. The 85 specimens were analyzed with the thin layer chromatography technique, (T.L.C.) (4). Ten samples were identified as *P. serrana* (3) and *P. ernstiae* (7). The morpho-chemical results were investigated with molecular analysis using two molecular-barcode markers ITS and β-tubulin.

In this study, we report new findings of *P. ernstiae* (12) and *P. serrana* (1), which are added to previous reports for the country (5, 6, 7).

Morphological and chemical analyses have proved to be useful for identifying cryptic specimens. However, the molecular approach was crucial to confirm the identification. Further investigations on larger and more representative samples are needed, in order to fully understand the distribution and ecology of *P.serrana* and *P.ernstiae* in Italy.

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5 = Effect of *Betula etnensis* Raf (Betulaceae) extract on breast cancer cells treated with adrontherapy

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Betula etnensis Raf is a medium-sized deciduous tree, typically reaching 5–20 m tall, which belongs to Betulaceae family. It is commonly known as Birch Etna and grows on the eastern slope of Etna, at an altitude between 1200 and 2000 m (1, 2). The bark is ivory-colored and there are numerous pelted resinous glands on the young branches. The bark of *B. etnensis* contains polyphenols, tannins, pentacyclic triterpenoids, such as betulin, betulinic acid and ursolic acid that showed antioxidant, antitumor and antiviral activities (3, 4). Betulinic acid and some of its most water-soluble derivatives *in vitro* resulted cytotoxic for neuroblastoma, melanoma, medulloblastoma and Ewing's sarcoma cells. Recently, there is a growing interest on flavonoids and triterpenic esters and their synthetic derivatives because of their possible applications in antitumor chemotherapy as anticancer and anti-inflammatory agents (5). It has been highlighted that conventional therapies (surgical intervention, chemoterapy, radiotherapy and endocrine therapy) can be associated with natural compounds in order to both enhance their therapeutic effects and improve the quality of life of cancer patients. Thus, the present study evaluated the effects of *B. etnensis* bark alcoholic extract on a human breast cancer cell line (MCF-7) after treatment with adrontherapy, an innovative technique that, using the same doses of conventional radiation therapy and the particle beams, targets the tumor cells in a targeted and precise manner, preserving healthy tissues. In the present research, we analysed some biochemical parameters (lactic dehydrogenase, non-proteic thiol groups, ROS, sirtuine levels) that allowed us to evaluate and to better understand the combined effect of *B. etnensis* and adrontherapy. MCF-7 cells were pretreated for 24 h with *B. etnensis* bark alcoholic extract (50 mg/ml, 100 mg/ml) and irradiated with ionizing radiations (2-4-6-9 Gys), proton beams at 62 MeV, in collaboration with the hadrontherapy facility of the Laboratori Nazionali del Sud – Italian National Nuclear Physics Institute (INFN). Obtained results showed that the combination of adrontherapy with *B. etnensis* bark alcoholic extract treatment significantly reduced cell viability of MCF-7 cells, inducing necrotic cell death in a concentration-depending manner. Moreover, results by clonogenic assay demonstrated that exposure of cells to the extract potentiated antiproliferative effects of ionizing radiations. Our results suggest that reduced cell viability is associated with necrotic cell death, also due to increased ROS levels although no significant modification in RSH levels was observed. In addition, an exposure of MCF-7 cell culture to different doses of adrontherapy induced a significant and dose-dependent increase in the activity of the nuclear SIRTs and the effect was more pronounced in cells pretreated with *B. etnensis* bark alcoholic extract.

These results confirmed the ability of *B. etnensis* to induce radiosensitizing effects that could have important therapeutic implications related to damage induced in tumor tissues of patients treated with radiotherapy.

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5 = Ethnobotanical investigation on the traditional uses of wild plants in the Kavre district, Kathmandu valley (Eastern Nepal)

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This study aims to document the traditional uses of plants from the Kavre District of the eastern part of Nepal, particularly among Tamang communities living in the rural villages of the hills (1000-2000 m) around Kathmandu Valley. Until now, only very few studies have been carried out on the Traditional Ethnobotanical Knowledge (TEK) of this district (1,2). The research seeks to understand which are the most important plants from the therapeutic point of view, with an antioxidant, antibacterial and antifungal value, as well as plants used in the care of farm animals. Such heritage of lore and natural resource must be preserved in the prospect of a sustainable economic development. The first step of the field research was conducted in hilly rural areas of Kavre District (Patar and Timal areas) among Tamang people (October 2016 and November 2017). According to the testimony of the elderly, in these rural areas no ethnobotanical research had been conducted before. We have collected data through informal and semi-structured interviews with shamans, local healers, plant traders and knowledgeable villagers, regarding local plant names and their traditional uses. Voucher specimens of plants were deposited at Tribhuvan University, Kirtipur, Kathmandu. Data showed the traditional uses of 55 plant species, belonging to 40 families. Of these, 36 plant species are used for medicinal purposes to treat 24 ailments (Fig. 1). The most represented families are Asteraceae and Lamiaceae. We have verified that some plant species are poorly documented by current literature on traditional medicine in Nepal. The indication for use, mode of preparation, dose, and administration of medicine are described for each species. Most common portions, used in these practices,were whole plants and leaves, followed by roots. The remedies were generally prepared by cold pressing with stone mortars, using hot or cold water as solvent. The different types of preparation included paste, decoction, infusion, while the routes of administration were mainly oral, topical, and nasal. The results reveal that a wealth of ethnobotanical knowledge still persists in rural areas of the Kavre District. However, due to land use change and migration, this ethnobotanical knowledge needs to be preserved for the future.

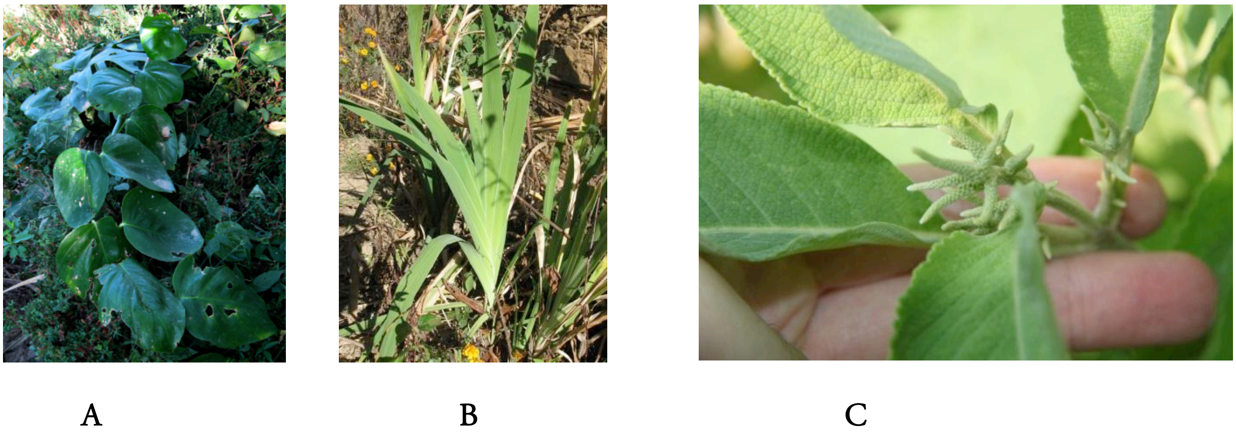

Fig 1. Some species of medicinal plants used in Kavre District. (A) *Raphidophorahookeri* Schott (Araceae), used for female fertility; (B)*Belamcandachinensis* (L.) DC. (Iridaceae), used for gastric disorders; (C) *Colebrookeaoppositifolia*Sm. (Lamiaceae), used for treatment of wounds and fractures (leaves) and for gastric problems (inflorescences).

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5 = Traditional use of plants in the Cogne valley (Gran Paradiso National Park, Aosta Valley, Italy)

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Most of traditional knowledge about plants and their uses is fast disappearing as a consequence of socio-economic and land use changes. This trend is also occurring in areas that are historically exposed to very few external influences, such as the Aosta Valley (Italy). From 2017 to 2018, an ethnobotanical investigation was carried out in the territory of the Gran Paradiso National Park (PNGP), an area of high naturalistic interest. The study aimed to collect new data from traditional knowledge on plant uses in Italian alpine areas, specifically in the Cogne valley. Mountainous valleys, due to their partial geographical and socio-cultural isolation, represent an ideal breeding ground for ethnobotanical research (1, 2, 3, 4). Typical traditions representative of the original population are still preserved in these areas, being scarcely affected by external influences. In addition, the existence of a Natural Park in the studied area, has allowed the safeguard and conservation of typical and endemic plant species.

Ethnobotanical data were gathered through questionnaires, extensive dialogues and semi-structured interviews with 20 native informants (10 men, 10 women), who had personal experience in the use of herbs. In these interviews, informants were requested to indicate vernacular names of plants, parts of the plant used, folk uses and preparation procedures. The final database included 1038 reports concerning 151 botanical species, which were used for medicinal (44%), food (33%), veterinary (8%), forage (3%), domestic (4%) and liquor making (7%) purposes.

The species (including 6 mushrooms and 1 lichen) belong to 62 plant families, of which Asteraceae (13%), Rosaceae (7%), Poaceae (6%), Lamiaceae (5%) and Apiaceae (5%) are the most represented. Collected data mainly referred to wild taxa, while cultivated or traded plants were infrequent (wild 82%, cultivated 21%, purchased 3%). Medicinal plants were used to treat 14 ailment categories, based on the International Statistical Classification of Diseases and Related Health Problems by the World Health Organization. Most species were used to treat respiratory (20%), digestive (17%), skin (14%), musculoskeletal (10%) and genito-urinary (10%) diseases. Most used parts were leaves, followed by fruits, flowers and roots. Major herbal preparations included infusion, decoction, syrup, poultice and maceration. Data were evaluated by quantitative parameters, such as Ethnobotanicity index (EI), Relative Frequency of Citation (RFC), Factor Informant Consensus (FIC), Fidelity Level (FL). Most cited species were *Peucedanum ostruthium, Juniperus communis,* and *Urtica dioica,* while relevant uses were also reported for some conifers (*Pinus cembra, P. mugo, P. sylvestris, P. abies, Larix decidua*), and in addition for *Viola calcarata, Tussilago farfara, Cetraria islandica,Artemisia genipi* and *Achillea herba-rota.*

The results show that the traditional knowledge of plants and their use are still alive in the Cogne Valley . However, many practices are no longer in use, only surviving as memories from the past in elderly people. This rapidly vanishing cultural diversity needs to be studied and documented before it disappears definitively.

Figs 1 - 4 Most reported medicinal, edible, liquoristic species: syrup of *P. sylvestris* (fig. 1). *P. ostruthium* leaves and roots (fig. 2). Edible plants: *P. bistorta, U. dioica, T. officinalis* (fig.3). Artisan distillery (fig. 4)

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5 = Ghirardi Botanic Garden, a "factory" of molecules

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Botanic gardens are primarily experienced as environments where the plants often lose their identity to give rise to a whole that welcomes visitors. Adopting a different inquiring approach, it turns out that each plant is different from the other. The presence of several species native of different countries attests that botanic gardens are primarily places devoted to preserve plant biodiversity. Another point of view is to perceive botanic gardens as *factories* of molecules, that means evaluating their characteristics at a higher level than that visually described. Indeed, each plant communicates with the environment synthesizing and emitting secondary metabolites. The aim of this project is to investigate botanic gardens under this novel perspective, as *factories* of molecules.

In this context, we planned a multidisciplinary research project on a pool of selected medicinal species preserved at the Ghirardi Botanic Garden (Toscolano Maderno, Bs) on the basis of their productivity in volatile compounds (VOCs), essential oils (EOs), epicuticular depositions and substances stored at tissue level. This heterogeneous set of molecules drives multiple functions for the plants themselves, and consequently for the ecosystem and the environment. The project involves multiple-scale analyses: *(i)* micromorphological description of the secretory structures; *(ii)* chemical characterization of the secondary metabolites; *(iii)* ecological study of the biotic interactions mediated by such chemicals; *(iv)* evaluation of their biological activity to assess their potential application in human nutrition as well as for therapeutic or cosmetic sectors.

As preliminary steps, we addressed our attention on the morpho-anatomy of the secretory structure and on the phytochemistry and antibacterial activities of the EOs obtained from the aerial parts of six target-species: *Cinnamomum camphora* (L.) J.Presl., *Laurus nobilis* L., *Myrtus communis* L., *Eugenia uniflora* L., *Santolina chamaecyparissus* L. and *Citrus japonica* Thunb.

The structures responsible for oil production and accumulation were found to be non-differentiated cells (Lauraceae), glandular hairs (Asteraceae) and secretory ducts (schizogenous in Myrtaceae and schizolysigenous in Rutaceae).

The analysis of EO composition allowed the overall identification of 165 different compounds, with the highest number detected in *S. chamaecyparissus* (72) and the lowest in *M. communis* (31). Oxygenated sesquiterpenes represented the most abundant chemical class in *C. japonica* and *E. uniflora*, whereas oxygenated monoterpenes dominated in the other examined species. Two common compounds were found in all the species: a-pinene and myrcene, most abundant in *M. communis* (39.0%) and in *S. chamaecyparissus* (3.7%), respectively. As a whole, a high level of chemical variability was found across the EOs, with *S. chamaecyparissus* exhibiting the most complex profile due to the occurrence of the highest number of exclusive compounds, accounting for 58.3% of the total.

The EO antimicrobial activity was evaluated towards *Staphylococcus aureus* ATCC 6538, *Escherichia coli* ATCC 15325, *Salmonella enterica thiphymurium* ATCC 14028, *Lysteria monocytogenes* ATCC 7644, *Enterococcus faecalis* V583E and *Pseudomonas aeruginosa* ATCC 27853. The most active oils resulted *S. chamaecyparissus* EO against *L. monocytogenes* (MIC 1:256 v/v) and *P. aeruginosa* (MIC 1:256 v/v) and *C. japonicum* EO against *S. aureus* (MIC 1:256 v/v) and *L. monocytogenes* (MIC 1:512 v/v).

These preliminary results were discussed in the light of the most recent literature contributions.

5 = Anticancer properties of *Myrtus communis* L. (Myrtaceae) berries: preliminary studies on breast cancer cell line MCF-7

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Myrtus communis L., belonging to Myrtaceae family, that includes around 100 genera and 3000 species, is an evergreen, perennial, aromatic shrub, from 1.5 to 3 m tall, with an erect stem and branches forming a full head, densely covered with dark green, leathery, twinned leaves. The flowers are white, fragrant and solitary, up to 3 cm in diameter, carried by long peduncles which in November show little oval berries with a dark blue epicarp and a tiny white pulp with a harsh and resinous flavor (1, 2). *M. communis* grows wild all around the Mediterranean regions and its therapeutic properties are known since before the ancient Greeks. Extracts and macerated of this plant have been largely used for the treatment of several diseases such as gastric ulcer, diarrhea, inflammation, anxiety, insomnia, diabetes, hypertension, pulmonary disorders, and skin diseases (3). The plant contains a number of biologically active constituents (phenolic acids, tannins, flavonoids, glycosides, and terpenes) with extensive pharmacological activities, including antioxidant, anti-inflammatory, and anticancer activities, among others. In particular, previous studies showed that compounds from *M. communis*. induced apoptosis and drastically reduced the viability in breast cancer cell lines with marginal cytotoxicity for non-transformed 3T3 fibroblast cell line (4). In order to achieve new insight into therapeutic activity and anticancer properties of *M. communis*, in the present study we evaluated the effects of *M. communis* berries ethanolic extract on a human breast cancer cell line (MCF-7) treated for 24 h with 50 mM, 75 mM, 100 mM myricitrin.

High performance liquid chromatography (HPLC-PAD analysis) was used to characterize and quantify phenolic compounds in *M. communis* berry alcoholic extract and determination of total phenolic and flavonoid contents was also performed. Cell viability was assessed using 3-(4,5-dimethyl-2 thiazoyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay and levels of reactive oxygen species (ROS) were determined by 2',7'-dichlorfluorescein-diacetate (DCFH-DA). Furthermore, LDH release, GSH content and radical scavenging activity (DPPH test) were also evaluated. By using nuclear extracts, SIRT activity was also measured. Members of this protein family are implicated in the development of many tumors, including breast cancer (5). Here, obtained results showed that berry extract significantly reduced cell viability of MCF-7 in a concentration-depending manner, inducing necrotic cell death already at 50 mM. Moreover, the extract was able to significantly reduce ROS levels and to increase GSH content at 50 mM and 75 mM; on the contrary, however, a dose 100 mM increased ROS level and depleted GSH levels. The evaluation of SIRT nuclear activity allowed us to confirm the hypothesis of sirtuin involvement in the cytotoxicity of the extract. Exposure of MCF-7 cell cultures to different doses of *M. communis* L. berries induced a significant and dose-dependent increase in the activity of these nuclear enzymatic proteins at 50 mM and 75 mM; conversely, at 100 mM the SIRT nuclear activity is significantly reduced. These interesting results may represent a stimulating point to deeper investigate the chemotherapeutic properties of *M. communis* L. berries extract in cancer therapy.

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5 = Anti-proliferative activity of *Thymelaea hirsuta* (L.) Endl. (Thymelaeaceae)

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Thymelaea hirsuta (L.) Endl. is a plant species belonging to Thymelaeaceae family, widespread in the Mediterranean region. It is a perennial, evergreen, shrub, growing up to 2 m tall. The leaves are scale-like, ovate to oblong and leathery. The stems are densely-branched and covered with small (2-6 mm long) overlapping leaves, which are adpressed against the stem. This plant is used in the traditional medicine of Tunisia as anti-inflammatory, anti-septic and anti-hypertensive (1). Several studies demonstrated its antioxidant, hypoglycaemic, and anti-melanogenesis potential activities (2-4). Phytochemical investigations showed the presence of daphnane diterpenes as main constituents (4, 5).

Cancer is a leading cause of death worldwide, accounting for 8.8 million deaths in 2015 (6). Approximately 70% of deaths from cancer occur in low- and middle-income countries. The most common cancer sites were cancers of the female breast, followed by colorectal, prostate, and lung. These four cancers represent half of the overall burden of cancer in Europe. The use of natural products alone or in combination with conventional anti-cancer agents has been shown to produce beneficial effects through actions upon multiple molecular pathways.

This work aimed to study the *in vitro* cancer cell growth inhibitory activity of four extracts of *T. hirsuta*.

The aerial parts of *T. hirsuta* were collected in February 2011 in Calabria (Southern Italy), Sovereto (Isola Capo Rizzuto, KR) at 5 m a.s.l. and authenticated by Dr. NG Passalacqua. The extraction was made by maceration using four solvent at different polarity. Extraction yields of 1.05, 2.42, 5.32, and 9.22% were obtained using *n*-hexane, dichloromethane, ethyl acetate, and methanol, respectively.

The anti-proliferative activity was investigated in four human cancer cell lines such as human breast cancer ER+ cells (MCF-7), triple negative breast adenocarcinoma cell line (MDA-MB-231), lung adenocarcinoma (A549), and lung large carcinoma (COR-L23) cell line, using sulforhodamine B (SRB) assay (7). SRB assay is one of the most widely methods used to detect cell viability or drug cytotoxicity. This assay relies on the ability of SRB to bind cellular protein components and measure the total biomass. SRB is a bright-pink aminoxanthene dye that can form an electrostatic complex with basic amino acid residues of proteins in slightly acidic conditions but it can dissociate under basic conditions. It has been widely used for drug toxicity screening against different types of cancerous and non-cancerous cell lines.

All extracts showed anti-proliferative activity in tested cell lines in a concentration-dependent manner. The methanol extract was the most potent against A549 cell line, presenting an IC_{50} value of 57.75 mg/ml. Dichloromethane extract showed the best anti-proliferative activity against COR-L23 cells with an IC_{50} value of 60.91 mg/ml. The obtained data provide a rationale for further studies to investigate the molecular mechanisms responsible for the bioactivity of the most active extract of *T. hirsuta*.

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POSTER N. 89

5 = Study of tricalcium phosphate solubilization by some soil microfungi and evaluation of their potential as efficient plant growth-promoting organisms for *Glycine max*

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The potential of use of fungi and bacteria as biological fertilizers is widely recognized and represents a key strategy to improve the phosphorous (P) availability for crops. P is both mobilized and sequestered in soil by the interaction of biological and geochemical processes. Exchangeable P represents a short-term storage pool that can replenish the soil solution. The aims of this research were to study P solubilisation mechanisms of some selected fungal strains with different life strategies and to evaluate their potential to promote efficiently soy growth. Saprotrophic fungi play very important geoactive roles in P biogeochemical cycle, for instance by leaching minerals and solubilizing insoluble P with different mechanisms in soils. The soybean is one of the most important plant for food production worldwide. P depletion is one of the most relevant abiotic constraints that threaten soybean production. Chemical analyses with SEM/EDXA, colorimetric quantification and ICP-MS were performed to quantify TCP solubilised by fungi in liquid medium and P concentration in fungal biomass (1–6). Tested fungi were able to solubilise tricalcium phosphate (TCP) in different extents, increasing P concentration in liquid medium and in biomass. Evaluation of soy growth promotion ability and fungal-plant interactions were studied by microscopy and histological evaluation of seedlings.

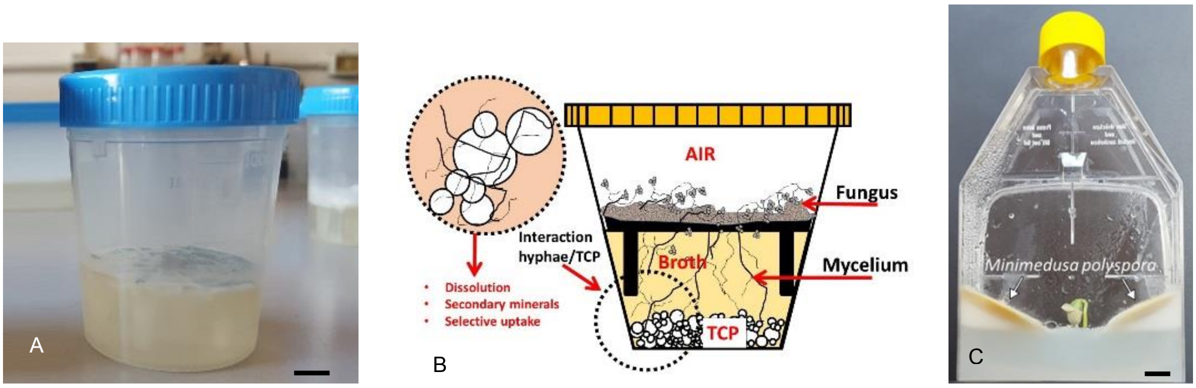

Fig. 1.A A container with *P. griseofulvum* grown on a perforated plastic support floating on 35 ml liquid medium. Scale bar = 1 cm; 1.B schematic representation of the cultural system; 1.C Flask with soy growing on Murashige and Skoog medium and *Minimedusa polyspora* (Hotson) Weresub & P.M. LeClair on Pikovskaya's medium for testing TCP solubilization and P mobilization for plant growth. Scale bar = 1 cm.

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5 = "Closing the loop" with Oyster Mushroom to recycle agricultural organic waste

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Circular economy aims at optimizing production process exploiting and reusing various wastes. Fungi play a key role in recycling lignocellulosic matter and thanks to their ability to produce several metabolite are the most promising organisms to "closing the loop" in several agricultural production processes. Furthermore, edible fungi are intensely used as nutraceutical and functional foods thanks to their ability to produce bioactive molecules such as antioxidants, β-glucans, triterpenoids.

Our study, framed in the European ALCOTRA project FINNOVER, has two aims: i) exploiting *Pleurotus ostreatus* to reuse the lavender solid waste from the production of lavender essential oil (extracted by steam distillation); ii) find new products to revamp the agriculture in the western Liguria coast.

More precisely, Pulsed Ultrasound-Assisted Extraction (PUAE) was employed to extract from 2 lavender solid wastes (*Lavandula angustifolia* Mill. and a hybrid called *Boscomare*) the liquid phenolic compounds whose valorisation as bioactive fraction is another goal of FINNOVER project not discussed here. After this extraction, a Second Solid Waste remains (SSW) was tested for mushrooms cultivations.

Two strains of *P. ostreatus* (one of which is autochthonous from Liguria) were selected to test different substrates in order to find the best spawn recipe for growing *P. ostreatus* on two different SSWs. More precisely we tested 5 types of substrates made with 2 SSWs mixed with 3 vegetal wastes (poplar sawdust, oak sawdust, and straw) in different percentages. The recipe with the major growth yield was used to prepare a spawn for a laboratory pilot experiment and, later, for a farm pilot experiment. The mycelium was treated for extracting different kinds of metabolites. Once individuated and standardized the extraction, we performed an analysis in HPLC coupled to medium and high resolution mass spectrometry for the quali-quantitative characterization of metabolites, being our work devoted to identify molecules of nutracetical and/or pharmacological interest.

The results related in vitro tests showed that lavender up to 30% in the recipe did not affect the miceliar growth rate. Other cultivation pilot tests confirmed these data and highlighted a fruitbody rate of about 20%.

5 = Ectomycorrhizal synthesis of *Tuber mesentericum* Vittad. A review of ECM descriptions and DNA characterization

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Tuber mesentericum Vittad., also known as the "black truffle of Bagnoli Irpino", is a lesser known ectomycorrhizal hypogeous fungus in the Pezizales. Known from the majority of European countries, this species characteristically lives in loose, easily warming up, calcareous soils. *Tuber mesentericum* has a developed market in southern Italy as well as in north eastern France, where it is more expensive than *Tuber aestivum* Vittad. (Summer truffle), being able to reach the price of 450 Euro/kg. In Italy, it is sold at a price ranging from 100 to 200 Euro/kg (1). However, from the Italian market, *T. mesentericum* is also exported and sold in Germany at higher prices than in Italy. Previous data suggested that *T. mesentericum* ECM have high similarity to those of *T. aestivum* Vittad. However, the high variability of the ITS region of the nuclear ribosomal DNA in *T. mesentericum* makes it impossible to design primers able to selectively amplify all the genotypes of this species. *Tuber mesentericum* ectomycorrhizae (ECMs) were obtained, after spore inoculation in laboratory conditions, by Rauscher et al. (2) and Zambonelli et al. (3,4). To the best of our knowledge, there were no literature regarding selective isolation and characterization of DNA from *T. mesentericum* ECM. The aim of this study was to propose a comprehensive morphological description of *T. mesentericum* ectomycorrhiza (ECM) supported by photographs and molecular data. Mycorrhization percentages on 16 *Quercus pubescens* Willd. seedlings inoculated with *T. mesentericum*, were assessed over a period of 9 and 14 months. Two different quantities of the spore-slurry were used to inoculate two batches of plants (A and B). After 9 months the presence of non-target ECM contaminant *Pulvinula constellatio* (Berk. & Broome) Boud. was detected with an average level of root colonization of 15 %. After 14 months *T. mesentericum* produced well-formed ECM with a rate of 20 % and 40 % in batches A and B, respectively. Morpho-anatomically *Tuber mesentericum* ECM showed a ramified monopodial-pinnate pattern and were ochre to brown at complete formation. The unramified ends were straight, cylindric, inflated and club-shaped, densely covered by the emanating hyphae. Septed, awl-shaped, brownish cystidia were present in the outer mantle; they were bristle-like (type A) with proximal ramification. ITS region from ECM and ascomata used for the inoculum were sequenced and compared with *T. aestivum* and *T. mesentericum* sequences available in GenBank. A maximum likelihood phylogenetic tree was produced showing the presence of four main clades belonging to the *Aestivum Clade* (5). Two clades include only sequences of *T. mesentericum*, other two clades include only sequences of *T. aestivum*. This is consistent with Benucci et al. (1), who showed the existence of different species within the *T. mesentericum* species complex. For future research to more precisely evaluate *T. mesentericum* development and mycorrhization trends, ecological features of natural environment must be investigated. Collecting data in natural sites is also necessary to define future strategies for *T. mesentericum* cultivation.

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5 = Exploiting saprotrophic soil microfungi in bioremediation of DDT: stress tolerance, oxidative stress and antioxidants

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DDT was extensively used worldwide as an organochlorine insecticide to control agricultural pests and vectors of several insect-borne human diseases; its use was banned in the early 70's in most industrialized countries due to toxicological concerns (1, 2). However, due to its persistence in the environment, accumulation in the food chain and long-distance transport in the upper atmosphere, residues still remain in environmental compartments becoming long-term sources of exposure affecting organisms (1, 2).

Several studies describe indigenous fungi as a promising tool for bioremediation of historically contaminated soils by hazardous chemicals (3, 4).

In this research we have isolated 51 saprotrophic fungal species from DDT-contaminated agricultural soils in Poland. Among these species, 24 were selected to test their tolerance (Fig. 1) to high concentration of DDT. In particular, *Trichoderma harzianum* Rifai and *Rhizopus stolonifer* (Ehrenb.) Vuill. (Fig. 2) were tested to evaluate fungal oxidative stress responses induced by the presence of the xenobiotic. Two species were chosen on the base of their sporulation capacity, diametric growth, biomass production and tolerance observed when growing on DDT enriched media.

The production of reactive oxygen species (ROS) and the activity of major antioxidant enzymes, namely superoxide dismutase (SOD), catalase (CAT), glutathione s-transferase (GST) and peroxidase (PRX) were used to evaluate the oxidative stress in the fungal cells (Fig. 3).

Two tolerance indexes based on fungal growth and mycelial biomass (Rt:Rc; TI) showed a high capacity of the two strains to tolerate high DDT concentration in the medium. On the other hand, high level of DDT promoted ROS formation in comparison to the untreated control. The specific activity of SOD increased in *T. harzianum* while the CAT activity increased in *R. stolonifer*. Lastly, the GST and PRX activity increased in both species. Our results suggest that the increased ROS production due to DDT induced a differential expression of ROS scavenging enzymes by the two fungal strains, which, in any case, allowed them to tolerate the xenobiotic and produce a strong growth.

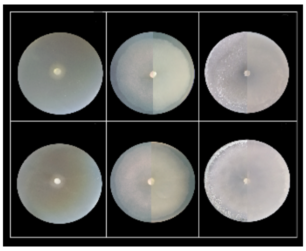

Fig. 1. Tolerance test

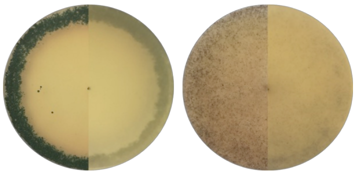

Fig. 2. Selected species

Fig. 3. Oxidative stress analysis

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5 = Red list of fungi from Umbria (Italy): the *Boletaceae* Chevall.

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This work is the second (after the "*Pezizomycotina* red list") of a series that will be produced as part of the "Fungal Conservation Assessment" project initiated by our research group in the 2016 (1). It aims at working towards assessing the status of all native fungal species against standard criteria based on the internationally accepted guidelines developed by the International Union for Conservation of Nature (IUCN) (2, 3, 4, 5).

The subject of this conservation assessment is the family *Boletaceae* Chevall. (Basidiomycota). Mushrooms of this family are mainly characterized by fleshy context and a tubulose, rarely lamellate hymenophore. They are extraordinarily diverse; in the Umbrian checklist the family *Boletaceae* is one of the most represented, with about 22 genera and 50 species (6). Some of these boletes have great economic, dietary, and health value (for example, *Boletus edulis* Bull. *sensu lato*). Ecologically, many species of *Boletaceae* are important ectomycorrhizal fungi in the ecosystem and can form ectomycorrhizal relationships with plants.

The decisions to conserve species should primarily be based upon an objective process of determining the degree of threat to the survival of a species. In the present work, it will be done by assigning the fungal species to one of the IUCN threat categories. This assessment of threats to survival should be separate and distinct from the subsequent process of deciding which species require action and what activities and resources should be allocated.

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5 = Guiding the guidelines for monitoring species under Habitats Directive surveillance: the case of *Crocus etruscus* Parl. (Iridaceae)

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The Habitats Directive requires Member States to monitor the conservation status of habitats and species of Community interest. For plant species, monitoring can be carried out at population level and the estimation of the population size is one of the key objectives. Generally, the measuring unit, required as a priority by the European Commission, is the "number of mature individuals", considered as the adult reproductive individuals solely (1).

In order to implement the surveillance of conservation status of plant species, ISPRA (Istituto Superiore per la Protezione e la Ricerca Ambientale) and Società Botanica Italiana have finalised a handbook aimed at developing the methodology and protocols for carrying out the monitoring (2). This handbook provides species-specific methods to ensure that field data are consistent with the overall European reporting system and to optimize monitoring and data collection at national level. The handbook gives indications on size unit to use for Italian endemic species, as they require a population size unit concordant at national level only. In the case of *Crocus etruscus* Parl., a geophyte endemic to Emilia-Romagna, Tuscany, and Umbria (3) included in Annex IV, the handbook suggests to plot the sites of occurrence on a 1 × 1 km grid, and to make an estimation of population size on 10% of the total cells (4).

To assess the reliability of this indication, we counted in the field the population size of *C. etruscus* in the occupied 1×1 km grid cells. Then, we randomly sampled, without replacement, an increasing number of cells from one to the total sample size (n = 1, 2, 3, to 59), with 1,000 iterations for each sampling. Then, we compared the distribution (mean and standard deviation) of the number of individuals in each sampling round to that calculated for the whole species (i.e., the whole sampling with n = 59), used as reference.

Our results show that selecting 10% of the total cells could cause a large underestimation of the true number of individuals, whereas the use of 50% of the cells provides a reliable estimation. In consideration of the potential difficulty to cover so large a number of cells, we suggest that 25% of the total cells can be considered a good compromise. Indeed, outliers disappear at this threshold, and the low associated underestimation fits well with the precautionary approach that has to be followed when dealing with species under protection. Accordingly, we recommend using a 25% threshold for conservation purposes.

Extending the case of *Crocus etruscus* to other species included in the Habitats Directive and applying the same evaluation method could not be straightforward, on account of biological differences occurring among species. However, we maintain that such an approach can be successfully applied to species with a similar range of occupied cells on the 1 × 1 km grid (ca. 50–100), with a number of individuals among cells strongly deviant from normal distribution, or with a similar habit (e.g., spring geophytes). However, our case testifies for potential biases that can be encountered if a full knowledge of species range and abundance is lacking. Therefore, for species of Annexes II and IV, we strongly advocate a large or full coverage of 1 × 1 km cells as a baseline (time 0) for subsequent monitoring surveys.

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5 = Contribution to the knowledge and valorization value of "copafam", a landrace of *Phaseolus coccineus* L. from the Brescia pre-Alps (northern Italy)

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The runner (scarlet) bean (*Phaseolus coccineus* L.) is an allogamous species of the Leguminosae family, native to Mesoamerica and widely cultivated in Europe as both an ornamental plant and a food crop. It is a perennial plant with showy red or white flowers (depending on the variety) and edible pods and seeds that are larger and more digestible than those of the common bean (*Phaseolus vulgaris* L.). A number of landraces of *P. coccineus* have been grown in Italy, with different seed sizes and seed coat colors. The landrace called "copafam" (Fig. 1), which is the focus of this study, is found in the pre-Alps of the Lombardy region in northern Italy, in particular in the Brescia pre-Alps (1). It was once cultivated by across a greater area but is now at risk of extinction as a result of depopulation and abandonment of mountain farms. According to hobby farmers in the province of Brescia, "copafam" can be grown only in the mountains, because in the plains (Po Plain), it would have problems both germinating and producing fruit. This study investigated the effect of elevation on the agronomic and bromatological characteristics of "copafam" beans. The goals of the study were to contribute to the knowledge of this little-known landrace, to identify the elevations most suitable for its cultivation (and thus conservation), and to explore its potential for inclusion in the Italian registry of agricultural products and for commercial exploitation by farmers in economically struggling mountain regions, such as the Lombardy pre-Alps. The agronomic characteristics of plants and the bromatological features of seeds harvested in 7 experimental fields, set up at different elevations in Northern Italy, were analyzed. Results showed that this landrace is most suitable for cultivation at higher elevations. As elevation increased from 110 to 1100 m above sea level, the plants became more vigorous and productive and the beans became larger and more digestible. This study, in addition to presenting knowledge regarding a little-known landrace, aims to be a stimulus for all those who are called to safeguard worldwide agrobiodiversity and in particular to encourage other researchers to commence similar research targeted not only at identifying and characterizing landraces and little-known traditional agricultural products but also at identifying a strategy for their valorization as an aid to sustainable development.

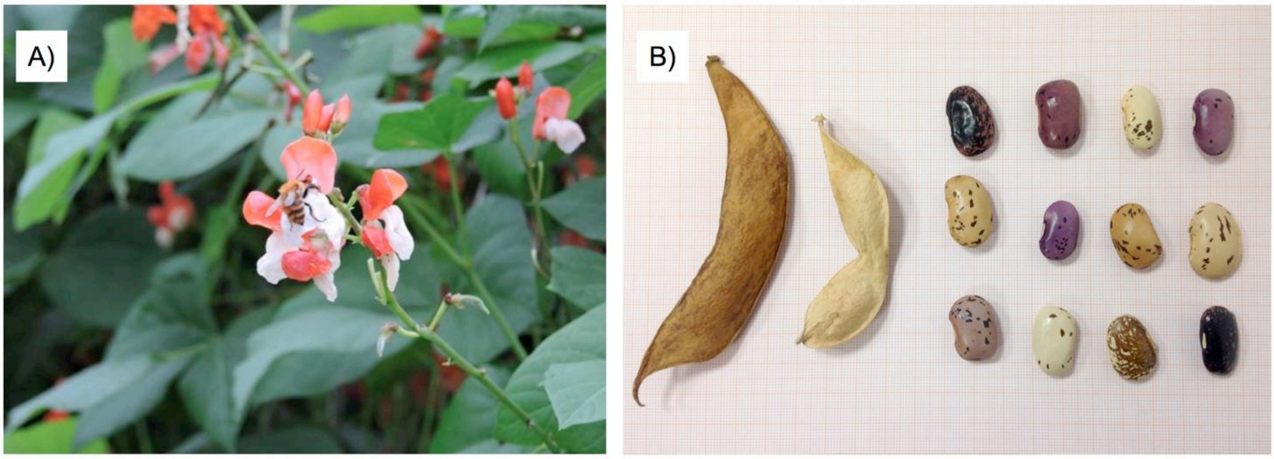

Fig. 1. Copafam landrace: flowers (A), poods and seeds (B).

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5 = Conservation status of the Aspromontana flora: monitoring and new stations of *Salvia ceratophylloides* Ard. (Lamiaceae) endemic species in Reggio Calabria (southern Italy)

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Salvia ceratophylloides Ard. is a valuable endemism of the western side of the Aspromonte. This species is located in the suburban surroundings of Reggio Calabria, on coastal strip hilly ridges between 250 and 450 m a.s.l. In the past, the species was present in several localities, as evidenced by papers that occurred from 1800 to the early 900s (1, 2), where, however, when it was described it was very rare. Afterwards, despite the research carried out by various botanists, the species was no longer found, disappearing in the places mentioned in literature due to the intense environmental transformations suffered by the territory surrounding Reggio Calabria. Therefore, the species since 1997 was included in the "Red Book of the flora of Italy" among the extinct species (3, 4). The successive researches carried out in 2008 made it possible to ascertain 4 micro-populations near Reggio Calabria at about 10 km distant from those reported in the literature, each of which consisting in a few dozen individuals, for a total surveyed of just few less of 100 mature individuals (5). Here we show the results of a study on the conservation status of *Salvia ceratophylloides* Ard., 10 years later from the last study. Known populations consistence has been analysed and field researches were started inorder to ascertain the presence of new stations. The monitoring was started up according to ISPRA manual (4, 6).

Actual population consists in 1.101 individuals occupying a total surface of 4.230m^2. The overall population is not increasing, in fact the young plants are only 216. Based on the data collected the IUNC Status "Critically Endangered" (CR) is confirmed.

Threats for surviving and diffusion of the species are several, and these are compromised essentially by habitat changes due to urbanization (E01) and devastating fires (J01.01); other pressure factors are the cultivated surfaces extension (A02.01) and the elimination of grasslands near cultivations(A02.03), artificial plantations (B01.02) and invasive species(I01).

This unique endemism could become the floral symbol of the city of Reggio Calabria and would deserve more safeguard by specific forms of protection as the establishment of a microriserve.

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5 = Development of a Sicilian bean core collection using morphological descriptors

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Different species and varieties of bean, spread in Sicily, are representative of local agricultural practices, as result of a careful exploration (1). Many landraces have become obsolete due to the spread of commercial varieties, but are still cultivated in small areas of Nebrodi Mountains (ME-Italy) and are endangered.

The Sicilian bean landraces are often poorly known but represent a genetic heritage to be preserve and to enhance. The e*x situ* conservation of Sicilian bean landraces was carried out in "Living Plants Germplasm Bank" of Ucria (ME-Italy), founded by the Nebrodi Regional Park, and in "Sicilian Plant Germplasm Repository" of STEBICEF Department - University of Palermo. Within *ex situ* germplasm conservation the "core collection" represents the maximum genetic diversity without redundancy stored in a gene bank, providing an overall view of the properties to be found in a whole collection and increasing the efficiency of characterization and utilization of collection.

The aim of this study was to develop a core collection of representative Sicilian bean genotypes, by morphological characterization.

Flowers, pods and seeds of *Phaseolus vulgaris* L., *Phaseolus coccineus* L. and *Vigna unguiculata* L. cultivars were collected from plants grown under same pedo-climatic condition at "Living Plants Germplasm Bank" of Ucria (Fig.1) and used for morphological characterization according to the IPGRI descriptors (2, 3). The analysis of the seeds has allowed to identify 35 morphotypes within the collection, indicated by a number code (5 descriptors). The 68,4% of landraces showed seed coat with pattern and the 46% exhibited a cuboid shape. The main representative seed-morphotype was the 27513 code (6 landraces) characterized by striped pattern coat, seed bicolor (purple and pink) with ligther prevalent and cuboid shape. In general, multiallelic control and environmental factors contribute to color expression variation in flower, pod and seed coat of bean (4, 5). The results, observed in field collection, shows that the purple flowers are often linked to darker seed development, while white or liliac flowers are linked to lighter seeds.

In summary, the results showed a redundancy of some bean accessions supporting the hypothesis that same cultivar is named in different way by the local farmers. Further genetic investigation will be needed to confirm the suggesting cases of synonymies between Sicilian accessions.

a) b) c)

Fig. 1. E*x situ* conservation field: a) "Living Plants Germplasm Bank"; b and c) different development stage of bean plant

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