

111° Congresso della Società Botanica Italiana  
III international Plant Science Conference

Roma 21-23 settembre 2016

**BOOK OF ABSTRACT**

KEYNOTE LECTURES, COMMUNICATIONS, POSTERS



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# 111° Congresso della Società Botanica Italiana onlus

## III INTERNATIONAL PLANT SCIENCE CONFERENCE (IPSC)

Roma, Tor Vergata, 21 - 23 September 2016

### Programme

#### Wednesday 21 September 2016

8:30-09:30 Registration and welcome coffee

9:30-11:00 Opening Ceremony

#### General Session

(chairpersons A. Canini and C. Siniscalco)

11:00-11:45 • **Federica Brandizzi**, Michigan State University, USA (40 + 5 min)  
Dynamic of secretory membrane traffic in plant cells

11:45-12:30 • **Carl Beierkuhnlein**, Bayreuth University, Germany (40 + 5 min)  
Plant responses to climatic changes - The role of extreme events

12:30-14:00 Lunch

#### Symposium 1

##### FILLING GAPS IN SYSTEMATICS AND CONSERVATION OF THE MEDITERRANEAN FLORA: RECENT OUTCOMES AND FUTURE CHALLENGES

(chairpersons D. Gargano and L. Peruzzi)

**Key words:** adaptation, DNA barcoding, evolution, evolutionary ecology, floristics, phylogeny, phylogeography, plant conservation, pollination ecology, Red Lists, systematics, taxonomy

14:00-17:30

- **Juan Arroyo**, University of Sevilla, Spain (30 + 5 min)  
Using systematics for conservation of biodiversity and underlying ecological and evolutionary processes
- **Félix Forest**, Royal Botanic Gardens, Kew, UK (30 + 5 min)  
Integrating evolutionary patterns in biodiversity science and conservation

#### Communications

- **E. Sulis**, G. Bacchetta, D. Cogoni, G. Fenu (12 + 3 min)  
Global and local demographic analysis of *Helianthemum caput-felis* populations along the western Mediterranean basin
- **G. Astuti**, L. Peruzzi (12 + 3 min)  
Providing identification tools for a proper conservation of European bladderworts (*Utricularia* L.)
- **S. Orsenigo**, D. Gargano, C. Montagnani, L. Peruzzi, T. Abeli, A. Alessandrini, N.M.G. Ardenghi, G. Astuti, G. Bacchetta, F. Bartolucci, L. Bernardo, M. Bovio, S. Brullo, A. Carta, M. Castello, D. Cogoni, F. Conti, G. Domina, G. Fenu, B. Foggi, M. Gennai, R. Gentili, M. Iberite, S. Magrini, G. Nicolella, L. Poggio, F. Prosser, A. Santangelo, A. Selvaggi, I. Vagge, M. Villani, R.P. Wagensommer, T. Wilhelm, C. Blasi, G. Rossi (12 + 3 min)  
New Red List of the Italian vascular flora: nearing the end
- **B. Marazzi**, M. Moretti, N. Schönenberger (12 + 3 min)

What about facultative ant-plant interactions mediated by extraflora nectaries in temperate zones? New insights from the southern Alps

- **G. Pellegrino**, F. Bellusci, A.M. Palermo (12 + 3 min)  
Effect on population structure of pollen flow, clonality rates and reproductive success in fragmented *Serapias lingua* populations
- **A. Trinco**, A. Carta, D. Ciccarelli, M. Ruffini Castiglione, L. Peruzzi, G. Astuti (12 + 3 min)  
Adaptive responses to human impacts in the threatened Webb's hyacinth (*Bellevalia webbiana* Parl., Asparagaceae)
- **T. Abeli**, S. Orsenigo (12 + 3 min)  
Recognizing the role of ecological studies in plant translocation: review and perspectives
- L. Rosati, E. Farris, S. Fascetti, A. Coppi, **F. Selvi** (12 + 3 min)  
Phylogenetic affinity and systematic position of the Italian populations of *Gymnospermium* (Berberidaceae)

17:30-18:00 Presentation of the book "Orti botanici, eccellenze italiane", Ed. Associazione Nuove Direzioni Cittadino e Viaggiatore, Firenze, (M. Clauser, P. Pavone)

18:00-19:30 Poster session (Botanic Gardens Tor Vergata University)

19:30- 22:00 Welcome Cocktail (Botanic Gardens Tor Vergata University)

## Thursday 22 September 2016

### Symposium 2

#### PLANT CELL PLASTICITY IN GROWTH AND STRESS RESPONSES

(chairpersons G. Falasca and L. Sanità di Toppi)

**Key words:** cell plasticity, stress responses, plant growth, morphogenesis, cell signaling

09:30-13:00

- **Aart van Bel**, University of Giessen, Germany (30 + 5 min)  
Phloem as a communication network between locations of emerging stress and sites of response
- **Michael Riemann**, Karlsruhe Institute of Technology, Germany (30 + 5 min)  
Jasmonate - a versatile phytohormone and its functions in the abiotic stress response of rice

#### Communications

- **M.C. Sorrentino**, F. Capozzi, S. Giordano, V. Spagnuolo (12 + 3 min)  
Genotoxic effects of cadmium and lead in the moss *Sphagnum palustre* L.
- **C. Faglierone**, C. Mosca, C. Verseux, D. Billi (12 + 3 min)  
Oxidative stress response in *Chroococciopsis* sp.
- L. Lombardi, E. De Vita, **A. Andreucci** (12 + 3 min)  
The Universal Stress Protein of *Populus alba* "Villafranca" clone: preliminary characterization
- **L. Bruno**, I. Forgione, M. Pacenza, A. Muto, M. Greco, F. Araniti, A. Chiappetta, M. R. Abenavoli, M. Van Lijsbettens, M.B. Bitonti (12 + 3 min)  
An omics approach to unravel the relationship between methylation status and plant growth plasticity in the *drm1drm2cmt3* mutant of *Arabidopsis thaliana*
- I. Aloisi, **G. Cai**, L. Navazio, S. Del Duca (12 + 3 min)  
The effects of polyamines during polarized cell growth of pollen tubes
- **A. Ceci**, F. Russo, O. Maggi, C. Riccardi, L. Pierro, M. Pietrangeli Papini, M. Cernik, A.M. Persiani (12 + 3 min)

Stress response and tolerance to hexachlorocyclohexane: soil fungal species isolated from contaminated areas of Italy and Czech Republic and their potential in fungal bioremediation

- **S. Novelli**, A. Gismondi, L. Canuti, G. Di Marco, A. Canini (12 + 3 min)  
Interactions between host plants and pathogens: the case of *Xylella fastidiosa*
- **M. Chialva**, A. Salvioli, S. Daghino, P. Bagnaresi, F. Neri, M. Novero, D. Spadaro, S. Perotto, P. Bonfante (12 + 3 min)  
A next generation approach reveals the impact of soil microbiota on gene and protein profile of tomato
- **L. Fattorini**, A. Ricci, E. Rolli, F. Brunoni, L. Dramis, E. Sacco, B. Ruffoni, C. Díaz-Sala, M. M. Altamura (12 + 3 min)  
Roles of 1,3-di(benzo[d]oxazol-5-yl)urea, an urea derivative, in carob and pine cuttings: adventitious rooting versus xylogenesis

13:00-14:30 Lunch

### Symposium 3

#### PLANT BIODIVERSITY AND ECOSYSTEM SERVICES WITHIN URBAN AREAS

(chairpersons C. Blasi and M. Marchetti)

**Key words:** urban areas, urban infrastructure, services of the nature, flora and vegetation in the cities, urban forest, mitigation of the pollution, well-being, health, urban pilot project

14:30-18:00

- **Francisco Diaz Pineda**, Complutense University of Madrid (30 + 5 min)  
Relationship current vegetation and soil uses-socioeconomy around a big metropolis
- **Stefan Zerbe**, Free University of Bozen-Bolzano (30 + 5 min)  
Flora and vegetation in urban ecosystems: services and disservices with particular regard to human health

#### Communications

- **F. Manes**, F. Marando, E. Salvatori, L. Fusaro, G. Capotorti, C. Blasi (12 + 3 min)  
Nature-based solutions to improve air quality in metropolitan areas
- **E. Roccotiello**, K. Perini, L. Cannatà, M.G. Mariotti (12 + 3 min)  
Air pollution mitigation via urban green interactions with particulate matter
- **L. Gratani**, M.F. Crescente, L. Varone, F. Tarquini, G. Puglielli, R. Catoni, F. Lucchetta, S. Sangiorgio (12 + 3 min)  
Ecosystem services: the role of plants in urban parks
- **G. Capotorti**, L. Zavattoni, B. Mollo, M.M. Alòs Ortì, C. Blasi (12 + 3 min)  
Proposal for an urban green infrastructure in the metropolitan city of Rome
- **M. D'Arco**, L. Ferroni, M. Speranza (12 + 3 min)  
How important is the choice of plant species for the green roof's cooling effect?  
Comparison of the behavior of two CAM-facultative species and two C3 species under drought conditions
- **M. Salinitro**, A. Alessandrini, A. Tassoni (12 + 3 min)  
The spontaneous vascular flora of the city of Bologna and its changes over 120 years
- **S. Ciccarello**, F. Scafidi, E. Di Gristina, G. Domina (12 + 3 min)  
Study of the alien flora of the urban area of Palermo (Sicily)
- **F. Bartoli**, L. Traversetti, G. Caneva (12 + 3 min)  
Ecological context of *Hedera helix* L. in Roman archaeological sites

- 18:30 Assembly of the Italian Botanical Society (members only)  
 20:30 Congress social dinner

## Friday, 23 September 2016

### Symposium 4

#### PLANTS: A RICH SOURCE OF HEALTHY COMPOUNDS (chairpersons N. De Tommasi and C. Bicchi)

**Key words:** medicinal plants, plant secondary metabolites, phytochemistry, chemotaxonomy, plant occurring compounds, food plants

09:00-12:30

- **Hernman Stuppner**, University of Innsbruck, Austria (30 + 5 min)  
Identification of novel hit and lead compounds inspired by nature
- **Orazio Tagliatalata-Scafati**, University Federico II, Naples, Italy (30 + 5 min)  
Malaria and plant metabolites: present and future of a long-standing relationship

#### Communications

- **L. Milella**, D. Russo, I. Faraone, L. Vignola, P.B. Andrade, E.C. Fernandez, N. De Tommasi (12 + 3 min)  
Biological activity and phytochemical profile of genetically different yacon genotypes
- **C. Villano**, A. Rinaldi, C. Lanzillo, D. Carputo, R. Garramone, R. Aversano (12 + 3 min)  
Metabolic and transcript profiling of phenolics accumulation in Aglianico grape berries
- **A. Marengo**, A. Maccioni, A. Maxia, C. Bertera, C. Cagliari, C. Sanna, P. Rubiolo (12 + 3 min)  
Phytochemical and biomolecular analyses of four wild edible *Carduus* species from Sardinia
- **C. Frezza**, A. Venditti, F. Maggi, S. Foddai, M. Nicoletti, A. Bianco, M. Serafini (12 + 3 min)  
Study on the evolution of some Lamiaceae based upon phytochemistry
- **C. Sanna**, M. Scognamiglio, E. Tramontano, M. Mandrone, M. Ballero, A. Maxia, A. Maccioni, A. Marengo, L. D'Aiello, F. Antognoni, F. Poli, A. Fiorentino, F. Esposito (12 + 3 min)  
*Hypericum scruglii*, a species endemic to Sardinia as a source of potential HIV-1 integrase inhibitors
- **F. Conforti**, M. Marrelli, M. Nicoletti, F. Menichini (12 + 3 min)  
*Capsicum annuum* L.: a new source of antioxidant compounds and inhibitors of pancreatic lipase
- G. Certo, D. Forestieri, **M.P. Germanò**, N. Malafronte, T.M. Bauab, B.V. Bonifácio, K.M. Silveira Negri, L. Perez de Souza, W. Vilegas, A. Rapisarda, N. De Tommasi (12 + 3 min)  
Effects on vivo angiogenesis of extracts from *Astronium fraxinifolium* Schott and *A. urundeuva* (Fr. All.) Engl. (Anacardiaceae)
- **M. De Leo**, D. Dolci, A. Braca, N. De Tommasi, G. Flamini, P.L. Cioni, L. Peruzzi (12 + 3 min)  
A systematic and chemical study of *Polygala flavescens* (Polygalaceae), endemic to Italy

12:30-13:30 Closure Ceremony

13:30-14:30 Lunch

## Post Congress, open conferences (in parallel)

### Sessione speciale

#### “COMUNICAZIONE E ORTI BOTANICI”

- 14:30-15:00 R. Braglia, F. Scuderi, E.L. Redi, L. Zanella, F. Imperi, P. Palermo, A. Montoro, A. Canini. (25 + 5 min)  
Orti Botanici e Comunicazione nell'epoca 4.0
- 15:00-15:30 G. Rinaldi, F. Zonca (25 + 5 min)  
Dall'Osmunda alla sicurezza alimentare esercizi di comunicazione da Orto Botanico.
- 15:30-16:00 P. Menegoni, S. Pignatti, R. Guarino, F. Colucci, M. D'Andrea, V. Inanilli, F. Lecce, A. Paolini, L. Pietrelli, M. Sighicelli, C. Trotta, A. Marotta, E. Redondi, F. Genovese. (25 + 5 min)  
Comunicare la scienza per conservare la natura: storie da un percorso di ricerca botanica e di comunicazione scientifica.
- 16:00-16:30 Comunicazione (FAI) (25 + 5 min)

### Simposio satellite

#### LE PIANTE MEDICINALI FRA BOTANICA FARMACEUTICA, AGRONOMIA E GENETICA: LE INTERAZIONI NECESSARIE

- 14:40-15:00 Introduzione: le piante medicinali, fra fitochimica, agronomia e genetica
- 15:00-15:20 Il progetto di filiera sulle piante officinali (MIPAAF)
- 15:20-15:40 Le principali esperienze agronomiche italiane sulle piante officinali (SOI)
- 15:40-16:00 Le principali esperienze genetiche italiane sulle piante officinali (SIGA)
- 16:00-16:20 Le principali esperienze italiane sulle piante officinali (SBI)
- 16:20-16:35 Comunicazione breve (12 + 3 m) (SOI)
- 16:35-16:50 Comunicazione breve (12 + 3 m) (SIGA)
- 16:50-17:05 Comunicazione breve (12 + 3 m) (SBI)
- 17:05-17:30 Tavola rotonda: Prospettive di collaborazione scientifiche nel settore delle piante officinali (MIPAAF, SBI, SOI, SIGA)

*Timetable may be modified*

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# INDEX

## Key Lecture

PAG. N. | TITLE | AUTHORS

### General Session

- |    |  |                    |
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| 1. | DYNAMIC OF SECRETORY MEMBRANE TRAFFIC IN PLANT CELLS             | Brandizzi Federica |
| 2. | PLANT RESPONSES TO CLIMATIC CHANGES - THE ROLE OF EXTREME EVENTS | Beierkuhnlein Carl |

### I Simposio

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| 3. | USING SYSTEMATICS FOR CONSERVATION OF BIODIVERSITY AND UNDERLYING ECOLOGICAL AND EVOLUTIONARY PROCESSES | Arroyo Marin Juan |
| 4. | INTEGRATING EVOLUTIONARY PATTERNS IN BIODIVERSITY SCIENCE AND CONSERVATION                              | Forest Félix      |

### II Simposio

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| 5. | PHLOEM AS A COMMUNICATION NETWORK BETWEEN LOCATIONS OF EMERGING STRESS AND SITES OF RESPONSE  | van Bel Aart   |
| 6. | JASMONATE - A VERSATILE PHYTOHORMONE AND ITS FUNCTIONS IN THE ABIOTIC STRESS RESPONSE OF RICE | Riemann Michael, Dhakarey Rohit, Peethambaran Preshobha Kodackattumannil, Raorane Manish, Treumann Achim, Singh Rakesh Kumar, Kohli Ajay, Nick Peter |

### III Simposio

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| 7. | RELATIONSHIP CURRENT VEGETATION AND SOIL USES-SOCIOECONOMY AROUND A BIG METROPOLIS | Pineda Francisco Diaz |
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8. FLORA AND VEGETATION IN URBAN ECOSYSTEMS: SERVICES AND DISSERVICES WITH PARTICULAR REGARD TO HUMAN HEALTH Zerbe Stefan

### IV Simposio

9. IDENTIFICATION OF NOVEL HIT AND LEAD COMPOUNDS INSPIRED BY NATURE Stuppner Hernman
10. MALARIA AND PLANT METABOLITES: PRESENT AND FUTURE OF A LONG-STANDING RELATIONSHIP Taglialatela-Scafati Orazio

## Communications

### I Simposio

11. GLOBAL AND LOCAL DEMOGRAPHIC ANALYSIS OF *HELIANTHEMUM CAPUT-FELIS* POPULATIONS ALONG THE WESTERN MEDITERRANEAN BASIN Sulis Elena, Bacchetta Gianluigi, Cogoni Donatella, Fenu Giuseppe
12. PROVIDING IDENTIFICATION TOOLS FOR A PROPER CONSERVATION OF EUROPEAN BLADDERWORTS (*UTRICULARIA* L.) Astuti Giovanni, Peruzzi Lorenzo
13. NEW RED LIST OF THE ITALIAN VASCULAR FLORA: NEARING THE END Orsenigo Simone, Gargano Domenico, Montagnani Chiara, Peruzzi Lorenzo, Abeli Thomas, Alessandrini Alessandro, Ardenghi Nicola M.G., Astuti Giovanni, Bacchetta Gianluigi, Bartolucci Fabrizio, Bernardo Liliana, Bovio Maurizio, Brullo Salvatore, Carta Angelino, Castello Miris, Cogoni Donatella, Conti Fabio, Domina Giannantonio, Fenu Giuseppe, Foggi Bruno, Gennai Matilde, Gentili Rodolfo, Iberite Mauro, Magrini Sara, Nicoletta Gianluca, Poggio Laura, Prosser Filippo, Santangelo Annalisa, Selvaggi Alberto, Vagge Ilda, Villani Mariacristina, Wagensommer Robert P., Wilhelm Thomas, Blasi Carlo, Rossi Graziano
14. WHAT ABOUT FACULTATIVE ANT-PLANT INTERACTIONS MEDIATED BY EXTRAFLORAL NECTARIES IN TEMPERATE ZONES? NEW INSIGHTS FROM THE SOUTHERN ALPS Marazzi Brigitte, Moretti Marco, Schönenberger Nicola
15. EFFECTS OF POPULATION STRUCTURE ON POLLEN FLOW, CLONALITY RATES AND REPRODUCTIVE SUCCESS IN FRAGMENTED *SERAPIAS LINGUA* POPULATIONS Pellegrino Giuseppe, Bellusci Francesca, Palermo Anna Maria
16. ADAPTIVE RESPONSES TO HUMAN IMPACTS IN THE THREATENED WEBB'S Trinco Alberto, Carta Angelino, Ciccarelli Daniela, Ruffini Castiglione Monica, Peruzzi

- HYACINTH (BELLEVALIA WEBBIANA PARL.,  
ASPARAGACEAE) Lorenzo, Astuti Giovanni
17. RECOGNIZING THE ROLE OF ECOLOGICAL  
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AND PERSPECTIVES Abeli Thomas, Orsenigo Simone
18. PHYLOGENETIC AFFINITIES AND  
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(BERBERIDACEAE) Rosati Leonardo, Farris Emmanuele, Fascetti  
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### II simposio

19. GENOTOXIC EFFECTS OF CADMIUM AND  
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L. Sorrentino Maria Cristina, Capozzi Fiore, Giordano  
Simonetta, Spagnuolo Valeria
20. OXIDATIVE STRESS RESPONSE IN  
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21. THE UNIVERSAL STRESS PROTEIN OF  
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PRELIMINARY CHARACTERIZATION Lombardi Lara, De Vita Elisa, Andreucci Andrea
22. AN OMICS APPROACH TO UNRAVEL THE  
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STATUS AND PLANT GROWTH PLASTICITY IN  
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*ARABIDOPSIS THALIANA*. Bruno Leonardo, Forgione Ivano, Pacenza  
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23. THE EFFECTS OF POLYAMINES DURING  
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24. STRESS RESPONSE AND TOLERANCE TO  
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27. ROLES OF 1,3-DI(BENZO[D]OXAZOL-5-  
YL)UREA, AN UREA DERIVATIVE, IN CAROB  
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ROOTING VERSUS XYLOGENESIS Fattorini Laura, Ricci Ada, Rolli Enrico, Brunoni  
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Barbara, Díaz-Sala Carmen, Altamura Maria  
Maddalena

### III simposio

28. NATURE-BASED SOLUTIONS TO IMPROVE AIR QUALITY IN METROPOLITAN AREAS Manes Fausto, Marando Federica, Salvatori Elisabetta, Fusaro Lina, Capotorti Giulia, Blasi Carlo
29. AIR POLLUTION MITIGATION VIA URBAN GREEN INTERACTIONS WITH PARTICULATE MATTER Roccotiello Enrica, Perini Katia, Cannatà Laura, Mariotti Mauro G.
30. ECOSYSTEM SERVICES: THE ROLE OF PLANTS IN URBAN PARKS Gratani Loretta, Crescente Maria Fiore, Varone Laura, Tarquini Flavio, Puglielli Giacomo, Catoni Rosangela, Lucchetta Francesca, Sangiorgio Silvia
31. PROPOSAL FOR AN URBAN GREEN INFRASTRUCTURE IN THE METROPOLITAN CITY OF ROME Capotorti Giulia, Zavattero Laura, Mollo Barbara, Alòs Ortì Marta Maria, Blasi Carlo
32. HOW IMPORTANT IS THE CHOICE OF PLANT SPECIES FOR THE GREEN ROOF'S COOLING EFFECT? COMPARISON OF THE BEHAVIOUR OF TWO CAM-FACULTATIVE SPECIES AND TWO C3 SPECIES UNDER DROUGHT CONDITION D'Arco Matteo, Ferroni Lucia, Speranza Maria
33. THE SPONTANEOUS VASCULAR FLORA OF THE CITY OF BOLOGNA AND ITS CHANGE OVER 120 YEARS Salinitro Mirko, Alessandrini Alessandro, Tassoni Annalisa
34. STUDY OF THE ALIEN FLORA OF THE URBAN AREA OF PALERMO (SICILY) Ciccarello Sebastiano, Scafidi Filippo, Di Gristina Emilio, Domina Giannantonio
35. ECOLOGICAL CONTEXT OF HEDERA HELIX L. IN ROMAN ARCHAEOLOGICAL SITES Bartoli Flavia, Traversetti Lorenzo, Caneva Giulia

### IV simposio

36. BIOLOGICAL ACTIVITY AND PHYTOCHEMICAL PROFILE OF GENETICALLY DIFFERENT YACON GENOTYPES Milella Luigi, Russo Daniela, Faraone Immacolata, Vignola Lisiana, Andrade Paula B., Fernandez Eloy C., De Tommasi Nunziatina
37. METABOLIC AND TRANSCRIPT PROFILING OF PHENOLICS ACCUMULATION IN AGLIANICO GRAPE BERRIES Villano Clizia, Rinaldi Alessandra, Lanzillo Carmine, Carputo Domenico, Garramone Raffaele, Aversano Riccardo
38. PHYTOCHEMICAL AND BIOMOLECULAR ANALYSES OF FOUR WILD EDIBLE *CARDUUS* SPECIES FROM SARDINIA Marengo Arianna, Maccioni Alfredo, Maxia Andrea, Berdea Cinzia, Cagliari Cecilia, Sanna Cinzia, Rubiolo Patrizia
39. STUDY ON THE EVOLUTION OF SOME LAMIACEAE BASED UPON PHYTOCHEMISTRY Frezza Claudio, Venditti Alessandro, Maggi Filippo, Foddai Sebastiano, Nicoletti Marcello, Bianco Armandodoriano, Serafini Mauro
40. *HYPERICUM SCRUGLII*, A SPECIES ENDEMIC TO SARDINIA AS A SOURCE OF POTENTIAL HIV-1 INTEGRASE INHIBITORS Sanna Cinzia, Scognamiglio Monica, Tramontano Enzo, Mandrone Manuela, Ballero Mauro, Maxia Andrea, Maccioni Alfredo, Marengo Arianna, D'Aiello Lidia, Antognoni Fabiana, Poli Ferruccio, Fiorentino Antonio, Esposito Francesca
41. *CAPSICUM ANNUUM* L.: A NEW SOURCE OF ANTIOXIDANT COMPOUNDS AND INHIBITORS OF PANCREATIC LIPASE Conforti Filomena, Marrelli Mariangela, Nicoletti Marcello, Menichini Francesco

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|-----|---|--|
| 42. | EFFECTS ON <i>IN VIVO</i> ANGIOGENESIS OF EXTRACTS FROM <i>ASTRONIUM FRAXINIFOLIUM</i> SCHOTT AND <i>A. URUNDEUVA</i> (FR.ALL.) ENGL. (ANACARDIACEAE) | Certo Giovanna, Forestieri Debora, Germanò Maria Paola, Malafronte Nicola, Bauab Taís Maria, Vidal Bonifácio Bruna, Silveira Negri Kamila Maria, Perez De Souza Leonardo, Vilegas Wagner, Rapisarda Antonio, De Tommasi Nunziatina |
| 43. | A SYSTEMATIC AND CHEMICAL STUDY OF <i>POLYGALA FLAVESCENS</i> (POLYGALACEAE), ENDEMIC TO ITALY  | De Leo Marinella, Dolci David, Braca Alessandra, De Tommasi Nunziatina, Flamini Guido, Cioni Pier Luigi, Peruzzi Lorenzo   |

## Poster

### 1.1

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## DYNAMIC OF SECRETORY MEMBRANE TRAFFIC IN PLANT CELLS

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Understanding how cells maintain efficient compartmentalization and control the delivery and integration of bio-molecules into specialized organelles is a fundamental challenge in eukaryotic cell biology. The secretory pathway is an exquisite example of complexity for biomolecule trafficking and compartmentalization. The plant secretory pathway is responsible for the biosynthesis of one third of the cellular proteome, essential lipids and protein-modifying sugars. The life of these biomolecules starts in the endoplasmic reticulum (ER). They are then shuttled to the Golgi apparatus and the trans-Golgi network for sorting and delivery to target compartments. Movement of proteins from the early secretory compartments towards the distal compartments requires precise trafficking mechanisms that are still largely uncharacterized. To learn about trafficking and efficient compartmentalization of proteins we have carried out forward and reverse genetic screens with fluorescent reporters to markers of proximal and distal compartments of the secretory pathway. Through these screens we have identified novel plant-specific as well as conserved factors that regulate the delivery of proteins between the Golgi and distal compartments as well as novel factors that control the morphological integrity of the ER and Golgi. Our most recent findings will be presented in this talk.

## PLANT RESPONSES TO CLIMATIC CHANGES - THE ROLE OF EXTREME EVENTS

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The effects of climatic changes on plants can be manifold. From pollination success over seed maturation, germination, growth and survival all phases of their life cycles and population dynamics can be affected and thereby influence the quantitative contribution of plant species and their functional performance in ecosystems. Shifts in distribution areas are likely to occur. However, it is unlikely that the speed of change and the speed of response will be synchronized. Mismatches can result in novel future ecosystems with high uncertainty about their structure and composition.

Uncertainty about plant species performance in the future is even increased through the fact that climate change will increase the frequency, intensity and duration of extreme weather events in many parts of the world. More energy in the atmosphere as a consequence of global warming will reduce the regularity and reliability of seasonal patterns and precipitation sums both, towards surplus supply or shortage of water. Such comparably short periods with stressful physical conditions can have stronger impacts on plant life than gradual shifts in average conditions. This is particularly to be expected if such events are repeated and also if the event has an intensity that lies beyond meteorologically recorded cases.

We approach knowledge gaps in plant ecology and ecosystem research through experiments and observational studies. Experimental treatments with different intensities of extremeness are compared for plant species and in communities with different degrees of control ranging from even aged individuals in defined spacing on constructed soils to old-growth stands and including grassland and tree species. Climatic manipulations include drought, heavy rain, and warming. In parts, these are combined with different degrees of soil fertility and with different intensities of agricultural management. We find a series of different processes and mechanisms of plants to be impacted. Astonishingly, in temperate grasslands biomass production seems to be very resilient towards short periods of extreme drought at the longer run. Species assemblages are modified specifically through the support or suppression of establishment. Individual exhibit memory effects to drought which can be seen as one adaptation strategy to a modified environment.

Observational studies include remote sensing and long-term time series to identify the effects of extreme events on plants and vegetation that occurred in the real world. Long-term observational studies show that successional trajectories can be influenced by one extraordinary season. Satellite imagery proves that one late frost event of a few hours can significantly reduce photosynthesis and carbon sequestration over large transnational scales.

Finally, gaps in current ecological climate change research are identified and discussed. Challenges and directions of future research are highlighted.

## USING SYSTEMATICS FOR CONSERVATION OF BIODIVERSITY AND UNDERLYING ECOLOGICAL AND EVOLUTIONARY PROCESSES

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It is currently well known that plant biodiversity in the Mediterranean Basin is outstanding, both in terms of species richness and endemism. Recent progress has been done on depicting patterns of biodiversity within this region, determining many hot and warm sub-spots there. Less is known about the processes underlying these patterns. However, there is some ecological and evolutionary research that has thrown some light. One of the main challenges to this goal is that it should be addressed at diverse spatial and biological scales, from microhabitats to the whole region, from populations and local communities to entire biotas. Here we present several studies that aimed to disentangle the drivers of current biodiversity in several plant groups and regions. The ultimate goal of our research programme is to gain knowledge that permits decision makers to preserve processes determining biodiversity, rather than adhering to a blind strategy addressed to patterns (regions and taxa of high diversity). These patterns are strongly dependent on current knowledge of sound biological systematics, which is in many cases under poor development, thus limiting the power of the approach.

Population level studies are a good option in those cases where systematics is confusing and over-splitting has caused an inflation of efforts devoted to conservation. We offer an example in *Narcissus tazetta* L. s.l., where a detailed account of range-wide reproductive biology has offered insight on the maintenance of the population genetic diversity. We show also how populations of some particularly old species, relict (*Laurus nobilis* L.) or widespread (*Pistacia lentiscus* L.), are particularly illustrative of the history of the building up of the Mediterranean vegetation.

Daffodils (*Narcissus*) also offer excellent opportunities to analyze the historical role of the palaeogeography of the Mediterranean in depicting the current distribution of biodiversity in a genus, which is frequently included in conservation programs, without a strict scrutiny of what lineages do represent. Here, problems of taxonomic over-splitting and high endemism of resulting taxa has had an effect.

Finally, a big challenge has been assumed for an ecological and historical account of plant biodiversity for the complete flora and plant communities of a Mediterranean hotspot, the Betic-Rifean arch in the Western end of the Region. Based in profound and relatively homogeneous floristic knowledge of regions at both sides of the Strait of Gibraltar, we use available phylogenetic information to determine patterns of phylogenetic diversity across an entire region.

In general, our results illustrate how important is to have sound floristic (both in terms of species lists and localities), systematic and phylogenetic information for a proper determination of current and past patterns of biodiversity, which should result in a more efficient use of conservation resources, in a period when natural history sciences are suffering deep cuttings.



## INTEGRATING EVOLUTIONARY PATTERNS IN BIODIVERSITY SCIENCE AND CONSERVATION

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Ever since the revolutionary ideas put forward by Darwin, evolutionary studies have played a fundamental role in our understanding of life and the mechanisms that led to its current diversity. Until relatively recently however, evolutionary biology and associated sub-disciplines had a relatively modest involvement in tackling conservation issues. This state of affairs has however shifted considerably in recent years, with numerous approaches being proposed to integrated evolutionary history and phylogenetic information in biodiversity science.

I will present a few examples of studies integrating evolutionary information in biodiversity science that exemplifies how such information can be crucial in conservation planning and decision making.

In a first study, we investigated the evolutionary patterns in the flora of the Cape region of South Africa, one of the five Mediterranean-type ecosystems of the world and a centre of high species diversity and endemism. Previous research using a genus-level phylogenetic tree of the region's flora showed that phylogenetic diversity is more clustered in the western part of the region, while it is more over-dispersed in the eastern part (1). These patterns were attributed to the fact that the western part acted as a refugium with high rates of speciation and low extinction, while the eastern part was the scene of more exchanges with neighbouring biomes. We examined phylogenetic diversity patterns in the region using modelled species distributions and phylogenetic trees from 21 "Cape floral clades", groups of plants that have mostly diversified in the Cape (2), representing more than 2,500 of the ca. 9,300 species found in the Cape (3). These new analyses provide a more refined account of the phylogenetic patterns uncovered in the genus-level study and identified new ones. In a second study, I will discuss the combinations of extinction risk data, obtained from conservation assessments made using the IUCN categorisation, and phylogenetic information to rank species according to how evolutionary distinct and globally endangered they are: the EDGE approach (4). I will present results from the first major group of plants that have been assessed using this approach, the Gymnosperms.

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## THE PHLOEM AS A COMMUNICATION NETWORK BETWEEN LOCATIONS OF EMERGING STRESS AND SITES OF RESPONSE

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Forisomes are giant, fusiform protein complexes that act as reversible stopcocks in the sieve tubes of legumes (1), which disperse and contract at high and low  $\text{Ca}^{2+}$  concentrations respectively. Sudden local stresses such as burning (2) or high salt concentrations (3) trigger distant sieve-tube occlusion by forisome dispersion which partly overlaps in time with callose sieve-pore deposition (2). Both events result from the propagation of electric potentials waves which are associated with  $\text{Ca}^{2+}$  influx which is potentiated by concerted action of diverse types of  $\text{Ca}^{2+}$  permeable channels on plasma membrane and ER membranes of the sieve elements (4,5). The clustered  $\text{Ca}^{2+}$  channels give rise to hotspots, where  $\text{Ca}^{2+}$  concentrations rise to exceptionally high values in the sieve-element microplasm (4,5). Forisome tips are located in the vicinity of  $\text{Ca}^{2+}$  hotspots (4). After some time, forisome re-contract and callose is degraded which probably depends on  $\text{Ca}^{2+}$  removal from the sieve-tube microplasm (8). The  $\text{Ca}^{2+}$  waves have been linked with parallel corroborative waves of ROS (reactive oxygen species) production along the sieve tubes (6). ROS may assist the release of  $\text{Ca}^{2+}$  from their ER stores via CICR channels (7).  $\text{Ca}^{2+}$  influx and ROS production show complex relationships with the synthesis of jasmonic acid and salicylic acid.

Along the pathway, the  $\text{Ca}^{2+}$  waves evoke temporary closure of the sieve pores and the plasmodesmal contacts (PPUs) between sieve elements and companion cells, and perhaps also those between companion cells and vascular parenchyma cells. This would lead to a temporary reorganization of the phloem symplasm with profound local consequences for long-distance signalling (8). On the whole-plant level, it has been speculated, that three waves of signalling emerge due to the reversible symplasmic reorganization (8). During the first wave preceding symplasmic reorganization, free  $\text{Ca}^{2+}$  and ROS propagate from the sites of stress to other responsive locations elsewhere in the plant.  $\text{Ca}^{2+}$  and ROS may in part readily bind to phloem specific proteins in the sieve-tube lumen. During the period of symplasmic isolation, cellular autonomy may enable the cells to produce low-molecular agents and to gene expression without plasmodesma-mediated corrective actions by the neighbouring cells. After re-opening of the symplasmic contacts, the  $\text{Ca}^{2+}$ - and ROS-conjugated proteins may be transported by mass flow to the target organs as well as the low-molecular substances (JA, SA, NO?) released from the companion cells. During the third wave, several species of macromolecules (newly synthesised proteins, sRNA, mRNA) are released into the sieve tubes.

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## JASMONATE – A VERSATILE PHYTOHORMONE AND ITS FUNCTIONS IN THE ABIOTIC STRESS RESPONSE OF RICE

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Jasmonic acid and its derivatives, collectively called jasmonates, are phytohormones with a multitude of functions. A large body of knowledge about jasmonates derives from *Arabidopsis thaliana*. The hormone jasmonic acid (JA) is synthesized in a linear pathway starting from the fatty acid linolenic acid and needs to be activated by conjugation with an amino acid in a reaction catalyzed by enzymes of the GH3 family. Although many basic mechanisms and functions of jasmonates are conserved, the pathway needs to be examined in different plant species to elucidate its role in a specific context. In rice jasmonates were found to be involved in classical jasmonate functions such as wounding responses or plant fertility. But also novel roles such as involvement in the abiotic stress responses or photomorphogenesis have been identified. An overview of these functions identified in mutant approaches will be provided highlighting novel findings in the response to salinity and drought. These results suggest that activation of the jasmonate pathway in wild type rice plants upon abiotic stress leads to more severe stress symptoms. On the molecular level, we observed that abiotic stress causes higher levels of oxidative stress in the wild type, presumably because antioxidative enzymes are more abundant in the mutant under stress. We also found evidence that JA biosynthesis mutant maintain a better root performance under drought, partially due to a higher abundance of cell wall biosynthetic enzymes. Hence targeted suppression of JA signalling might contribute to a better stress tolerance.

ECOLOGICAL RELATIONSHIPS 'CURRENT VEGETATION AND NEW  
LAND USES-SOCIOECONOMY' AROUND A BIG METROPOLIS

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In most of the Planet, human population growth is concentrated in large metropolis. This happen especially in the last five decades. Natural vegetation is the main spatio-temporal indicator of the biological components of the rural landscape. Nowadays we can better explain the current variation of this vegetation according to the dispersion of urban settlements than by the usual changes in agrarian land uses -development of agriculture, pastoralism and forestry-.

The research group 'ADAPTA' (Complutense University of Madrid), is developing some not easy, but simple, numerical models to relate the rural landscape structures and the socioeconomics of local populations. Case studies developed in the Madrid Region try to explain the current changes, and simulate future scenarios, in the landscape and ecosystem services through a sequence of influences 'metropolis - dispersed rural urban nuclei - local socioeconomics - heterogeneity and complexity of the rural cultural landscape - biodiversity - new rural cultural landscape'.

## FLORA AND VEGETATION IN THE URBAN ECOSYSTEMS: SERVICES AND DISSERVICES WITH PARTICULAR REGARD TO HUMAN HEALTH

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Since more and more people are living in cities, urban development and planning, living comfort, and health are of growing concern worldwide. Urban flora and vegetation as an essential part of a city structure play an increasing role in providing services for humans, making cities greener, healthier, and more livable. They can be found on private or public ground, be located in parks, urban places, gardens, or along streets. Urban green provides a habitat for wildlife, improve air quality, mitigate climate change by sequestering carbon dioxide, contribute to climate change adaptation, improve water quality, mitigate water runoff, reduce erosion, attenuate noise, increase city dwellers' well-being and positively impact social relations, save energy by providing shade and thus cooling buildings, and they create "green" jobs. However, urban flora and vegetation can also negatively affect human well-being and the socio-economic system, respectively. These negative effects have been defined as ecosystem disservices. For example, some tree species can contribute to the formation of ozone due to the emission of volatile organic compounds and plants can cause health problems via allergenic pollen. The relationship of urban ecosystems and human health in cities is outlined referring to the literature and own studies in Central European cities.

## IDENTIFICATION OF NOVEL HIT AND LEAD COMPOUNDS INSPIRED BY NATURE

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The treatment of inflammatory disorders in Western medicine relies heavily on the use of non-steroidal anti-inflammatory drugs (NSAIDs) and corticosteroids (1). However, currently available treatment options are often unsatisfactory. Natural products (NPs) have always been an important source of new drug leads. Almost half of the drugs currently in clinical use are of natural product origin and even today, in the post genomic era, plants, fungi, marine organisms, and microorganisms are still an important source for the development of new drugs (2).

In the course of a national research network project involving scientists of six Austrian universities we aimed to identify and characterize anti-inflammatory NPs capable to combat inflammatory processes specifically in the cardiovascular system. The combined use of computational techniques with traditional knowledge, high-tech chemical analysis and synthesis, and a broad range of in vitro, cell-based, and in vivo pharmacological models led to the identification of a series of promising anti-inflammatory hit and lead compounds. Mechanistic studies contributed to a better understanding of their mechanism of action and delivered new knowledge on the molecular level of inflammatory processes. Highlights of this interdisciplinary project which started in 2008 will be presented (3).

Acknowledgements: This work was financially supported by grant no S107 „Drugs from Nature Targeting Inflammation” from the Austrian Science Fund (<http://www.uibk.ac.at/pharmazie/pharmakognosie/dnti/>).

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The histories of plant metabolites and malaria treatment are strictly intertwined. Indeed, malaria can be regarded as the first disease to be treated with an active principle isolated from a natural source, namely quinine obtained from Cinchona plant. The isolation of pure quinine from the bark of this plant founded the modern natural product chemistry and, at the same time, inaugurated the chemotherapeutic approach to the management of malaria cases. Since then and until 2006, when quinine was withdrawn by WHO as a first-line treatment for malaria (1), this alkaloid not only has been used as such but it has also inspired the preparation of a plethora of synthetic antimalarials based on its aminoquinoline scaffold.

The second connection between malaria and plant metabolites has emerged with the discovery of artemisinin (2), a major breakthrough in the fight against malaria. Artemisinin is a rearranged cadinane peroxide, isolated from sweet wormwood (*Artemisia annua*), a plant used in China as an anti-fever remedy for more than two thousand years, and later discovered to contain this potent, fast-acting antimalarial compound, active also against chloroquine-resistant strains. At the moment, almost all the artemisinin supply derives from the cultivation of the plant on an estimated area of 20,000 hectares in Africa, India and China. In the last years, hundreds of semi-synthetic derivatives of artemisinin have been prepared, but also their production relies on the isolation of natural artemisinin. The introduction and nowadays wide availability of ACTs, together with the mass use of insecticide impregnated bed nets, can be recognized as the main reasons for the significant decrease in the number of yearly deaths by 25-30% over the last decade. However, the current estimated 660,000 deaths per year and 220 millions of cases are still unacceptable. In addition, the emergence of *Plasmodium* strains resistant to artemisinin (3), is raising severe concerns and the possible diffusion of resistant strains to sub-Saharan Africa would further worsen the situation, creating a tremendous therapeutic void.

Thus, there is a urgent need of new drugs to fight malaria and the role of natural products to meet this need is beyond doubt. An integrated elimination/control strategy would need antimalarials able not only to kill malaria parasite in the erythrocyte stage but also to target the non-pathogenic *Plasmodium* stages responsible for transmission (e. g. gametocytes and early sporogonic stages).

The Author will present an account of the personal contribution in this field and a critical overview of the plant-derived antimalarial compounds in current development.

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## 1.8. = GLOBAL AND LOCAL DEMOGRAPHIC ANALYSIS OF *HELIANTHEMUM CAPUT-FELIS* POPULATIONS ALONG THE WESTERN MEDITERRANEAN BASIN

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Population dynamics is the area of science which tries to explain in a simple mechanistic way the time variations of the size and structure of biological populations (1). Determining whether a population is growing or declining is central to conservation biology and species' range dynamics (2); in particular, it is crucial to evaluate the current conservation status and possible future management of plant populations.

This study focussed on population dynamics of *Helianthemum caput-felis* Boiss., a long-lived coastal plant distributed throughout the western Mediterranean Basin (SE Iberian Peninsula, Majorca, Sardinia and NW Africa), characterized by a highly fragmented areal isolated from the distribution centre (3).

Specific aim of the study was to determine the population dynamics by the analyses of populations' vital rates, both at global level than a local scale.

Plant performance and population dynamics of *H. caput-felis* populations were investigated in plants sampled within 98 permanent plots randomly established among the overall distribution range of the species; within these plots, 821 plants were marked and surveyed three times a year from 2013 to 2015. Data were analysed by Integral Projection Models (IPMs; 4, 5), which describe how a population structured by a continuous individual-level state variable (as plant size) changes in discrete time (4). The IPMs were constructed both considering the global population and analysing six localities, separately.

The global population showed a general stability in the first year transition, after which it present a decline of 8%. This trend was probably due to the higher shrinkage of plants in the last year of study, which correlates negatively with reproductive traits. Despite this decline, stochastic population growth rate highlighted that *H. caput-felis* global population oscillated around the equilibrium. Elasticity analysis indicated the higher importance of large individuals and the determinant role of the survival-growth transitions for the population growth rate. Moreover, plants reached high generation times and mean long life span, which, with the limited seedling recruitment rate and the presence of a consistent seed bank, reflect patterns that are typical both of many long-lived plants and of calcareous rupicolous Mediterranean species (6).

At local level, the typical pattern of long-lived Mediterranean coastal plants was also confirmed; in fact, populations were mainly composed from large individuals, characterized by slow growth. *H. caput-felis* showed vital rates and population dynamics varying among populations which should be correlated to particular microclimate and ecological conditions; in particular, the high aridity of Cabo Roig (Alicante, Spain) and the sand substrate of Sa Ràpita (Majorca, Spain) could be negatively affect the population growth rate, while the ecological optimum in Sardinia could increase the size and the vital rates of plants.

The results of this study gave new findings for the populations' dynamics of *H. caput-felis*, and demonstrate the importance to considerate both global than local level in demography studies, which play an important role in planning the management of *in situ* conservation for this threatened species.

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## 1.11. = PROVIDING IDENTIFICATION TOOLS FOR A PROPER CONSERVATION OF EUROPEAN BLADDERWORTS (*UTRICULARIA* L.)

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In Europe only seven native species of *Utricularia* (bladderworts) occur: *U. australis* R.Br., *U. bremii* Heer, *U. intermedia* Hayne, *U. minor* L., *U. ochroleuca* R.Hartm., *U. stygia* Thor, and *U. vulgaris* L. Considering systematic aspects, these species are mainly distinguished by flowers (1, 2). Concerning the vegetative parts, they can be easily subdivided in three aggregates: *U. intermedia* aggr., also including *U. ochroleuca* and *U. stygia*, *U. minor* aggr., also including *U. bremii*, and *U. vulgaris* aggr., also including *U. australis*. Within each aggregate, species share almost identical shoot morphology, so that their distinction is very hard in absence of flowers. Unfortunately, many populations of the mostly sterile species (e.g. *U. bremii*, *U. ochroleuca* and *U. stygia*) rarely flower. Probably, these species represent vegetative apomicts derived from *U. intermedia* and *U. minor* or, alternatively, the product of hybridization between species of *U. intermedia* and *U. minor* aggregates (2, 3). European bladderworts are all aquatic, often inhabiting threatened environments. All these species are included in the European Red Lists as DD, *Data Deficient* (*U. bremii*, *U. ochroleuca*, *U. intermedia*, and *U. stygia*) or as LC, *Least Concern* (*U. australis*, *U. minor*, and *U. vulgaris*) (4). It is not surprising that *U. bremii*, *U. ochroleuca*, *U. intermedia*, and *U. stygia* are all listed as DD, since they are the most taxonomically critical units. However, in such systematically complex situation, more information can be recovered at regional level. Indeed, at national level (5), *U. bremii* is reported as *Endangered* (EN) in Switzerland, *Critically Endangered* (CR) in Italy, Germany, Austria, Hungary, Czech Republic and Slovakia. Similarly, *U. ochroleuca* is assessed as DD in Great Britain, CR in Czech Republic, Germany and Switzerland, where its presence is doubtful because of possible confusion with *U. stygia*. Because of possible confusion with *U. ochroleuca* and the subsequent difficulty to delimit its distribution, *U. stygia* lacks an adequate assessment of its conservation status also at regional levels. Indeed, it is reported as DD in Great Britain, NE (*Not Evaluated*) in Switzerland, where it occurs with certainty. It has been assessed as VU in France, whereas in Germany, Czech Republic and Italy as CR. Finally, *U. intermedia* is considered VU in France, EN in Switzerland and CR in Germany, whereas few data are available for other countries. For a proper conservation status assessment of these species, a correct definition of their geographic distribution is needed. However, to achieve this, it is crucial to correctly and safely identify these taxa, even in absence of flowers (which is a frequent case). Aimed to provide valuable tools for species identification using vegetative portions of the plants, morphometric and DNA barcoding analyses were carried out.

Geometric morphometrics on quadrifid glands resulted useful within the *U. intermedia* aggr., particularly if combined with other morphometric analyses, despite an overlapping among species. The ITS marker discriminates between *U. intermedia* and *U. stygia*. On the contrary, the large intraspecific variability found in *U. ochroleuca* and *U. stygia* may be due to their possible hybrid origin (3), thus ITS is not a good marker for identify these taxa. Barcoding with *trnL-trnF* IGS and *rps16* intron (cpDNA) revealed inapplicable for most of the critical species, but may be useful for the distinction of *U. bremii* and *U. minor*, even if a little proportion of the haplotypes (barcodes) found in *U. bremii* can match those found in *U. minor* and viceversa.

Thereafter, our results confirmed that these taxa are difficult to distinguish, either on morphological or molecular ground, also raising doubts about their taxonomic status. Indeed, the populations of *U. bremii* could fall within the variability of *U. minor* and, similarly, *U. ochroleuca* and *U. stygia* could fall within the variability of *U. intermedia*. Despite this, *U. bremii*, *U. ochroleuca* and *U. stygia* represent an important source of variability. In fact, all populations of these taxa show distinct genetic assets, as witnessed by their molecular profile, and, sometimes, distinct morphological traits (e.g. flowers). For these reasons, they deserve protection, along with *U. intermedia*, in virtue of the suggestions for a right conservation practice derived by systematic data (e.g., 6).

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## 1.10. = NEW RED LIST OF THE ITALIAN VASCULAR FLORA: NEARING THE END

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In 2011, MATTM and SBI started a collaboration for the New Red List of Italian Vascular Flora (1, 2), according to the IUCN Categories and Criteria. IUCN Red Lists represent the most widely applied protocol for evaluating species extinction risk, because they facilitate objective and replicable assessments (3). National assessments provide a basis for conservation planning, representing an important starting points for further conservation actions.

Data on species distribution gathered, from recent field surveys, herbarium specimens, published and unpublished data, were assembled with the collaboration of a large number of SBI members. All georeferenced records were validated by a selected group of botanists and organized in a 2 × 2 km cells-grid in GIS environment. A database including distribution data, population trends and the main threats affecting taxa was developed. All the assessments were validated during several collective workshops. During 2016, the New Red List of the Italian Vascular Flora will be completed with the evaluation of more than 2000 taxa including Italian Policy species (152 taxa, listed in the annexes of Directive 92/43/CEE "Habitat" and Bern Convention) (4), all Italian endemics (more than 1200 taxa, most part of taxonomically critical genera as *Alchemilla*, *Hieracium*, *Pilosella*, *Ranunculus*, *Rubus* and *Taraxacum* excluded) and all those species of potential conservation interest listed in 2012 for MATTM (more than 1500 taxa, some endemics included). Here, we present the updated status, in view of the ultimate goal that will be reached by the end of 2016. The publication of a book that will summarize all the work done is foreseen for 2017.

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#### 1.4. = WHAT ABOUT FACULTATIVE ANT-PLANT INTERACTIONS MEDIATED BY EXTRAFLORAL NECTARIES IN TEMPERATE ZONES? NEW INSIGHTS FROM THE SOUTHERN ALPS

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Many plants have evolved traits to use ants, one of the most ecologically relevant insect groups, for seed dispersal and protection (1). For instance, one common facultative and often mutualistic interaction between plants and ants is mediated by extrafloral nectaries (EFNs). These structures secrete a sugary reward that attracts ants, which, in return for this valuable food source, defend the plant from herbivores (2). These interactions are well documented in the Tropics and Subtropics, where EFN-bearing plants can make up to half of the surveyed flora and are thus ecologically relevant components of these ecosystems (1, 3). In contrast, these interactions are less-well studied in plant communities of the temperate zone, where they appear absent or nearly absent (4), or have been overlooked.

In this study, we provide new insights into the diversity of EFN-bearing plants in European temperate regions, focusing on the flora of the Swiss Southern Alps, where native ants were found to visit EFNs of cultivated exotic species (5). We explored which plant species bear EFNs, and where on the plant they bear them, whether they are functional (i.e. secreting) and attracting ants, and whether there is a seasonal pattern in EFN activity and interaction with ants. Finally, we discuss implications of our results and outline future research perspectives.

We first prepared a working list of target species based on published records of EFNs (6) and compared with species lists of the native flora as well as the exotic flora of Cantone Ticino (7). Presence of functional EFNs (i.e. with visible nectar droplet) and ants visiting them was confirmed in the field. In selected taxa subjected to the same weather conditions ("insubric climate"), activity of EFNs and ants was surveyed in natural populations, starting in Winter (February 2016), i.e. when plants were still dormant.

We currently confirmed EFNs presence in at least 22 species (half of them are alien including several invasive aliens) from 13 plant families, 1 fern genus (*Pteridium*) and 16 angiosperm genera (*Acacia*, *Ailanthus*, *Albizia*, *Fallopia*, *Impatiens*, *Ligustrum*, *Opuntia*, *Passiflora*, *Populus*, *Prunus*, *Pueraria*, *Reynoutria*, *Salix*, *Sambucus*, *Viburnum*, *Vicia*). The EFNs are found on internodes, stipules, leaves (on the petiole, between leaflets on the rachis of compound leaves, on the leaf lamina), and inflorescences. EFNs were functional and attracting ants in all but three species (*Albizia julibrissin*, *Ligustrum lucidum*, *Viburnum opulus*), where we did not (yet) observe nectar (or ants). Activity of EFNs coincided with the development of the organs subtending the EFNs. The three native and four exotic species surveyed for seasonal patterns in the interactions differed in their secretion period due to different phenology of their EFNs. EFNs were active and ants present as early as late Winter (early March; *Acacia dealbata*), though most activity in native species was observed in Spring, while that of non-native extended into Summer.

In conclusion, current results confirmed the presence of EFN-ant interactions in the flora of the temperate zone, involving not only native but also at least as many alien plant species. These aliens likely represent an additional food source for local ant communities, raising new questions about the ecological role of aliens in the ecosystems where they occur. Whether the EFN-bearing plants actually benefit from the ants' presence, still needs to be tested. Furthermore, to increase our understanding of the role of these interactions at the ecosystem level, one of the next research steps could be that of investigating the abundance and cover of EFN plants, the identity and abundance of nectarivore ants, and the resulting interaction networks in selected plant communities. We hope that these new insights set the stage for and inspire similar research in EFN-mediated ant-plant interactions in Mediterranean ecosystems.

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#### 1.4. = EFFECTS OF POPULATION STRUCTURE ON POLLEN FLOW, CLONALITY RATES AND REPRODUCTIVE SUCCESS IN FRAGMENTED *SERAPIAS LINGUA* POPULATIONS

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Fragmentation of habitats by roads, railroads, fields, buildings and other human activities can affect population size, pollination success, sexual and asexual reproduction (1) specially in plants showing pollinator limitation, such as Mediterranean orchids (2). In this study, we assessed pollen flow, selfing rates, vegetative reproduction, female reproductive success and their correlations with habitat characters in nine fragmented subpopulations of *Serapias lingua*. To improve understanding of population structure effects on plant biology, we examined the genetic differentiation among populations, pollen flow, selfing rates and clonal reproduction using nuclear microsatellite markers.

Smaller populations showed a significant heterozygote deficit at all five nuclear microsatellite loci examined in this study, the coefficient of genetic differentiation among populations was 0.053 and pairwise  $F_{ST}$  was significantly correlated with the geographical distance between populations. Paternity analysis of seeds showed that most pollen flow occurred within a population and there was a positive correlation between percentage of received pollen and distance between populations.

The fruit production rate varied between 5.10% and 20.30% and increased with increasing population size, while the percentage of viable seeds (78-85%) did not differ significantly among populations. The extent of clonality together with the clonal and sexual reproductive strategies varied greatly among the nine subpopulations and correlated with the habitats where they occur. The small, isolated populations tended to have high clonal diversity and low fruit production, whereas the large populations with little disturbance had reduced clonal growth and increased sexual reproduction.

We found that clonality offers an advantage in small and isolated populations of *S. lingua*, where clones may have a greater ability to persist than sexually reproducing individuals (3). Since clonal growth is associated with a progressive reduction in genotypic diversity, sexual reproduction might be indispensable to the long-term success of a species and clonal growth may play an important role in prolonging the time to extinction when sexual fertility is reduced or absent.

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1.1. = ADAPTIVE RESPONSES TO HUMAN IMPACTS IN THE THREATENED WEBB'S HYACINTH (*BELLEVALIA WEBBIANA* PARL., ASPARAGACEAE)

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Biodiversity is facing significant loss due to global climate change and to human activity, causing habitat loss and degradation, pollution, overexploitation and invasive species introduction (1).

*Bellevalia webbiana* Parl. is a bulbous geophyte endemic to a restricted, highly anthropised area of pre-Appenninic Tuscany and Emilia-Romagna. By considering the significant reduction of the species range, the bad condition and low number of its populations and the threats they face (2), *B. webbiana* is currently listed as *Endangered* (EN) in the Global IUCN Red List (3).

This species occurs in open habitats, especially at the margins of oak forests, olive tree groves, fields and meadows. Changes in land use associated with natural wooded vegetation recovery are related with the reduction and loss of several populations. Moreover, most part of the stands are included within private lands, leading to a high management unpredictability. Nevertheless, the species successfully survives in early successional stages after abandonment and, having a bulb buried deep in the soil, it seems able to cope with most farming practices (e.g. ploughing). However, accurate field surveys at the population level and the species adaptive responses have never been investigated.

Given the uniqueness and vulnerability of this species, our study aims to understand the impact that anthropic disturbance may have on populations of *B. webbiana*.

In order to cover as best as possible the range of the species, five different populations have been selected, three in Tuscany (Province of Florence) located in Pratolino (Vaglia), Uccellatoio (Vaglia) and Villa Antinori (Impruneta) and two in Emilia-Romagna (Province of Ravenna) located in Casola Valsenio and Faenza. In both regions, the populations were selected according to different degrees of disturbance. The analysis was based on the comparison of several functional traits through the LHS (Leaf-Height-Seed) model, seed set, vegetative and inflorescence heights, number of flowers per inflorescence, number of fruits per individual, number of seeds per fruit and number of pollen grains per flower (4). In addition, soil analyses were carried out, searching for differences between the sites and to test correlations with the functional and reproductive features of the populations.

From the very first data acquired, disturbed and undisturbed populations appeared to be fairly different in the fitness of single individuals, in favor of the undisturbed sites (i.e. Villa Antinori and Faenza). In these undisturbed sites taller and healthier plants with high amounts of flowers, fruits and seeds were found. On the other hand, the bad conservation status of the disturbed sites appeared evident, even more increased by grass-cutting activity in Pratolino, by change in land use in Casola Valsenio, and by predation (i.e. wild boars) in Uccellatoio. Indeed, non-parametric tests (Kruskal-Wallis and Mann-Whitney pairwise comparisons) confirm these patterns with significant differences among area and dry mass of sampled leaves respectively with value  $p < 0.01$  and  $p < 0.001$ .

Overall, we conclude that the plants show different responses in growth and fitness according to their site of occurrence and environmental variations.

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## 1.8. = RECOGNIZING THE ROLE OF ECOLOGICAL STUDIES IN PLANT TRANSLOCATION: REVIEW AND PERSPECTIVES

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A recent paper by Abeli & Dixon (1) highlighted the role of ecological studies applied to plant translocation, naming this peculiar application as Translocation Ecology. Although, prevention and *in situ* conservation measures should be preferred, reinforcement, reintroduction and introduction of species at risk of extinction are valuable (but risky) conservation options. Indeed, translocations are rarely the simple exercise of moving species and/or populations for conservation purposes with a 'gardening approach'; behind each translocation the support of science is essential to increase the probability of success of actions with an intrinsic high rate of failure (2).

Key questions in plant translocation concern the selection of suitable release sites both as macro-areas and microsites, plant-plant and plant-pollinator relationships, identification and mitigation of threats, source populations and propagation protocols, tolerance to local abiotic and biotic stress and so on (3). Ecological studies may help to answer all of these questions or contribute to the decision process in a translocation project.

Here, the role of ecology in plant translocation is discussed and a review of cases is presented.

Moreover, a special focus is given to some best practices specifically concerning: (i) the issues related to the relationships between host and parasite in parasitic plants translocation, (ii) the effect of microsite conditions on translocation success (iii) the choice of source populations in presence of confounding dispersal patterns, (iv) the use of paleo-ecological data in translocation planning.

It results that a greater integration of ecology in plant translocation is a key to reduce uncertainty in such type of conservation actions and that Translocation Ecology, hence ecological research applied to translocation, is a growing science, that will have a great impact in future conservation actions (1).

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## 1.12. = PHYLOGENETIC AFFINITIES AND SYSTEMATIC POSITION OF THE ITALIAN POPULATIONS OF GYMNOSPERMIUM (BERBERIDACEAE)

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*Gymnospermium* Spach (Berberidaceae) is a small genus of tuberous herbs, characterized by fruits with exposed seeds when the membranous pericarp splits (from the Greek “gymnos”, naked, and “sperma”, seed). Together with the related genera *Leontice* L. and *Caulophyllum* Michx., it is part of tribe Leonticeae (Spach) Kosenko, which is characterized by succulent staminodia, eu-reticulate pollen exine, utricular gynoecea and a basic chromosome number of  $x = 8$  (with exceptions). Although *Gymnospermium* is the most diverse genus in Leonticeae, it only includes around twelve specific or subspecific taxa, inhabiting semiarid steppes, montane shrublands and mesic forests across Eurasia. These taxa are mostly allopatric diploid endemics, forming three distinct geographic groups in eastern China, in central-western Asia and in the Balkan peninsula (1, 2). In spite of such a vast distribution range, the degree of morphological differentiation in the group is low and this has caused different interpretations of the limits, taxonomic status and number of species.

In 2014 populations of *Gymnospermium* were discovered by the first two authors of this work in a small forest area of the Maddalena mountains, a massif of the southern Apennines (Salerno province). Biogeographically, this finding allowed to extend much to the west the limit of the genus range. Based on macro-morphological characters, the Italian populations were first identified as *G. scipetarum* E.Mayer & Pulević, a species endemic to central Albania and south Montenegro. Other closely related species are *G. maloi* Kit Tan & Shuka, endemic to south Albania, and *G. peloponnesiacum* (Phitos) Strid, the third Balkan species endemic to the Peloponnese (3). The discovery of *Gymnospermium* in Italy prompted further studies on the species-level systematics of this little-known group, about which neither taxonomic investigations nor phylogenetic analyses have been conducted to date. We aimed at filling this gap using a combined molecular and morphological approach, and performing a phylogenetic analysis of the genus based on a taxon sampling including all the Balkan taxa plus other species from Europe, western and central Asia, and the Far East. Both nrDNA (ITS region) and cpDNA (*trnL-trnF* IGS) markers were used to infer interspecific relationships and better understand the affinities of the Italian populations. The rate of variation of both markers was relatively low, so that not all the nodes were resolved in the Bayesian and Maximum Parsimony trees. This result matches the low degree of morphological differentiation among species, despite their, often wide, geographic isolation. However, the Albanian and Italian accessions were retrieved as a monophyletic group by both markers, and the populations from the southern Apennines resulted closer to *G. maloi* than to *G. scipetarum*. The Italian accessions, however, were characterized by a few Single Nucleotide Positions (SNPs) and formed a distinct, well supported group in the combined ITS- *trnLF* analysis. The morphometric study revealed a somewhat intermediate position of the Italian plants between the two above species, from which they differ by a peculiar combination of character-states. Karyological analysis revealed the chromosome number  $2n = 14$ , as in *G. maloi* (2), while most of the other investigated species of *Gymnospermium* have  $2n = 16$ . In the light of these results, the Italian populations are provisionally referred to a new species which is currently under description.

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2.2 = GENOTOXIC EFFECTS OF CADMIUM AND LEAD IN THE MOSS *SPHAGNUM PALUSTRE* L.

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Evaluation of toxic effects of heavy metals is one of the most important topics in environmental studies; indeed, it is known that high concentration of heavy metals can damage morphology and physiology of cells and tissues (1). In last decades, mosses have been largely used to evaluate accumulation of heavy metals (2), as well as metal induced morpho-physiological changes; but genotoxic effects of heavy metals have been poorly investigated in these organisms so far (3). The aim of this study is: i) to assess the genotoxic effects of cadmium and lead in a clone of *Sphagnum palustre* L. using inter-simple sequence repeats (ISSRs); ii) to evaluate the pathway of uptake of these two elements by X-ray SEM microanalysis. *S. palustre* clone was cultured in axenic conditions and exposed to different concentrations of Cd and Pb for 30 days; the DNA was extracted and amplified using 12 ISSR primers. ISSR profiles for each primer were analyzed using a software (4) for band counting and size assignment (Fig.1). Genomic Template Stability was calculated for each treatment to estimate genotoxicity, and the values obtained compared to GTS for control sample assumed as 100%. Metals induced loss of normal bands, appearance of new bands, and changes in band intensity compared to control samples; both heavy metals decreased GTS in a dose dependent manner. Observation by X-ray SEM microanalysis highlighted Pb particles on moss surface (Fig.2), whereas Cd was never found in form of particles (Fig.3), but diffused in the tissues suggesting that this element can move to cell compartments. Future studies are needed to clarify the way of uptake and genotoxicity of Pb and Cd in *S. palustre* and related species.

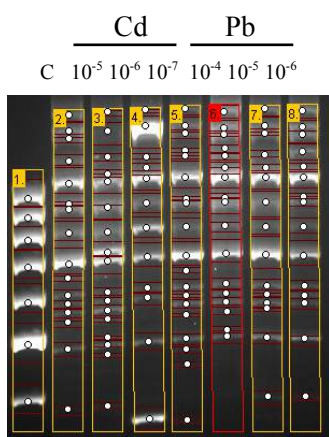


Fig. 1. ISSR profiles of *S. palustre* DNA treated with different Cd and Pb concentrations.

C = control.

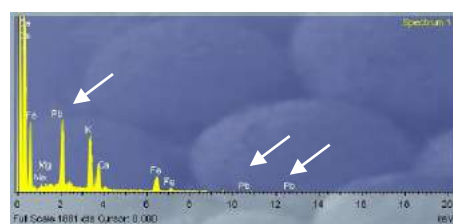
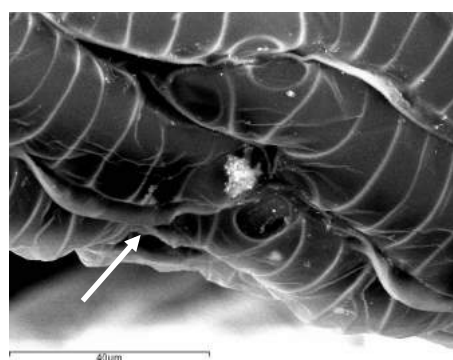


Fig. 2a-2b. SEM photograph of *S. palustre* treated with Pb  $10^{-5}$  and relative spectrum of a particle; arrows indicate Pb peaks.



Fig. 3. SEM photograph of *S. palustre* treated with Cd  $10^{-5}$

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2.2 = OXIDATIVE STRESS RESPONSE IN *CHROOCOCCIDIOPSIS* SP.CLAUDIA FAGLIARONE<sup>1</sup>, CLAUDIA MOSCA<sup>1</sup>, CYPRIEN VERSEUX<sup>1,2</sup>, DANIELA BILLI<sup>1</sup><sup>1</sup>Department of Biology, University of Rome "Tor Vergata", Via della Ricerca Scientifica snc, 00133 Roma, Italy<sup>2</sup>NASA EAP Associate, NASA Ames Research Center, Moffett Field, California 94035, USA

Oxidative stress is caused by reactive oxygen species (ROS), which are produced physiologically as metabolic derivatives. However as a result of exposure to physical and chemical agents such as ionizing radiation, desiccation or hydrogen peroxide, these free radicals can accumulate within the cell with consequent damage of proteins, lipids, carbohydrates and nucleic acids (1, 2, 3). Some bacteria such as those of the genus *Chroococcidiopsis* and *Deinococcus* are able to evade oxidative damage induced by radiation and desiccation (4, 5). In particular, members of the genus *Chroococcidiopsis* are characterized by a pronounced ability to withstand the lethal effects of desiccation (6). In fact in nature these cyanobacteria survive in the most extreme arid environments remaining in a desiccated state for most of the time (7). Moreover it was shown that *Chroococcidiopsis* is capable to survive ionizing radiation up to 15 kGy and UVC doses as high as 13 kJ/m<sup>2</sup> (8). The molecular mechanisms underlying the extraordinary irradiation and dehydration resistance of this cyanobacterium remain poorly understood, however there are evidences that suggest a strong correlation between desiccation and radiation resistance. In fact it seems that radiation resistance is a consequence of desiccation resistance (9). For a long time it was thought that the main target of ROS was DNA, however recent studies have shown that *Deinococcus radiodurans* is susceptible to DNA damage as all the other species, but its proteome is better protected against free radicals respect to susceptible bacteria such as *E. coli* (10). Thus the amount of protein damage, and not DNA damage, together with the cellular ROS-scavenging capacity determine the radiation survival of bacteria (11). Therefore in this work we want to verify if *Chroococcidiopsis* is able to protect its proteins against oxidative damage induced by different stress conditions by analyzing the protein carbonylation as a marker of oxidative stress. So we have treated *Chroococcidiopsis* with increasing concentrations of hydrogen peroxide using *E. coli* as a susceptible control. We also treated *Chroococcidiopsis* and *Synechocystis* (sensitive control) with increasing doses of radiation up to 25 kGy. Finally the protein carbonylation was evaluated for both cyanobacteria after a year of desiccation. For each experiment was also performed survival and DNA damage analysis.

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## 2.2 THE UNIVERSAL STRESS PROTEIN OF *POPULUS ALBA* VILLAFRANCA CLONE: PRELIMINARY CHARACTERIZATION

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The Universal Stress Proteins (USPs) are a group of proteins encoded by a superfamily of genes that are present in bacteria, archaea, fungi, protozoa and plants (1). These proteins are characterized by a conserved domain of 140 – 160 residues (2). The USPs are known to provide to the organism the ability to tolerate many kinds of biotic or abiotic stresses, such as nutrient starvation, extreme temperatures, drought, high salinity, osmotic stress, hypoxia, exposition to toxic chemicals and UV damage (3, 4). Therefore the USPs have an important role to improve the survival rate of the organism when it is exposed to environmental stressing agents (5), but, up to now, the molecular and biochemical mechanism of the USPs is still not known. Initially the USPs were found in bacteria, and the first USP identified was C13.5, but the name was after changed in USP for underline the fact that the bacteria overexpressed these proteins when the organisms have to affront a plethora of environmental stresses (3). The plant USPs the derive from a 1MJH – like ancestor, referred to the USP A of *Methanocaldococcus jannaschi*, that binds ATP (6). The ability of binding ATP suggests that these USPs can have an activity has a molecular switch, but this role was not already shown (6).

In this study we have identified a poplar *UPS* gene, encoding a protein of 179 aminoacids similar to *Populus trichocarpa* USP (XP\_002302760.2) containing the USP and the bound ATP domains.

Our results showed that the expression of *PaUSP* has been found in roots, stem and leaves of *P. alba* *in vitro* grown plants; expression is induced by gibberellins in roots and leaves and by auxin in leaves and stem. Expression is also induced by sub-lethal concentration of zinc. Interestingly, *PaUPS* expression in leaves and in roots is highly induced by oxidative stress caused by hydrogen peroxide and by the herbicide methyl viologen (Paraquat).

We demonstrated, also, that bacteria (*E. coli*) expressing *PaUSP* are more resistant to oxidative stress induced by hydrogen peroxide and to salt stress induced by NaCl. Our results suggest that the Universal Stress Protein may be involved in the resistance to environmental stress in *P. alba*.

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### 2.3 = AN OMICS APPROACH TO UNRAVEL THE RELATIONSHIP BETWEEN METHYLATION STATUS AND PLANT GROWTH PLASTICITY IN THE DRM1DRM2CMT3 MUTANT OF *Arabidopsis thaliana*.

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Plants are sessile organisms that evolved a high degree of plasticity to adapt and optimize their growth and reproduction in relation to fluctuating environmental conditions including light, temperature, water content, but also to different stressful conditions. Therefore, the efficient perception, interpretation, and transduction of external signals allow plants not only to synchronize their development with seasonal change, but also to cope with even increasing stresses. It has been suggested that nuclear DNA methylation, together with other epigenetic marks, is responsible of the genome plasticity (1). Accordingly, nuclear DNA methylation has been found to regulate gene transcriptional activity in response to both endogenous stimuli and external factors (2). In order to understand how the DNA methylation status can act as an interface between external stimuli and plant development, we investigated a loss of function *Arabidopsis* mutant, *drm1 drm2 cmt3* defective in both maintenance and de novo methylation processes (1,3,4). By using several transgenic lines, we showed through confocal and PCR analysis that the pleiotropic phenotype abnormalities exhibited by this mutant, from embryogenesis to reproductive phase, are related to an alteration of auxin distribution and to an affected expression of specific auxin-related genes. MeDIP analysis conducted on some of these genes did not revealed any direct relationship between gene expression and methylation level. Moreover, we also observed a higher tolerance of this mutant to different stressful factors such as Cd and farnesene, compared to the wild type. In order to get a general overview on the genetic networks and metabolic pathways affected by the defective DNA methylation status, an Omics approach was applied. In particular, we sequenced and analyzed by RNA seq technologies the transcriptome of *drm1 drm2 cmt3* triple mutant vs WT. All together the obtained results, besides confirm the alteration in hormone pathway, support the hypothesis that an epigenetic control of photosynthetic process, energy efficiency and homeostasis is a component of the different growth response and plasticity observed in the *drm1 drm2 cmt3* mutant of *Arabidopsis thaliana*. Interestingly, our results also showed an up-regulation of genes involved in light signal perception, suggesting a role of the DNA methylation status in the light perception and in the light signaling pathways. With the aim to verify such hypothesis, we performed the hypocotyl assay, a conventional method to compare the response of plants grown under different light wavelengths or in darkness conditions, and also useful to identify signaling pathway(s) mediated by the product of the mutated gene. The *drm1 drm2 cmt3* mutant (Col-0 background) and the WT (Col0) were tested under all light conditions such as red, far-red and blue light, for four and six days. A shorter hypocotyl was observed in the mutant vs WT under all light qualities analyzed, while in the dark the hypocotyl length was the same to the WT, thus proving a hypersensitivity of the mutant to light. This result, together with bioinformatic analysis of the methylation level of genes involved in light signaling perception, strongly support our hypothesis. Work is in progress to go further insight these aspects.

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## 2.2= THE EFFECTS OF POLYAMINES DURING THE POLARIZED CELL GROWTH OF POLLEN TUBES

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Pollen tube growth is a rapid process restricted to the only tip region and depends on an elaborate mechanism that integrates several molecular and cytological sub-processes and ensures a cell shape adapted to growth. Many factors cooperate to allow this apical growth, creating an intricate signalling network. The continuous rebuilding of the cell wall and apical migration of the cytoplasm sustained by cytoskeleton re-organisation are the most important driving forces needed for growth, but many other factors are involved in this process, among which ions gradients (mostly Ca<sup>2+</sup> and H<sup>+</sup>) and ROS, that support the apical growth, at physiological concentration. The growth mechanism is also controlled by external signaling molecules such as polyamines (PAs). We analyzed the PAs effects on the growth of pear pollen tubes; results showed that PAs, in particular spermine entered through the pollen tube tip, then diffused in the sub-apical region. The same region underwent drastic morphological changes, showing loss of polarity leading to apical swellings that momentarily stopped the growth process. After this initial critical phase, pollen tubes were able to resume growth. The presence of swelling was characterized by drastic modifications of different parameters among which pH and cytosolic Ca<sup>2+</sup> concentration. The effects of PAs concerned, at least in part, also their ability to modulate ROS concentration, by altering the activity of ROS-producing and ROS-scavenging enzymes. The new scenario consecutively caused a major reorganization of actin filaments that were randomly organized at the swelling phase but that resumed their typical organization as soon as pollen tubes started to grow again. This led to alteration of cytoplasmic organelle movement and finally, to deep alteration in cell wall organization, mostly through modification of pectin and callose deposition; the latter process was closely related to rearrangement of the enzyme callose synthase. In summary, PAs are able to deeply affect pollen tube growth and therefore they could play important roles in the relationship between pollen tube and pistil in flowering plants during fertilization.

## 2.2 = STRESS RESPONSE AND TOLERANCE TO HEXACHLOROCYCLOHEXANE: SOIL FUNGAL SPECIES ISOLATED FROM CONTAMINATED AREAS OF ITALY AND CZECH REPUBLIC AND THEIR POTENTIAL IN FUNGAL BIOREMEDIATION

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Fungi play important roles in soil, providing and supporting ecological services for ecosystems and human wellbeing (1, 2, 3). To date, the metabolic and morphological versatility of fungi underpins practically all bioremediation applications, but often is still untapped (1, 4, 5). They can tolerate extreme environmental conditions and survive in high concentrations of persistent organic pollutants (e.g. pesticides) by possessing mechanisms for the degradation, utilization and transformation of organic substrates (1, 4, 5). The study of soil microbial community represents an important step to shed further light on the environmental contest. In this research, we have isolated fungal species occurring in samples from sites of Italy and Czech Republic with high concentration of hexachlorocyclohexane. Isolates belonging to Ascomycota, Zygomycota and anamorphic fungi have been identified. Some of them have been reported in literature for the biotransformation of organic pollutants, such as polycyclic aromatic hydrocarbons and pesticides (1, 4, 5, 6). Moreover, we have investigated the tolerance of selected fungal species to different isomers of hexachlorocyclohexane by using tolerance indices (Rt:Rc; T.I.). For their adaptation to stressful environmental conditions, fungal species isolated from contaminated sites may provide opportunities for new environmentally-friendly, integrated and cost-effective approaches for environmental management and restoration.

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2.2 = INTERACTIONS BETWEEN HOST PLANTS AND PATHOGENS: THE CASE OF *XYLELLA FASTIDIOSA*

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At the moment, a very dangerous plant disease, that targets *Olea europaea* L. trees, has invaded our country, causing a significant economical loss. The disease, known as "quick decline syndrome of olive trees" (OQDS), appeared, for the first time in Italy, on the 15<sup>th</sup> October 2013 in Salento peninsula (Apulia) and interested the 10% of 90.000 hectares of land purposed to cultivation of olive trees.

The etiopathology of OQDS seems to be very complex and includes the action of three different agents: the wood leopard moth (*Zeuzera pyrina* L.); the fungus *Phaeoacremonium parasiticum* and the bacterium *Xylella fastidiosa* W. In particular, this last microorganism is considered the principal responsible for the desiccation that characterized infected plants, through xylematic vessel occlusions and phloematic damages. Since 1981, it has been also introduced in the EPPO (European Plant Protection organization) list of quarantine pathogens.

*X. fastidiosa* transmission occurs through the help of xylem-fluid feeding vector insects: *Philtaenus spumarius* L., *Neophilaenus campestris* F., *Euscelis lineolatus* B. Currently, the only intervention strategies applied in the contaminated area include agronomical approaches, as bordering of contaminated zones, isolation of infected materials, pruning of desiccated plants and application of preventive and phyto-sanitary strategies (such as use of pesticides against vector insects and antibiotic directly on trees).

The collection of infected and healthy plant materials of different cultivars was accomplished in January and June of 2015 from two small towns in province of Lecce: Sannicola and Alezio.

The presence of *X. fastidiosa* in these samples was confirmed through ELISA and PCR analyses. The genetic characterization identified the *pauca* subspecies as the one that infects *O. europaea* trees in Salento. Moreover, to clarify the colonization ability of the bacterium in target plants, the disposition of *X. fastidiosa* in host tissues was carried out through confocal fluorescence and scanning electron microscopy.

An interesting prospective comes from the different response of *Olea europaea* cultivars to the infection. A wide comparison between Cellina di Nardò susceptible cultivar and Leccino resistant one was performed to explain the alternative response of these ecotypes to the current biotic stress.

Encouraging data have been obtained analysing ROS expression in infected and healthy *O. europaea* plants. In fact, since Cellina di Nardò healthy cultivar produced less basal ROS than the Leccino healthy one, during the infection Cellina di Nardò plants were not able to guarantee a sufficient ROS concentration to reject pathogen's attack. On the contrary, higher ROS levels produced by Leccino plants could represent the reason of their successful response to the infection.

A general proteomic analysis did not give significant difference, even if detection and quantification of carbonylated proteins revealed a variance among samples, supporting previous oxidative results.

Samples were also subjected to a deep biochemical study, by spectrophotometry and HPLC-DAD-MS; it demonstrated how specific secondary metabolites might have a key role in olive tree defence against *X. fastidiosa*.

Further genetic investigations will be carried out, monitoring the expression of several host resistance plant genes, to support the existence of an alternative bacterial stress sensibility of different olive tree varieties.

In conclusion, this kind study will hopefully permit to find reproducible factors that can improve the resistance of *O. europaea* plants to this dangerous infection that threatens one of the most productive global plant species.

## 2.2 = A NEXT GENERATION APPROACH REVEALS THE IMPACT OF SOIL MICROBIOTA ON GENE AND PROTEIN PROFILE OF TOMATO

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Root-associated microbiota, in addition to soil characteristics, play a major role in shaping plant physiology and agronomic traits under diverse environmental conditions. While several studies have investigated microbiota biodiversity (1, 2), highlighting the impact of plant genotype (3, 4), the comprehension of the mechanisms related to plant response is still at its infancy.

To begin to untangle this complex skein, we investigated in tomato (*Solanum lycopersicum*), a model plant of economic interest, how plants respond when grown on a natural soil. Two tomato genotypes, Cuore di Bue and Battito, respectively susceptible and resistant to *Fusarium oxysporum* f. sp. *lycopersici* (FOL), were maintained in two soils different for geographical origin, history and microbiota: RO sampled in Rosta (TO, Italy), AL sampled in Albenga (SV, Italy), as well as a sterile peat-moss soil (CONT).

As a first explorative analysis, we grew tomato plants in glasshouse on the three different substrates, extracted RNA and proteins from roots and performed i) Illumina HiSeq deep-transcriptome sequencing on 18 cDNA libraries and ii) global proteome profiling for the susceptible genotype only (6 libraries) using LC-MS/MS on Q-Exactive Orbitrap. Transcriptome datasets showed that soil, with its biotic and abiotic components, is the main driving force that induces the plant to activate its defense pathway. Interestingly, KEGG pathway analysis of both transcriptome and proteome profiling datasets highlighted that soil-grown plants activate more efficiently generic defense mechanisms, when compared to plants growing in a sterile substrate, including those related with lignin deposition and plant-pathogen interactions, such as pathogenesis-related proteins (PRs). AL soil, characterized by partial disease suppression (5) was particularly effective in priming elicitation. As a second step, sequencing data were technically validated using qRT-PCR; lignin and total phenol content were assessed in a second experiment performed on root and leaf tissues confirming the transcriptome and proteome results.

Finally, a third experiment was performed by adding a FOL inoculum to the experimental setup. Without pathogen inoculation, the two plant genotypes responded in a similar way, as also observed in the previous experiments, but when FOL was introduced into the system, the resistant genotype showed higher yield and lower disease severity, as expected. Moreover, under pathogen attack, the susceptible genotype activated stronger molecular defenses.

In conclusion, our work is starting to reveal some mechanisms operating in tomato living in natural soils.

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## 2.1 = ROLES OF 1,3-DI(BENZO[D]OXAZOL-5-YL)UREA, AN UREA DERIVATIVE, IN CAROB AND PINE CUTTINGS: ADVENTITIOUS ROOTING VERSUS XYLOGENESIS

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Adventitious root (AR) formation is often a limiting step for vegetative plant propagation, specially for woody species of economic value. There are evidences showing that adventitious rooting and xylogenesis, both auxin-mediated programs, may compete in different plant experimental systems, and the prevalence of xylogenesis vs rhizogenesis may negatively influence the results of a rooting protocol. Previous results demonstrate that two synthetic urea derivatives, named 1,3-di(benzo[d]oxazol-5-yl)urea (5-BDPU) and 1,3-di(benzo[d]oxazol-6-yl)urea (6-BDPU), combined with the auxin indole-3-butyric acid (IBA), enhance AR formation in cuttings of different woody species, e.g. *Malus pumila* (1) and *Pinus radiata* (pine; 2). Present research is the first attempt to utilize these two unusual chemicals for improving adventitious rooting in stem cuttings of *Ceratonia siliqua* L. (carob), a woody angiosperm of high environmental and commercial value (3), but with rooting recalcitrance. Another aim was to identify morphogenic roles of the most effective urea compound in common between carob and pine, to verify the persistence of the same actions in distant species. To the aims, firstly, different protocols were applied in carob, varying the time of chemicals application/dark exposition, in the presence/absence of different IBA concentrations, in order to stimulate AR formation. The results showed that 5-BDPU (10  $\mu$ M), combined with IBA (1  $\mu$ M), highly enhanced rooting in carob cuttings in comparison with IBA alone, better than 6-BDPU (10  $\mu$ M), further reducing callus formation in comparison with IBA alone. Neither of the two urea derivatives showed rooting inductive ability *per se*, confirming previous results in pine (2) and that IBA was the central factor for AR induction in both species. However, the histological analyses on carob cuttings revealed a new morphogenic role for 5-BDPU, i.e., when used alone the compound favoured xylogenesis from the cambial cells, which were instead able to initiate the adventitious rooting process when also IBA was present. The histological analyses in pine hypocotyl cuttings confirmed that 5-BDPU alone (at the same concentration used in carob) favoured xylogenesis, starting from the AR-competent cells, which in this species were those bordering the resin ducts. In conclusion, in two distantly related species, this urea derivative exhibits a dual morphogenic role, being involved in the switching between rhizogenesis and xylogenesis depending on the presence/absence of exogenous auxin input. The results open the way for understanding xylogenesis as an alternative program inhibiting AR formation and successful micropropagation.

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## 3.6= NATURE BASED SOLUTIONS TO IMPROVE AIR QUALITY IN METROPOLITAN AREAS

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Improving air quality in cities is one of the main challenges for the European Union (EU). In particular air pollution due to particulate matter (PM) and tropospheric ozone (O<sub>3</sub>) poses a significant health risks for European citizens (1). The European Environmental Agency reports that, in 2011, around 33% of the urban population has been exposed to coarse PM (PM<sub>10</sub>) levels exceeding the daily limit value imposed by the Directive 2008/50/CE (50 µg m<sup>-3</sup>) and, if the WHO annual air quality guidelines (20 µg m<sup>-3</sup>) are considered, the percentage rises to 88% (1). This scenario of increasing environmental risks in cities calls for the new solutions to improve the urban environmental quality. In this regard, the European Union recently suggested that the properties of natural ecosystems, and the Ecosystem Services (ES) they provide, may become the focus of specific research and innovation policies in order to find new viable solutions to challenges faced by society. These so-called "nature-based solutions" may exert a positive environmental impact, which could form the basis of sustainable urban planning, by reducing energy requirement costs and mitigating climate changes and the causes of stress conditions (2, 3). In this context, urban and periurban forests, which are integrated within the concept of Green Infrastructure (4), exert a pivotal role, since they provide important ES, including air quality amelioration through pollutant removal (5, 6).

In this study, we quantified the ES of particulate matter (PM<sub>10</sub>) and Ozone (O<sub>3</sub>) removal from urban and periurban forests in some metropolitan cities in Italy, and its total monetary value. In particular, the pollution removal of forest ecosystems, which are classified into different Physiognomic-Structural Vegetation Categories, was mapped using a remote sensing and GIS approach, by applying a deposition model and a stomatal flux model (6). We estimated, for the considered metropolitan cities, an overall pollution abatement of 7150 Mg of PM<sub>10</sub> and 30014 Mg of O<sub>3</sub> in the year 2003, which was an extremely hot year. Our findings indicate that structural characteristics (i.e. Leaf Area Index) and functional diversity, linked to stomatal conductance, exert a marked influence on the provision of this regulating ES. The total monetary value of this ES was estimated to be equal to 47 and 297 million USD for PM<sub>10</sub> and O<sub>3</sub> removal, respectively, thus underlining the crucial role played by nature-based solutions in human well-being in urban areas. This study represents the first national-scale assessment of the ES of air pollution mitigation in Europe, and provides information that may be useful to stakeholders for a better development and management of the urban and periurban Green Infrastructure.

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## 3.6 = AIR POLLUTION MITIGATION VIA URBAN GREEN INTERACTIONS WITH PARTICULATE MATTER

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Air quality in urban areas is strongly affected by the noteworthy presence of traffic-induced emissions like nitrogen oxides (NO<sub>x</sub>), carbon monoxide (CO) and dioxide (CO<sub>2</sub>), hydrocarbons (HC), and particulate matter (PM<sub>x</sub>) (1). Specifically, fine and ultra fine dusts with a diameter of less than 2.5 μm (e.g., PM<sub>2.5</sub>) represents an inhalable suspended fraction which can cause cardiopulmonary diseases, especially for long-term exposure (2). On the other hand, it is also known that vegetation can help restoring the environmental quality of dense urban areas by reducing the "heat island effect", improving energy performance of buildings, managing storm-water and realizing a trap effect on air pollutants (3, 4). This latter is getting a growing interest thanks to the potential ability of plant species to decrease the level of particulate matter in street canyons. Plant characteristics (e.g., species used, plant shape, leaf surfaces, leaf area index, porosity, etc.) play a key role in potential air quality improvement (5).

The goal of the present study was to evaluate the interactions between PM and leaf epidermis and estimate the potential ability of PM removal in plant species commonly used for greening urban areas in Genoa (NW Italy).

Four specie at different sampling height were selected: *Cedrus libani* A. Rich., *Pittosporum tobira* (Thumb) W. Aiton, *Photinia × fraseri* (*P. glabra* (thumb) Maxim. × *P. serratifolia* (Desf.) Kalkman, and *Hedera helix* L. For each species an ESEM-EDS analysis was performed to evaluate PM composition, disposition and dimension using the counting method (6). The evaluation of PM deposition revealed a different removal ability by the considered species: *C. libani* > *P. × fraseri* > *H. helix* > *P. tobira* (Fig. 1). The removal ability is species-specific and depends on leaf epidermis, morphology and disposition.

Our results show the mitigation potential of plants in limiting near-road PM exposure. Finally, the measured data can provide useful input data for the evaluation of theoretical models to estimate the effect of different greening systems with selected species in urban areas.

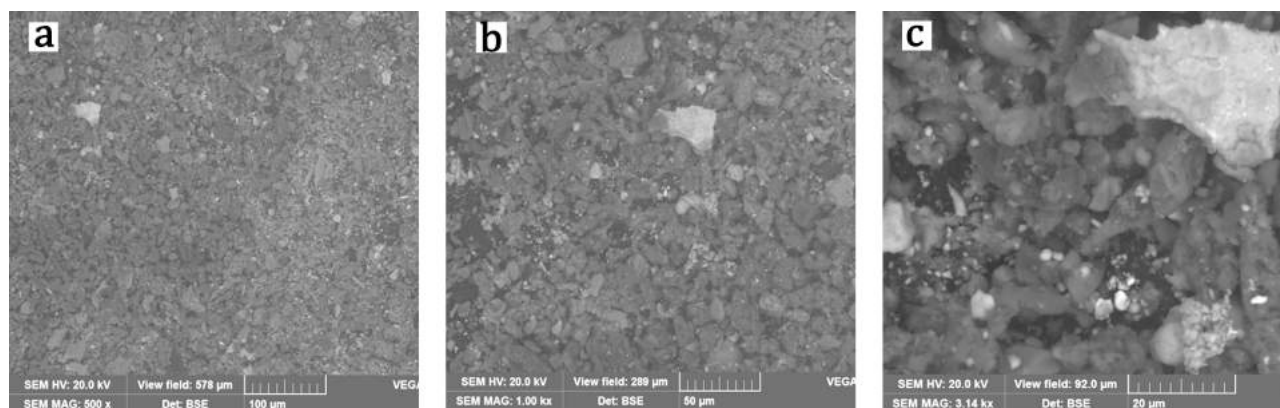


Fig. 1. PM specifically binds to leaf epidermis in *C. libani*. ESEM micrographs.

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### 3.1 = ECOSYSTEM SERVICES: THE ROLE OF PLANTS IN URBAN PARKS

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The increasing urbanization process radically modifies the landscape ecology including soil and water degradation, and loss of species diversity (1). Urban areas are estimated to consume 67% of the global energy and emit 71% of the energy-related CO<sub>2</sub> emissions (2). A current estimate of the expected rise in global average air temperature due to greenhouse gas is between 1.4 and 4.0 °C by the year 2100 (3). Taking into account that over 50% of the world population lives in cities and more than two thirds are expected to increase by 2050, the problem of mitigating the atmospheric CO<sub>2</sub> concentration is considerable. Moreover, after air and water pollution, noise is the third most serious kind of pollution in cities affecting human health unfavorably both physically and psychologically (4). Urban parks could generate significant ecosystem services, such as offsetting carbon emissions, removing air pollutants, reducing noise, regulating microclimate, and favoring recreation and amenity (5) contributing both to physical and mental well-being of people (6). In this context, the main objective of our research was to evaluate the role of urban parks in delivering ecosystem services and highlighting the contribution of different vegetation types, size and position of the considered urban parks in Rome. In particular, we analyzed the effects of plants in decreasing CO<sub>2</sub> concentration and noise and improving microclimatic conditions inside the parks and in the surrounding areas. Moreover, since the presence of buildings within urban parks determines CO<sub>2</sub> emissions closely related to their purpose of use, the role of trees, hedges and grass in decreasing the use of energy was analyzed. In fact, vegetation reduces solar radiation from heating buildings, cools the area through transpiration, reduces wind speed and consequently the use of air-conditioning systems. The results highlight that plants in the parks represent an excellent regulator of air temperature by moderating urban heat islands. Nevertheless, the range of the effects of the vegetation in the surrounding park areas is a function of the park size and trees coverage. Moreover, the results highlight that plants have a significant role in decreasing noise level. A set of possible solutions for energy use reduction in the historical buildings to support all human activities in urban parks are proposed. Understanding the relationship among urban vegetation, people, and the environment can facilitate future urban designs to enhance environmental and social benefits (e.g. building energy conservation, individual and community well-being, wildlife habitats). Our data concerning urban green areas and plant traits, including their specific air amelioration capability could be used as urban inventory for tree planting programs to ameliorate urban air quality.

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## 3.2 = PROPOSAL FOR A URBAN GREEN INFRASTRUCTURE IN THE METROPOLITAN CITY OF ROME

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Target 2 of the EU Biodiversity Strategy for 2020 requires member states to Map and Assess the state of Ecosystems and their Services (MAES), and to maintain and enhance ecosystems and their services by establishing Green Infrastructure (GI) and restoring at least 15% of degraded ecosystems.

In keeping with the implementation of the MAES process in Italy (1), we present a proposal for urban GI in the metropolitan area of Rome. According to the specific requirements of a metropolitan city, we therefore attuned at a proper scale for intervention the criteria for setting biodiversity priorities, the understanding and spatial recognition of key demands for ecosystem services and the opportunity of integrating GI into existing policy and planning tools that have been defined at the national level.

Moving from the available scientific knowledge as regards typification, mapping and assessment of ecosystems and their services in the study area (2-6), a GI proposal was developed that is able to effectively combine ecosystem services provision with restoration of biodiversity components and enhancement of ecological connectivity. Moreover, by means of an estimate of both the environmental and socio-economic expected benefits, the proposal was adopted as a pilot case study at the national level (7) for promoting the actual inclusion of GI into land development planning and for encouraging investments into 'green' rather than 'grey' solutions against challenges that affect urban and peri-urban contexts.

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### 3.2 = HOW IMPORTANT IS THE CHOICE OF PLANT SPECIES FOR THE GREEN ROOF'S COOLING EFFECT ? COMPARISON OF THE BEHAVIOUR OF TWO CAM-FACULTATIVE SPECIES AND TWO C3 SPECIES UNDER DROUGHT CONDITION

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High temperature and substrate moisture deficit are common on green roofs during the summer season. Crassulacean acid metabolism (CAM) allows succulent species growing on green roofs to survive under drought, due to the day-time stomatal closure and malate production (Dark CO<sub>2</sub> fixation). This reduces the transpiration (Tp), but, consequently, the cooling effect due to the transpiration itself. In this study we compared the transpiration rates of two CAM-facultative species (*Sedum lydium* Boiss and *Sedum kantschaticum* Fisch.) and two C3 species of semi-xeric grasslands (*Lotus corniculatus* L. and *Bromus erectus* Huds.). CAM-facultative species switch between C3 and CAM photosynthesis to respond to environmental conditions, for instance lowering of substrate moisture content (SMC) (1, 2). Our aims were to understand the capacity of selected species to continue the evapo-transpiration process, as the substrate moisture content declines and to establish when the CAM behaviour occurs in CAM-facultative *Sedum* species. Experiment was carried out in the glasshouses of the DipSA Department, with 14°/26°C night/day temperature regime. Plants were grown in boxes (40 cm x 30 cm x 22 cm) filled with 10 kg of green roof substrate (Harpo/SEIC intensive substrate). In addition to plant species (three replicate boxes each) three evaporation control boxes, containing just bare substrate were used. At the start of the experiment all boxes were watered to field capacity (average SMC 26% weight/weight) and no supplementary water was provided until the SMC reached < 3.5% w/w. In *L. corniculatus* and *B. erectus*, this was on the day 15th from the start of the experiment, while for the two *Sedum* species it was on the day 30th. The following parameters were measured: daily evapo-transpiration rate and daily SMC, through gravimetric method; leaf Relative Water Content (RWC) three times a week for all the species (3); nocturnal malate accumulation through titration method with NaOH in *Sedum* leaves three times a week (4). SMC declined over the course of the experiment that ended when the SMC of the substrate reached extremely low values (around 2.5 – 3.5% w/w). Malate concentration in *S. kantschaticum* leaves rose sharply when SMC was around 11% w/w, while in *S. lydium* two malate peaks were observed when SMC was respectively 14% w/w and 6.5% w/w. We hypothesized that the increase of the malate concentration in the *Sedum* leaves at 10 - 14% w/w of SMC could be associated with the switch from C3 to CAM metabolism (4). This hypothesis is supported by the evapo-transpiration rates significantly lower in the boxes of *Sedum* species, compared to ones with *L. corniculatus* and *B. erectus* as well as by the relatively high RWC values under drought (the minimum RWC value recorded for the *Sedum* species was the 75.8 %). The C3 species, instead, continued to transpire at the 10 - 13% w/w SMC values when both *Sedum* species changed their metabolism, but this caused a rapid decline of RWC (*L. corniculatus* reached the 38.5 % and *B. erectus* the 55 %). We conclude that until the 10 – 13% w/w of SMC, which support the Tp of C3 species considered, *L. corniculatus* and *B. erectus* have a potential to improve the cooling effect of a green roof than the two *Sedum* species used for the experimentation. Green roofs with *L. corniculatus* and *B. erectus* will however require more frequent irrigation than one with *Sedum* species which lose this cooling capacity sooner, but they show a greater resistance to drought. Further experiments will focus on the continuous monitoring of leaf and substrate temperature and linking this with the leaf stomatal conductance ( $g_s$ ) and the net assimilation rates (A). This will allow us to further elucidate the mechanisms underlying cooling provision and the actual differences in the extent of cooling between these species.

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### 3.4. = THE SPONTANEOUS VASCULAR FLORA OF THE CITY OF BOLOGNA AND ITS CHANGES OVER 120 YEARS

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The aim of the study was to investigate the urban flora of the city of Bologna, in order to produce an updated floristic list and to compare it with the only previous study performed by Gabelli in 1894 [1]. Another purpose of the study was to monitor the variation of urban flora, in relation to climate and urban change from the end of '800 up to now.

The study area was identified with the old town of Bologna, enclosed within the remaining of old city walls nowadays identifiable with the circle of avenues. This area is perfectly coincident with the area studied by Gabelli [1]. The floristic survey was carried out through three rounds of samplings: the first preliminary round was conducted with the aim of identifying the investigation area. Finally 10 transects (5 East-West and 5 North-South) subdivided into 162 segments, were chosen based on the feature of the streets and their main exposition. In addition also 22 green areas were monitored. All the transects and green areas were investigated twice: in spring (from March to May, 2016) and in autumn (from September to November), in order to collect all the species growing in different seasons. During the samplings, 478 species were found, many more than in the historical study of 1894, in which only 187 species were identified. The survey led also to detect 7 new naturalised species for Emilia-Romagna region (e.g. *Chlorophytum comosum*) and two new naturalised species for Italy (*Cotoneaster hissaricus*, *Eragrostis barrelieri*). In comparison to the Gabelli's study, the percentage of exotic plants increased from 12.5 to 30%, moreover, many of the species of 1894 are not anymore present. To investigate how the climatic and urban changes influenced urban flora, Ellenberg's ecological indices [2], were applied. Homogeneous categories were created based on the need of light, humidity and temperature stability of the different species. Chorological and biological spectra of the two floras, were also compared. With respect to the study of 1894, a sharp increase of phanerophytes (from 8 to 25 %) was detected, given the introduction of new woody exotic species primarily for ornamental purposes. The comparison of the chorological spectra showed an increase of naturalized adventitious plants (9 to 15 %), cultivated species (5 to 14 %) and of steno-mediterranean species. The rise of therophytes and steno-mediterranean species, suggests an adaptation of the present flora to a warmer weather than in the past (because of global warming and the urban heat island effect). Further confirmations of this hypothesis were also given by the comparison, between the present and past studies, of brightness, temperature and humidity indexes.

By analysing the distribution of species in different city habitats, it was pointed out that of the 478 total species, 222 were exclusive of not cemented areas, while a much lower number of exclusive plants was in the other types of habitats (25 on pavements, 16 on the walls, 2 in roofs, 2 in manholes).

Other factors that can also affect the biodiversity of urban flora were: width, exposure and material present on the ground in the streets. The streets with cobblestone pavements were found to be richer in species than asphalt or sampientrini streets. Larger streets hosted greater diversity than narrower ones. Moreover, NW-SE or NE-SW exposed streets were characterised by maximum diversity.

In conclusion, the urban flora of the city of Bologna seems unequivocally to reflect climate change, urban planning and floricultural preferences of the last 120 years. A general total increase of the species and a slow tropicalization of the flora were detected. This trend will most probably become more severe over the next few decades.

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2) H. Ellenberg (1979) Zeigerwerte der Gefäupflanzen Mitteleuropas (Indicator values of vascular plants in Central Europe), Scripta Geobotanica 9, 1-122

### 3.4 = STUDY OF THE ALIEN FLORA OF THE URBAN AREA OF PALERMO (SICILY)

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Alien plants are an integral part of the Mediterranean agricultural and urban landscape.

Taking into account that man is an active voluntary or involuntary carrier of plant diaspores, cities and areas where human activity is predominant represent preferential targets for the study of new plant introductions.

In addition, some species as *Pennisetum setaceum* (Forssk.) Chiov. and *Opuntia ficus-indica* (L.) Mill., initially exclusive to high disturbed habitats, succeeded in penetrating in cliffs, degraded maquis and garrigues characterizing them. Thus the importance of studying these areas to predict future colonization of more natural habitats.

Starting from literature and integrating it with field observations we prepared a list of alien species occurring in the urban area of Palermo. This list includes a categorization of the non native species occurring in the city according to their origin, their behaviour and the habitat where these plants were recorded.

The starting points were the contributions about the flora of Sicily (1, 2, 3), the alien flora of Italy (4) and the flora living on trees of the city of Palermo (5). Literature sources were followed by intense field work from September 2013 to May 2016, that allowed to include new species that only recently showed their tendency to naturalization and to exclude species reported more than 100 years ago that have not be found anymore or taxa occurring in different parts of Sicily but not in the perimeter that defines the study area.

About the categories, relevant literature gives different categorization depending on whether the point of view adopted is anthropocentric, biological, ecological or biogeographic.

Here we adopted the categories suggested by Raimondo & al. (1) dividing the studied taxa in *Adventive* or *Cultivated* depending on whether the introduction was accidental or voluntary, and subdividing them further in *casual*, *naturalized* and *invasive* depending on whether their permanence and development into the new territory.

On the whole, 145 specific and infraspecific taxa have been recorded. Neophytes are 133: 43 adventive and 90 coming from cultivation; 42 are casual, 94 naturalized and 9 invasive. Archaeophytes (cfr. 6, 7) are 12: 6 casual, 5 naturalized and 1 invasive.

This study allowed to record recent changes in the alien flora of the city. These are mainly due to:

- the popularity of the plants that are grown for ornament (e.g. *Nephrolepis cordifolia* (L.) K. Presl widely cultivated until 30 years ago now its cultivation is almost entirely disappeared inside the city);
- the variation of construction techniques and materials with the rarefaction of roof tiles and rough walls in limestone in favour of more modern covers that do not allow the establishment of plants (comporting an evident reduction of *Crassulaceae* observable on the roofs);
- the arrival of new pollinators that allowed the production of fertile fruits e.g. in *Ficus microcarpa* L. and *F. watkinsiana* F. M. Bailey.

An example of the spreading of new taxa is *Sesamum indicum* L., reported as only cultivated plant without tendency to naturalize (8) in the last year, several individuals inside the city of Palermo have been recorded in ruderal habitat. This could have been due to the presence of an increasing number of not Italian of birth citizens who grow this plant for food purposes.

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2) F. M. Raimondo, G. Domina, V. Spadaro, G. Aquila (2005) Quad. Bot. Amb. Appl., 16 (2005): 219-220.

3) F. M. Raimondo, G. Domina, V. Spadaro (2010) Quad. Bot. Amb. Appl., 21 (2010): 189-252.

4) L. Celesti-Grapow & al. (2009) Pl. Biosyst., 143(2): 386-430.

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3.4= ECOLOGICAL CONTEXT OF *HEDERA HELIX* L. IN ROMAN ARCHAEOLOGICAL SITES

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Plants are an integral part of urban environments, as furnishing weed presence, but their growth on buildings, monuments and cultural heritage remains an ongoing question (1).

*Hedera helix* L. has been used for centuries as greenery element due to its aesthetical value, evergreen characteristic and for its ability to grow on vertical surfaces, although its vigorous habitus in the archaeological and monumental area can be dangerous for the buildings. Ivy is an Atlantic element of the European flora, and it is classified by Ellenberg (2) as a semi-shade plant, typically growing in gallery forests. In Roman archaeological sites, this species is widespread and sometimes it can completely cover the ruins leading to structural damage and to loss of stability as confirmed by previous studies (3, 4).

The difficulty to manage the growth of Ivy is due to several aspects as the common bird dispersion of its seeds, its efficient sprouting and rooting, and its ability in vegetative reproduction. Then, this work aimed to study the distribution of this species within the most important Roman archaeological areas to evaluate its growth preferentiality as a useful tool to define management plans and preventive conservation strategies.

A total of 28 archaeological sites were selected by a literature database of phytosociological relieves from 42 Roman archaeological sites, according to the availability of botanical data. Particularly sites were grouped into 4 subsets according to their geographical location: Imperial Fora (F), Caracalla Thermal Bath (C), Villa of Massentius (M) and Ostia Antica (O). For each subset, Ivy presence (as number of literature's relieves) and cover, in the different expositions, were evaluated, considering also its relation to the phytosociological context. In particular, the analysis of variance (ANOVA) was performed in order to evaluate the significant differences between exposure and coverage among the 4 subsets.

Both Ivy occurrence in relation to the expositions and average coverage were compared showing a linear relation. In general, with increasing presence results an increase of the cover, as confirmed for sites M and C. Whereas, for the O and F the correspondence is only partially observed. It is probably due to the ruling occurrence, in these sites, of *Parietaria judaica* Oberd. 1977 and *Adiantum capilli-veneris* Br.-Bl. in Br.-Bl., Roussine & Nègre 1952 phytosociological classes where the Ivy is recognized as mate (5). Regarding the preferentiality of Ivy growth for exposure, NE, N and NW showed the highest presence and coverage. This result is also confirmed overlaying information regarding the analyzed phytosociological classes. The only case of preferentiality growth for the SW exposure (M) is associated with *Quercus-Fagetum* Br.-Bl. et Vlieg. 1937 class, on which Ivy is characteristic (5).

It is possible to conclude that due to the extreme ecological plasticity of Ivy, for its management it is important to characterize the archaeological site vegetation in order to be able to intervene preventively in those areas that might be appropriate to sprouting, rooting, and growth of this species.

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- 2) H. Ellenberg (1988) Cambridge University Press, Cambridge, UK.
- 3) M. A. Signorini (1996) *Informatore botanico italiano*, 28, 7-14.
- 4) F. Bartoli, F. Romiti, G. Caneva (in press) *Plant Biosyst.*
- 5) S. Ceschin, F. Bartoli, G. Salerno, V. Zuccarello, G. Caneva (2014) *Plant Biosyst.*, 1-10.



#### 4.5 = BIOLOGICAL ACTIVITY AND PHYTOCHEMICAL PROFILE OF GENETICALLY DIFFERENT YACON GENOTYPES

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Plants are potential sources of bioactive compounds showing health-promoting effects on humans due to their biological properties. Yacon (*Smallanthus sonchifolius* [Poepp. et Endl.] H. Robinson) is an ancient Andean root crop considered a functional food because it is an important source of healthy compounds, including fructo-oligosaccharides and antioxidants. Nowadays yacon is cultivated in other countries due to the traditional use and medicinal properties of both roots and leaves (1).

The objective of this study was to determine the metabolomic and biological differences among 14 yacon genotype extracts. All genotypes were grown under the same field conditions in Czech Republic. Preliminarily, yacon genotypes were investigated for their genetic diversity, assessed by ISSR and AFLP molecular markers; consequently the ripe leaves were collected and used for further studies.

Dried leaf tissue of all samples was extracted using solvents with increasing polarity (*n*-hexane, CHCl<sub>3</sub>, CHCl<sub>3</sub>:MeOH 9:1 and MeOH). All extracts were tested to evaluate their polyphenolic, flavonoid and tannin content by Folin-Ciocalteu, AlCl<sub>3</sub> and protein precipitation methods, respectively. Methanol extracts, the richest in term of phenolics, were tested to evaluate their antioxidant activity by using different assays (radical-scavenging, reducing power and inhibition of lipid peroxidation) and their inhibition ability vs enzymes involved in postprandial hyperglycaemia ( $\alpha$ -amylase and  $\alpha$ -glucosidase) (2). Quali-quantitative analysis of all methanol extracts was performed by HPLC-DAD system. Yacon genotypes showed different secondary metabolite content and different biological activity. Relative Antioxidant Capacity Index was calculated to compare results obtained from different antioxidant assays (Fig. 1). Qualitative HPLC-DAD analysis confirmed the presence of rutin, caffeic acid, chlorogenic acid (5-*O*-caffeoylquinic acid), 3,5-*O*-dicaffeoylquinic acid, 4,5-*O*- di caffeoylquinic acid (cynarin). The compound 1,5-*O*-di caffeoylquinic acid was identified for the first time in yacon leaves. Yacon chromatograms showed similar phenolic profiles, but remarkable quantitative differences were observed (Fig. 2). Yacon can be considered a natural source of secondary metabolites and the selection and propagation of specific yacon genotypes could ensure their potential use in pharmaceutical and nutraceutical areas.

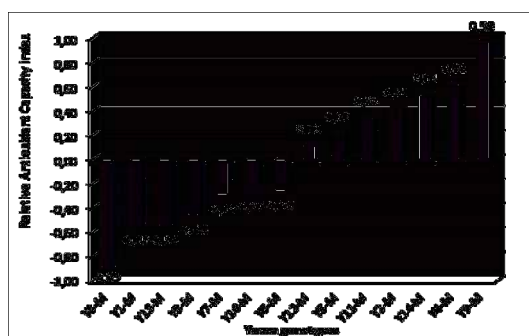


Fig. 1 Relative Antioxidant Capacity Index

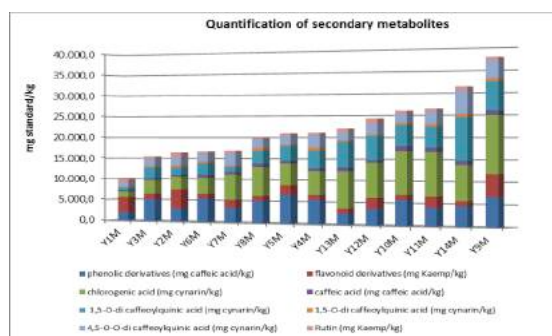


Fig. 2 Quantification of secondary metabolites in yacon genotypes

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## 4.2 = METABOLIC AND TRANSCRIPT PROFILING OF PHENOLICS ACCUMULATION IN AGLIANICO GRAPE BERRIES

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Polyphenols have many useful biological functions in plants, including defence against biotic stresses (1). They are produced through the phenylpropanoid pathway (Fig. 1), in which the amino acid phenylalanine is transformed into a variety of molecules, including flavonoids such as anthocyanins, flavonols and proanthocyanidins (PAs). The specific regulation of PAs biosynthesis in plant species other than *Arabidopsis* is not well characterized (2). The aim of this work was to study the accumulation of polyphenols in grape during berry ripening and to correlate it with gene expression in order to shed light on the specificity of PAs enzyme activities in relation to compounds responsible for the astringent sensation. The genotype used in this study is Aglianico del Taburno (Fig. 2), which is characterized by a high content of total flavonols, anthocyanins and resveratrol conferring high positive nutraceutical properties (3). Aglianico berries were collected during five developmental stages and dissected in skin and seed. The same material was used for metabolic and genetic analysis. PAs were extracted, fractionated into monomers, oligomers and polymers and quantified using spectrophotometric techniques, while the expression of 14 structural genes and two transcription factors (MybPA1 and MybPA2) was evaluated using the RT-qPCR approach. The results provided evidence that PAs were more abundant in seeds than in skins, with differences in the structural composition between tissues. In skin, significant differences were observed between fractions, with the polymeric fraction being the most abundant, on the counterpart seed PAs were principally composed by monomers. Expression analysis showed that PAs genes were highly activated at ripening and that Aglianico is able to accumulate polyphenols due to its ability to modulate key genes in a tissue-specific manner. Among PAs key structural genes, LAR1 and ANR enzymes provide two separate pathways for the synthesis of the starting units for PA polymers. In our study, those genes showed a tissue-specific behaviour, as their expression levels varied in whole berries, skin and seed. We believe that three main factors emerge from this research. First, Aglianico berry tissues contain PAs with structural characteristics that greatly affect astringency. From a technological perspective, the management of seed tannins in the last phases of ripening may be important to reduce the astringency of Aglianico wine. Second, from a molecular standpoint, the expression level of genes involved in the first steps of the phenylpropanoid pathway in Aglianico appears similar to that of other red grape cultivars, both in skin and seeds.

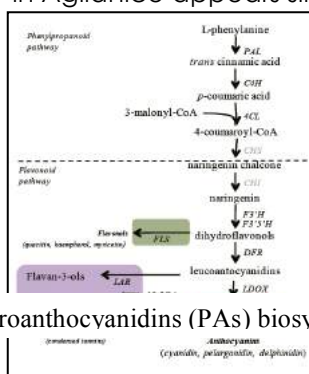


Fig. 1. Proanthocyanidins (PAs) biosynthesis pathway.



Fig. 2. Aglianico del Taburno vineyard used as sampling area (left) and an example of the grape clusters sampled (right).

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- 2) L. Jaakola (2013). *Trends in plant science*, 18(9), 477-483.
- 3) M. De Nisco, M. Manfra, A. Bolognese, A. Sofo, A. Scopa, G.C. Tenore, F. Pagano, C. Milite, M.T. Russo (2013). *Food Chemistry*, 140(4), 623-629

## 4.2 = PHYTOCHEMICAL AND BIOMOLECULAR ANALYSES OF FOUR WILD EDIBLE *CARDUUS* SPECIES FROM SARDINIA

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The genus *Carduus* L. belongs to the *Cardueae* tribe (*Asteraceae*) and is widely distributed in the Mediterranean area (1,2). In the Sardinian tradition, different parts of wild *Carduus* plants (stems, leaves, inflorescences) are consumed, both raw and cooked, for their taste and effects on health (1,3,4,5). Several species have been reported to treat liver disorders or generic diuretic and digestive problems (1,5,7).

The goal of this work is to characterize four wild edible *Carduus* species collected in Sardinia (*Carduus argyrea* Biv., *Carduus nutans* subsp. *macrocephalus* (Desf.) Nyman, *Carduus pycnocephalus* L., *Carduus cephalanthus* Viv.) by chemical and biomolecular analyses. Few literature data are available until now, especially on plants deriving from the island (1,6). At the same time, for their relevance as local and traditional food and remedy, we consider valuable to collect more information about these plants.

Two different analytical techniques, HPLC-PDA-MS/MS and PCR amplification and sequencing of the nrDNA internal transcribed spacer (ITS), have been used to verify similarities and dissimilarities in the chemical and molecular profiles among the four species. Ten individuals for each species (aerial parts) were submitted to ultrasonic extraction and then analysed. Among the thirty components detected, fifteen were identified as O-glycosides of Quercetin, Luteolin, Kaempferol, and Apigenin as well as Caffeoylquinic Acid derivatives, in accordance with previous works (1,6,7). Principal component analysis and hierarchical clustering analysis were used to evaluate the differences in metabolite profiles across species and reveal that individuals cluster into four distinct groups according to taxonomic classification. Furthermore, hierarchical clustering analysis on ITS sequences indicates an interspecific nucleotide variation.

The combination of the two different techniques allows the discrimination of the four species within the same genus, by genetic and chemical point of view, giving more information on poorly investigated plants used in the Sardinian traditional culture. Additionally, this approach could be useful in the species identification in thistles-based commercial local products.

- 1) L.A. Al-Shammari, W.H.B. Hassan, H.M. Al-Youssef (2015) *Journal of Saudi Chemical Society*, 19, 410-416
- 2) S. Pignatti (1982) *Edagricole*
- 3) P.M. Guerrero, V. Savo (2016) *Journal of Ethnopharmacology*, 185, 202-234
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- 7) R. Li, S. Liu, W. Song, Y. Wang, Y. Li, X. Qiao, H. Liang, M. Ye (2014) *Analytical Methods*, 6, 7181-7189

## 4.4.= STUDY ON THE EVOLUTION OF SOME LAMIACEAE BASED UPON PHYTOCHEMISTRY

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Lamiaceae is a family of plants belonging to the Eudicot clade of Angiosperms, comprising 236 genera and 7534 species.

These plants are spread all over the world growing on rocky, calcareous and sandy soils in hot and temperate climates which make them perfectly suitable for the Mediterranean habitat.

The overall phylogenetic tree inserts this family among the most derived ones (1).

In this work, we present a study on the evolution of some Lamiaceae species according to phytochemical data using secondary metabolites such as iridoids as metabolic markers.

We focused our attention on two sub-families and their genera: Ajugoideae (*Ajuga* and *Teucrium*) and Lamioideae (*Lamium*, *Melittis* and *Stachys*).

Our scope was to ascertain if the phylogeny of Lamiaceae based on phytochemical data is in agreement with that deriving from DNA sequencing which represents the traditional way of building phylogenetic trees.

The basilar concept of phytochemical evolution is that the more oxidized is the compound, the more evolved is that biogenetic pathway.

Previous works (1,2) placed Ajugoideae as an early branching subfamily in Lamiaceae (with *Teucrium* diverging before *Ajuga*) while Lamioideae are indeed the most derived ones with *Lamium*, *Melittis*, and *Stachys* as following grades.

During our phytochemical analysis, conducted by means of classical Column Chromatography for the separation procedure of the ethanolic crude extracts and by means of NMR Spectroscopy and MS spectrometry for the identification of the natural compounds present in the samples, we evidenced the presence of several iridoids.

Among these, the chemotaxonomic markers of the family were present but also some which are actually more typical of other families.

Moreover we found iridoids which are biosynthesized according to different pathways thus providing a more accurate and general view of the family.

These results allowed us to take the first steps into the formulation of a new phylogenetic tree concerning the studied genera of Lamiaceae.

1) R.M. Harley, S. Atkins, A.L. Budantsev, P.D. Cantino, B.J. Conn, R.J. Grayer, M.M. Harley, R.P.J. de Kok, T.V. Krestovskaja, R. Morales, A.J. Paton, P. OlofRyding. (2004) In: K.Kubitzki, J.W. Kadereit(Eds.) Families and genera of vascular plants 7.Springer-Verlag, Berlin, Germany, pp. 167-275

2) S.J. Wagstaff, L. Hickerson, R. Russ, P.A. Reeves, R.G. Olmstead. (1998)Plant Syst.Evol., 209(3-4), 265-274

#### 4.2 = *HYPERICUM SCRUGLII*, A SPECIES ENDEMIC TO SARDINIA AS A SOURCE OF POTENTIAL HIV-1 INTEGRASE INHIBITORS

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Earlier research on the development of effective anti-human immunodeficiency virus type 1 (HIV-1) agents has been focused on inhibitors of the critical viral enzymes, reverse transcriptase (RT) and protease (PR), but there is an urgent need to develop new and more effective therapeutics and the drug targets have been extended to include HIV-1 integrase (IN) inhibitors (1,2). IN is the enzyme responsible for integration of viral provirus into the human genome, a critical event for HIV permanent infection that is then irreversible (3). Till date, there are only three integrase inhibitors approved by US-FDA and resistance to them is known (4,5). Thus, the search for new integrase inhibitors with novel mechanism of action and effective on HIV drug-resistant strains is still a worldwide health care issue (6,7).

Hence, in our ongoing research of bioactive natural products inhibiting the replication of HIV-1 from the Sardinian endemic flora, some compounds obtained from aerial parts of *Hypericum scruglii* Bacch., Brullo et Salmeri have been assayed to evaluate their HIV-1 IN inhibition ability.

*Hypericum scruglii* is a perennial herb belonging to Hypericaceae family, endemic and exclusive of Sardinia (Italy), where it grows generally linked to calcareous substrates (8). For a long time the Sardinian populations of this species have been identified as *Hypericum tomentosum* L., but a detailed analysis of living material reported that they differ from typical specimens of *H. tomentosum* respect to the shape and size of leaves, floral structures and capsules (9).

The lyophilized aerial parts of *H. scruglii* was extracted using a H<sub>2</sub>O/MeOH (1:1) solution. The obtained extract was purified by column chromatography (SiO<sub>2</sub>, Sephadex LH-20, RP, C8 and C18) and six compounds (3-Geranyl-1-(2'-methylbutanoyl)phloroglucinol, 3-Geranyl-1-(2'-methylpropanoyl)phloroglucinol, 1,3,5-benzotriol 2-[(2S,3R)-3-(3,4-dihydroxyphenyl)-2,3-dihydroxypropyl], 3-(13-hydroxygeranyl)-1-(2'-methylbutanoyl)phloroglucinol, 3,4-dihydroxybenzoic acid and quercitrin) were isolated. All compounds were further purified by HPLC and their structures were established on the basis of physical and spectroscopic analysis. The 3-(13-hydroxygeranyl)-1-(2'-methylbutanoyl)phloroglucinol is reported for the first time.

All isolated compounds have been tested for their ability to inhibit the HIV-1 IN strand-transfer catalytic activity in Homogeneous Time Resolved Fluorescence assay (10). Five of them showed a significant inhibition activity with IC<sub>50</sub> values between 1.58 and 13 µg/mL; only 3,4-dihydroxybenzoic acid was found to be inactive. In particular, the most active compound, quercitrin, inhibited the HIV-1 IN catalytic activity with IC<sub>50</sub> value of 1.58 ± 0.16 µg/mL.

In our research on novel HIV-1 enzyme inhibitors, we report compounds that display strong inhibition against HIV-1 IN and that could be used for further drug development.

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#### 4.6 = *CAPSICUM ANNUUM* L.: A NEW SOURCE OF ANTIOXIDANT COMPOUNDS AND INHIBITORS OF PANCREATIC LIPASE

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Pepper is a vegetable of key importance in human nutrition. Currently, one of the most interesting properties of natural products is their antioxidant content. In recent years, peppers have grown in popularity, and a wide number of varieties are now available in the grocery stores, as well in nutraceuticals as fat burner. This taxon includes both sweet cultivars eaten mainly as vegetables and hot ones, often used as a spice. Hot pepper, genus *Capsicum*, belongs to the Solanaceae family [1]. Chemical composition of pepper fruit has been studied fairly well, mainly with respect to vitamin (C, E),  $\beta$ -carotene and carotenoid pigments content [2], and capsaicinoids [3].

Metabolic syndrome had become a global epidemic (globesity), defined as a cluster of three of five criteria: insulin resistance and glucose intolerance, abdominal obesity, hypertension, low high-density lipoprotein cholesterol and hypertriglyceridemia. The prevalence of metabolic syndrome in adults has been increasing rapidly in the past decades in most western countries. Many studies have been carried out on the use of natural food materials in the prevention and treatment of obesity, as it is known that food not only supplies the calories and the nutrients but also helps to prevent and treat diseases with the natural functional materials it contains [4].

Numerous trials have been conducted to find and develop new anti-obesity drugs through herbal sources, in order to minimize side effects associated with the present anti-obesity drugs. One of the most important strategy in the treatment of obesity includes the development of nutrient digestion and absorption inhibitors, in an attempt to reduce the energy intake through gastrointestinal mechanisms without altering any central mechanisms. Pancreatic lipase is a key enzyme for triglycerides absorption in the small intestine.

This study was designed to evaluate the effect of a hydroalcoholic extract of hot pepper flower on nutrient digesting enzyme, lipid slicing enzyme pancreatic lipase in particular. The radical scavenging activity and the potential in inhibiting nitric oxide production and lipid peroxidation were assessed as well.

The inhibition of lipase activity was measured by monitoring the hydrolysis of p-NPC, which releases the yellow chromogen p-nitrophenol. The inhibitory action was investigated by measuring the enzyme activity at a fixed substrate concentration and varying concentrations of the extract. The extract inhibited the enzyme with a clear concentration dependence, reaching 82% inhibition at the highest concentration. The effects of hot pepper flower extract on lipopolysaccharide (LPS)-stimulated NO production were evaluated on RAW 264.7 macrophage. The highest tested concentration was able to induce 94% inhibition of NO production. The extract also inhibited lipid peroxidation.

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#### 4.1 = EFFECTS ON *IN VIVO* ANGIOGENESIS OF EXTRACTS FROM *ASTRONIUM FRAXINIFOLIUM* SCHOTT AND *A. URUNDEUVA* (FR. ALL.) ENGL. (ANACARDIACEAE)

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*Astronium fraxinifolium* Schott (Gonçalo-alves), *Astronium graveolens* Jacq. (guaritá) and *Astronium urundeuva* (Fr. All.) Engl. (aroeira-do-sertão), arboreal species typical of northeast brazilian savanna belonging to Anacardiaceae family, are used as folk medicine for their anti-inflammatory, antimicrobial, anti-ulcerogenic and wound healing properties (1). A previous study reported that the methanolic extract of *A. graveolens* leaves shows anti-angiogenic properties by regulating the placental growth factor (PIGF), an angiogenesis modulator, and allowed identification of 1,2,3,4,6-penta-O-galloyl-D-glucopyranose as the most active compound (2). Basing on these scientific evidences, our study was carried out to evaluate the effects of the ethanolic extracts from *A. fraxinifolium* (AF) and *A. urundeuva* (AU) stem barks and leaves on the chick embryo chorioallantoic membrane (CAM) model that is commonly used to study angiogenesis and anti-angiogenesis *in vivo*.

*A. fraxinifolium* and *A. urundeuva* samples were collected, respectively, in Bálsamo and Votuporanga and in Tocantins (Brazil). The CAM assay was performed following the method of Germanò et al. (3); fertilized eggs of *Gallus gallus* were previously maintained in a humidified incubator at 37 °C and, after four days of incubation, a small window was created on the broad side of the eggs to apply different doses of AF and AU ethanolic (70%) extracts (30, 50, and 80 µg), previously suspended in albumen, directly on the CAM surface. Retinoic acid (2 µg) and VEGF (250 ng) were used as antiangiogenic/proangiogenic reference compounds. After treatment, the eggs were re-incubated for two days, then they were observed under a stereomicroscope (Zeiss Stemi 2000-c) equipped with a digital camera (Axiocam MRc 5 Zeiss) and photographed for the morphological analysis. The anti-angiogenic effects on the CAMs were quantified by counting the number of blood vessels branch points/mm<sup>2</sup> and expressed as percentage of inhibition by means the following equation: anti-angiogenic activity (%) = 1 - (T/C) x 100, where T = number of blood vessel branch points/mm<sup>2</sup> in the CAMs treated with extracts and C = number of blood vessel branch points/mm<sup>2</sup> in the CAMs treated with albumen.

The results (means ± standard deviation) for AF evidenced that both the stem bark and the leaves extracts showed a strong anti-angiogenic activity (49 % and 61% of inhibition, respectively at 80 µg). Conversely, the extract from AU stem bark weakly inhibits the vessel formations in treated CAMs (20% of inhibition at the highest dose) while the extract from the leaves induces a remarkable increase of vessel formation.

Frequently, in the scientific literature it's possible find similar biological activities of plant complex matrices obtained from different species of the same genus or from different plant organs of the same species. The results obtained in this study, instead, prove that the anti-angiogenic effects of *A. fraxinifolium* extracts are comparable to those of *A. graveolens* leaves extract (2) while *A. urundeuva* leaves extract is able to interact with the 'targets' of angiogenesis in the opposite way.

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#### 4.4 = A SYSTEMATIC AND CHEMICAL STUDY OF *POLYGALA FLAVESCENS* (POLYGALACEAE), ENDEMIC TO ITALY

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*Polygala* is the largest genus of Polygalaceae, comprising about 350 species (one third of the entire family), including trees, shrubs and herbs distributed throughout the world (1).

In Italy, this genus only consists of herbaceous plants, with a significant number of specific and subspecific taxa endemic to the country (2). Among them, there is *Polygala flavescens* DC., the only Italian species with yellow flowers, whose geographical distribution ranges from Liguria to Basilicata, across Italian peninsula. Very recently, three subspecies were recognised within *P. flavescens*, based on qualitative morphological characters (3): *P. flavescens* subsp. *flavescens* (covering most of the geographic range of the species), *P. flavescens* subsp. *maremmana* (Fiori) Arrigoni (with range limited to the coastal portion of Tuscany), and *P. flavescens* subsp. *pisauensis* (Caldesi) Arrigoni (with range limited to the coastal portion of Emilia-Romagna and Marche). The latter taxon was long time considered as a distinct species (4). A chemotaxonomic study was carried out in order to contribute to the systematics and correct taxonomic setting of these taxa.

To this aim, we selected five different populations: three of *P. flavescens* subsp. *flavescens* (i.e. the species with the largest range), collected in Tuscany (PFF-T), Molise (PFF-M) and Abruzzo (PFF-A); one of *P. flavescens* subsp. *maremmana* collected at Monte Argentario (PFM), and one of *P. flavescens* subsp. *pisauensis* collected at Fano (PFP), i.e. the areas from which they were originally described (*loci classici*).

Aerial parts were dried, defatted with *n*-hexane, and extracted at room temperature with methanol. Each MeOH extract was partitioned between *n*-BuOH and H<sub>2</sub>O and the *n*-BuOH soluble fraction was subjected to LC-PDA-ESIMS and LC-PDA-ESIMS/MS analyses to compare the chromatographic profile and provide wider information concerning the secondary metabolites contents. A preliminary qualitative analysis showed that all plants displayed an identical flavonoid and oligosaccharide pattern, while the profile of saponins showed little differences among the five populations.

Then, a more in-depth chemical study was carried out on the selected population PFF-T collected in the highest amount. PFF-T *n*-BuOH extract was so separated by Sephadex LH-20 followed by CPC and RP-HPLC. Flavonoids and oligosaccharide esters, including some new compounds, were purified and fully characterized by spectroscopic techniques such as 1D and 2D-NMR, and mass spectrometry. Results were in agreements with those reported in the literature for other *Polygala* species, being flavonoids, oligosaccharides, and saponins widely distributed in the genus (5, 6).

In addition, also the volatile profile of fresh flowers was compared among populations, by means of Head Space-Solid Phase Micro Extraction (HS-SPME) technique (7) combined with GC-MS. The results were submitted to Multivariate Statistical Analysis. According to the SPME emission pattern, the most similar ones resulted to be PFF-M and PFP that formed a cluster in the HCA dendrogram. Another cluster was provided by the three remaining ones, with PFF-A and PFM more similar each other than PFF-T. The PCA confirmed this behavior. The solitary placement of PFF-T is due to its high production of *cis*- $\alpha$ -ambrinol and the exclusive emission of caryophyllene oxide and dendrolasin.

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## 1.1. = GENETIC AND EPIGENETIC STUDY OF ARUNDO SPECIES

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The genus *Arundo* includes five taxa distributed from the Mediterranean Basin to tropical Asia (1). *Arundo donax* is the most widely distributed among the five *Arundo* species, but neither pollen nor seeds have been studied in detail (2, 3). Its vegetative reproduction reduces the genetic biodiversity, even though the plants show different phenotypic and phenological features (4). The presence of *A. donax* in Sardinia is well documented by many studies (5, 6), and this species is generally considered as an invasive archaeophyte. The presence in Italy of other *Arundo* species has been reported, but their taxonomic status is debated (7, 8). In this study, the genetic diversity of 60 plants collected in Sardinia and mainland Italy was investigated by means of AFLP analysis. The results highlighted a very limited biodiversity, even though the plants showed some phenotypic differences and capacity to live in different habitats. Recent evidence has suggested that phenotypic variation is not only caused by genetic mutations, but it might be due in part to different DNA methylation status. In fact, patterns of DNA methylation are strongly associated with transcriptional variations. To investigate the epigenetic stability of the *Arundo* populations, MSAP analyses were performed. The data were analysed through Bayesian and statistical approaches. The results, compared with those obtained by AFLP markers, showed that the diversity within the population increased when the *Hpa*II profile was considered. Moreover, we demonstrated that inner cytosine hemi-methylation was the most abundant methylation in *Arundo* populations, and, moreover, that the *Hpa*II profile was more closely related to geographic position rather than to genetic profiles.

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## 1.1. = STUDY ON *POSIDONIA OCEANICA* (L.) DELILE ROOTS GROWING ON DIFFERENT SUBSTRATA BY HISTO-ANATOMICAL AND MICRO-MORPHOLOGICAL ANALYSIS

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*Posidonia oceanica* (L.) Delile grows on different substratum types, ranging from sand, which is easily penetrable by the roots, to rock, in which they are able to enter through crevices (1). In this study a survey was carried out on roots of plagiotropic rhizomes growing on sand (*matte*), and rock (dolonstone and calcarenite), to assess difference in morphological and anatomical features. In particular, for each substratum, nine rhizomes were randomly sampled by scuba diver at 10 meters of depth. In each rhizome histological and morphometric data (2, 3) were recorded on roots up to the second lateral order. Roots on rock were isolated from their substratum by chemical dissolution. Data analysis exhibited differences in roots anatomy and histology between substrata. The adventitious and second order lateral roots showed larger diameter on sandy sediment (from  $3,111.88 \pm 571.76 \mu\text{m}$  to  $924.64 \pm 146.11 \mu\text{m}$ ) compared to ones growing on rocky substratum (from  $2,695.14 \pm 703.75 \mu\text{m}$  to  $786.36 \pm 168.18 \mu\text{m}$ ). The outer cortex of roots was on average thicker on sand ( $229.41 \pm 263.66 \mu\text{m}$ ) than rock ( $123.49 \pm 198.42 \mu\text{m}$ ). Conversely, rhizodermis and mechanical hypodermis were thicker on rock (from  $37.17 \pm 5.97 \mu\text{m}$  to  $233.19 \pm 81.55 \mu\text{m}$ ) than sand (from  $30.66 \pm 5.34 \mu\text{m}$  to  $139.29 \pm 74.04 \mu\text{m}$ ). Similar trends in inner cortex and stele thickness were also found. In adventitious roots, the medulla was rich of well-lignified sclerenchymatic fibers, as they are responsible of the tensile strength, both on rock and sand, while variable results were obtained in first and second order lateral roots. Structures of adhesion have been detected (Fig. 1), very similar to ones discovered in seedlings (4), allowing to identify micro adhesive patterns. In particular, the root hairs genesis was reconstructed on rhizodermis, highlighting the attachment strategies to the substratum based on distal part of root hairs. The penetration strategy of adventitious roots (Fig. 2) was finally observed. Biochemical dissolution of carbonate cements in dolonstone or disintegration of particle components in calcarenite are used by the roots to penetrate into the rock for improving the anchoring capacity and the substrate exploration.

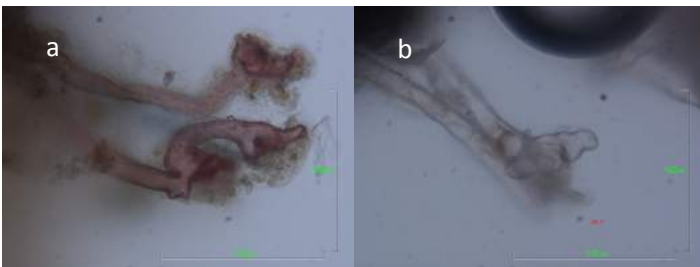


Fig. 1. Adhesion structures of *P. oceanica* root hairs: distal part of root hair on calcarenite (a) and on sand (b).



Fig. 2. Cross-section of calcarenite with roots system above

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#### 1.4. = EFFECTS OF ABANDONMENT OF LAND TRADITIONAL MANAGEMENT ON SPECIES AND PHYLOGENETIC DIVERSITY. A CASE STUDY FROM THE NORTHERN APENNINE OF EASTERN TUSCANY

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Low altitude hay meadows (code 6510) and *Festuco-Brometea* dry grasslands (code 6210, habitat of priority interest) represent two habitats worthy of conservation according to the Annex I of the Habitat Directive (Council Directive 92/43/EEC). The abandonment of the traditional agro-pastoral activities exposes these habitats to strong transformations, often with the ingress of shrub species that lead these communities toward very different vegetation types that are of less relevance from a conservation point of view. This study aims to investigate the floristic changes in these habitats related to both the kind of management and the different stages of vegetation evolution after the abandonment of agro-pastoral activities in the eastern Tuscan Apennines. In addition, we also investigated the phylogenetic processes connected to the evolution of these habitats. The floristic data were recorded on 60 plots (10 × 2 types of habitat × 3 different stages of evolution) and 147 species were identified. A Bayesian phylogenetic tree was obtained by using nrDNA sequences available at the NCIB database and by performing new sequences for the 13 species not present in the world database. We compared the differences in species richness, species composition and Phylogenetic Diversity of the plots according to the different types of habitat and stages of evolution, also searching for eventual correlations between these two parameters. Species richness in the two grassland types appears comparable and consistently decreases with the increase of the abandonment level (Fig. 1B). On the other hand, the two habitats appear strongly separated regarding the species assemblages, while tend to be more similar in the transitional phase that follows the abandonment (Fig. 1A). The two habitats tend to segregate again in the more dynamically evolved stage characterized by a high shrub cover (Fig. 1A). Data from the phylogenetic studies reveal a strong loss in phylogenetic diversity related to the loss in species richness, although the final stages of hay meadow host a higher Phylogenetic Diversity and Mean Pairwise Distance than those derived from the dry grassland (Fig. 1C). The standardized effect size on MPD reveals a progressive phylogenetic overdispersion passing from the managed stages to the abandoned ones.

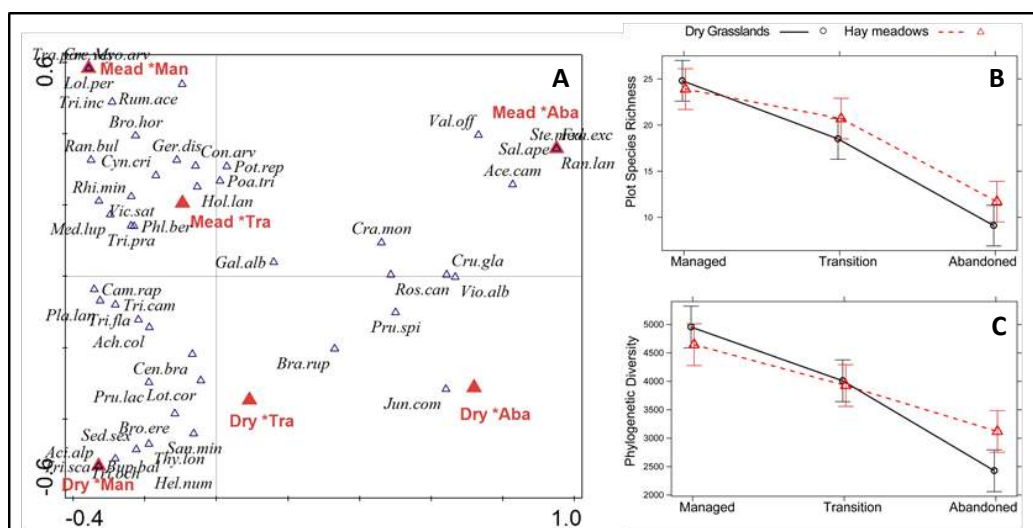


Fig. 1: (A) CCA plot for species composition of plots according to the kind of management and stages of evolution. Total variation is 4.98, explanatory variables account for 25.2%. Permutation Test Results: pseudo-F = 3.6, P = 0.0002. (B) Mean species richness and phylogenetic diversity (C) according to the kind of management and stages of evolution.

## 1.5. = ANNOTATED CHECKLIST OF UMBRIAN MACROFUNGI (CENTRAL ITALY)

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From a geobotanical point of view, Umbria (central Italy, 28,456 km<sup>2</sup>, 50-2,436 m a.s.l.) belongs to the middle-European floristic region with some 'islands' of Mediterranean flora producing a patchiness at regional scale, characterized by a high species richness and great diversity of habitats and vegetation (1). We present here an annotated check-list, based on 5,232 records and 1,016 species of Ascomycota (106) and Basidiomycota (910) recorded in 95 different localities of Umbria, from 1990 to 2015. This checklist represents the first comprehensive listing of macrofungi for Umbria and was compiled from data of Umbrian records published in past scientific publications, from recent researches in 'Natura 2000' Sites of Community Importance (SCI) (2), and from unpublished lists and personal observations.

Among the most interesting results of this work, we observed 32 species of Basidiomycota that were not reported in the previous Italian checklist (3). Furthermore, it is interesting to note the occurrence of: a) nine taxa included in the preliminary list of 23 species considered rare and/or threatened in Italy, as reported by the Mycology working group of the Italian Botanical Society (4); b) seven taxa included in the list of 33 fungal species threatened at European level proposed for amendment to Annex 1 of the Bern Convention, document T-PVS (2001), 34 (5); c) four taxa recently included in the red-list of the Italian Flora (6). Moreover 12 species of the Umbrian mycobiota are considered as critically endangered, endangered or vulnerable in the nearby Toscana, the only Italian region where a fungal Red List was compiled (7).

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1.5. = NEW RECORDS OF *MALUS CRESCIMANNOI* (ROSACEAE) IN SICILY

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*Malus crescimannoi* Raimondo (Rosaceae), a tree firstly described from Nebrodi Mts. (N Sicily, Messina Province) (1), has been recently found in the neighboring area of Madonie Mts. (N Sicily, Palermo Province).

The species is originally known from the mountain area near Floresta (Messina), where it is rather common on siliceous soils, within *Quercus cerris* L. and *Fagus sylvatica* L. deciduous communities (1). The same applies to the new sites of Madonie Mts., where the species is found at the edge of *Quercus petraea* (Matt.) Liebl. and *Fagus sylvatica* woods, growing on quartzarenitic substratum at 1200-1800 m of elevation.

These new localities are placed at the south-western limit of *Fagus sylvatica* distribution area, on both north-facing [Contrada Sempria (Castelbuono) and slopes of Madonna dell'Alto (Castellana Sicula)], and south-facing slopes [Locality Prato (Polizzi Generosa)].

In the Madonie Mts. *Malus crescimannoi* comes in contact with the allied species *Malus sylvestris* (L.) Mill., while these two species are very rarely found together on the Nebrodi Mts. In this respect, it must be emphasized that distinctive features of *M. crescimannoi* from *M. sylvestris* mainly concern the leaf outline, which is ovate-lanceolate in the former and ovate in the latter (2), the flower size, smaller in *M. crescimannoi*, and the pome shape, oblong in *M. crescimannoi*, but globose-depressed in *M. sylvestris*. Another relevant feature is the tree foliage shape, which is assurgent in the former species and expanded in the latter one.

As far as taxonomy is concerned, *Malus crescimannoi* belongs to *Malus* sect. *Malus* and is differentiated from the two related species, *M. sylvestris* and *M. domestica* Borkh., by relevant morphological characters including its ascending branches, the length and thickness of fertile branchlets, leaf shape and serration, petiole length, flower size, with particular reference to petals, which are shorter and differently shaped and coloured, length of stamen filaments in relation to the styles, and both shape and size of the pome, which also shows a longer stalk.

*Malus crescimannoi* is strictly endemic to northern Sicily. First reports marked it as confined to the sub-montane belt of the basin of the river Flascio, a tributary of the Alcantara river, in the areas of Floresta and Randazzo, and to the northern slopes of Monte Soro, between 1000 and 1600 m of elevation. In that area, the species is rather well represented, with several different aged individuals and even long-standing specimens more than 300 years old, which often grow together with other Rosaceae such as *Pyrus spinosa* L., *P. pyrastrer* (L.) Du Roi, *Pyrus ciancioi* Marino & al., *Sorbus torminalis* Crantz, *Crataegus monogyna* Jacq., *C. orientalis* M.Bieb., *Prunus spinosa* L. and *Rosa canina* L.

The new records of *M. crescimannoi* from Madonie Mts., where few and much localized individuals occur, enlarge the geographic area of this species from Nebrodi Mts westwards to the whole North Sicilian mountain ranges populated by the common beech. Specimens of *M. crescimannoi* from new localities are deposited in PAL.

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## 1.5 = PRELIMINARY INVESTIGATIONS ON THE FLORA OF THE PERMANENT BANQUETTES IN WESTERN SICILY

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The banquettes, landed clusters of dead leaves of *Posidonia oceanica* (L.) Delile, represent a deposited biomass from sea to land that protects the shoreline from wind and sea erosion as well as constitutes a reserve of nutrients and organic matter for sea prairies. These wracks can become a potential biodiversity hotspot as indicated by a recent investigation on invertebrates that has shown high levels of consumption of detritus (1). This biodiversity, greater than that found in coastal areas without such accumulations, can be explained by the margin effect between the marine and the terrestrial environment. The banquette can therefore be considered an ecotone (2, 3) where communities of supralittoral and inland areas coexist in the same transition system.

Studies on terrestrial invertebrates of the banquettes have not shown a clear relation between the trophic beached debris and terrestrial biological communities settled on them (4).

Aim of this research is to investigate the ecological role played by such accumulations in the coastline of western Sicily, with particular reference to the terrestrial plant communities that are settled on them.

In western Sicily 327 km of residues were detected, 24 km of little mounds or temporary banquettes (shifted naturally by wind or storm surges) and 29 km of permanent banquettes (5). The latter are usually very rare, because beach tourism involves mechanized cleaning activities of the accumulations of dead leaves that favours the coast erosion (6, 7).

In spring 2016 surveys were carried out on the flora and vegetation of permanent banquettes in the Lagoon of Marsala (Stagnone) where the largest accumulations in Sicily are found. Here were detected, in fact, more than 10 km of banquettes with a beached material layer up to about 50 meters wide, and 1 meter above the sea level.

The substrate of the banquettes changes its composition depending on the distance from the shore line: near the sea it consists exclusively of organic matter, away from the coastline there is a higher sand content, with values that reach up to 33.9%. Behind the banquettes brackish marshes and rocky coasts are found.

The flora occurring on the banquettes includes a low number of plants specialized for psammophilous and halophilous habitats. Near the shoreline there is a pioneer, halo-nitrophilous flora with therophytes as *Cakile maritima* Scop., *Matthiola tricuspidata* (L.) R.Br., *Salsola soda* L., *Senecio leucanthemifolius* Poir., *Spergularia salina* J.Presl & C.Presl, and some perennial plants as *Beta vulgaris* subsp. *maritima* (L.) Arcang. and *Sonchus bulbosus* (L.) N.Kilian & Greuter than can also cover large areas. Then, a belt follows where the above listed taxa are mixed with species typical of brackish marshes and nitrophilous habitats. Among them: *Crithmum maritimum* L., *Dittrichia viscosa* (L.) Greuter, *Halimione portulacoides* (L.) Aellen, *Limbarda crithmoides* (L.) Dumort., *Polypogon monspeliensis* (L.) Desf., *Sarcocornia fruticosa* (L.) A.J.Scott, *Sonchus oleraceus* L., and *S. tenerrimus* L.

On the Isola Lunga the occasional presence of *Calendula suffruticosa* subsp. *maritima* (Guss.) Meikle, endemic to Sicily and Sardinia, was also observed. In fact, this taxon is associated to the coastline but linked to sandy-gravelly or rocky substrates (8).

These preliminary observations have further emphasized that the ecological role of the banquettes is to be carefully evaluated in planning the coastal management in Sicily and the Mediterranean, in order to promote activities with low impact and protect these deposits, including the widening of protected areas when necessary.

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## 1.5. = BRYOFLORA OF MEDITERRANEAN TEMPORARY PONDS: CHOROLOGICAL AND ECOLOGICAL FEATURES

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The "Mediterranean Temporary pond" is an important habitat for biodiversity in the Mediterranean region, classified as a priority natural habitat (3170\*) under the Habitat Directive 92/43/EEC (1). The damp habitats with a seasonal presence of surface water are potential bryophyte habitats, they show high species richness including several species of conservation interest. Despite their important ecological functions, there are very few studies on the chorological and ecological characteristics driving bryophyte diversity. The scarce interest in the bryophyte component in the study and conservation of Mediterranean damp habitats seems incongruous: the success of bryophytes in these ephemeral habitats is largely due to their unique and very effective physiological water relation system that allow species to tolerate long periods of dehydration without undergoing physiological damage at cell level (2, 3).

To describe the bryoflora of Mediterranean temporary ponds, we compared the available bibliographical data on temporary ponds in the Mediterranean (4, 5, 6, 7, 8). We created a georeferenced database of 214 bryophyte species, ranging from Spain to Sicily. We analysed the bryoflora in terms of species richness and composition, chorological and ecological elements.

We can define here a group of bryophytes typically occurring in Mediterranean temporary ponds: *Archidium alternifolium* (Hedw.) Mitt., *Trichostomum brachydontium* Bruch., *Imbricbryum alpinum* (Huds. ex Wiyh.) N.Pedersen, *Tortella flavovirens* (Bruch) Broth., *Ptychostomum pseudotriquetrum* (Hedw.) J.R.Spence & H.P.Ramsay, and *Riccia sorocarpa* Bisch.

Four out of 30 reported families (Pottiaceae, Brachytheciaceae, Bryaceae and Ricciaceae) include 55% of the total species. The genus *Riccia* is the most common taxon. The most frequent species is *Archidium alternifolium*, an acrocarpic moss that shows a high ecological plasticity, growing on open, moist and for long periods submerged soils.

The chorological data with mostly Oceanic species (36%) confirm the presence of species associated with humid environmental conditions. The abundant presence of xerophytic (21%) and hygro-mesophytic (19%) bryophytes is indicative of the high diversity found in temporary ponds, caused by a typical small-scale zonation. The predominance of turf life form (34%) is linked to the ability of the species to tolerate extreme aridity during the most critical period. The major presence of species with colonist life strategy (35%) is important for the habitat conservation because they prevent high substrate desiccation and encourage the establishment of other species.

Although ephemeral, limited to peculiar and scattered conditions and quantitatively small in area, this habitat hosts several species of high conservation interest, such as *Riccia huebeneriana* (9).

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1.5. = NOVELTIES IN THE DISTRIBUTION OF LONG-STYLED, SPRING-FLOWERING *CROCUS* SER. *VERNI* (IRIDACEAE) IN ITALY

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According to Harpke et al. (1), seven species of *Crocus* ser. *Verni* occur in Italy: the autumn flowering *C. longiflorus* Raf., occurring in S Italy and Sicily; the short-styled, spring flowering *C. siculus* Tineo and *C. vernus* (L.) Hill, endemic to Sicily the former, very common above 1500 m in N Italy, the latter; the long-styled, spring flowering *C. etruscus* Parl., *C. ilvensis* Peruzzi & Carta, *C. neapolitanus* (Ker Gawl.) Loisel. (= *C. vernus* auct. pro parte), and *C. neglectus* Peruzzi & Carta (= *C. vernus* auct. pro parte). While the first two taxa, both narrow endemic to Tuscany, are not particularly problematic (1, 2, 3), the remaining species (*C. neapolitanus* and *C. neglectus*) are more difficult to identify. *Crocus neapolitanus* has been recorded generically for "Italian peninsula", but it is absent from Emilia-Romagna, Liguria, Tuscany, where *C. neglectus* occurs (1). Given that records of long-styled plants are known also for other regions in Northern and Central Italy, we carried out herbarium and field studies in order to clarify the distribution of these taxa. According to our results, it was possible to highlight that the plants so far referred to as "*C. neapolitanus*" or "*C. vernus* auct." from Friuli Venezia Giulia actually belong to a eighth species, not previously recorded for Italy: *C. heuffelianus* Herb. (4). The plants referred to as "*C. vernus* auct." or "*C. neapolitanus*" in Lombardia, Piemonte, Marche, and a single locality in Northern Abruzzo actually pertain to *C. neglectus*. In central-southern Abruzzo, Lazio, Molise, Campania, Puglia, Basilicata and central-northern Calabria, *C. neapolitanus* occurs. Incidentally, the rejection of the name *C. purpureus* Weston, which should have nomenclatural priority for the latter species, was recently proposed (5). Gaps in distribution (and/or in knowledge) of these plants are apparent between Lombardia and Veneto, and in territories of Central Italy between northern Lazio/Abruzzo and southern Tuscany/Umbria/Marche. Further field investigations should be carried out in these areas, to check if these taxa are completely allopatric, as the data available today seem to suggest.

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## 1.5. SIGNIFICANT SPECIES OF THE VASCULAR FLORA OF THE NUOVA GUSSONEA BOTANIC GARDEN OF MT. ETNA

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The Nuova Gussonea botanic garden have been founded in 1979. It is located on the southern slopes of Mt. Etna, at 1700 m a.s.l. in the B zone of the Etnean natural Park. It lies in the Regional forest property on an area of about 10 hectares. In the garden, since its foundation, many species are annually introduced. They are collected in various Etnean sites, located at different altitudes and slopes.

The aim of this study is to point out the floristic richness of the garden since its foundation and highlight the species which are particularly significant within the Etnean flora and the flora of Sicily.

The data have been collected sampling specimens and through field observations. Moreover annual census-papers and literature data (2, 3, 6, 8) have been consulted.

The results obtained allow to highlight the floristic diversity on the garden in different periods, since its foundation. Such flora is made up by native and introduced species, which are in total more than 800 entities.

Introduced species are the best represented group; they are more than 660 (more than 80% of the total of the species) while the native species reach the 18 % of the total.

Among the native species there are: endemic species (as *Senecio squalidus* L. subsp. *aetnensis* (DC.) Greuter, *Rumex scutatus* L. subsp. *aetnensis* (J. et C. Presl) Ciferri e Giac.); rare species (as *Sternbergia colchiciflora* Waldst. et Kit. subsp. *aetnensis* (Raf.) P. Fourn.); a new ibryd (*Saponaria sicula* Raf. x *Saponaria officinalis* L.).

The introduced species and subspecies present a wide diversity and include many significant species as: endemic species (as *Betula aetnensis* Raf., *Scleranthus perennis* L. subsp. *vulcanicus* (Strobl) Bég.); rare species, which reach a percentage of 23% (as *Carex pendula* Huds., *Thymus spinulosus* Ten., *Ilex aquifolium* L.); new records (as *Cymbalaria pubescens* (C. Presl in J. et C. Presl) Cufod., *Salix purpurea* L. var. *lambertiana* (Sm.) Macreight, *Salix alba* L. subsp. *vitellina* (L.) Arcang.); species which are largely widespread in Sicily, but rare or very rare on Mt. Etna (as *Cistus salvifolius* L., *Ampelodesmos mauritanicus* (Poir.) T. Durand & Schinz, *Pistacia lentiscus* L.); species, considered as at risk of extinction or endangered in the Etnean territory (as *Quercus suber* L., *Crambe hispanica* L.). There are moreover intraspecific entities not indicated on the Italian Flora of Pignatti (1) and on the checklists of the flora of Italy (4) and of Sicily (5,7) (*Vicia cracca* L. var. *aetnensis* Fiori, *Chamaenerion dodonaei* (Vill.) Schur ex Fuss var. *palustre* (Burn.) Poli Marchese & Turrisi nom. prov., *Viola aetnensis* (DC.) Strobl subsp. *messanensis* (W. Becker) Merxm. & Lippert var. *gracilis* (Sibth. & Sm.) Poli Marchese & Turrisi nom. prov.); species of which the Sicilian sites are only on the Etnean territory (as *Asplenium septentrionale* (L.) Hoffm. subsp. *septentrionale*).

About the introduced species new distributive references are indicated; new literature data and new herbarium references are indicated for the introduced species which are rare.

This work allow to update the floristic knowledge of Mt. Etna and in some cases of the Sicily.

The Nuova Gussonea botanic garden, due to its rich and significant flora, could be considered as an optimal area for the *ex situ* conservation of species which are particularly significant for the Etnean and Sicilian flora.

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## 1.5. = TOWARDS A CHECKLIST OF THE ITALIAN GYPSOPHILOUS VASCULAR FLORA

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The strict relationship between plants and particular types of substrate has long been known to botanists and plant ecologists who have dealt with this issue. The peculiar flora growing on Italian gypsum substrates has been underlined since the nineteenth century (1, 2, 3). The concept of plant gypsophily can be defined as the exclusiveness or marked preference for living on gypsum outcropping rocks. Thus, only those plant species which show preference, or even exclusivity, for gypsum substrates, should be classified as gypsophilous (4).

The main aim of this research has been to provide a checklist of the Italian gypsophilous flora. The study was made possible through international collaboration between the "Mediterranea" University of Reggio Calabria (Italy) and the University of Almería (Spain).

During this first step, an extensive literature review has been carried out in order to collect all available information about Italian gypsophilous flora and to compile a preliminary list.

Afterwards, 18 regional botanists expert on Italian gypsum flora have been asked to rank the gypsum preference of the species from this preliminary list. Following the methodology proposed by Mota et al. (5), the degree of gypsophily was ranked on a scale from 1 to 5 (corresponding to low and total dependence on gypsum substrates, respectively). The species list was subsequently amended and improved twice on the basis of peer opinions to obtain a final evaluation. More than 360 species have been taken into account. According to the obtained values, all the plant species, ranking between absolute or preferential gypsophytes (with median values between 5 and 3), will figure in the final checklist of the Italian gypsophytes.

A more detailed study on the gypsophilous flora and plant communities would be required to support an effective conservation action for the Italian gypsum areas and their natural heritage.

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1.6. = MORPHOLOGICAL, KARYOLOGICAL AND MOLECULAR ANALYSES ON THE *ADENOSTYLES ALPINA* GROUP IN SOUTHERN ITALY: PRELIMINARY DATA

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*Adenostyles* Cass. (Asteraceae, Senecioneae) is a genus endemic to Europe, prevalently distributed throughout the Alps, but with its range extending also to northern Spain, the Balkans, Corsica, doubtfully Greece, and along the entire Apennine in Italy, including the mountains of Calabria and Sicily (1).

Within the genus, particularly interesting results were obtained for *A. alpina*, that on the basis of recent molecular data (2) includes five subspecies: *A. alpina* Bluff & Fingerh. subsp. *alpina*, *A. alpina* subsp. *pyrenaica* (Lange) Dillenb. & Kadereit, *A. alpina* subsp. *briquetii* (Gamisan) Tutin, *A. alpina* subsp. *macrocephala* (Huter, Porta & Rigo) Dillenb. & Kadereit, and *A. alpina* subsp. *nebrodensis* (Wagenitz & I.Müll.) Greuter.

The taxa of this group are characterized by high morphological diversity which occurs mostly in southern Italian populations, recently attributed to *A. australis* (3), which in the past were treated as varieties of *A. alpina* or as various different species or subspecies (4, 5, 6).

In particular, morphological variation is found in characters that have been considered taxonomically important: number of flowers per capitulum, size of the involucre, flower and achene morphology, leaf margin and presence or absence of auriculate upper leaves.

The present study aims to clarify phylogenetic relationships among taxa belonging to this group, especially those in southern Italy, by means of morphological, karyological and molecular investigations using DNA sequences of nrITS, nrETS and the plastid marker *ndhF-rpl32* IGS.

First analyses of *ndhF-rpl32* IGS, with indels treated as missing data, revealed seven haplotypes: haplotype A is exclusive to Corsica, haplotype B occurs in France, northern Italy, Switzerland, Austria and Germany, haplotype C is represented by a single specimen from Sila (Calabria, southern Italy), haplotype D is restricted to the Pyrenees (Spain), haplotype E is represented by two specimens from Aspromonte (Calabria, southern Italy), haplotype F is exclusive to central-southern Italy, with one specimen from the Dolomites (northern Italy) and three specimens from the Madonie Mountains (Sicily), and haplotype G is represented by two specimens from the Apuan Alps (northern Italy) and the Alpes Maritimes (France).

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1.8. = *IN VITRO* AND *IN VIVO* GERMINATION OF *VIOLA ELATIOR* FRIES: IMPLICATIONS FOR CONSERVATION

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*Viola elatior* (Violaceae) is one of the most rare and endangered European species and occurs in Italy in small fragmented populations situated at the SW margin of its range. It is a river corridor plant, typical of humid grasslands, banks, field margins and woodlands, growing in places where the water table shows notable annual level variations (flooding in winter and strong summer aridity). From late 1800 onwards, it underwent a strong decline due to drainage, urban sprawl, soil eutrophication, pollution: today it is *Endangered* at a national level (1). To cope with such a decline, which is still running, we studied the *in vitro* and *in vivo* propagation by seed, in order to obtain a number of individuals for population reinforcing. A first preliminary *in vitro* experiment was performed in 2015 on 25 seeds collected in late May of that year (chasmogamous flowering) from the plants maintained at the Botanic Garden of Modena. Seeds were sterilised for 14' in 5% NaOCl, then posed on a 1/2 MS + 6 g/l agar cultivation terrain. After sowing, they were kept in the dark, for 11 days at 25 °C, then 35 days at 4 °C and then again at 25 °C for 4 days. In a second experiment, we took into account 3 populations of the province of Mantua, which are particularly notable for number of individuals and vegetative strength. Seeds were collected during spring and summer 2015, sampling separately the production of chasmogamous and cleistogamous flowering. Seeds were kept in the dark at 18 °C until the sowing (January 2016). Two tests were carried out: sowing *in vitro* after 30 days of chilling at 4 °C; sowing outdoor in pots without chilling, in an area near the collection places. Germination occurrence was recorded for 50 days after sowing. Totally, in the second experiment 1724 seeds were sowed. In the first experiment (*in vitro*) we obtained a germination rate by 96%. In the second one, the highest germination percentage was observed *in vivo*, with seeds coming from cleistogamous flowering (85.9%); the lowest percentage was recorded *in vitro*, for seeds taken from chasmogamous flowering (14%). Germination percentage was higher for the seeds coming from the cleistogamous flowers (56.4% *in vivo*, vs. 34.3% *in vitro*), in comparison with seeds coming from the chasmogamous flowers (50% and 23%, respectively). Such findings are conflicting with the literature data, taken from central European populations (2), which refer lower germination rates for seeds from cleistogamous flowers and practically no germination under controlled conditions (3). The *in vivo* germination values are quite similar to what is known for *V. elatior* (2); the *in vitro* germination values are instead always notably different both from literature data and from precedent *in vitro* experiments (4) performed on *Viola pumila* Chaix, which is ecologically and biologically similar to *V. elatior* (5). Results similar to those obtained from the populations of the province of Mantua were found also for *Jacobaea paludosa* (L.) G.Gaertn., B.Mey. & Scherb. subsp. *angustifolia* (Holub.) B.Nord. & Greuter (6), another river corridor species living in marshes and wetlands. Briefly, in conditions of peripheral rarity (Mantuan sites) our tests show a higher germination of the seeds coming from the cleistogamous flowering, both *in vitro* and *in vivo*; in more central areas of the distribution range, data from literature show a higher germination for the seeds from chasmogamous flowering. These results could be correlated to the climate of the Mantua province, which is warmer than in central-eastern Europe, and could be exacerbated by the fact that 2015 has been the warmest year since 1860. The nearly total germination obtained *in vitro* in the first experiment (seeds from the chasmogamous flowers) may have been influenced by mother plants cultivation in the botanic garden. At present, the *ex situ* protocols for *V. elatior* seed germination should be improved, individuating more properly the ecological conditions to which seeds are subjected in the central zones of the species' distribution area, where a high germination occurs (2).

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1.8. = SAVING THE LAST ITALIAN POPULATION OF *CISTUS LAURIFOLIUS* L. SUBSP. *LAURIFOLIUS*: PLANT FUNCTIONAL TRAITS AND REPRODUCTIVE STRATEGIES UNDER THE LIGHT OF A CONSERVATION PERSPECTIVE

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One of the most interesting Italian woody taxa from a conservation point of view is the laurel-leaved rockrose (*Cistus laurifolius* L. subsp. *laurifolius*). This taxon is a heliophilous shrub with large white flowers, whose range scatters across Morocco, Portugal, Spain, S France, Italy, towards the Black Sea region, Turkey (1). Currently, in Italy this species occurs only in Tuscany, where it is distributed in a single population (with five subpopulations) near Florence. According to the IUCN protocol, it has been recently assessed as vulnerable (VU) at national level (2). Our aim was to estimate its population size, density and demographic structure in Italy, and to study the reproductive success for different densities and for each subpopulation. Furthermore, we investigated plant functional traits in two contrasted habitats: garrigue vs. underbrush. According to methodological approaches exposed in (3), the following parameters were measured for both ecological contexts: leaf area, wet and dry leaf weight, height of individuals and seed mass. In addition, a soil analysis was performed to evaluate the following parameters: texture, pH, electrical conductivity, N, K and soil organic matter content. Demographic results showed that the Italian population of this species is composed by ca. 10.000 individuals with a mean density of 0.116 plants/m<sup>2</sup>. The smallest subpopulation has currently just one adult plant left, so that it could be considered as almost extinct, whereas the largest subpopulation contains more than 90% of all the Italian plants. The subdivision of investigated plants into three age classes highlighted that 2% of the individuals are juvenile plants (i.e. with no more than 6 leaves), most of the plants (86%) are adult and the rest (12%) belong to an intermediate developmental stage. Our results revealed that plants occurring in high-density plots produced a number of flowers, fruits and seeds significantly higher than plants growing in medium- and low-density plots (Wilcoxon-Mann-Whitney with Bonferroni correction test;  $p < 0.05$ ). In addition, at subpopulation level, the largest one produced a significantly higher number of seeds per fruit than others ( $p < 0.01$ ). These preliminary results led us to hypothesize an Allee effect for the Italian population of this species: small or sparse subpopulations may suffer fitness reduction. Values of Specific Leaf Area (i.e., leaf area (mm<sup>2</sup>)/dry leaf weight (mg)), expressed as mean  $\pm$  st. dev., were  $4.58 \pm 0.47$  in garrigue and  $7.83 \pm 1.79$  in underbrush context, whereas those of Leaf Dry Matter Content (i.e., oven-dry mass (mg)/water-saturated fresh mass (g)) were  $377.61 \pm 33.36$  in garrigue and  $317.06 \pm 42.20$  in underbrush context, revealing significant differences (Student's *t* test;  $p < 0.01$ ). Our results are in agreement with the typical sun-shade morphological responses, in which thinner leaves with higher leaf area were produced under low light conditions. These parameters suggest an acclimatization to maximise the carbon gain under shade conditions. Concerning future perspectives, our work aims to obtain a better understanding of the adaptive responses and optimal ecological requirements of laurel-leaved rockrose in Italy in order to plan, if necessary, efficient and taxon-calibrated conservation measures.

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## 1.8. = ANALYSIS OF DIASPORE MORPHOLOGY AND SEED GERMINATION IN *BUBON MACEDONICUM* L., A RARE SPECIES IN ITALY

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*Bubon macedonicum* L. is a scapose hemicryptophyte that belongs to the Apiaceae family (tribe Scandiceae), that is of particular phytogeographical interest both for its rarity and for its distribution in severely disjunct populations. This species is monocarpic, as it lives 4 or 5 years on average, and dies after fruiting. *Bubon macedonicum* is an obligate chasmophyte with entomochorous dissemination. It grows at very variable altitudes (0-1500 m a.s.l.) in more or less shady rock crevices, cliffs, walls (Greece) or, rarely, in coastal cliffs (Valona district, Albania). In Italy, *B. macedonicum* has only been reported for the Rocca Monforte locality (Campobasso, Molise) with a population of about 300 individuals and for that reason classified as Critically Endangered (CR) in the IUCN Red List of Italian Flora.

*Bubon macedonicum* has been studied mainly for the chemical composition of its essential oils or in coenological terms in Greece and Albania, while there have been no studies relating to its biology.

For this reason, it was deemed necessary to start coordinated studies on the reproductive biology of this species (e.g., pollination, fruiting, germination), with the aim of identifying the critical elements and the most appropriate and effective measures to take to avoid its extinction in the unique Italian site.

In the present study, the investigations focussed on the following:

- studies into the morphological parameters of *B. macedonicum* diaspores, and their ability to germinate under controlled conditions (e.g., temperature, humidity, photoperiod);
- definition of a seed germination protocol aimed at *ex situ* cultivation of this species, for its reintroduction *in situ*.

According to international protocols (ISTA, 2012), about 3,000 seeds were collected in August 2013, which constituted less than 10% of the seeds that were available in the existing populations.

The mother plants were randomly selected, and mature seeds of *B. macedonicum* were collected during the fruiting period, immediately before their dispersal, last 10 days of August. Fifty diaspores were measured. The measured parameters were: seed length (L, major axis), width (W, intermediate axis) and thickness (T, minor axis). From these data, the following were calculated: diaspore surface ( $S = L \times P$ ), volume ( $V = L \times W \times T$ ), density ( $D = SM/V$ ) and S/SM ratio (surface/ mass). To characterise the shapes of the diaspores, the index of eccentricity was used ( $EI = L/W$ ).

The morphometric parameters that were examined for the *B. macedonicum* diaspores showed morphological dormancy, where a short summer period is necessary for embryo growth and seed germination. For the data analysis we used one-way ANOVA, followed by post-hoc Tukey's tests, to compare the differences between the means. The analysis of the results obtained showed high germination percentages under the different conditions of temperature, pH, GA3 and photoperiod. The germination rates at the six temperatures tested (5, 10, 15, 20, 25, 25/10 °C) showed that the seeds had good germination ability at almost all of these temperatures. In particular, at 20 °C the germinating percentage of the seeds was greater, while at 5 °C there was no germination. For the pH tests (4, 5.7, 7, 8, 11), the germination rate of 100% was reached for those seeds exposed to pH 5.7, while maintaining values that were always higher than 80% under the other conditions. Treatment with GA3 at concentrations of 250 ppm and 500ppm also gave high germination rates. The analysis obtained for the comparison of a photoperiod of 12 h of light with 24 h of dark did not provide significant differences between the two treatments, with high germination capacity seen both in the dark and in the light, although a slightly higher germination rate was achieved with the exposure to the light. The results relative to the cold-storage treatment are of considerable conservation interest, as the seeds subjected to this process retained high germination percentages after both 130 days and 390 days, at 98.4% and 91.3%, respectively. This shows that these seeds retain good germination ability over time, a factor that is of great importance for the conservation of this species over the medium and long term.

## 1.8. = USING EXTINCTIONS IN SPECIES DISTRIBUTION MODELS TO EVALUATE AND PREDICT THREATS: A CONTRIBUTION TO THE PLANT CONSERVATION PLANNING IN THE ISLAND OF SARDINIA

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Estimates and documentation of recent extinction rates suggest that humans are now causing the sixth mass extinction. In this context, Mediterranean islands are at the forefront of many of the environmental issues that the world is facing. This study provides an alternative approach for investigating documented local plant extinctions that occurred in Sardinia (W-Mediterranean Basin) during the last half century. The local extinctions of 62 plant species were used to investigate the independent effects of eight ecological and anthropogenic variables and to model the areas of potential extinctions where plant conservation efforts could be focused. In most of cases, both anthropogenic and environmental factors explained local extinctions. Furthermore, areas potentially rich in species with conservation interest, but which are particularly anthropized, were highlighted by our analysis. Building upon this practical case study in Sardinia, this paper suggests a reproducible, operational framework to analyse which extinction factors may play an important role in similar contexts and where they might occur.

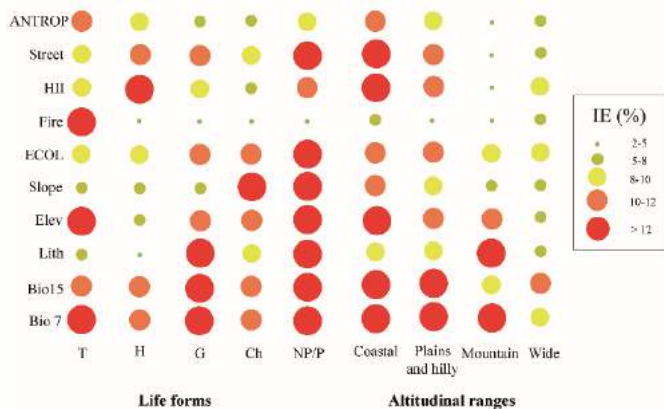


Fig. 1 Scatterplots of the percentage of independent effect of each variable utilized and the mean of ecological (ECOL) and

anthropogenic (ANTROP) groups. Results were subdivided according to the life forms proposed by Raunkiaer (1934) and their altitudinal ranges of past and present occurrences.

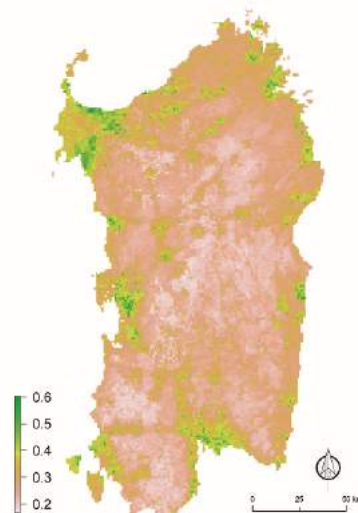


Fig. 2 Average map of 32 singularly modelled extinction cases. Values from

0 to 1 measured the probabilities of extinction in all the Sardinian territory.

## 1.8. = ENDEMIC VASCULAR FLORA OF MT. ETNA (SICILY): ELEVATION GRADIENT AND CONSERVATION PERSPECTIVES

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Mount Etna is a huge polygenic basaltic volcano covering a broad sub-rounded surface of 1,178 Km<sup>2</sup> from sea level, along the Ionian coast of Sicily, up to a height of 3,328 m. It is characterized by an almost continuous eruptive activity from its summit craters and fairly frequent lava flows from lateral fissures. Despite its recent origin (late Quaternary), Mt. Etna is the highest peak of Sicily and the highest active volcano of Europe.

Our research allowed to perform an in-depth survey on the distribution patterns of vascular plant species richness and endemism along the elevation gradient. We used all available data (literature, dried collections and field data) on distribution of the Etna plant species to interpolate their presence between minimum and maximum elevations in 100 m elevational intervals. Overall, against a flora of about 1050 taxa (roughly corresponding to one third of the Sicilian flora), 112 taxa are endemic to Sicily (ca. 11%, ES), 30 of which are exclusive of Mt. Etna (i.e. narrow endemic species, NES). Area is a crucial factor influencing species richness, and it generally declines with increasing elevation. However, the area available for plants on high mountains is not constant through time. This is particularly true for an active volcano whose lava flows or tephra falls may suddenly cover huge surfaces (e.g. up to 40 km<sup>2</sup> during the 1669 eruption) or catastrophic natural events may reduce available area as a consequence of huge collapses as happened 15,000 years ago. In addition, such variations may evenly affect areas at low, mid or high elevation and, subsequently, cause variations in total plant species richness, degree of isolation and thereby the extent of endemism.

Floristic richness has its maximum between 600 m and 900 m a.s.l., and hence there is a steep decrease towards the highest peak of Mt. Etna. This would agree with the hard boundaries theory which predicts that total species richness will have a peak close to the mid-elevation, rather than to the Steven's theory which extends the Rapoport's latitudinal rule to altitude. The highest endemic richness is recorded between 800 m and 1000 m a.s.l. and, except for a plateau between 1200 and 1600 m a.s.l. it progressively decreases towards the top of the volcano, while the highest narrow endemic richness (NES) is recorded between 1500 and 1900 m a.s.l, well above the interval of maximum total floristic richness.

Species richness is decreasing from about 900 m a.s.l. and upwards; this confirms the rule of reduced species number with higher elevation. This is likely caused by eco-physiological constraints (e.g. reduced growing season, low temperatures, strong winds, etc.), by the lack of ruderal, (sub)nitrophilous and alien species mostly linked to anthropogenic environments, as well as by the absence of weedy species linked to the different local crops.

As regards the conservation issues, apart the well documented extinction of *Limonium catanense* (Tineo ex Lojac.) Brullo, a halophyte described at the beginning of the XX century from the rocky coast of Catania (E Sicily), most of the NES are located within a protected area and, most relevant, at higher altitudes (above 1500 m). Actually, since 1987 Mt. Etna has been established as regional park ("Parco Regionale dell'Etna"), 13 Sites of Community Importance (SCIs) are within the regional park and are managed by the park authority, and lastly since 2013 the highest part of the volcano (about 192 km<sup>2</sup>) has been included in the UNESCO World Heritage List. Despite this, some endemic species, such as *Sisymbrella dentata* (L.) O.E.Schulz, *Crassula basaltica* Brullo & Siracusa, *Asparagus aetnensis* Tornab., *Celtis tournefortii* Lam. subsp. *aetnensis* (Tornab.) Raimondo & Schicchi, *Kali basalticum* C.Brullo, Brullo, Gaskin, Giusso, Hrusa & Salmeri, occur on very restricted areas, more or less severely affected by agriculture or grazing animals and, likely most worrying, at lower altitudes. In addition, some endemic species may result particularly sensitive to human-induced climatic variations which may cause not only highest yearly mean temperatures, but also reduced precipitations or reduced snow cover. This phenomenon could be further exacerbated by the altitudinal rising of trivial or invasive alien species, such as *Ailanthus altissima* (Mill.) Swingle which is moving from the coastal and hilly stands of Mt. Etna towards the higher altitudes.

Concluding, scientifically based monitoring plans should be implemented both for the most endangered species (endemic or not), but also for assessing the conservation status of the habitats where they are found. Concrete in-situ actions, including population translocations or reinforcement, pest control, passive protections, etc. are equally necessary.



1.8. = *ZANTHOXYLUM ARMATUM* (RUTACEAE) A NEW INVASIVE SPECIES IN ITALY?

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The genus *Zanthoxylum* (Rutaceae) comprises more than 200 species naturally distributed from tropics to temperate latitudes of East Asia and East North America. The genus is widely known for its economic importance due to pharmacological and ethnobotanical uses.

*Zanthoxylum armatum* DC., commonly called winged prickly ash, is native to South and East Asia and it is an important plant in traditional herbal medicine (e.g. China, India), beyond being an ornamental and alimentary plant cultivated in different part of the globe (1, 2). It is a deciduous erect or scandent, thorny small tree or large shrub and a dioecious species (3), even if asexual reproductive strategies are plausible according to observations and literature data (4, 5). Winged prickly ash lives in different habitats as valleys and thickets in mountains, wastelands as well as the understory of mixed forests, but it is also found in open slopes or rocky ledges, in full sun or semi-shade, not over the 3100 m a.s.l. (2, 6). Because of its ecological characteristics *Z. armatum* has been used in national project of afforestation of degraded slopes in China (7).

Mainly due to its nutraceutical qualities, *Z. armatum* was introduced to Italy at Hanbury Botanical Gardens (Ventimiglia, IM) in 1868 (8), and then cultivated in other Italian botanical institutions. No evidences of naturalization in Italy were made official until 2012, when a naturalized population of *Z. armatum* was found in the mesophilous mixed woods of Blevio (CO) (3). Progressively other records of naturalization have been collected in Italy. Episodes of naturalization and expansion are known also in Argentina (Cordoba province), where the species is cultivated and classified as naturalized neophyte (1).

As biological invasions are one of the worst threats to biodiversity at global level (9), in view of ecology, biology, history and trade of the species, it is urgent defining if *Z. armatum* is a potential threat to Italian native ecosystems and flora. Basing on field and literature data, the species has been assessed in order to evaluate its invasiveness. The assessment followed EPPO and A-WRA protocols, two of the most widely used tools providing consistent evaluation of invasive plant (10) in order to identify species deserving management measures. Here we present the results of the assessment with perspectives for future studies and actions.

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1.8. = VIABILITY AND LONG TERM STORAGE OF *CORYLUS AVELLANA* L. POLLEN

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European hazel is one of the most important fruit tree crop in the world. The demand of nuts is growing and the cultivation area of this species has extended outside its native range. Hazel cultivars are self-incompatible and the flowering period of male and female flowers only partially overlaps, so hazel requires compatible and good pollinizer cultivars in order to obtain a good yield (1). Nevertheless, the flowering period can vary from year to year and so artificial or supplementary pollination can be used as a solution to improve the final yield and optimize the cross-pollination, in particular outside the native range, where the wild type is absent (2). Therefore, the aim is to evaluate the best temperature conditions (20, 4, - 30°C) for long-term storage of pollen in order to maintain a high viability level. Pollen coming from three cultivars ("Tonda Gentile delle Langhe", "Tonda di Giffoni" and "Tonda Gentile Romana") and wild hazel were collected during two winters (2015 and 2016). Several methods were used to assess a more objective estimation of pollen viability: *in vitro* pollen germination with hanging-drop method, 2,3,5-triphenyltetrazolium chloride (TTC+sucrose) method and fluorescein diacetate and propidium iodide (FDA+PI) method (3). Pollen was desiccated over dehydrated silica gel at room temperature and the petri dishes were then sealed. The samples of pollen coming from different cultivars were stored at room temperature (20°C), in a fridge at 4°C and in a cooled incubator at - 30°C. The three different viability tests started from the 3<sup>rd</sup> or 10<sup>th</sup> day from harvesting and were carried out every seven days during the first month and then every fifteen days. All the samples were divided into different vials in order to reduce the stress linked with thawing. The pollen was rehydrated by placing the samples in humid chambers for a measured time (60-90 min) before performing the different tests. Each test was composed of two repetitions for the same sample of pollen of "Tonda Gentile delle Langhe" (TGdL), "Tonda di Giffoni" (TdG), "Tonda Gentile Romana" (TGR) and wild hazel (WH). Each test was repeated every fifteen days until 150 days from the harvesting for each cultivar.

In germination tests each sample was observed under microscope (250×) by locating ten random fields and score it in order to evaluate the percentage of alive and anomalous grains. When the pollen tube length was greater than the diameter of the pollen grain, a germination event was considered.

In TTC test the pollen grains were incubated in 2,3,5-triphenyl tetrazolium chloride and sucrose for 24 hours in dark and humid chambers. Following incubation, each sample was observed under microscope (250×) by locating ten random fields and pollen grains that presented a red color were scored as viable.

In the last experiment, the pollen coming from different storage conditions was incubated in fluorescein diacetate (FDA) and propidium iodide solution. The pollen was observed under a fluorescence microscope with green filters. Thereafter, images of the pollen grains were taken and were analyzed with the CellProfiler Software (4).

The results have shown that the pollen viability varied between cultivars, years of sampling and on the base of the method used to assess the viability. However this study demonstrated that the -30°C temperature storage condition was the best in order to maintain a high viability level. At this temperature a percentage of about 50% of the pollen grains were viable after 150 days from harvesting.

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## 1.8. = THE BOTANICAL GARDEN "BERNARDINO DA UCRIA" IN THE NATURAL PARK OF THE NEBRODI (SICILY) AND ITS MISSION TO CONSERVE, EXPLOIT AND SPREAD LOCAL AGROBIODIVERSITY AND OFFICINAL PLANTS

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In Sicily, the academic botanical gardens of Catania, Messina and Palermo have been historically exerting a multiplicity of activities ranging from maintenance of *ex situ* collections to plant conservation policy, practice and ecological restoration, along with more traditional functions related to education and academic research. In the last decade's, two new botanical gardens the "Nuova Gussonea" and the "Bernardino da Ucria", were created in Sicily, with the aim to play more delimited, yet modern and complementary roles. The garden "Nuova Gussonea", within the Etna Natural Park, is mostly devoted to collect and preserve the native flora of the Mount Etna, with a special focus on endemic and rare plants and their promotion to a wider public. The garden "Bernardino da Ucria", in the Nebrodi Natural Park, is mainly specialized in promoting the local culture and to collect and preserve the agro-biodiversity of officinal plants of the Nebrodi territory. It is located in the homonymous village in the Messina district where the famous "demonstrator of plants" and co-founder of the Botanical Garden of Palermo University was born. Noteworthy, within Sicily the Nebrodi area is undoubtedly the widest and richest in traditional activities regarding agriculture, sheep farming and silviculture, which produced an agrobiodiversity heritage often representative of individual community cultures. Initially promoted by Palermo University and sustained by the Nebrodi Regional Park, this garden comprises the "Banca vivente del Germoplasma vegetale dei Nebrodi". Accessions conserved *in vivo* so far include cultivars of crops (*Corylus*, *Pyrus*, *Malus*, *Prunus* sp. pl., *Ficus*, *Juglans*), vegetables (*Phaseolus* and *Lycopersicum*) and officinal plants, that are in part representative of local agricultural practices. Among crops, the collections of *Ficus*, *Pyrus* and *Corylus* are remarkable; while among vegetables, the beans (*Phaseolus*) are represented by over 65 distinct cultivars, comprising 57 climbing and 8 dwarf species (1). Additionally, the garden's bank hosts several botanical collections unrelated to the local flora, representative of officinal and ornamental genera, such as *Salvia*, *Helleborus*, *Paeonia* and *Camellia*. The cryopreserved accessions so far encompass the bean cultivars, mainly found within the Nebrodi Park area. The aim of this particular collection is to further exploit the local agricultural and food heritage by recovering and spreading among local farmers of autochthonous cultivars under extinction risk. Moreover, the garden has recently been equipped with a molecular biology laboratory, performing studies on the genetic diversity of the accessions, as well as activities instrumental to the creation of a related DNA bank, interconnected with the Palermo Botanical garden bank (HBP-Bank). In 2011, the botanical garden of Ucria hosted the Summer School "Knowledge, conservation and management of plant biodiversity of the Mediterranean mountain systems", organized by Palermo University together with OPTIMA, and financially supported by the Nebrodi Park. Similarly, the Summer School "Management of plant biodiversity in the Mediterranean: *ex situ* conservation and germplasm banks" will be held in 2016. The activities of the new Ucria botanical garden and its germplasm bank, is supported by a dedicated Consortium, established by the Nebrodi Park together with Palermo University. The botanical garden "Bernardino da Ucria" is open to public in the spring and summer period. It is also offering didactic visits for educational purposes to several schools.

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## 1.8. = THE TEMPORARY WETLAND COMPLEX OF ANGUILLARA AND ITS KEY ROLE FOR THE BOTANICAL HERITAGE OF WESTERN SICILY

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Temporary wetlands are endangered throughout the entire Mediterranean area (1). Contrary to the permanent wetlands, that (usually) cover larger surfaces and are now protected after a long period of siege and 'reclamation' attempts, temporary ponds and wetlands are small, scattered areas still under pressure and threatened by human activities. This fact represents a serious threat for biodiversity, since many species only occur in these peculiar sites. To secure their protection Mediterranean temporary ponds are listed as "priority habitat" in the EU "Habitats" Directive (92/43/EEC). The temporary ponds of Anguillara, located near Calatafimi in Western Sicily, host a huge amount of rare plants. This site was still unexplored until few years ago, and it falls outside the regional nature reserves and the Natura 2000 network. Although in 2011 an attempt was made to obtain a legal protection of the area (2), this proposal was not accepted by regional authorities, and few months later some of the ponds were destroyed to build greenhouses with solar panels. In the framework of a multidisciplinary study promoted by the 'Società Siciliana di Scienze Naturali' in order to improve the knowledge and awareness on the biological importance of the site, the wetlands were visited many times by bryologists, botanists and vegetation scientists in different seasons. The preliminary results of these still ongoing investigations are presented here. Several hygro-hydrophilous plant communities have been detected: the *Ranunculetum peltati* Horst, Krausch & Müller-Stoll 1966 em. Weber-Oldenoc 1969 (class *Potametea pectinati* Klika in Klika et Novák 1941) is linked to the deepest part of the main seasonal pool, where species such as *Alisma lanceolatum* With. and *Glyceria notata* Chevall. occur. On the edges of the ponds several communities referred to the classes *Phragmito australis-Magnocaricetea elatae* Klika in Klika & Novák 1941 and *Isoëto-Nanojuncetea* Br.-Bl. & Tüxen ex Westhoff, Dijk & Passchier 1946 have been observed. The local vascular flora includes many species of high biogeographic and conservation interest, such as *Isoetes longissima* Bory (= *I. velata* Auct.) and *Ipomoea sagittata* Poir., the latter included in the European Red List (3), and a number of regionally or globally rare species such as *Myosotis sicula* Guss., *Elatine* cf. *macropoda* Guss., *Solenopsis laurentia* (L.) C.Presl, *Cicendia filiformis* (L.) Delarbre, *Lysimachia arvensis* (L.) U.Manns & Anderb. subsp. *parviflora* (Hoffmanns. & Link) Peruzzi. Local 'highlights' are with no doubt the recent discoveries of the only Sicilian population of the globally endangered *Pilularia minuta* Durieu (3), included in the European Red List (4), and of the only Italian population of *Trifolium isthmocarpum* Brot. subsp. *jaminianum* (Boiss.) Murb. (5), which was considered extinct at the national level. The site hosts also some interesting mosses such as *Ephemerum crassinervium* (Schwägrichen) Hampe subsp. *sessile* (Bruch) Holyoak, included in the National Red List (6) and confirmed a century after the collection in one single site of eastern Sicily, and *Enthostodon fascicularis* (Hedw.) Müll.Hal., new to western Sicily; and the liverwort *Riccia bicarinata* Lindb., rare in Italy.

Due to the richness of its communities and the conservation value of its flora at the local, regional and global level, the site of Anguillara with its network of temporary wetlands and pools results to play a strategic role for biodiversity of western Sicily, thus it urgently deserves appropriate protection measures.

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## 1.8. = EX SITU CONSERVATION OF LIGURIAN ENDANGERED SPECIES

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The main objectives of the Laboratorio per la Conservazione della Diversità vegetale ligure (the Ligurian Germplasm Bank) are to conserve and to study endemic, rare, threatened and policy species (Annexes to Directive 92/437 EEC, Bern Convention) from Liguria Region. In order to achieve these goals, we collected and stored accessions representative of morphological and genetic diversity of the studied taxa.

In 2015, within the project Natura2000 AdM Progres (ALCOTRA Program) about the consolidation of cross-border operational models of management of the knowledge of the biodiversity status and the tangible results for natural heritage conservation (1), requirements for seed germination of most vulnerable species were investigated. The studied taxa and their categories of conservation interest are reported (2).

	Status IUCN Italy Ass. 2013	Liguria Regional
<i>Polygonum oxyspermum</i> C.A.Mey. & Bunge subsp. <i>robertii</i> (Loisel.) Akeroyd & D.A.Webb	CR	X
<i>Acis nicaeensis</i> (Ardoino) Lledó, A.P.Davis &	CR	X
<i>Brassica montana</i> Pourr.	VU	X
<i>Crocus versicolor</i> Ker Gawl.	under assessment	X
<i>Limonium avei</i> (De Not.) Brullo & Erben	under assessment	X
<i>Limonium cordatum</i> (L.) Mill.	under assessment	X
<i>Malva subovata</i> (DC.) Molero & J.M.Monts.	under assessment	X
<i>Silene badaroi</i> Breistr.	under assessment	X
<i>Fritillaria involucreta</i> All.	under assessment	X
<i>Saxifraga cochlearis</i> Rchb.	under assessment	X
<i>Euphorbia canuti</i> Parl.	under assessment	X
<i>Rhaponticum coniferum</i> (L.) Greuter	-	X
<i>Iris lutescens</i> Lam.	-	X

In 2013, 2014, 2015, at the time of dispersal, seed accessions were collected from wild populations, randomly selected within groups of populations defined according to morphological and genetic criteria (3). The accessions were stored at room temperature until the start of germination trials. The tests were carried out in 4 replicates of 25 seeds each, incubated in a germination chamber under different temperature and photoperiod treatments.

Fig.1. *Acis nicaeensis*Fig. 2. *Polygonum oxyspermum* subsp. *robertii*Fig. 3. *Saxifraga cochlearis*

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2) G. Rossi, C. Montagnani, D. Gargano, L. Peruzzi, T. Abeli, S. Ravera, A. Cogoni, G. Fenu, S. Magrini, M. Gennai, B. Foggi, R.P. Wagensommer, G. Venturella, C. Blasi, F.M. Raimondo, S. Orsenigo (Eds.) (2013) Lista Rossa della Flora Italiana. 1. Policy Species e altre specie minacciate. Comitato Italiano IUCN e Ministero dell'Ambiente e della Tutela del Territorio e del Mare.

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 1.9. = LIMITATION TO REPRODUCTIVE PERFORMANCE IN THE DIOECIOUS ENDEMIC SHRUB *OREOHERZOGIA GLAUCOPHYLLA* (SOMMIER) W.VENT (RHAMNACEAE)

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Dioecy is a relatively rare sexual system occurring in approximately 7–10% of angiosperm species (1). As the consequence of separate sexes is the impossibility of selfing, the avoidance of inbreeding has traditionally been invoked as an important selective force in the evolution of dioecy (2). However, other correlations of dioecy with several ecological and life-history attributes have been found, including tropical distribution, woody growth form, abiotic pollination, small inconspicuous flowers and fleshy fruits (3). Despite the multiple ecological roles that dioecious species play in the landscape, experimental studies of their reproduction have not received considerable attention compared to hermaphroditic plants (4, 5). Here, we examined the effect of ecological and population traits (including topography, pollination environment, neighbouring competition and plant size) on reproductive performance of *Oreohertzogia glaucophylla* (Sommier) W.Vent, a dioecious shrub endemic to Apuan Alps and a restricted range of Tuscan Apennine (6, 7). To this end, 60 females, designated as focal females, were randomly selected in two contrasting environments located at the extremes of its altitudinal range for monitoring their reproductive performance between April and September 2015.

Generalised linear mixed models revealed that elevation, aspect and plant size have no significant effect on fruit set. On the other hand, the distance to nearest male resulted the single most important factor affecting fruit set, with number of fruits significantly decreasing with increasing distance to nearest male. Neighbouring competition (presence of other females) and floral competition (number of flowers within the same inflorescence) also negatively affect the fruit set. By contrast, fruit set was not limited by all the above mentioned factors at the low altitude site, where they might be overridden by other undetected factors. In both sites, seed set was unaffected by all considered factors. That is to say, in any single flower, ovules fecundation is not limited by pollen load in the light of the low number of ovules (four) per ovary.

Overall, the results of this study support the importance of pollen limitation on fruit set in *O. glaucophylla*, however, the effect of year on resource availability cannot be ruled out, especially on seed quality and viability.

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## 1.9. = HABITAT-DRIVEN SHIFTS IN PLANT-INSECT INTERACTION AND THEIR EFFECT ON PLANT REPRODUCTION

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Plant biotic interactions (PBIs) (i.e. plant functional relationships with mutualistic and antagonist organisms) are a key driver of population processes in angiosperms, the second group of biological diversity on the Earth. In flowering plants, PBIs are mainly founded on associations with insects, which may act as pollinators or herbivores (1, 2). The ecological heterogeneity found at forest edges promotes spatial shifts in insect fauna, which can influence patterns and strength of PBIs and, then, plant reproduction (3). This may have relevant implications for the preservation of plant diversity, especially in highly fragmented landscapes (4). Nonetheless, there are little empirical data showing the functional relationships among patterns of insect visit, intensity of mutualistic and antagonistic plant-insect interactions, and plant reproductive fitness over structured ecological gradients.

We investigated how changes in flower-visitor abundance, identity, and behavior over a forest-open ecological gradient can affect plant biotic interactions (i.e. pollination and herbivory), and quantitative and qualitative fitness components in the ecotone-specialist *Dianthus balbisii* Ser. Field data showed that composition and behavior of the insects visiting flowers of *D. balbisii* varied strongly over the study gradient, influencing strength and patterns of plant biotic interactions, as well as the relationships between pollinators and plant traits involved in insect attraction. Seed set analyses on free- and manually pollinated flowers revealed spatial variations in the extent of quantitative pollen limitation. This was congruent to the patterns of flower visit and plant biotic interactions. Data on seed and seedling viability suggested that spatial variations in amount and type of pollinators, and frequency of herbivory affected qualitative fitness of *D. balbisii* by influencing selfing and outcrossing rates.

Our findings highlight the role of plant biotic interactions as a fine-scale mediator of plant reproductive fitness in forest ecotones, an integral ecological component of fragmented landscapes. Based on our data, optimal plant reproduction can take place into a limited interval of the ecological gradient found at forest edges. Therefore, any reduction of the ecological complexity typical of such transition contexts may be detrimental for the maintenance of viable populations of ecotone-adapted plants.

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1.11. = LEAF MORPHOLOGY IN *MALUS CRESCIMANNOI* AND *M. SYLVESTRIS* (ROSACEAE)

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The genus *Malus* Mill. includes woody plants characterized by petioled, herbaceous, deciduous leaves, with lamina entire and more or less deeply crenate or serrate margin, the teeth apically bearing hydathodes and mucilaginous epidermis. The petiole, of moderate length, is provided with apically showy, leathery sheath.

This contribution aims to further characterize taxonomically a recently described species endemic to Sicily (*Malus crescimannoi* Raimondo), with respect to *M. sylvestris* (L.) Mill. The latter is a Central European species, also occurring in Italy and in Sicily, while *M. crescimannoi* is so far known only for Nebrodi and Madonie Mountains (2). The study shows that leaves are usually ovate to round with crenate margin in *M. sylvestris*, while are ovate to elliptic in *M. crescimannoi*. In the latter species, leaves are narrower and longer with margins deeply crenate or serrate. The petiole is usually longer and thin in *M. crescimannoi*, while it is shorter and thick in *M. sylvestris*.

The study of leaf morphology shows that anatomical, micromorphological and architectural characteristics are quite similar in the two species; nevertheless, they differ in some peculiarities:

- size and shape: leaves are larger and longer in *M. crescimannoi*;
- leaf margins are serrate in *M. crescimannoi*, crenate in *M. sylvestris*;
- petiole is usually longer in *M. crescimannoi*, shorter and thickened in *M. sylvestris*;
- epidermic trichomes are more frequent in leaves of *M. crescimannoi*;
- apical protrusion is stressed in leaves of *M. crescimannoi*, attenuate in *M. sylvestris*;
- cells × mm<sup>2</sup> on the adaxial epidermic surface are more numerous in *M. crescimannoi*;
- sinuate cell mixed with stomata, × mm<sup>2</sup> in the abaxial epidermic surface are more numerous in *M. crescimannoi*;
- hydathodes occur on leaves of both species, but poricidal excrescences are more frequent in leaves of *M. crescimannoi*;
- xylematic pattern is more lignified in leaves of *M. crescimannoi*.

All these leaf features point towards a better adaptation of *M. crescimannoi* to xeric and sunny environments.

Therefore, *M. crescimannoi* is confirmed as a more thermophilous and heliophilous species, frequently occurring at the margins of *Quercus cerris* L. woods, rather than *Fagus sylvatica* L. woods, where *M. sylvestris* occurs (2).

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2) E. Di Gristina, F.M. Raimondo, C. Salmeri (2016) Abstracts 111° Congresso SBI, Roma



1.12. = *TRICHOLOSPORUM GONIOSPERMUM*, GENETIC DIVERSITY AND PHYLOGENETIC RELATIONSHIP WITH THE TRICHOLOMATINEAE [FORMERLY TRICHOLOMATOID CLADE]

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*Tricholosporum goniospermum* (Bres.) Guzmán ex T.J.Baroni is mainly distributed in northern and central Europe. The first Italian record of this species is reported from northern Italy, in the region of Trentino-Alto Adige (1); only a few and scattered localities in northern Italy were known until 1995, when it was reported also on high altitude grasslands in central Italy (2, 3, 4).

In this study, we examined samples collected in central Italy that showed spore size smaller than that typically reported for this species. In agreement with morphological observations, we found genetic polymorphisms in the ITS region suggesting that populations from central Italy are genetically different from those of northern Italy. Moreover, we detected intra-individual polymorphism in the ITS region. These findings may be explained with the extremely fragmented habitat of this species, that may represent a limit for gene flow. Based on morphology, the genus *Tricholosporum* is currently classified in the *Tricholomataceae*, however the presence of cruciate basidiospores represents a distinctive character with respect to other *Tricholomataceae*. Thus, we used nuclear ITS, LSU, SSU and *rpb2* DNA sequences to evaluate the phylogenetic position of this genus within the clade of "tricholomatoid" fungi. This analysis showed a low affinity of *Tricholosporum* with the clade of the *Tricholomataceae* and an isolated position of this genus within the *Tricholomatineae*. Thus the analyses performed in this study provided further insights on the ongoing process of revision of the species and family in the *Tricholomatineae*.

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## 2.1 = THE ANALYSIS OF XYLOGENESIS HELPS UNDERSTANDING THE FORMATION OF INTRA-ANNUAL DENSITY FLUCTUATIONS IN TREE RINGS OF *PINUS HALEPENSIS* AND *ARBUTUS UNEDO*.

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Mediterranean tree and shrub species cope with abrupt seasonal variations in climate conditions harmonizing their anatomical wood traits in order to optimize the efficiency and safety of water transport (1). Variations in environmental conditions are thus recorded in wood anatomical functional traits often leading to the formation of Intra-Annual Density Fluctuations (IADFs) (2). The analysis of IADF genesis and of the factors triggering their formation can help encoding the anatomical intra-annual rings variability and obtain information on the response of Mediterranean species to environmental changes with seasonal resolution. This assumes considerable importance in the framework of upscaling information to understand and forecast growth and adaptation capability of trees and thus forests under environmental changes (3). In this study, we monitored xylogenesis in *Pinus halepensis* and *Arbutus unedo* plants co-occurring at a site in southern Italy characterized by a Mediterranean climate. Cambial production was monitored in the year 2014 during the period of summer aridity, considered a trigger for IADF formation (4). Cambial production was also analyzed at the end of the calendar year. Microcores were collected weekly from eight trees of both species to assess the different phases of wood formation with the aim of evaluating whether and which type of IADFs were formed. The samples were prepared for light microscopy to observe the following phases of cell development: cambial cells (C), post cambial cells (P), cells with developing secondary wall (S) and mature cells with lignified secondary wall (M) (Figure 1). Moreover, we applied traditional dendrochronological analysis for IADFs identification and tree-ring dating.

We found that both species formed the same type of IADFs (earlywood-like cells within latewood) although IADFs in *A. unedo* were formed earlier in the season and were more frequent than in *P. halepensis*. The analysis of temperature and precipitation data suggested that such IADFs were triggered by a temporary restoration of growth after rain events during the period of summer drought. The overall data suggested that *A. unedo* is more sensitive than *P. halepensis* and promptly responds to seasonal variations. The study approach, combining the analysis of tree-ring series and of xylogenesis proved to be suitable to detect the timing of IADF formation and to make hypotheses on the reasons for their formation in both species. This is the first report of such an analysis in a Mediterranean hardwood species forming frequent IADFs.



Fig.1: Developing xylem in *Pinus halepensis*. Moving from the cambial zone toward the center of the stem, the following cells are encountered: cambial cells (C), enlarging post cambial cells (P), cells developing secondary walls (S), and mature cells with lignified secondary wall (M) (5).

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## 2.2. PHYSIOLOGICAL AND BIOCHEMICAL ADJUSTMENTS OF MORINGA OLEIFERA TO DROUGHT

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*Moringa oleifera* is a native tree of the northern sub-Himalayan tract of India, a region characterized by a humid subtropical climate. It has a high nutritional value and several medicinal properties<sup>1</sup>. *M. oleifera* has been described as a drought tolerant species and the agricultural environments in which this plant is cultivated often present strong ecological contrasts with its native environments. *M. oleifera* is now widely cultivated in many arid countries, where inadequate and variable precipitation are usually accompanied by excessive heat and high light radiation. Our study attempts to elucidate the physiological and biochemical adjustments that allow *M. oleifera* plants to cope with prolonged drought period. We performed a long-term experiment with progressive water stress simulating field conditions. Physiological and biochemical measurements were conducted at different fractions of transpirable soil water (FTSW 100%, 60%, 40%, 25%). Firstly, our study shows that *M. oleifera* is an isohydric species with a water saving behaviour. Secondly, this is the first report on isoprene emission by *M. oleifera* and we hypothesize a protective role of this molecule during drought periods<sup>2</sup>. In addition to increase isoprene emission, water stress induced in *M. oleifera* a significant allocation of carbon also in non-volatile isoprenoids and phenylpropanoids. During water stress carotenoids underwent large variations: DES (de-epoxidation state of xanthophylls cycle) increased during early stages of drought but declined at severe stress, whereas lutein increased significantly. This change in lutein might play an important role in NPQ (non-photochemical quenching) whereas zeaxanthin may act as a membrane strengthener<sup>3</sup>. Lastly, the accumulation of phenylpropanoids in water stressed plants may offer a further protection against oxidative damages<sup>4</sup>. In conclusion, this study draws a comprehensive picture of the effects of a progressive drought on the performance of *M. oleifera* and highlights the metabolic plasticity of this plant during environmental stress events.

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## 2.2 = BALANCE OF CALLOSE METABOLISM IN PHYTOPLASMA/PLANT INTERACTION

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Callose deposition is a calcium-mediated process occurring in sieve elements in response to both abiotic and biotic stresses, such as wounding or pathogen infection. On the other hand, callose synthesis normally takes place in plant tissues during growing conditions, playing an important role in the process of phloem cell maturation. Even if callose accumulation at the sieve plates has been widely documented in plants affected by phytoplasmas, fine distribution of callose barriers in the phloem, as well as callose quantification, have not been investigated in detail till now.

In this work, an integrated approach, based on the combined use of microscopic, biochemical and immunological analyses, has been adopted to investigate callose presence and distribution in leaf midribs of *Solanum lycopersicum* ('cv. Micro-Tom') infected by '*Candidatus Phytoplasma olani*' (group 16 SrXII, subgroup A) and *Arabidopsis thaliana* (ecotype Col-0) infected by '*Candidatus Phytoplasma asteris*' (group 16SrI, subgroup B).

The results demonstrated site-specific changes in callose distribution and metabolism of the infected tissues, expressing a fine balancing between plant-defense strategies and growth under biotrophic interaction.

## 2.2 = STUDY OF CADMIUM : AND LEAD INDUCE DIFFERENT LEVEL OF PROTEINS AND GENOTOXIC EFFECTS IN ZEA MAYS

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The problem of soil pollution with heavy metals (HMs) is becoming increasingly urgent. When such elements enter the cell, they can interfere with many physiological functions, inactivating key enzymes responsible of many metabolic processes. Among heavy metals, cadmium (Cd) and lead (Pb) are especially hazardous non-nutrient, since their contamination results from many human activities, including soil-applied chemicals (e.g. fertilizers).(1)

Phytoremediation represents one of the new frontier in plant science with the aim to investigate the plant capability to restore polluted soils; *Zea mays* L. is reported among the most resistant species to HM injuries being suitable to uptake these pollutants from the soil (2). The aim of this study is to analyze the physiological response of *Z. mays* to HM stress induced by known concentrations of Cd and Pb salts added to soil (3). In particular, we tested the level of Hsp70, Rubisco and D1 by western blotting analysis in leaves and roots from plants grown for 35 days in soil added with CdCl<sub>2</sub> and Pb(NO<sub>3</sub>)<sub>2</sub>. We also tested the genotoxic effects of Cd and Pb by ISSR analysis in *in vitro* cultured plantlets exposed for 7 days to these metals.

The results showed an increase of the levels of Hsp70 in roots grown with both HMs, compared to control samples; at parity of metal concentration, a higher increase was induced by Cd rather than Pb. A decrease of the levels of Rubisco and D1 in treated leaves was also observed. ISSR analysis showed alterations of the amplification profiles in plants grown with Cd, indicating a genotoxic effect induced by this HM. These preliminary results indicate that Cd and Pb affect the concentrations of Hsp70, Rubisco and D1 in *Z. mays* and that Cd has a genotoxic effect in the exposed plants. Further studies are needed to clarify the mechanisms of action of these pollutants and their location /interaction at tissue and cell level.

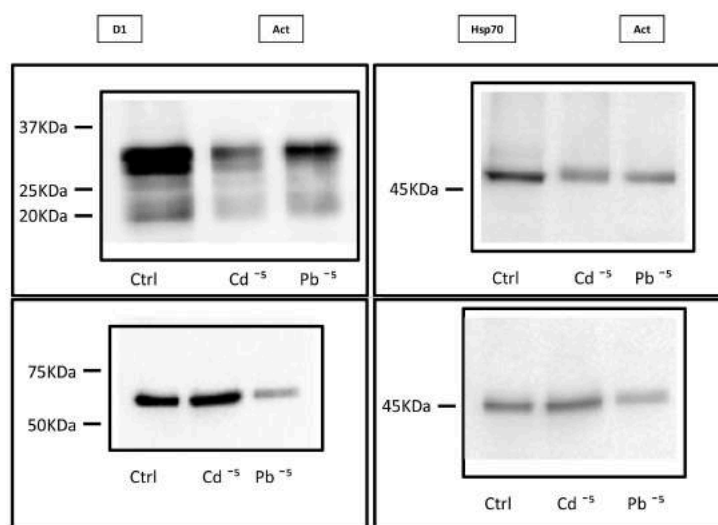


Fig. 1a-1b. D1 Variation in leaves

Fig. 2a-2b. Hsp70 variation in roots

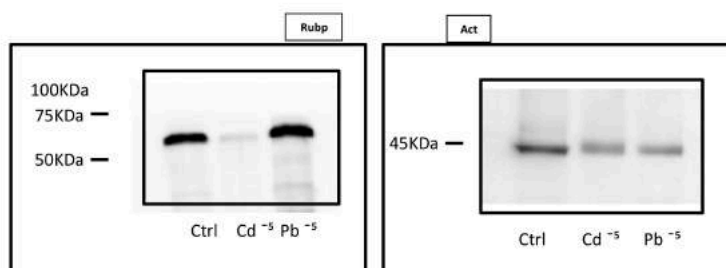


Fig. 3a-3b. Rubp variation in leaves

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## 2.2 = CHANGES IN THE LEAF EMISSION LEVEL OF VOLATILE COMPOUNDS (VOCs) FROM *PINUS HALEPENSIS* PLANTS SUBJECTED TO MECHANICAL INJURY

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Plants are relevant producers of biogenic volatile compounds (VOCs) which, beside affect chemical and physical properties of the atmosphere (1), are of strong ecological importance and may confer protection to the plants being repellent/inhibitors for herbivores and different pathogens (2).

In general, VOCs emission has been largely investigated in mediterranean plant species and it has been demonstrated that they emit a great variety of these compounds (3,4). It has also been shown that mechanical stress is able to induce large bursts of VOCs from these plants (2-4).

In this context, in the present study we aimed to investigate the variations induced, through mechanical injury of branchlet, on the level and spectrum of volatile organic compounds (VOCs) emitted from five-years old plants of *Pinus halepensis*, co-implanted in a fig orchard.

VOCs were trapped by adsorption on "radiello" trap-system (SUPELCO – USA), recovered by thermal desorption and analysis was performed by capillary gas chromatography and GS-MS detection.

Our results showed significant differences in the level of emitted VOCs in relation to both the age of *Pinus halepensis* plants (3) and the imposed stress. Moreover a relationship has been observed between the level and spectrum of emitted VOCs and the repellent activity against the major pathogenic insects of fig fresh fruits.

The obtained results are discussed also in relation to their potential positive relapse on sustainable management of fig orchard.

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## 2.2 = ON THE METAL TOLERANCE OF *HELICHRYSUM ITALICUM* (ROTH) G. DON GROWING ON MINE SOILS OF TUSCANY: MICROMORPHOLOGY OF LEAVES AND ROOTS

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Heavy metal accumulation produces significant physiological and biochemical responses in vascular plants. Plants growing on abandoned mine sites are of particular interest, since they are genetically tolerant to high metal concentrations. In this work we examined the effects of heavy metals (HM) on the morphology of *Helichrysum italicum* growing on mine soils, with the following objectives:

- to determine the fate of HM within the soil-plant system;
- to highlight morphological modifications at anatomical and cytological level;
- to ascertain the plant tolerance to heavy metals, and their resilience capacity.

Wild specimens of *H. italicum*, with their soil clod, were gathered from sites with different contamination levels by heavy metals (Cd, Cu, Fe, Pb, Zn) in the abandoned Niccioleta mine (Tuscany, Italy). Plants were brought to the botanical laboratory of the University of Florence, and appeared macroscopically not affected by toxic signals (e.g. reduced growth, leaf necrosis) induced by soil HM concentration. Leaves and roots taken at the same growing season were observed by light microscopy (LM) and transmission electron microscopy (TEM).

Light microscopy observations show a clear difference in the cell organization of not-contaminated and contaminated samples. In particular, the secreting trichomes, which are responsible for the characteristic flavour of the plant, present a different morphology in the polluted specimens with respect to the not-polluted ones. Indeed, the latter present the typical trichomes of the Asteraceae family, with two lines of cells bearing the secretion accumulated on the apical subcuticular space. Trichomes of the polluted plants, instead, present a completely different morphology, with a stalk of 3-4 cells and a large secreting apical cell (i.e. they are capitate hairs).

Samples from contaminated sites, moreover, present a palisade parenchyma less organized, and a reduction of leaf thickness proportional to HM concentration, as observed also in *Taraxacum officinale*. The poor structural organisations, and the reduced foliar thickness of the contaminated plants, are related to soil contamination.

A gradual restoration of cell organization suggests that somewhat resilience occurred in plants. Moreover, the presence of stress-tolerant mycorrhizal fungi could contribute to reduce metal toxicity. The resilience capacity suggests that *H. italicum* could be a useful species in remediation projects.

## 2.2 = A MULTIDISCIPLINARY STUDY OF SOME TRITICALE ELITE LINES: AGRONOMIC AND GENETIC CHARACTERIZATION.

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The promotion of renewable energy sources represents a target of the European 2020 strategy for economic growth and sustainable competitiveness, in the frame of solving increasing global energy needs. Plant biomass and organic wastes from agriculture represent an effective resource to be exploited for a sustainable rural development, optimizing the land use, diversifying rural entrepreneurship and producing energy and new income.

Among crops, triticale is considered particularly promising as biomass source, showing a number of advantages such as high grain yield even in marginal environments, tolerance to drought, tolerance to more acid soils, lower production costs and lower susceptibility to biotic and abiotic stresses. Triticale (X Triticosecale Wittmack) is a human-made crop produced crossing *Triticum* (wheat) and *Secale* (rye), thus combining interesting traits from both, such as high productivity, protein content, hardiness and resistance to different causes of stress.

Triticale is present in the pipeline of various Italian seed companies and lines specifically developed for energy applications are included in their sales catalogue. Through a long collaboration with CIMMYT, some Mexican elite lines were grown in Italy on marginal lands, in order to identify the most adaptable lines, able to improve the biomass production in different environments [1],[2] (Figs. 1-2). Beside agronomic evaluations, an attempt was carried out to genotype the lines using RAPD technique and preliminary results are presented (Fig. 3).

This interdisciplinary approach of such a research will allow to identify and characterize various haplotypes with the aim to associate them with agronomic data, as a tool for selection of new lines in the frame of the full exploitation of this energy crop.



Fig. 2. Example of triticale spike

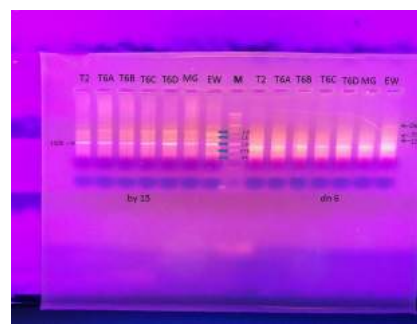


Fig. 3. Fingerprinting of triticale

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2.2 = ALTERED ROOT PATTERNING AND GROWTH IN Cd-TREATED SEEDLINGS OF *Arabidopsis thaliana* IS ACCOMPANIED BY CHANGES IN AUXIN/CYTOKININ SIGNALLING.

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Cd<sup>2+</sup> is one of the most highly toxic and accumulative heavy metals and is largely spread in soils and water through atmospheric deposition, urban-industrial activities and agricultural practices (1). Moreover, Cd<sup>2+</sup> is rapidly absorbed by plant roots and subsequently transferred into the food chain, presenting a threat to human health (2).

During the evolution, plants evolved several strategies to avoid Cd<sup>2+</sup> toxicity, from the restriction of Cd entry into the xylem to its compartmentalization and detoxification (3). Notwithstanding, Cd<sup>2+</sup> reduces plant growth and crop yields, even at low concentrations, because Cd interferes with numerous processes, such as metabolic, physiological and developmental ones (4). The plant root, as the first organ sensing soil Cd, is also affected by Cd, which inhibits the growth of the primary root (5,6,7). However, the mechanisms underlying Cd mediated inhibition of plant primary root growth remain largely unclear.

Our aim was to study the effect of Cd<sup>2+</sup> on meristem architecture and root growth in seedlings of *Arabidopsis thaliana* exposed to Cd, at the concentrations of 25 µM and 50 µM since eight days after germination.

The obtained results showed a significant and dose-dependent reduction of proximal meristem size (length and width) in roots of seedlings exposed to Cd compared to the control ones. Moreover, an incorrect stratification on staminal cell niche and an increase in the size of Quiescent Centre cells (QC) was also observed.

In order to understand the molecular basis of Cd toxicity, many transgenic lines were used and analysed through Laser Confocal Microscopy (LCF) under Cd treatment. LCF analysis of the *proSCR:GFP:SCR* (SGS) line revealed that SCR is expressed in QC cells, but exhibit some ectopic SCR expression in root under Cd 50 µM compared to wild type. Furthermore, the analysis of the PINOID:GFP lines and the cytokinin signalling Sensor TCSn::GFP line allowed us to verify that polar auxin transport and cytokinin signalling were impaired, respectively. In particular, LCF analysis evidenced a restriction of the histological expression domains of PIN3 and PIN7, and in some cases an alteration of their polarity distribution. By contrast, TCSn::GFP cytokinin sensor showed an increase of GFP signal. These results are supported by transcriptomic analysis performed on treated plants, which also suggest an epigenetic basis for Cd toxicity.

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## 2.2 = SEED GERMINATION ECOLOGY IN *SISYMBRELLA DENTATA* IS AFFECTED BY NITRATE AVAILABILITY AND SEED DESSICATION

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Seed dormancy is a complex trait which has evolved differently across species through adaptation to various habitats and environments, so that germination occurs when conditions for establishing a new generation are likely to be suitable (1). Bioactive gibberellins and abscisic acid (ABA) act antagonistically to control seed germination and dormancy release (2). Whereas, nitrate has been described as a way to release dormancy in a range of Brassicaceae species (3). Furthermore, after-ripening was shown to affect seed dormancy degree in *Arabidopsis thaliana* through fluctuation cycles that cause induction and release of dormancy (4), factors that likely also operates in temporary ponds where seed germination is triggered by typical environmental cues as water availability, light, and temperature regimes (5).

Here, the germination physiology of two different populations of *Sisymbrella dentata*, an endemic plant to Sicily found in wet muds and shallow temporary pools was examined. Upon harvest, seeds from the two populations shown a primary weak dormancy degree, with significant difference between the two populations in the germination pattern where seeds from one population (Pantano) evidently exhibited a higher *de novo* gibberellic acid production respect to the other one (Gurrida). Indeed, with light deprivation even small amount of exogenous gibberellic acid provoked a germination response ten times higher in Pantano respect to the other population.

Observation of germination dynamics revealed that seed coat and endosperm rupture take place in different times where testa rupture always preceded endosperm breakage. Treatments with nitrate in light enhanced final germination and its rate by stimulating seed coat rupture.

Dry storage and imbibition in dark resulted in an induction of seed dormancy, which was released with a dose-response relationship by the addition of exogenous gibberellic acid, even at lower concentrations.

Germination physiology in *S. dentata* could be the result of the adaptation to a temporary environment, where, taking advantage to its very short time life cycle, after dispersion, water availability and light promptly trigger germination allowing multiple life cycles within the same year; on the other hand, if the desiccation is more pronounced, because of the dry season, dormancy is inducted, and the seed would maybe undergo fluctuation cycles before dormancy breakage.

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## 2.2. TRANSCRIPTIONAL CHANGES IN QUINOA SEEDLINGS EXPOSED TO HIGH SALINITY: FOCUS ON GROWTH, ION HOMEOSTASIS, ABA, POLYAMINE, AND STRESS-RELATED GENES

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Quinoa (*Chenopodium quinoa* Willd) is the only halophytic crop species producing edible seeds with highly nutritious properties [1]. It, therefore, represents an interesting model species for studies on salt stress responses [2]. The species' high genetic diversity has led to the identification of five ecotypes. Amongst these, the *salares* ecotype is adapted to the extremely arid highland deserts (*altiplano*) of the Andes while the coastal-lowlands ecotype is found in rainier central-southern Chile. Strong differences in salt tolerance between quinoa genotypes have been documented both in terms of agronomic features and physiological responses [3]. Genes likely to be involved in salinity tolerance fall into three main functional categories: those that control salt uptake, translocation, and compartmentalization; those that have protective functions against drought, salt, osmotic, and oxidative stresses; those that regulate growth. In the present study, changes in transcript levels of the following genes were investigated: (i) genes involved in growth, *cyclinD3* (*CycD3*) and  $\beta$ -*expansin* ( $\beta$ -*EXP*); (ii) ion homeostasis genes *NHX*, *HKT* and *Salt Overly Sensitive1* (*SOS1*); (iii) polyamine (PA) biosynthetic and oxidative genes (and PA levels), and (iv) a proline biosynthetic gene *1-pyrroline-5-carboxylate synthase* (*P5CS*). We also investigated ABA biosynthetic (*NCED*), perception (*PYL*, *PYR*), and conjugate cleavage (*BG1*) genes, as well as ABA-responsive and other stress-related transcription factors (TFs): *ABA-binding factor* (*ABF3*), *bZIP24*, *Dehydration Responsive Element Binding* (*DREB2a*), and *Dehydration Responsive Protein* (*RD22*). Analyses were conducted on two Chilean landraces of quinoa, R49 (*salares* ecotype) and Villarrica (VR, coastal-lowlands ecotype) whose different responses to high salinity were previously reported [4]. Seeds were germinated on agar plates and seedlings transferred to saline (300 mM NaCl) or non-saline media for 0.5, 2, 24 and 120 h. The two genotypes displayed both common and specific responses to short-term salinity. High salinity reduced root elongation growth in both genotypes; this was accompanied by a down-regulation of *CycD3* transcript levels;  $\beta$ -*EXP* was instead up-regulated. Under salt stress, a PA response in terms of content (decrease in Put, increase in Spm) was observed starting from 2 h in culture; this was accompanied by changes in PA biosynthetic (especially *arginine decarboxylase 2*) and oxidative (*diamine oxidase*) gene expression. The overall up-regulation of *P5CS* transcript levels in the presence of salt is in accord with the known protective functions of proline during salt/osmotic stress and its accumulation also in quinoa [5]. In both R49 and VR, decreased expression of *PYR/PYL* transcript levels under salinity relative to controls indicated that strongly enhanced ABA biosynthetic capacity, i.e., dramatically increased *NCED* expression, negatively regulated the expression of genes encoding for its receptors. Transcript levels of *ABF3* increased early after transfer to saline medium and followed the same pattern as *NCED*. *DREB2a*, a TF involved in ABA-independent stress response pathways, exhibited, together with *NCED* and *ABF3*, the strongest up-regulation (up to 6-fold) in response to salinity. *bZIP* transcript levels were decreased by salt in agreement with its role as negative regulator of adaptation to salt stress. *RD22*, a drought-inducible gene whose expression is ABA-dependent, was up-regulated by salt in roots and shoots of R49 starting from 2 h, while in VR its up-regulation in response to salt was limited to 24 h after transfer and only in the roots. Although in R49 the response began earlier (2 h) than in VR, *SOS1* and, especially, *NHX* and *HKT* were all up-regulated by salt in both landraces. The positive correlation with enhanced *NCED* transcript levels suggests that increased ABA production may have contributed to higher expression of these ion transporters.

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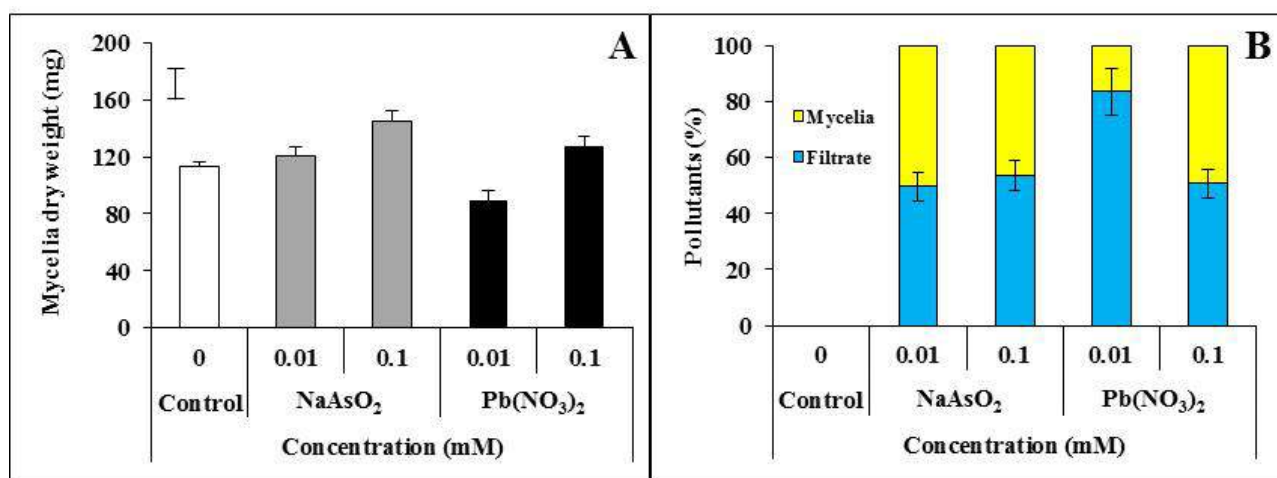
2.2 = Further studies on fungal endophytes from *Nephrolepis cordifolia* (L.) C. Presl and their interaction with inorganic pollutants

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*Nephrolepis cordifolia* had been proposed as a possible candidate for phytoremediation in polluted environments (1) because of its resistance to abiotic stress, ability to accumulate heavy metals (1,2,3) and tolerate some rare earth elements (4). The presence of microbial endophytes (5,6) in roots, tubers and rhizomes could helping accumulation of inorganic pollutants in plants. This research reports *in vitro* studies on responses of *Trichoderma harzianum* Rifai associated with *N. cordifolia* to NaAsO<sub>2</sub>, and Pb(NO<sub>3</sub>)<sub>2</sub>. The strain Th1, isolated from rhizomes of *N. cordifolia* populations grown in Bari, was exposed at 0.01 and 0.1 mM of NaAsO<sub>2</sub>, and Pb(NO<sub>3</sub>)<sub>2</sub> on liquid cultures performed on Potato Dextrose Broth at 130 rpm, 25 ± 1°C, in the dark for 14 days. A suspension of 2 × 10<sup>4</sup> conidia per µl was used as an inoculum. Fresh and dry mycelia mat were used to evaluate fungal growth. The presence of As and Pb was detected on culture filtrates and mycelia.

Our results demonstrated that *T. harzianum* strain Th1 tolerates the two pollutants tested. Under arsenic stress, biomass production increased of 6.9 and 28.4% when tested at 0.01 and 0.1 mM of NaAsO<sub>2</sub>. At the end of the incubation period, lead increases (about 12%) the mycelia production at 0.1 mM of Pb(NO<sub>3</sub>)<sub>2</sub>. No alterations were observed on hyphae morphology. Moreover, conidia production was increased from both pollutants when tested at 0,1 mM concentration. After 14 days of incubation, spectroscopic analysis indicated a reduction of As and Pb concentration in the culture filtrate. At the same time, both pollutants were detected on mycelia. At the tested concentrations, at least the 50% of arsenic and lead added to the media were recovered from mycelia. On the basis of these data and of previous researches, *N. cordifolia*, in association with *T. harzianum*, should be fully considered in bioremediation process against arsenic and lead.



Effect of 0.01 and 0.1 mM of NaAsO<sub>2</sub> or Pb(NO<sub>3</sub>)<sub>2</sub> on *Trichoderma harzianum* strain Th1 grown in liquid Potato Dextrose Broth, and pollutants distribution between mycelia (■) and culture filtrate (■). Each histogram represents the mean of three replicates ± ds

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## 2.2 = VARIABILITY OF FUNCTIONAL TRAITS IN TREE RINGS OF *QUERCUS ILEX* L. AND *PINUS PINEA* L. COPING WITH INTRA-ANNUAL CLIMATE FLUCTUATIONS IN A MIXED FOREST STAND

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Wood anatomical features, such as intra-annual density fluctuations (IADFs), imprint the environmental conditions that have occurred during the growing season, providing information on plant responses to biotic and abiotic factors at a sub-seasonal level. IADFs are an expression of cell plasticity in growth as changes of wood density within a tree ring, and appear as latewood-like cells within earlywood or earlywood-like cells within latewood (1). Their formation is frequent in Mediterranean species (2), is driven by climate seasonal variability and is dependent on the age and size of the tree (3, 4, 5). Little is known about broadleaf species which have been less studied than conifers (6). Moreover, the discussion on IADF functionality as a trait of the adaptive capacity of the species to climate fluctuations is still open.

The aims of this study are: 1) to anatomically characterize the IADFs of a broadleaf and a conifer cohabiting a typical low elevation Mediterranean forest stand, and 2) to analyze how climate parameters influence the formation of IADFs and the competitive ability of the two species. The study site was a mixed holm oak (*Quercus ilex* L.) and Stone pine (*Pinus pinea* L.) stand located in Southern Italy on the Vesuvius volcano (Fig. 1). Tree-ring growth and basal area increment chronologies of the two species were developed by coring, measuring and cross-dating 20 trees per species (two cores per tree) (Fig. 2). The same dated cores were used to identify the presence/absence of IADFs to develop the chronologies of IADF frequency. Moreover, chronologies of tracheid/vessel size, wood density and vessel frequency were developed through the application of digital image analysis on microphotographs of tree rings of 5 cores per species. Time-series climate analyses were performed using average monthly data of mean, minimum and maximum temperature and total precipitation.

The study showed the high frequency of IADFs within the rings of both species and the significant correspondence between IADF-frequency chronologies, suggesting that they can be considered as a common response to the environment rather than growth anomalies. Quantitative wood anatomy evidenced that two different types of IADFs occur in the two species. Consequently, IADFs were better evidenced by different functional anatomical traits in the two species.

The sub-seasonal oscillations between wet and dry periods triggered the formation of IADFs in both the species, showing the high plasticity in wood growth of these two Mediterranean trees in coping with climatic stress. The used approach allowed the comparison of intra-annual responses to climate between two coexisting species. Characterizing the responses of typically spread Mediterranean species to climate fluctuations may provide useful information to forecast future forest responses to the forecasted increasing climatic variability.



Fig. 1. Study site.



Fig. 2. Coring and developing tree-ring growth chronologies.

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## 2.3 = INFLUENCES OF MICROCLIMATE ON FLOWERING TIME IN ALPINE SNOWBEDS

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Snowbed communities are among the alpine habitats most threatened by the current climate change. Warmer growing seasons, coupled with longer snow-free periods, will influence flowering phenology, which is a key process in species adaptation and plant population dynamics. However, we know little about the effects of changing micro-climate on flowering time in these habitats. In addition, the mechanisms underlying the phenological responses of snowbed plants are poorly understood. The flowering phenology of species inhabiting alpine snowbeds was assessed with weekly observations over the period 2010-2014. We analyzed flowering time in relation to micro-climatic variation in snowmelt date, soil and air temperature, and experimental warming during the snow-free period. Plants inhabiting alpine snowbeds did not rely on the same environmental cue to flower. At the growing season time-scale, plants commonly tracked air temperature by means of cumulative heat perception to enter anthesis, but at the annual time-scale snowmelt time was the main controlling factor of the blooming period (Fig. 1), even in warmer climate. Moreover, plant populations from later snowmelt sites showed faster phenological development compared to populations from earlier snowmelt sites. In addition, phenological responses to experimental warming differed among species (Fig. 2), were proportional to the pre-flowering time-span of plants, and did not show consistent trends of change over time. Finally, higher levels of flowering synchrony were found under experimental warming.

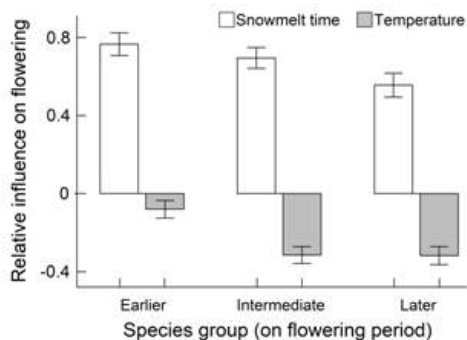


Fig. 1. Relative importance of snowmelt time and temperature

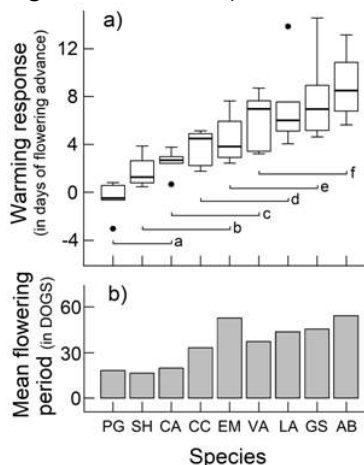


Fig. 2. Species-specific (a) warming-induced changes in flowering time and (b) pre-flowering time-spans duration

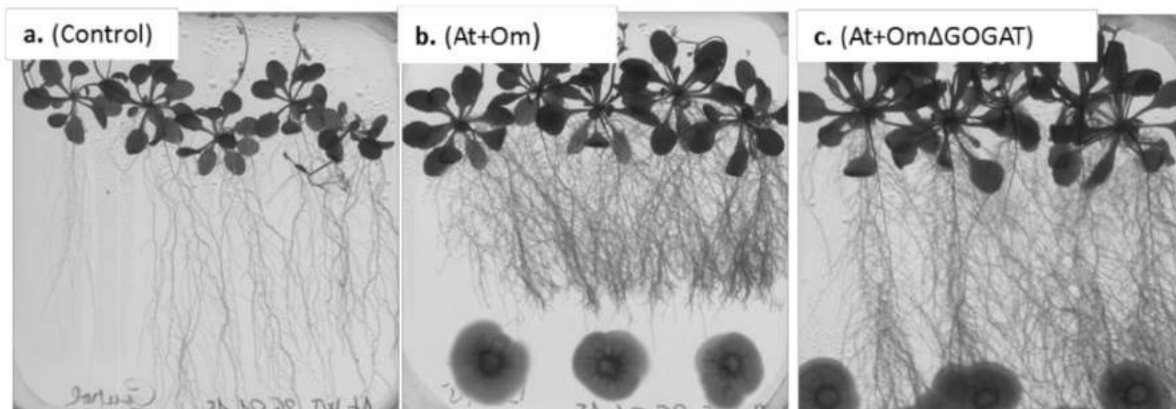
## 2.3=FUNGI WITH DIFFERENT ECOLOGICAL STRATEGIES AFFECT ARABIDOPSIS DEVELOPMENT IN VITRO: CONTRIBUTION OF SOME SOLUBLE AND VOLATILE COMPOUNDS

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Plant growth and development can be influenced by mutualistic and non-mutualistic microorganisms [1]. We investigated the ability of the ericoid endomycorrhizal fungus *Oidiodendron maius* to influence growth and development of the non-host plant *Arabidopsis thaliana*. Different experimental setups (non-compartmented and compartmented co-culture plates) were used to investigate the influence of both soluble and volatile fungal molecules on the plant phenotype. *O. maius* promoted growth of *A. thaliana* in all experimental setups. Moreover, we were able to distinguish a peculiar clumped root morphology in the non-compartmented plates (Figure 1b), with shorter primary root and longer lateral roots. The absence of this root phenotype in the compartmented plates, together with auxin measurements, suggest the involvement of soluble diffusible molecules independent of the auxin signaling pathway. Although the nature of these fungal compounds is as yet unknown, the inability of the *O. maius*ΔGOGAT [2] (Figure 1c), carrying a partial deletion of the glutamate synthase gene, should link this peculiar root phenotype to the production and metabolism of some nitrogen compounds. In both compartmented and non-compartmented plates, we observed a significant increase in the plant biomass. The use of different gas traps suggested a nonspecific CO<sub>2</sub> mechanism of plant growth promotion, rather than specific VOC signals. These findings were further supported by experiments with fungi featuring mycorrhizal, saprotrophic and pathogenic lifestyle. Altogether, these results should raise awareness for the interpretation of the results of plant-fungus co-cultivation experiments in vitro, and shed light on the mechanisms through which rhizospheric fungi can influence plant growth and modify root architecture.

1) P.



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## 2.3. A NEW PROCESS-BASED MODEL OF XYLOGENESIS

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In vascular plants heterogeneity in cell size arises during xylem growth (i.e. formation of early- and latewood, Fig. 1A). While environmental conditions are known to influence this phenomenon, no biologically sound hypothesis explaining the difference among early- and latewood cell size has been either proposed or tested yet. Here we introduce a new model for xylogenesis, based on cell stretching, secondary cell wall synthesis, lignin biosynthesis, and cell wall lignification. These processes are linked by the following assumptions: 1) both cellulose and lignin biosynthesis are dependant on sugar availability; 2) cell stretching is restricted by secondary wall formation; 3) death of a cell occurs when its secondary wall is fully lignified. Based on this conceptual framework, we built a computer simulation using the VirtualLeaf (1) modelling environment. The simulation is initialized with a proliferating cambial cell and a phloematic cell that distribute sugars to the tissue by diffusion. During the simulation, proliferation produce daughter cells that will differentiate in either phloem or xylem precursor, depending on cell position. If a cell is a xylem precursor it will enlarge, thicken and lignify at a rate regulated by sugar availability. Simulation outputs (e.g. Fig. 1B) shows that heterogeneity in xylem cell size at tissue level is correctly rendered by differences in sugar availability among cells, thus suggesting that size differences between early- and latewood can be explained by seasonal variations in sugar availability.

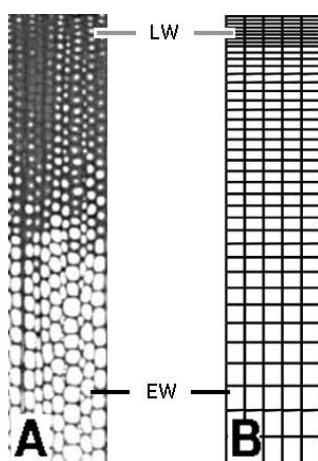


Figure 1: [A] Stem cross section of a wood ring in eastern larch (2). [B] Simulated section produced by a typical model run. (LW stands for 'latewood', EW stands for 'earlywood').

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### 3.1 = IL CENTRO SERVIZI DI ATENEO "HORTUS BOTANICUS KARALITANUS" (HBK) A 150 ANNI DALLA FONDAZIONE DELL'ORTO BOTANICO DI CAGLIARI

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Il prossimo 15 novembre 2016 l'Orto Botanico di Cagliari festeggerà i suoi primi 150 anni. Dalla nascita ai giorni nostri, sono tanti gli eventi che si sono susseguiti e che hanno nel tempo portato all'evoluzione della struttura e delle collezioni. Questa ricorrenza arriva in un momento critico per gli Orti Botanici italiani, chiamati ad una profonda riorganizzazione per superare difficoltà di carattere gestionale ed economico. Per perseguire i propri obiettivi gli Orti sono oggi obbligati a porre in atto strategie volte ad incrementare le risorse economiche necessarie per la cura del proprio patrimonio, non solo botanico. A questo scopo, per favorire una migliore gestione l'Università degli Studi di Cagliari, con D.R. n. 192 del 9 novembre 2015 ha istituito il Centro Servizi di Ateneo denominato HBK (Hortus Botanicus Karalitanus).

L'HBK è costituito dall'Orto Botanico, dalla Banca del Germoplasma della Sardegna (BG-SAR) e dal Museo Botanico (MBK). Finalità del centro sono la protezione, conservazione e incremento delle collezioni botaniche e di quelle museali, nonché la tutela del patrimonio archeologico in esso presente. HBK promuove inoltre le attività di servizio a favore del territorio, curando i rapporti con enti pubblici e privati a cui fornisce supporto tecnico e scientifico per la gestione della biodiversità e del verde urbano.

Notevole attenzione viene riservata alle strategie di divulgazione e comunicazione volte in particolare alla conoscenza dei diversi settori dell'Orto come, ad esempio, le gimnosperme, l'Orto dei Semplici, il settore mediterraneo, le roccaglie della biodiversità, il palmeto o il deserto con numerose piante succulente. Attraverso visite guidate e laboratori didattici rivolti a scuole di ogni ordine e grado, promuove attività educative allo scopo di fornire le basi scientifiche per la conoscenza della diversità vegetale e il rispetto dell'ambiente naturale. Mentre i laboratori didattici utilizzano una metodologia di "didattica attiva" attraverso esperienze pratico-creative che consentono agli utenti di "imparare facendo", le visite guidate si ispirano invece all'interpretazione ambientale. I ragazzi possono così trasformarsi in pittori e dipingere utilizzando i colori estratti dai vegetali, andare a caccia di semi per comprendere gli affascinanti meccanismi di dispersione oppure scoprire come una pianta vive senza radici. L'offerta formativa permette quindi di scoprire una ricca biodiversità utilizzando un approccio emozionale che permette di sensibilizzare, coinvolgere e stimolare curiosità per il mondo vegetale. L'offerta formativa è inoltre studiata e progettata per raggiungere tutti i target di età, in particolare le nuove generazioni in quanto sarà loro il compito di preservare la biodiversità del pianeta.

Altre strategie volte al miglioramento della comunicazione nei confronti degli utenti dell'Orto riguardano la progettazione e la realizzazione di un sito Web, il rifacimento della cartellonistica di servizio e di approfondimento delle tematiche botaniche, operazioni attualmente in corso che si inquadrano tra gli interventi di qualificazione della struttura e delle collezioni.

### 3.1 “UniCaVerde” – A PROJECT OF ANALYSIS AND ENHANCEMENT FOR THE GREEN AREAS OF THE UNIVERSITY OF CAGLIARI

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 Centro d'Ateneo Hortus Botanicus Karalitanus, Università degli Studi di Cagliari

The University of Cagliari, founded in 1620 by Philip III of Spain, counts approximately twenty green areas within its spaces and buildings destined to research and teaching. The oldest areas are presumably those near the first settlements - like the Botanical Garden, the Biology Station and the Belgrano palace, which today hosts the Rectorate. Starting from 2013 a number of projects began to develop, with the aim of taking a census of the botanical heritage, assessing its conservational status, and proposing management measures. These projects included a number of bachelor degree and master degree theses, which were meant to investigate the Botanical garden and the green areas of the University Campus in Monserrato and of the campuses of Engineering, Animal Biology, Classical Studies, Geology, Economy and Law. For each area were compiled a checklist of the flora, a chorological analysis, and numerous proposals in order to enhance both their management and their use by the students, the academic personnel and the public.

The major area is the Botanical Garden with its 5 hectares. Founded 150 years ago, it preserves many vegetal entities and testifies a long history of acclimation of exotic species. Providing competence and practical solutions (1, 2, 3), it plays a pivotal role in the management and enhancement of all the other green areas of the University. The area in the campus of Economy and Law, known as “garden of the deaf-mutes”, has a flora of 62 *taxa*. Selecting 12 of these, following chorological criteria, a didactical route has been created (4). The campus of Engineering presents a green area which host 59 *taxa* and is divided in sectors. For this area there are some proposals concerning planting new species, protecting the current specimens and containing the invasive flora (5). The census of the plants in the campus of Classical Studies “Sa Duchessa” resulted in 88 *taxa*, with a predominance of asiatic species. The proposal for the enhancement of this area aims to introduce Mediterranean species (6). The Animal Biology's area contains 84 *taxa*, mostly exotics. Among the seven management proposals there is a project of didactic route focused on the ecological relationship and geographical distribution between the botanical species in the garden and the animal species of the zoological museum hosted in the building (7). The only area outside of the town is the University Campus, placed in Monserrato, where 109 *taxa* were identified. The proposals concern: the management of the green areas; and the introduction of new species which would be fitter for the bio-climatic profile. Furthermore, the project proposes the creation of new green spaces, destined to sports activity and to the psychophysical rehabilitation of the patients of the near Hospital (8).

The “UniCaVerde” project aims to the construction of a network of all green areas of our University, while keeping in mind the importance of the urban green and its ecological and environmental functions, its value as social and recreational space, and its cultural and didactical importance.

- 1) F. Mascia, 2014 Analisi comparativa di parametri climatici per la gestione di specie critiche dell'Orto Botanico dell'Università degli Studi di Cagliari. Tesi di Laurea magistrale.
- 2) A. Argiolas, 2015 Realizzazione di un nuovo settore presso l'Orto Botanico di Cagliari: Il Giardino delle Salvia. Tesi di Laurea triennale.
- 3) A. Lallai, 2016 Censimento e revisione nomenclaturale di settori e collezioni botaniche conservate presso l'Orto Botanico degli Studi di Cagliari. Tesi di Laurea magistrale.
- 4) L. Abis, 2014 Progettazione di un percorso didattico per la valorizzazione di un area verde dell'Università degli Studi di Cagliari: “ex giardino sordomuti” (Polo Giuridico-Economico). Tesi di Laurea magistrale.
- 5) S. Farris, 2015 Componente floristica e proposte di valorizzazione del verde della Facoltà di Ingegneria e Architettura (sede Piazza D'Armi) dell'Università degli Studi di Cagliari. Tesi di Laurea magistrale.
- 6) G. Concu, 2016 La componente floristica dell'area verde del Polo Umanistico “Sa Duchessa” dell'Università degli Studi di Cagliari. Tesi di Laurea triennale.
- 7) F. Corona, 2016 Proposte di valorizzazione e gestione dell'area verde della sezione di Biologia Animale del DISVA dell'Università degli Studi di Cagliari. Tesi di Laurea magistrale.
- 8) A. Sordo, 2016 Analisi della flora delle aree verdi della Cittadella Universitaria di Monserrato, proposte di gestione e valorizzazione. Tesi di Laurea magistrale.

## 3.2 – NATIVE PERENNIAL HERBACEOUS SPECIES FOR NEW COVERS OF GREEN ROOFS

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The European Communication on Green Infrastructures (1) encourages the use of reedbed, wildflower verge, green walls, green roofs, etc. to mitigate the consequences of the artificiality of the urban and rural environment. As far as the green roofs are concerned, they are engineered ecosystems occupying underutilized urban spaces, that rely on the plant cover to provide services, such as reduction of roof temperature, retention of stormwater, enhancement of urban biodiversity (2). Researches aiming at quantifying the green roof performances, depending on the physio-ecological characteristics of the plants used in their realization, are not yet fully developed (3). The *Sedum* species have been widely employed because of the extreme abiotic conditions they can tolerate. Recently, however, the importance to distinguish between the ability of a species to survive on a green roof and his ability to provide valuable services, starts to be considered. In the harsh conditions of green roofs, *Sedum* species may have the highest survival, even without some irrigation. However, other species are more likely to provide multiple, effective green roof services compared to a *Sedum* monoculture, if a moderate irrigation is foreseen. Irrigation during very dry periods would allow to employ a more diversified plant community and the improvements in green roof performances, could offer a high return on the investment of time and money (4). In this framework we explored the ability of some wild herbaceous perennial species of the arid and semi-arid grassland communities (*Xerobromion* and *Mesobromion* communities), to survive and colonize the substrate of a commercial extensive green roof system, adopted by the University of Bologna in the realization of the green roofs of the Agricultural Sciences School. The native plant species represent a rich heritage, to now not fully explored, which can be profitably used for the production of plant cover in green roofs of high environmental quality, well adapted to the conditions of the site where they are realized. The plant material was collected in natural and semi-natural plant communities of the Emilian Apennines, then cultivated and reproduced at the Azienda Agraria of the Bologna University (AUB) and finally utilized for the realization of the plant cover of an experimental green roof. For each species, the same number of young plants was planted in plots of 50x60 cm, at the mid June 2015, irrigated during the overall 2015 summer season. The irrigation was then suspended at mid September 2015. We report here the main results of a monthly monitoring (from June 2015 to April 2016) of the growth and colonization capacity of four of the species under study. For each species, different parameters were monitored, depending on its particular way of growth, in order to better describe the ability to cover the plot surface. *Festuca ovina* L. a perennial caespitose, showed a great capacity of shooting. During the 2015 summer, under irrigation, the number of shoots increased very quickly. Shooting capacity slowed in autumn, but at mid-end winter 2016 restarted. Water availability greatly influenced the shoot production. *Hieracium pilosella* L. is a stoloniferous creeping species. Stolons are important structures for the vegetative propagation as well as for the space colonization; they elongate mainly in the autumn and winter months. New leaves arranged in a basal rosette and floral axes are produced at the rooting points of the stolons. *Sanguisorba minor* Scop. is a medium size plant with a cluster of basal leaves that progressively enlarges all around. We measured the increase of the projection of the canopy on the horizontal surface. Maximum increase occurred at the start of autumn 2015 and in spring 2016, whereas in the winter months its cover progressively decreases. *Achillea millefolium* L. has solid, mat-forming rhizome/root system and fine feathery leaves which make it drought resistant. Both sexual and vegetative reproduction coexist and both are very efficient. The number of individuals produced through vegetative propagation regularly increases all over the observed period. After a period of acclimatation, all the species under study survived in the green roof artificial environment, even without particular cares. Each of them has a particular strategy and period of maximum development. Undoubtedly they represent an interesting pool of species whose eco-physiological characteristics could be explored, in order to better define their utilization for different performances and environmental services of urban green roofs.

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### 3.3 = CONVENTIONAL VS ORGANIC VS CONSERVATION AGRICULTURE: ASSESSMENT OF SOIL QUALITY BY CO<sub>2</sub> AND FUNGAL COUNT

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Conservation agriculture (CA) aims to achieve sustainable and profitable agriculture through the application of three principles (1):

1. minimal tillage and soil disturbance;
2. permanent soil cover with crop residues and live mulches;
3. crop rotations and intercropping.

CA is an improvement on conservation tillage, where no-till, mulch and rotations significantly improve soil properties and other biotic factors. CA is considered a more sustainable and environmentally friendly management system for cultivating crops than those presently practiced (2). The quality of a specific kind of soil indicates the measure of its capacity to sustain plant and animal productivity, maintain or enhance water and air quality, support human health and habitation within natural or managed ecosystems. A soil with high quality can accomplish its main functions: sustaining biological activity, diversity, and productivity, regulating the water and solute flow; filtering and buffering, degrading, immobilizing, and detoxifying organic and inorganic materials, storing and cycling nutrients and other elements (2).

In this work the soil quality of a plot managed for conservation agriculture has been compared with 2 plots managed for organic or conventional one.

Soil samples from conventional, organic and conservation agriculture, were collected in the fields of the "Calvenzana" farm, and located in a flat area near Rivanazzano Terme (PV), Oltrepo Pavese, Italy. Soil texture was siltloam in the organic and conservation agriculture fields while it was silty-clay in the conventional one. CO<sub>2</sub> production and Colony Forming Units per g of soil dry weight were analyzed in each plot. The CO<sub>2</sub> flux ( $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ ) was measured with CO<sub>2</sub> portable analyser (ADCPro-sd with soil chamber).

Colony forming units were counted by soil dilution plates using Sabouraud agar as growth medium, using 10<sup>-5</sup> as dilution factor. CO<sub>2</sub> value was 3  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$  in the soil from the conventional, 7 in the organic, and 11 in the conservation agriculture area. CFU/g of soil dry weight were 2×10<sup>5</sup> in the conventional, 3×10<sup>5</sup> in the organic, and 8×10<sup>5</sup> in the conservation agricultural area.

Non-Parametric MANOVA (PERMANOVA) was applied on the CO<sub>2</sub> data and showed that there are significant differences ( $p < 0.001$ ) between the different plots and methods of management. The mycological data showed a similar statistical trend. The soil from the conservation agriculture showed the highest levels of respiration rate and fungal abundance. Analysis based on the results obtained underlined that CO<sub>2</sub> and fungal count are useful descriptors of soil condition and they can be helpful to monitor its quality. The data presented in this work, contribute to highlight that CA is a more sustainable and environmentally friendly management system for maintaining a high soil quality.

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2) C.A. Seybold, M.J. Mausbach, D.L. Karlen, and H.H. Rogers. 1998. Quantification of soil quality. p. 387-404. In: R. Lal, J.M. Kimble, R.F. Follett, and B.A. Stewart (Eds.) Soil Processes and the Carbon Cycle. CRC Press, Boca Raton.

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## 3.4 = BRYOPHYTE INVESTIGATIONS IN URBAN AREAS: DIVERSITY OF BRYOFLORA OF ERICE TOWN

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This work is part of a study of bryophyte floras of Sicilian towns that has highlighted the presence in these environments of interesting taxa from the taxonomic and phytogeographical point of view (1, 2, 3, 4).

Given its location, perched at 750 m a.s.l. on the S. Giuliano Mount at the northwestern tip of Sicily, the town of Erice has a climate which, although typically Mediterranean, has a strong wet connotation for the frequent presence of fog, especially in the winter time. These features contribute to creating conditions particularly suited to host bryophytes that grow both on artifacts and on phorophytes present in the city and specially in the Balio urban park.

The study has allowed to find 53 taxa including 43 mosses and 10 liverworts. Taxonomic, chorological and ecological features of this bryoflora well accord with the geographical position and mesoclimatic characters of site. A comparison with the bryoflora of other Sicilian towns shows it is a quite rich bryoflora with a high incidence of oceanic-Mediterranean taxa.

Finally, the presence of the taxon, *Scorpiurium sendtneri* (Schimp.) M.Fleisch., rare moss in Europe, is noteworthy.

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### 3.4 = BIOLOGICAL STUDIES PREPARATORY FOR THE KENTRIDGE'S ARTWORKS ON STONE EMBANKMENTS OF LUNGOTEVERE (ROME): IDENTIFICATION OF RUDERAL FLORA AND APPROPRIATE TREATMENTS METHODS

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In April 2016, the artist William Kentridge, together the foundation "TEVERETERNO", has created on a grand-scale along a stretch of the embankment of the Tiber River (Rome), illustrating the "Triumphs and Laments" of the history of Rome. More than eighty black figures, up to 10 meters high have been created through selective cleaning of the black biological patina (1).

In the stretch where the artistic intervention have been carried out, occurred a rural flora that interfered with the final project execution and for this reason has been investigated with the aim to clean the entire wall (Fig.1).

The vegetation can induce negative effects on the conservation of the architectural or some floristic elements could be of natural interest, and therefore should be protected and valued in order to preserve the presence of rare species (2, 3). Hence, a careful study of the vegetation is necessary before any intervention.

The purpose of this work was to 1) detect and map the rural vegetation in artistic intervention area, detailing the morphological and structural characteristics of the detected species and to 2) give indication of areas to be treated and of the most appropriate treatment methodologies. The challenge of this study was to find a suitable method for the removal of species that did not induce any change to the biological patina, which is the base of artwork. Our results have shown that the most abundant and more distributed species over this stretch especially in higher areas was *Erigeron karvinskianus* DC., followed by *Erigeron sumatrensis* Retz., *Pteris vittata* L., and *Parietaria judaica* L. In detail, prior to the selective cleaning of the stone surface, a mechanical removal of the annual plants (therophytes) *E. sumatrensis*, *Sonchus tenerrimus* L., *Stellaria media* (L.) Vill., *Fumaria capreolata* L., *Symphyotrichum squamatum* (Spreng.) G.L. Nesom, *Arenaria serpyllifolia* subsp. *leptocladus* L. have been carried out (Fig. 1a). After, in the case of the most damaging woody plants (phanerophytes), *Capparis spinosa* var. *inermis* Zohary, *Ficus carica* L., *Rubus ulmifolius* Schott, *Rhamnus alaternus* L., and *Alnus glutinosa* (L.) Gaertn. has been applied a devitalization of the root with suitable herbicide (Fig.1b).

Finally, in different points of the stretch explored the presence of a rare tropical fern (*P. vittata*) has been detected. It has been considered as species of conservation interest, as included in the National Red List (4) and in the Regional Red Lists (5) being endangered at the national and / or regional level. *P. vittata* has been left in the areas, which it occurs.



Fig. 1  
Different phases of the rural vegetation removal (a-b) and cleaning for the realization the artistic work (c-d).

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## 3.4 = A GOOD EXAMPLE OF A HISTORICAL GARDEN: THE "CARLO RUGGIERO" MUNICIPAL PARK IN CITTANOVA (RC) - ITALY

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In the recent past, historical gardens have become a destination that attracts, as well as experts and enthusiasts of architecture and botany, also a wider public related to cultural tourism, together with students and citizens looking for places to relax. Visiting historic gardens is a moment of cultural enrichment, a chance to discover the botanical, historical and artistic places that host them.

Proper valorization of the historical gardens occurs through a careful analysis of the botanical heritage.

The "Carlo Ruggiero" Municipal Park in Cittanova founded in 1880 (1), built by Mayor Carlo Ruggiero and designed by Swiss agronomist engineer Henry Fehr, is one of the few historical gardens in Calabria. The garden covers an area of 2.5 ha and is divided into two parts: the so-called "botanical garden" and the "outdoor gardens". The park has maintained its original floristic and architectural structure.

Cittanova has a Mediterranean Pluviseasonal Oceanic bioclimate, with a low Mesomediterranean thermotype and low humid ombrotpe.

The aim of this study was a census of all plant species present in the "Carlo Ruggiero" Municipal Park, analysing systematic and chorological aspects, as well as the realization of a graphical project by CAD software for easier localization of each plant present.

The work carried out for the preparation of this study was divided into several phases: A) Localization *in situ* of the species; B) Sampling and identification of the species (2, 3, 4, 5, 6, 7) and realization of herbarium samples; C) archiving and digitalisation of data; D) CAD project of the Municipal Park.

This work produced a census of 888 plants, including 696 in the so-called "botanical garden" and 192 in the "outdoor gardens", belonging to 130 species and 59 families. Each species was assigned an identification code, useful for localizing it in CAD.

This Park is particularly precious for the diversity of species recorded and the mix of native and non-native plants. These include *Ilex canariensis* Poir. and *Phoenix canariensis* Chabaud (Canary Islands), *Ilex cornuta* Lindl. & Paxton, *Trachycarpus fortunei* (Hook.) H.Wendl., *Cephalotaxus sinensis* (Rehder & E.H.Wilson) H.L.Lio and *Ginkgo biloba* L. (China), *Feijoa sellowiana* (O. Berg) O. Berg (Brazil), *Liriodendron tulipifera* L. and *Pseudotsuga menziesii* (Mirb.) Franco (North America), *Phormium cookianum* Le Jol. (New Zealand) and *Cedrus deodara* (Roxb. ex D.Don) G. Don (Himalayan region).

The study showed that the "Carlo Ruggiero" Municipal Park in Cittanova, represents as a whole a green area of particular interest for species richness, the abundance and magnificence of the plants and the architectural design.

It therefore represents an historical garden of great value, a park of exceptional beauty and rarity and one of the most important of Calabria.

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### 3.5 = MONITORING OF RIPARIAN VEGETATION OF THE SAVENA RIVER (BOLOGNA, ITALY) IN THE SIC IT4050012, AFTER THE TREE LAYER CLEAR CUTTING

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In August 2014, a stretch of riparian forest of the Savena river included in the SIC IT4050012 (near the town of Pianoro Vecchio - BO) was subject to clear cutting, with the declared aim to avoid any risks arising from possible floods. While the potential benefits of this intervention are still the subject of lively discussion, its negative impact on the vegetation were found to be strong. In order to feed into future policy making, a monitoring campaign on plant communities concerned was therefore initiated, verifying the resilience, the variations of structure and phytodiversity, as well as the turn-over of plant species. A number of georeferenced sample plots were selected within a range of forest transects along the river channel. The plots, 100 square meters sized, were spaced approximately 70-100 m and have been used to undertake physiognomic-structural and phytosociological surveys in order to produce an impact assessment of the clear cutting. Neighbouring riparian woods upstream of the cutting area were used as comparison.

A first survey campaign was initiated in June 2015, approximately one year after cutting (1) and a second campaign was carried out in June 2016. The first results show an increase in diversity of cut areas, mainly due to a massive invasion of heliophilous and nitrophilous plants (because of the large quantities of plant debris) together with ruderal ones (taking advantage of the high level of disturbance): therophytes, cosmopolites, exotic adventitious. In the more humid areas, the growing resilience of some woody species is remarkable: in particular Poplars and *Robinia pseudacacia*. Furthermore there was a significant expansion of the brambles, *Rubus caesius* (incl. *R. corylifolius*) and *Rubus ulmifolius*, which make large areas almost impenetrable. In 2016, in some plots, the rapid increase in shrub cover has already led to a contraction of herbaceous diversity reached in 2015. In summary, the high disturbance caused by the drastic structural changes broke the existing ecological balance, triggering profound changes in the species composition of riparian communities. In fact, the chaotic resilience of wood, together with the fast turn-over of species, indicate a high level of instability of the plant communities, which probably will last for a long time to come.

1) F. Di Luzio, (2015), thesis University of Bologna, supervisor prof. A. Chiarucci



### 3.6 BIOLOGICAL CONTROL OF PESTS IN BOTANIC GARDEN "GIARDINO DEI SEMPLICI" OF FLORENCE

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The presence in the human body of different synthetic chemical residues increases the negative effects that are "aggravating contributory causes" of health risk especially in populations already exposed to other forms of environmental pollution. So the Botanical Garden of Florence, which is located in the city center, began the conversion of cropping system to the biological control of pests to reduce and possibly eliminate the chemical control. The project is very complex: it began with two training courses for the staff of the Italian botanical gardens during which the most advanced biological control techniques were illustrated. The second step was the activation of an agreement with Agernova – Servizi per l'Agroecologia e la Ricerca - to identify pests and organize a series of launches of antagonists and interventions with natural pesticides. Then we monitored parasites in the greenhouses and in the garden, with visual inspections and sticky traps, highlighting a heavy presence of cochineal, mites, thrips and aphids (the latter already parasitized by naturally present wasps), in addition to the inoculation of powdery mildew and black rot and other plant diseases (*Botrytis*, etc.). The project started in February 2016 by a spray with a compost of Neem oil and potassium soap to reduce the initial population of mealy (*Planococcus* spp. *Pseudococcus longispinus*), blocking the suit young nymphs and washing the sooty mold. On mealybug outbreaks were introduced larvae *Cryptolaemus*, a very active predator Coccinellide, with a first launch of "inundative" type, added to the parasitoid *Anagyrus pseudococci*, active on *Planococcus* spp. Against mites and thrips it was launched the Multipla Preventive inoculation method, with small doses of phytoseiid predatory mites. Roses, suffering from cancer control, were treated with innovative cupric to lingering absorption, cupric with zinc and iron in order to promote scarring and copper and sulfur, to prevent Oidi and Black spot. In the following months were repeated the *Cryptolaemus* launches localized on the scale insect outbreaks, particularly *Pseudococcus longispinus*. For red spider mite we introduced predators of *Phytoseiulus persimilis* species, very fast in the activity and in the reproduction. Three months later the cochineal considerably diminished; at this point the pest control involves the launch of *Anagyrus fusciventris* specific parasitoid, to counter the species *Pseudococcus longispinus*, by a treatment with Azadirachtin and oil-soap mixture to reduce possible outbreaks, plus the introduction of spider beetles of *Nephus includens* species. As regards the thrips, at present there are no major outbreaks therefore it continues the launches with *Amblysejus cucumeris* which preys thrips and red spider mite and *Amblyseus swirskii* which preys thrips and the rare white fly, the latter now monitored with yellow sticky traps and towards which there was a first launch of *Encarsia formosa* and *Eretmocerus eremicus*.

Results and conclusions: the experiment is giving good results and we try to achieve the ambitious goal in collecting data and informations for the scientific community of reference; in fact, in the botanical gardens has increased the sensibility to the use of alternative systems to chemistry for the control of pathogens; but apart from the Giardini botanici Hanbury where since many years they are making a biological control against several diseases and few other situations (such as the biological control of the red palm weevil in the Orto botanico of Catania) there are not many Botanical Gardens which have adopted a rigorous program of biological control for plant diseases.

In addition to sensitizing the staff working in Botanical Gardens, we also aim to motivate the staff who is in charge of public green spaces to pursue the reduction if not the complete elimination of chemical croppings used to contrast plant diseases in urban contexts.

At the same time the Botanical Garden of Florence is testing regenerative organic growing techniques with very important results in strengthening plants health. This will also let us eliminate rameic products in the future, for the toxicological studies indicate them as dangerous for the human health, though it is allowed in biological control.

### 3.6 = PHOSPHORUS AND HEAVY METALS BIOREMEDIATION COMBINED WITH LIPID PRODUCTION BY THE GREEN MICROALGA *DESMODESMUS* SP.: AN INTEGRATED APPROACH

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Widespread applications of microalgal-based systems in wastewater treatment (WWT) have been proposed since the 50s [1]. Anthropogenically derived wastewater can significantly increase the nutrient and heavy metal water load posing hazards to the ecosystem and human health [2]. The combination of algal growth with WWT is a green biotechnology that cuts down the cost of mass production of microalgae and potentially increases the environmental and economic sustainability of WWT process. WWT facilities can exploit microalgae's ability to reduce the concentration of excess nutrients, such as dissolved nitrogen and phosphorus, as well as heavy metals in wastewater treatment effluents, which is important in view of guaranteeing the good chemical status of the receiving water body (EU Water Framework Directive 2000/60/EC), but also biomass to harvest and process for the bio-product market [3].

In this scenario, we isolated a strain of *Desmodesmus* sp. (VRUC281) from the secondary tank of a municipal wastewater treatment plant. We evaluated its efficiency in removing phosphorus (P), copper (Cu) and nickel (Ni) from the liquid growth medium. After 4 days, total P was lower than 2 mg L<sup>-1</sup> complying with the European limits for effluent discharge into water bodies, and in 10 days over 90% of P was removed from the liquid medium that contained up to 4.5 mg P L<sup>-1</sup>. In addition, the strain was incubated for 48 hours in a mixed solution containing 9.8 and 7.4 mg L<sup>-1</sup> Cu and Ni, respectively. After exposure, only 20% of the initial Cu and 60% of the initial Ni were measured in the liquid solutions by Inductively Coupled Plasma Optical Emission Spectrometry (ICP-OES) analysis. Heavy metal adsorption tests were also conducted by using inactivated biomass of the green microalga *S. obliquus* (strain VRUC280) to analyse the biosorption of copper (Cu) and nickel (Ni). *S. obliquus* (0.5 gDW) was incubated in 5.0 and 3.9 mg L<sup>-1</sup> Cu and Ni solutions, respectively (contact time 0-120 min) and Cu and Ni concentrations were analysed in the solution and also in the microalgae after ultrasound assisted acid extraction [4]. After 15 minutes, up to 51% of the initial Cu (407 mg Cu kgDW<sup>-1</sup>) and 47% of Ni (349 mg Ni kgDW<sup>-1</sup>) were passively adsorbed on the cell surface.

The biomass obtained was harvested by sedimentation and centrifugation and used for FAME (fatty acids methyl esters) extraction to evaluate the effects of the presence of heavy metals on lipid accumulation. Our results encourage the research in this field for an efficient and less expensive water clean-up process, by integrating phosphorus and heavy metal removal using microalgae with biomass production, which can be used for biodiesel extraction. This technology can offer an alternative solution to problems of environmental pollution and to the traditional power generation based on fossil fuels, which are generally considered to be unsustainable in the long term.

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### 3.6.= IN VITRO SCREENING EVALUATION OF ABSORPTION CAPACITY, TOLERANCE AND TRANSLOCATION TO CADMIUM IN AUTOTROPHIC *POPULUS ALBA* CLONES "VILLAFRANCA"

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In vitro model system of micropropagated microshoots of the commercial clone 'Villafranca' of *Populus alba* (L.) was used to explore the metal phytoremediation potential of poplar. The aim of this work was to investigate accumulation, translocation and tolerance of autotrophic clones *Populus alba* in response to high concentrations of cadmium (Cd). For this purpose, increasing concentrations of Cd (0, 5, 50 and 250  $\mu\text{M}$ ) were administered to micropropagated poplar plantlets exposed to metal treatments for 15 days. To evaluate Cd influence on Poplar after Cd exposure, we reviewed the following morphometric and chemical parameters: number of leaves, length of roots and stems, leaf area index (LAI), shoot and root dry biomass, shoot and root Cd content as well as cadmium influence on essential nutrients content in the vegetable tissues. At the end of the treatment, the seedlings were sampled, weighed and finally mineralized after drying in a stove for the determination of Cd content in the respective underground and aboveground portions. Results obtained in this *in vitro* screening showed various potential of investigated clone for cadmium phytoremediation. Stability of investigated parameters was most expressed by 'Villafranca' clone at low and moderate amounts of metal (5 $\mu\text{M}$  and 50 $\mu\text{M}$  CdSO<sub>4</sub>) with which Poplar reacted with highest growth and lowered accumulation of Cd. Seedlings exposed to the highest pollution concentration (250  $\mu\text{M}$ ) show more severe effects. Results show that cadmium has affected the development of the seedlings especially at the highest concentrations. The contaminant has influenced drier biomass plants although the plants generally showed good tolerance to metal treatments. However, the tolerance indexes (Ti) were usually up the value 1, suggesting a good tolerance mechanism of poplar under Cd-exposure. Whereas, the plants had higher translocation factor (TF). Cadmium also seemed to affect absorption of some essential nutrients such as Ca, K, Mg, Mn, S, Fe, Ca, Na, Zn and P, limiting and/or changing their absorption. According to these results, it was shown *Populus alba* L. had strong tolerance and accumulation capability to Cd, therefore it is a potential Cd-hyperaccumulator.

## 3.6 A RHIZOSPHERE APPROACH TO MITIGATE SOIL EROSION AND POLLUTION

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Environmental pollution by metals represents a severe risk to human health and to the environment in urban and peri-urban areas, where the main sources of metal pollution are mining, municipal and industrial wastes and agricultural activities [1, 2]. In a few plant taxa called hyperaccumulator, the concentration of metal(loid)s in aboveground biomass is up to four orders of magnitude higher than in non-hyperaccumulator species and it is associated with a strongly enhanced metal hypertolerance [3]. The rhizosphere, defined as the soil-root interface, is the micro-ecosystem where roots access soil trace elements [4] and represents the first area of potential metal uptake. However, there is a general lack of knowledge about hyperaccumulators, particularly with respect to rhizosphere processes [4].

The aim of this study is to characterize the rhizosphere of selected hyperaccumulator and non-hyperaccumulator species in order to improve metal hyperaccumulation via root uptake.

Metal-tolerant plant and fungi were selected in metalliferous sites with high concentration of potentially toxic elements (i.e. Ni, Cr Co and Zn) exceeding the law limits (D. Lgs. 152/2006). Among the studied species, the nickel-hyperaccumulators *Alyssoides utriculata* (L.) Medik. and *Thlaspi caerulescens* J. & C. Presl. were selected as target species. *A. utriculata* is able to accumulate over 1.000 mg Ni kg<sup>-1</sup> (DW). Nickel uptaken by roots is adsorbed to rhizoplane (20%) or absorbed (80%) [5].

Besides, soil and rhizosphere samples were examined and some fungal strains were isolated. Among these, a *Trichoderma harzianum* Rifai strain exhibits high Ni tolerance, up to 500 mg l<sup>-1</sup> and high uptake ability, up to 11.000 mg Ni kg<sup>-1</sup> [6].

Seed germination tests were carried out in veg-box with germination substrate spiked with Ni (0, 10, 50, 100, 200, 400 mg Ni kg<sup>-1</sup>, respectively) on target species using non-hyperaccumulator species (i.e., *Alyssum montanum* L. and *Thlaspi arvense* L.) for comparison.

The same substrates were used in mesocosm 3D experiment in pots to evaluate the root elongation and the root anatomy under Ni hyperaccumulation.

Improving root surface increases metal uptake, prevent soil erosion and reduces the spread of metal pollutants, favoring the remediation of peri-urban dismissed industrial sites.

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## 3.8 = AEROBIOLOGICAL MONITORING IN L'AQUILA AND MADRID: PRELIMINARY DATA COMPARISON

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The aim of the present study is to detect allergenic pollens present in two different cities: L'Aquila, Italy (University, Coppito) and Madrid, Spain (Ciudad Universitaria) to guarantee a safe and pleasant stay to sensitive subjects who intend to move from a place to the other.

For this purpose we compared the aerobiological data of the above mentioned areas relating to the first six months of 2014. Time series of pollen concentration were collected using a volumetric type Hirst (Lanzoni VPPS 2000 in L'Aquila and Burkard in Madrid), placed on the roof of the Faculty of Science in L'Aquila and the Faculty of Pharmacy in Ciudad Universitaria in Madrid. The analyses of the samples were made following the usual procedures laid down by the two Aerobiological Networks, the Italian Monitoring Network in Aerobiologia (RIMA®) (1) and the Red Española De Aerobiologia (REA). The most representative families and genera in L'Aquila were found to be, in order of percentage of the total number: *Cupressaceae/Taxaceae*, *Populus*, *Gramineae*, *Urticaceae*, *Pinaceae*, *Quercus*, *Euphorbiaceae*, *Corylus*, *Betula* and *Salix*. Madrid instead presented *Platanus*, *Quercus*, *Cupressaceae/Taxaceae*, *Populus*, *Pinaceae*, *Gramineae*, *Fraxinus*, *Olea*, *Plantaginaceae* and *Moraceae*. The town of L'Aquila is located in the Aterno river valley and is surrounded by the mountain chains of the Gran Sasso (with the highest peaks in the Appennines) and Velino-Sirente (national and regional parks, respectively), both rich in vegetation. The landscape is made up of oak wood (*Quercus pubescens*), pine wood (*Pinus nigra*) and a community of pastures. Typical communities along the rivers are willows (*Salix* sp.) and poplars (*Populus* sp.) (2). Invasive plants are present in the surroundings of L'Aquila, especially in the uncultivated fields, road, path margins and dumps. The public and private green is made up of plants adapted to its "temperate sub-continental" climate, with very cold winters and hot summers. The aerobiological station in Madrid is situated in a semi-urban area, which presents tree-lined avenues, the largest city public park in Casa de Campo and the Botanical Garden. The climate is dry, characterized by low rainfall, a feature common to the Southern Meseta. The territory bears a strong human pressure. Therefore, its flora is rich in nitrophilous and ruderal plants, derived from the presence of infrastructure, and in ornamental and non-native plants in the gardens and lawns of the campus, near the monitoring station (3). Other observations are currently underway.

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### 3.8 = FIRST OBSERVATIONS IN ROME ON COMBINED EFFECT OF HIGH LEVEL OF ATMOSPHERIC POLLUTION AND POLLEN CONCENTRATION USING SYMPTOM MEDICATION SCORES

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Research and international regulations on air pollution are, even today, typically addressed to the evaluation of individual substances and their specific effects: the knowledge of the cumulative effect that more pollutants present in atmosphere near the ground with high concentrations in same interval of time can have on human health is low. Even less is studied, in a systematic way, the possible combined effect of traditional pollutants with the fraction of atmospheric particulate of biological origin, typically with an aerodynamic diameter close to or greater than 10  $\mu\text{m}$ , composed of pollen and spores that seems to have increasing consequences, in terms of allergies and asthma on the citizenship of the urban areas [1].

In this work has been studied, for the city of Rome over a period of six years (2010-2015), the synergistic effect of  $\text{PM}_{10}$ ,  $\text{PM}_{2.5}$ ,  $\text{NO}_2$  and  $\text{O}_3$ , airborne pollens (belonging to 5 families allergenic: Betulaceae, Cupressaceae/Taxaceae, Graminaceae, Oleaceae and Urticaceae and fungal spore *Alternaria*).

For air pollutants we used the concentration values recorded by 14 stations of the city's air quality monitoring network (ARPA Lazio), for pollens and spores data detected by Aerobiological Monitoring Centre of the University of Rome Tor Vergata.

The effects on human health have been estimated on a group of 100 patients through the Free Application AllergyMonitor™ [2] that allows to record daily symptoms of hay fever and bronchial asthma.

The data collected in the clinical diary were then processed in four different Symptom Medication daily Scores (RTSS, ACS, ADSS and Asthma score) and compared with the time series of concentration in the air of the air pollutants mentioned, pollen and spores.

The statistical analysis allowed to highlight the times of year when most occur concomitant high levels of allergenic species and air pollution, the influence of meteorological parameters, the flowering calendar, the intake of drugs and how these aspects reflect on the symptoms of stakeholders.

Conclusions:

1. Maximum concentrations in the air of pollen and air pollutants occur in different seasons of the year (the greater overlap period between February and March);
2. The analysis of the trend from 1999 to 2015 shows a statistically significant decrease for  $\text{PM}_{10}$ ,  $\text{PM}_{2.5}$  and  $\text{NO}_2$ , and growth for Urticaceae (all other parameters are stable);
3. The partial least squares discriminant analysis, based on atmospheric concentrations of air pollutants, pollen and weather data, showed good accuracy in the prediction of allergy symptoms;
4. The patient-specific models predict with accuracy the presence or absence of symptoms up to 4 days before the event;
5. The performance of predictive models improves when taken into account the degree of the individual's allergic predisposition;
6. The individual cases of the worst results in the comparison between 2012 and 2016 are found in correspondence to the lowering of the patient's awareness.

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#### 4.1 ANTIOXIDANT AND ANTIMICROBIAL ACTIVITIES OF *CASTANEA SATIVA* MILL. EXTRACT: NEW THERAPEUTIC PERSPECTIVES

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*Castanea* spp. (Fagaceae) is a genus consisting of plants ranging from small shrubs to large trees in the forests and orchards in Europe, Asia and North America (1). *Castanea sativa* Mill. is the only one species exploited in Europe, particularly in the Mediterranean basin, for fruit and wood production. *C. sativa* attains a height of 20–35 m with a 2 m diameter trunk. The oblong-lanceolate, boldly toothed leaves are 16–28 cm long and 5–9 cm broad. The flowers of both sexes are 10–20 cm long, in upright catkins, the male flowers in the upper part and female flowers in the lower part; the female flowers develop into spiny cupules containing 3–7 brownish nuts that are shed during October. The popularity of many *Castanea* spp. is based on several attributes of various species, including the production of nutritious, low fat nuts and decay-resistant and high-quality wood. Chestnut fruits contain various nutrients (starch, free sugars, proteins, lipids/fat), vitamins and minerals important for health (2). Chestnut flowers have an outstanding antioxidant capacity, also displaying antimicrobial and antitumor effects. Studies on chestnut by-products revealed a good profile of bioactive compounds with antioxidant, anticarcinogenic and cardioprotective properties (3). Recent studies on chemical composition revealed that *C. sativa* is characterized by betaines, flavonoids, tannins, pentacyclic triterpenes (2, -5). Investigation of botanical folk medicines used in the treatment of skin and soft tissue infections led us to study the plant for its potential antibacterial activity (6, 7). Tannins, flavonoids, and antioxidants in shell extracts of Chinese chestnut (*C. mollissima* Blume) appeared inhibitory to bacteria and fungi (8). Recently, Donis-Gonzalez reported that the greatest antimicrobial activity was found in the pellicle, a thin fibrous tissue found between the shell and kernel (9). When chestnut pellicle, shell, and nut kernel were directly placed on agar media, the pellicle tissue was the only tissue found to inhibit fungi (including yeast) and bacteria. In view of the interesting biological properties reported for *Castanea* species, objective of this study was to determinate the total phenolic and flavonoid contents of extract of pellicle of *C. sativa* and to investigate the *in vitro* antibacterial activities of extract of the plant against pathogenic bacteria. Furthermore, being commonly accepted that reactive oxygen species play an important role in the pathogenesis of various diseases, the antioxidant activity of the same extract was also evaluated. Total phenolic and flavonoid contents of the extract was evaluated by spectrophotometric assay; antiradical activity of *C. sativa* extract was performed by DPPH test and by superoxide anion scavenging capacity. The effects of chestnut extract on the growth of pathogenic bacteria (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecium*, *Enterococcus faecalis*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*) were determined using a microdilution method. Results obtained showed that the extract has both antioxidant *in vitro* activity and antimicrobial ability against Gram-positive and Gram-negative strains but with different effectiveness probably due to its active compounds. This study allowed us to enhance knowledge about the type of chestnut by-products produced, their chemical composition and biological activities in order to explore other possible applications of this extract.

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4.1 = *SEDUM TELEPHIUM* L.: FROM TRADITIONAL USE TO PHARMACOLOGICAL RESEARCH

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*Sedum telephium* L. is one of those species that have received the attention of scientific research many years after its clinical use derived from centuries of tradition and descriptions provided by various ancient medical texts. Although it has been mostly cultivated for ornamental use, it was also widely mentioned in the Italian ethno-botany. The traditional use of the species has been reported throughout Italy (1), from Alpine regions (2) to Central and Southern areas of the peninsula (3, 4). The species has been frequently used for various kinds of indications, including the treatment of insect bites, burns, corns, ulcers, warts, abscesses and wounds. The fresh leaves are applied as poultice or ointment for topical application, whereas the juice from crushed leaves is used to heal scars. The macerated aerial parts of *S. telephium* sub. *maximum* are also used as poultice for the same indications.

The main chemical constituents of the species are flavonoids and polysaccharides, but phenolic and organic acids are also present, together with coumarins, amino acids and triterpenes. Overall, at least fifteen different flavonoids have been identified, belonging to the group of glycosides of kaempferol and quercetin with one, two or three sugar units. The freeze-dried juice of *S. telephium* contains about 3% of total flavonoids expressed as kaempferol, and more than 5% of soluble polysaccharides (5). In modern herbal medicine, *S. telephium* has been used in clinical practice even in serious emergency cases, such as for treating open wounds and deep burns of II and III degree, or for major damage from traumatic events involving bone (6). More recently, *in vitro* and *in vivo* pharmacological studies have investigated the mechanisms of action, with the aim of proposing stable preparations with a standardized chemical composition.

Our data have shown that the whole phytocomplex is responsible for the biological activity, with a prominent role of both polysaccharides and flavonoids. Tissue repair is ascribed to a complex series of cellular and molecular events, including *in situ* tissue regeneration, reduction of pathogenic bacteria, and reshaping of the new tissue in its final structure. *S. telephium* shows bacteriostatic activity especially against *Streptococcus* spp. and *Staphylococcus* spp. Moreover, thanks to its anti-radical and antioxidants properties, it also inhibits the accumulation of reactive oxygen and nitrogen species in the site of action (in particular species produced by monocytes as antibacterial agents). Preparations obtained from *Sedum* leaves show radical scavenging and photoprotective ability *in vitro*, also confirmed by *in vivo* experiments (7). In addition, these preparations are able to modulate the inflammatory response, that is considered the ideal goal of a therapeutic healing agent (8). As another key mechanism of action, *S. telephium* promotes the proliferation of fibroblasts and collagen production. Finally, working with primary human fibroblasts, we highlighted that dry extracts of *S. telephium*, standardized in flavonoids and polysaccharides, are able to enhance the levels of sirtuin 1, which represents an interesting cell repair signal.

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## 4.1 = ESSENTIAL OILS: EFFECTS ON CENTRAL NERVOUS SYSTEM

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Essential oils are used in aromatherapy due the effects of their constituents in the treatment and prevention of certain diseases related to the Central Nervous System (1,2); the available literature reports their anticonvulsive, antinociceptive, antiviral, antioxidant and anticancer effects (3,4). The aims of this study are to determinate the chemical composition of *Lavandula angustifolia* Mill., *Coriandrum sativum* L., *Citrus medica* L. var. *liscia* and *C. medica* L. var. *rugosa* essential oils; to evaluate their cytotoxic effects in SH-SY5Y cells; to investigate whether an alteration of adenylate cyclase 1 (ADCY1) and of extracellular signal-regulated kinase (ERK) expression can take part in the molecular mechanisms of the essential oils; to study their possible effects in cellular electrophysiology.

The essential oils were obtained by hydrodistillation, and studied by GC and GC-MS. SH-SY5Y cells were incubated with different concentrations of essential oils, and of linalool and limonene that are major components of the four essential oils. Cell viability and effects on ADCY1 and ERK expression were analyzed by MTT and Western blot, respectively.

Variation in cellular electrophysiology was studied in primary cultures of rat cortical neurons with a Multi Electrode Array (MEAs)-based approach. Hydrodistillation of the aerial parts of *L. angustifolia*, of fruits of *C. sativum* and of fruit peels of *C. medica* var. *liscia* and *C. medica* var. *rugosa* were 5%, 2.1%, 0.9% and 0.7% yield on a dry mass basis, respectively. In the oil from *L. angustifolia* and *C. sativum*, linalool (33.1 % and 67.8 %, respectively) was the main component, whereas in *C. medica* var. *liscia* and *C. medica* var. *rugosa* the main constituent was limonene (67.2%–62.8%). Treatment of SH-SY5Y neuroblastoma cells with linalool resulted in a strong cytotoxic activity with 92% cell death. However, treatment with *L. angustifolia* and *C. sativum* essential oils resulted in 78% and 63% cell death, respectively. Limonene, *C. medica* var. *rugosa* and *C. medica* var. *liscia* essential oils showed higher IC<sub>50</sub> than the other essential oils. Moreover, our results show that linalool inhibited ADCY1 expression and *L. angustifolia* essential oil showed a similar effect also on ERK protein expression. High concentration of limonene appears to increase ADCY1 expression in SH-SY5Y cell. Neuronal networks subjected to *L. angustifolia* and *C. sativum* essential oils induced a concentration-dependent inhibition of spontaneous electrical activity (mean firing rate, MFR). In particular, *L. angustifolia* essential oil showed an IC<sub>50</sub> value comparable to that obtained with linalool, while *C. sativum* essential oil was the least effective, although its higher content of linalool than *L. angustifolia* essential oil.

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## 4.1 = ANTIBACTERIAL ACTIVITY OF MORUS ALBA L. LEAF EXTRACTS

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Moraceae family comprises about 40 genera and over 1000 species of flowering plants (1). The genus *Morus* (Moraceae) is widely distributed in Asia, Europe, North America, South America, and Africa, and is cultivated extensively in the Eastern, Central, and Southern Asia for silk production (2). *Morus alba* L., known as white mulberry, is a species native to China and now widely cultivated in other countries. It has white and purple fruits with a very sweet taste and low acidity. Its fruits are perishable and mostly used for fresh consumption (3). The different parts of this plant have been used in the traditional Chinese medicine for many purposes. The white mulberry leaves, an important food for silkworm, are used to treat hypertension, arthritis, and the fruit is a diuretic and a tonic agent. The root bark of the plant is considered as an important medicine to treat cough, inflammation, diabetes, cancer, hepatitis and heart diseases (4). The root is astringent, bark is anti-helminthic, decorked bark is used against chronic bronchitis and emphysema (5). *M. alba* mainly contains polyphenolic constituents including prenylated flavonoids, benzofurans and Diels–Alder type adducts with important biological activities such as hepatoprotective, cytotoxic, antioxidant, inhibitory of NF- $\kappa$ B and LOX-1. The glycosidase inhibitory activity of several alkaloids in the plant has also been reported (4). The root bark contains an alkaloid, 1-deoxynojirimycin, and its derivatives have great therapeutic potential for the treatment of viral infections, diabetes, obesity and cancer (3). *M. alba* L. leaves contain alkaloids, steroids, flavones, triterpenes, amino acids, vitamins and trace minerals (6, 7). The use of plant as an antimicrobial may inhibit bacteria by a mechanism different from that of antibiotic. Medicinal plants have a great positive impact on the treatment of infectious diseases caused by bacteria. Exploration of newer antimicrobials in plants brings about a different approach in minimizing antibiotic resistance, and thus offers potential benefits. The current study aimed to investigate the activity of acetonic, ethanolic, ethyl acetate and methanolic *M. alba* L. leaf extracts on the growth of Gram-positive (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecalis*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Proteus mirabilis*) pathogenic bacteria. Assays were performed by 96-well microplates method and antibacterial activity was compared with standard antibiotic (8). Acetonic extract was observed to be more effective than both ethanolic and methanolic extracts against Gram-positive strains. Acetonic, ethanolic and ethyl acetate extracts didn't inhibit the growth of Gram-negative bacteria. Ethyl acetate extract was inactive against all tested microorganism. Different activity could be related to the variability in contents of polyphenolic compounds. The results obtained in this study provides an important basis for the use of *Morus alba* L. extracts for the treatment of infections associated to the tested microorganisms. The crude extract found active could be useful for the development of new antimicrobial topic formulation. However, further studies are needed to confirm these findings.

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## 4.1 = CONTRIBUTION TO THE KNOWLEDGE OF THE TRADITIONAL USES OF NATIVE PLANTS IN SICILY

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The Sicilian territory as a whole presents orographic, geopedological, hydrographic characters and climate conditions that determine a wide diversity of environments that host a rich vascular flora consisting of over 3250 specific and intraspecific taxa (subspecies, varieties and forms), belonging to 880 genera of 134 botanical families (1). Of the above mentioned flora, about 500 entities are endemic and in several cases their populations are constituted by a small number of individuals and / or distributed on limited areas.

Within this great biodiversity, which places Sicily among the regions with greater floristic diversity of both Italy and the Mediterranean area, it is noteworthy that there is a conspicuous contingent of species used for food, aromatic, cosmetic, handicraft, agricultural, forestry and medicine interest, with the purpose of preserving health and well-being of humans and animals (2-6).

In this paper we present the preliminary results of a study aimed at identifying the traditional uses of wild (herbaceous and woody) taxa in several communities of the island. Particularly, the medicinal, veterinary and food uses are reported. The taxa hitherto surveyed were about 500, belonging to 89 families; the most represented families resulted Asteraceae (18.3% of species), Lamiaceae (9.3%), Apiaceae (6.2%), Fabaceae (5.7%), Rosaceae (3.4%) and Poaceae (3.4%).

The plants utilized for medicinal purposes constitute the largest group that we detected (270 taxa); the contingent of the plants used as vegetables is very big as well (205 taxa); 53 entities were surveyed for their veterinary use.

Among veterinary species it is significant to mention: *Helleborus bocconeii* L. subsp. *intermedius* (Guss) Greuter & Burdet, for diagnosing and treating pneumonia of horses and bovines; *Teucrium scordium* subsp. *scordioides* (Schreb.) Arcang., as a disinfectant and soothing when applied on wounds of animals; *Sideritis italica* (Mill.) Greuter & Burdet, employed as cicatrizing and hemostatic in the territory of the Madonie Mt.; *Calamintha nepeta* (L.) Savi, whose crushed leaves mixed with olive oil are applied to the areas of body affected by swelling and infection due to bites of poisonous animals and insect and against the parasites of animals.

The data obtained so far highlight the "knowledge" of the traditional uses of plants in the Sicilian rural communities that have been essentially passed down orally. Such knowledge potentially constitutes a source for effective economic enhancement of these species.

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4.1 = ULTRASTRUCTURAL EVALUATION OF THE GLANDULAR *INDUMENTUM* IN SELECTED LAMIACEAE SPECIES

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This work is part of a two-year research project (IRIS identification - project 2014-PDF-0363) focused on the plant heritage preserved at the Ghirardi Botanic Garden of Toscolano Maderno (Bs), which has a long tradition in the study and conservation of medicinal species.

We presented the results of an extensive ultrastructural investigation on the glandular *indumentum* of both the vegetative and reproductive organs in selected target-species belonging to the Lamiaceae family: *Ballota acetabulosa* Benth., *Ballota rupestris* Vis., *Lavandula angustifolia* Mill., *Lavandula dentata* L., *Salvia greggii* Grey, *Salvia uliginosa* Benth., *Salvia verticillata* Benth., *Scutellaria sieberi* Benth., *Scutellaria altissima* L. and *Scutellaria caucasica* A.Ham.

The investigation showed the occurrence of different types of glandular trichomes:

(i) peltate trichome, present on leaves and inflorescences of all of the species. It is constituted by a basal epidermal cell, a neck-cell and by a multicellular glandular head surrounded by a large subcuticular space in which the secretion is stored. The histochemical tests proved the production of terpenoidic, polysaccharidic and flavonoidic compounds. The presence of both lipophilic and hydrophilic components appears as osmiophilic droplets immersed in an abundant granular matrix. The secreted material is released after cuticle rupture.

(ii) short capitate hair widespread on both the vegetative and the reproductive organs of all the examined species. It is constituted by a basal epidermal cell, a neck-stalk cell and by a glandular head of 2-4 cells surrounded by a thin subcuticular space. The secreted material exhibits a strong positive response only to the hydrophilic dyes. The most striking ultrastructural feature is the presence of abundant Golgi bodies, sites of the synthesis and storage of polysaccharides.

(iii) long capitate trichomes observed only on *Salvia* species. It is composed by 1-2 epidermal cells, 2 stalk cells, 1 neck cell and by a globose head of 1-2 secretory cells surrounded by a storing chamber. The secretion is exclusively constituted by terpenes. The cytoplasm of the secreting cells is rich in plastids containing starch granules and a well-developed smooth endoplasmic reticulum, cellular compartments typical of a terpenoidic secretion.

(iv) long capitate trichomes observed only on the inflorescences of *Scutellaria* species. It is composed by 2 epidermal cells, 2-4 stalk cells, 1 neck cell and by 8 up to 18-20 secretory cells. Each secretory cells present a small subcuticular space at the apex. The secretion is characterized by a complex composition, positive to lipophilic and hydrophilic dyes. Therefore, the secretory products are constituted of polysaccharides, terpenes and polyphenols. On the apex of each secreting cell a small portion of the cuticle layer raises, originating a small chamber in which electron-dense fibrillar or granular material is stored. Occasionally some prearranged openings for the release of the secretion were observed, but most part of the secreting material seems to be extruded through the outer periclinal wall.

These highly-specialized secretory structures are characterized by a wide morphological and chemical polymorphism and by different mode of release of the secretory products. This extensive range of variability is probably crucial in determining different ecological roles.

## 4.1 = CHEMICAL COMPOSITION AND SYNERGISTIC BIOACTIVITIES OF AMAZONIAN ESSENTIAL OILS

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Thanks to a collaboration with two Ecuadorian Universities, studies about chemical and biological properties of Amazonian plants essential oils were performed. The antimicrobial and antifungal activities of essential oils are well known (1), and Amazon rainforest is indeed one of the most important biodiversity basin of the world. Based on the indication of the traditional ethnomedicine, we chose *Chenopodium ambrosioides* (Chenopodiaceae), *Cymbopogon citratus* (Poaceae), *Ocotea quixos* (Lauraceae), *Piper carpunya* (Piperaceae) and *Schinus molle* (Anacardiaceae) essential oils, obtained by leaves hydrodistillation. Each sample was chemically characterized by HPTLC, GC-MS, HS-GC for more than 95% of total composition. The major component detected for the essential oils were respectively: limonene (41,48%); geraniol (41,49%); eugenol (27,74%); piperitone (26,22%) and  $\alpha$ -phellandrene (13,62%).

Antibacterial activity analyses were performed, with microdilutions method, on 5 Gram+ and 5 Gram-human pathogens, where the most sensitive were *Staphylococcus aureus*, *Enterococcus faecalis*, *Proteus vulgaris*, *Micrococcus luteus* and *Klebsiella oxytoca*. The best results were obtained by *S. molle* (MIC<500  $\mu$ g/ml on 5 strains) and *O. quixos* (MIC<500  $\mu$ g/ml on 4 strains). Antifungal activity evaluation was carried out with agar vapour assay on two dermatophytes strain, *Trichophyton mentagrophytes* and *Nannizzia gypsea*. In this case *C. citratus* and *O. quixos* completely inhibited the fungal growth at concentration of 1 mg/ml. Anti-candida activity analyses were also performed on 3 strains, two sensitives (one clinical isolated and one commercial strain) and one resistant to fluconazole. The most promising results were obtained by *O. quixos* (MIC<200  $\mu$ g/ml), *C. citratus* (MIC<200  $\mu$ g/ml) and *C. ambrosioides* (MIC<400  $\mu$ g/ml) essential oils against all strains. Studying the synergism between essential oils and fluconazole, the most effective samples were *P. carpunya* (FIC<sub>index</sub> = 0,375 on resistant strain and 0,281 on a sensitive strain) and *S. molle* (FIC<sub>index</sub> = 0,141 on the clinical isolated sensitive strain).

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#### 4.1 = INTERACTION BETWEEN ENDOPHYTIC BACTERIAL COMMUNITIES ISOLATED FROM THE STEM/LEAF AND ROOT COMPARTMENTS OF THE MEDICINAL PLANTS *ECHINACEA PURPUREA* AND *ECHINACEA ANGUSTIFOLIA*.

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*Echinacea* has arisen as a herbal medicine in the treatment of common cold and upper respiratory infections, reducing duration and/or severity of symptoms [1]. Two species, *Echinacea purpurea* (L.) Moench and *Echinacea angustifolia* (DC.) Hell, are widely used; they are rich in various phytochemicals including phenolic compounds, such as phenylpropanoids or caffeic acid derivatives (CADs), flavonoids, terpenoids, lipids, nitrogen-containing compounds and polysaccharides. The concentrations of these bioactive compounds are species-specific and they vary due to geographical location, stage of plant development, time of harvest, growth conditions, processing and extraction methods. Therefore, plant cell and organ cultures have been transformed into appealing options for the production of biomass and phytochemicals. To fix the variability of crucial active substances in *Echinacea* spp, different *in vitro* methods have been developed. Recently, several studies have focused on the presence of bacterial endophytic communities in many species of aromatic and medicinal plants [2-4] to shed some light on the role that endophytes might have in the production of (plant) bioactive molecules. A previous study conducted in the Department of Biology allowed to achieve a collection of more than 500 cultivable bacterial isolates from the stem/leaf and root compartments of *Echinacea purpurea* and *E. angustifolia*, grown in the same soil and collected in the botanical garden of Casola Valsenio (Italy) [2]. The molecular analyses have showed that different communities inhabited the two plant species and different compartments of the same plant, and the low degree of strain sharing suggested the existence of a strong selective pressure within plant tissues.

The present work aimed to understand if these distinct bacterial communities could account for the differences in the medicinal properties of the two plants. To this purpose, cultures of axenic plants (in the absence of endophytes) derived from sterilized seeds were set up. After two months from germination, *E. purpurea* and *E. angustifolia* plants were infected with different bacterial endophytic strains, isolated from the stem/leaf and/or roots of *E. purpurea*. For each species, a suitable group of control plants (not infected) was provided. Plants were examined at different times for the presence of bacteria at the level both of the roots and the aerial part. Furthermore, in order to determine the possible role of bacteria as plant growth promoting, growth and physiology of plants were evaluated analyzing different parameters such as plant height and fresh weight, length of the roots and number of leaves. The same experiments were carried out on *Nicotiana tabacum* L. cv 'Xanthi' chosen as non-host species.

The overall analysis of the preliminary results suggested that the endophytic strains tended to recolonize the native niche (*i.e.* strains isolated from the leaf came back in the leaf compartment of axenic plants of the same species after *in vitro* infection). Several endophytes had a beneficial effect on the growth of the plant due to a significant increase in the number of leaves. Non-host plants did not show similar effects. In particular, the bacterial strains colonized mainly the roots of *E. angustifolia* infected with *E. purpurea* stem/leaf endophytes suggesting that the colonization of endophytic bacteria might depend on specific physiological characteristics of the host plant. On the other hand, the analysis of the *in vitro* morphogenetic behaviour indicated that the two *Echinacea* species had a different content of endogenous plant growth regulators. *E. purpurea* was able to regenerate new shoots in culture media enriched with high content of cytokinins while *E. angustifolia* produced only clusters of undifferentiated cells (callus). Then, both the presence of different endophytic communities and a different composition in the secondary metabolites (strictly related to the therapeutic properties) in the two species could depend on differences in plant primary metabolism. In this regard, experiments to characterize the chemical profile of the control and infected plants will be set up.

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#### 4.1 = VALORIZATION OF MEDICINAL PLANTS SUITABLE TO CULTIVATION IN THE AREA OF CASTRO AND BARRAÙ MT. (CORLEONE, PALERMO)

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The project Biovamed (Valorization of medicinal plants suitable to cultivation in the area of Castro and Barraù Mt.) is born in 2015 from the collaboration between the society Bona Furtuna LLC and the Interdepartmental Centre C.I.R.I.T.A. It continues the census of the flora already started in 2014 by the same company, which led to the identification of 530 infrageneric taxa (1), of which a significant part is of medicinal and / or aromatic interest. The estate, extended over 300 hectares in Castro, Giardinello and Valle Fredda lands of Corleone, spread between 610 and 1,300 m a.s.l. and is bordered to the north by Rocche di Mezzogiorno (885 m a.s.l.) and to the east from Barraù Mt. (1,420 m a.s.l.). It is an intact rural setting, of great natural and environmental interest, rich of spring waters, where there are also a large number of phytocenotical expressions correlated to wide altitudinal range and the variation of substrates, consisting of Tertiary clay deposits and Mesozoic carbonate successions of Sicano-Imerese domain (2). Also from the bioclimatic point of view (3), the study area is quite diversified and denotes different gradients ranging between lower mesomediterranean (upper arid) and the lower supramediterranean (lower sub-humid).

In particular, the project concerns:

- a) identification and assistance to the collection of plants of vascular flora for the staff of the company, particularly with respect to that present in the company land suitable for cultivation to extract oils and / or plant compounds of potential use at industrial level and / or pharmaceuticals;
- b) assistance to the implementation of the experimental fields and survey of environmental and phenological data;
- c) the drafting of protocols for the cultivation, the collection and storage for the extraction of oils and / or phytocomplexes;

As regards to herbaceous and shrubby species, indigenous in the area, particular attention has been given to the genera *Acanthus*, *Achillea*, *Agrimonia*, *Allium*, *Althaea*, *Anthemis*, *Athamanta*, *Artemisia*, *Bellis*, *Borago*, *Buglossoides*, *Centranthus*, *Chamomilla*, *Crataegus*, *Epilobium*, *Eryngium*, *Hypericum*, *Malva*, *Mentha*, *Micromeria*, *Nasturtium*, *Nepeta*, *Origanum*, *Papaver*, *Phagnalon*, *Potentilla*, *Prunus*, *Pulicaria*, *Rosa*, *Ruta*, *Salvia*, *Sambucus*, *Teucrium*, *Thymus*, *Tussilago*, *Urtica*, *Verbascum*. For some species, such as *Origanum vulgare* subsp. *viridulum* (Martini-Donos) Nyman e *Thymus spinulosus* Ten., the first extraction of oils in steam current in the company laboratory has already been performed. Within this group, other species not native to the territory of reference, are about to be introduced in culture. They are *Matricaria chamomilla* L., *Echinacea angustifolia* DC., *Gentiana lutea* L., *Lavandula angustifolia* Mill., *Lavandula hybrida* Rev. 'Grosso', *Symphytum officinale* L., *Valeriana officinalis* L. As regards to the arboreal plants, particular interest takes the manna ash, whose cultivation in Sicily is practiced only in the Madonie, and in particular in the territories of Castelbuono and Pollina (4). The experimental field involves the use of 400 plants of *Fraxinus angustifolia* Vahl cv. 'Verdello', grafted on *F. excelsior* L., positioned at regular sixth and provided with sub-irrigation system.

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#### 4.1 = STUDY OF POLYPHENOLS LOCALIZATION IN THE FRUIT OF DIFFERENT *SILYBUM MARIANUM* GENOTYPES

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*Silybum marianum* (L.) Gaertn. (Asteraceae; common name: milk thistle) is a plant species native to the Mediterranean area. At present, *S. marianum* is cultivated as a medicinal plant in Eastern Europe as well as in Asia, and is among the top-selling herbal products in the U.S. and in other markets. The diverse and important medicinal properties of *S. marianum* are determined by its ability to accumulate the complex of bioactive polyphenols, referred to as silymarin (1). Silymarin is a mixture of six different flavonolignans (silychristin, silydianin, silybin A, silybin B, isosilybin A and isosilybin B) that have been localized in the seed integument. In addition, the flavonoid taxifolin, a silymarin precursor, has been tentatively localized in the pericarp (2). Besides silymarin constituents and precursors, the fruit also contains other polyphenolic compounds such as condensed tannins, flavonoids and flavonols (2, 3, 4).

The aim of the present research is to analyse the different groups of polyphenols in the fruit through histochemical and quantitative methods. The study was conducted on 3 different *S. marianum* genotypes: genotype a, characterized by high silybin content; genotype b, characterized by high silydianin content; genotype c, a mutant line characterized by light-coloured fruit (Fig. 1). The histochemical analyses were performed on transversal fruit sections and quantitative analyses were carried out on cotyledons, seed integument and pericarp after mechanical separation.

The HPLC analyses of these different samples confirm that silymarin is localized only in the seed integument of the analysed genotypes. Taxifolin is localized only in the seed integument and is not present in the pericarp. The dark layer in the subepidermic cells of the pericarp is positive to condensed tannin colouration (vanillin-hydrochloric acid method) and is not present in the genotype c (Fig. 2). This result shows that, in contrast to *S. marianum* wild types (genotypes a and b), the light-coloured mutant line c contains a reduced amount of condensed tannins in the pericarp.



Fig. 1. Fruits of the studied *S. marianum*

genotypes (a, b and c)

Arrows indicate the subepidermic cell layer in the genotype with high silybin

content (a) and in the light-coloured mutant line (c). Bar = 50 mm

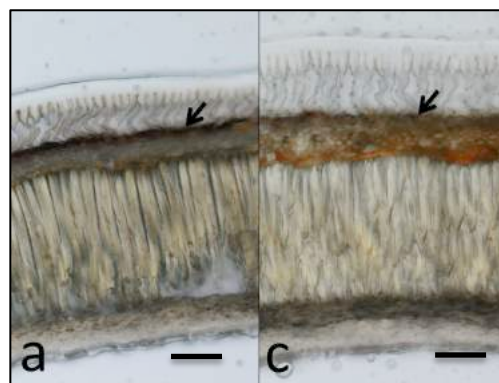


Fig. 2. Transversal sections of *S. marianum* fruit.

Arrows indicate the subepidermic cell layer in the genotype with high silybin content (a) and in the light-coloured mutant line (c). Bar = 50 mm

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#### 4.1 = ANTIOXIDANT AND ANTI-PROLIFERATIVE PROPERTIES OF THE PHENOLIC-RICH FRACTION OBTAINED FROM CAULINE LEAVES OF *ISATIS TINCTORIA* L. (BRASSICACEAE)

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*Isatis tinctoria* L. (Brassicaceae) is a biennial species with erect stem, basal leaves oblong-lanceolate and cauline sagittate; the yellow flowers are clustered in racemes. In Italy the plant grows wild mainly in Southern and North-western parts of the country, as well as on the major islands.

Our previous investigations highlighted some biological properties of polar extracts from the leaves (cauline and basal) collected in different times of the year, and the flowers of *I. tinctoria* grown wild around Acireale (Catania, Sicily, Italy) (1, 2). The obtained results indicated the good antioxidant and cytotoxic potential of the hydroalcoholic extract from the cauline leaves collected in January (It-J).

In continuation of our studies, the present work has been focused on the evaluation of the antioxidant and anti-proliferative activities of the phenolic-rich fraction, phytochemically characterized, obtained from cauline leaves of *I. tinctoria* picked in January.

The leaves were lyophilized and sequentially extracted with dichloromethane and 70% methanol at a temperature of 50 °C; then, the hydroalcoholic extract was suspended in distilled water and subjected to solvent-solvent partitioning with ethyl acetate in a separating funnel to obtain the phenolic-rich ethyl acetate fraction (ItJ-EAF). The total phenolic, total flavonoid and condensed tannin contents of ItJ-EAF were determined spectrophotometrically (3). The HPLC-PDA-ESI-MS qualitative analysis of the fraction led to the identification of 20 compounds belonging to the groups of flavonoids and phenolic acids, and revealed a phenolic profile superimposable to that of the crude extract. Flavone glycosides were quantitatively the most abundant constituents, and vicenin-2 resulted the main flavonoid.

The antioxidant properties of ItJ-EAF were examined by different *in vitro* systems: DPPH, reducing power, and ferrous ions chelating activity assays (3). Butylated hydroxytoluene (BHT) and ethylenediaminetetraacetic acid (EDTA) were used as reference standards. The fraction exhibited radical scavenging activity ( $IC_{50} = 0.6657 \pm 0.0024$  mg/ml) and reducing power ( $3.87 \pm 0.71$  ASE/ml), whereas it did not show any chelating activity.

The potential cytotoxicity of ItJ-EAF was tested using *Artemia salina* lethality bioassay (3); the fraction resulted non-toxic to brine shrimp larvae ( $LC_{50} > 1000$  µg/ml). The anti-proliferative effect of ItJ-EAF was evaluated *in vitro* on three human anaplastic thyroid carcinoma (ATC) cell lines (C643, CAL-62 and 8505C) by MTT assay. The fraction showed low activity against C643 cells, whereas a reduction of proliferation rate was observed in 8505C and CAL-62 cells in a concentration- and time-dependent way. At 48 h after treatment, about 65% growth inhibition was found in 8505C cells ( $p < 0.001$  vs untreated cells). The strongest anti-proliferative effect was observed in CAL-62 cells; particularly, after 48 h exposure, ItJ-EAF markedly inhibited cell growth, causing nearly 80% reduction of viability at the highest tested concentration (100 µg/ml) ( $p < 0.0001$  vs untreated cells).

Taken these results together, it can be concluded that *I. tinctoria* cauline leaves collected in January represent a source of phenolic compounds with antioxidant and anti-proliferative activity, which could be potentially used as natural agents for health promotion.

#### Acknowledgments

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#### 4.1 = PLANT EXTRACTS FROM TWO DIFFERENT POPULATIONS OF SULLA (*HEDYSARUM CORONARIUM* L.) SHOW DIFFERENT BIOACTIVITIES ON HUMAN KERATINOCYTES AND FIBROBLASTS

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Fabaceae are used from ancient time as forage, food, and crop rotation, but are underexploited as regards to their medicinal potentials. Plants belonging to this family are generally rich in secondary metabolites so they are a possible source of bioactive compounds with possible applications for human health. However, it is known that the phytocomplex of a plant can show significant variations in relationship to different year periods or climatic conditions (1).

In a screening of Fabaceae with potential applications for human health, we evaluated the bioactivity of extracts from aerial portions of the forage legume *sulla* (*Hedysarum coronarium* L.), which has been poorly studied from this point of view. Moreover, in order to evaluate the influence of geographical factors, we compared samples from two different Italian plant populations growing in proximity of Pisa and Ventimiglia.

Aerial portions of plants, collected at the two locations in the same period of year, were extracted with MTBE-EtAc-acetone (2). Extracts were then tested *in vitro* on matrix-degrading enzymes, human keratinocyte (HaCaT) and fibroblast (SC587) cell lines, and on differentiated adipocytes (Zen-Bio, Inc.).

A first assay of antioxidant power, based on the DPPH assay, showed stronger activity by the extract of Ventimiglia with respect to that of Pisa. Cell viability assays, conducted with MTT on keratinocytes and fibroblasts, showed low cytotoxicity of both extracts.

A series of cell-free assays showed lack of inhibition by both extracts on *jaluronidase*, while significant inhibition was observed on *collagenase* (3) at doses of 2.5 and 25 µg/mL, and on *elastase* at 250 µg/mL.

Type I collagen production, evaluated on fibroblasts by ELISA, revealed a significant induction by both extracts at 10 and 20 µg/mL, with a stronger effect of Pisa with respect to Ventimiglia.

Induction of lipolysis was evaluated on adipocytes by measuring glycerol release following triglyceride degradation (ZenBio Lipolysis Assay, Zen-Bio Inc.). The results showed significant induction in the range 50-200 µg/mL, with a stronger effect of Pisa over Ventimiglia at the lower doses.

The complex of data suggests possible applications of *sulla* in skin care treatments and as a food additive. However, data also highlight a series of differences in the strength of these effects, depending on the geographical origin of plants. Ventimiglia was more active in antioxidant activity and cytotoxicity, possibly depending on a collective effect of the phytocomplex. By contrast, Pisa was more effective in specific activities that might involve single active principles or particular classes of compounds.

Our results indicate that, although a plant species may possess specific bioactivities, the geographical origin of source materials can be a determinant factor for a fruitful exploitation of these virtues in clinical or industrial applications.

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1.1 = SESQUITERPENE COMPOSITION OF *DAUCUS VIRGATUS* (POIR.) MAIRE, A TUNISIAN ENDEMIC PLANT

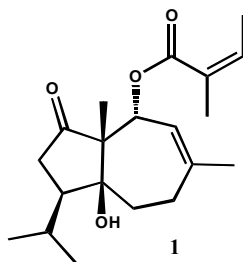
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The genus *Daucus*, belonging to the family Apiaceae (Umbelliferae), includes about 25 species widely distributed and commonly cultivated for their fleshy edible roots, among which the most notable cultivated member in terms of economic importance and nutrition is *Daucus carota* L. ssp. *carota*. Plants of the Apiaceae are rich in a number of secondary metabolites such as sesquiterpenes and phenylpropanoids with demonstrated therapeutic properties, primarily antibacterial, antioxidant, hepatoprotective, anti-tumoral and anti-inflammatory effects (1,2).

*Daucus virgatus* (Poir.) Maire is a species endemic to Northern Africa (Algeria and Tunisia). For our study, aerial parts of this species were collected in Tunisia and then sequentially extracted by cold maceration with solvents of increasing polarity: *n*-hexane, dichloromethane (CH<sub>2</sub>Cl<sub>2</sub>) and BuOH. Phytochemical investigation on CH<sub>2</sub>Cl<sub>2</sub> extract afforded the known daucane sesquiterpene vaginatin (**1**), identified by comparison of its spectroscopic data with those found in literature (3) and a number of new sesquiterpene derivatives, belonging to daucane, germacrane and elemanolide types.

In this communication, we will report the detailed stereostructures of the new compounds, elucidated on the basis of HR-ESIMS and 1D and 2D NMR experiments, and some preliminary data on their pharmacological activities.



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#### 4.1=VARIATION IN PHYTOCHEMICAL COMPOSITION AND ANTIOXIDANT PROPERTIES OF *CISTUS* SP.

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*Cistus* is a genus of rockrose plants (Cistaceae) containing about 20 species of perennial shrubs [1]. They are mostly considered native and endemic of Mediterranean flora. However, some common species, like *C. incanus* L. and *C. monspeliensis* L., are present in several parts of Europe wherever the favourable climate conditions make them similar to the Mediterranean ones. In particular, in Southern Sweden, Northern Danimarca, Bulgarian Black Sea and internal regions of Turkey, large populations have been found.

In the present study, the antioxidant properties of different ethanol extracts from some *Cistus* species, i.e. *C. creticus*, *C. incanus*, *C. salvifolius* and *C. monspeliensis*, collected in Turkey and Italy, were evaluated and related to their total polyphenol and flavanol contents [2]. The chemical composition was further determined by HPTLC (High Performance Thin Layer Chromatography) fingerprinting analysis in order to reveal the possible polyphenolic constituents involved in the antioxidant activity (Fig. 1) [3].

We report now that all the *Cistus* extracts possessed similar interesting antioxidant properties, being active in scavenging of both DPPH• and ABTS•+ radicals. Thereafter, according to data from HPTLC, the correlation analysis suggested that each *Cistus* extract possessed a peculiar composition in both polyphenols and flavanols, making a significant contribution to the antioxidant properties of the extracts. This methodological approach allows to hypothesize the involvement of specific constituents in the biological activity found.

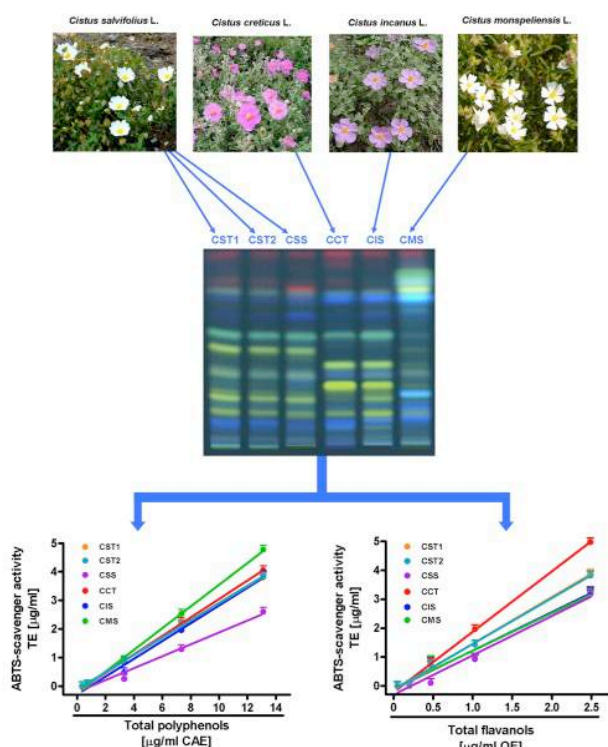


Fig. 1 Variation in phytochemical composition and antioxidant properties of *Cistus* sp.

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#### 4.1 = ANGIOTENSIN-CONVERTING ENZYME (ACE) INHIBITORY ACTIVITY OF *CITRUS MEDICA* L. CV. DIAMANTE (RUTACEAE)

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*Citrus medica* L. cv. Diamante (Rutaceae) is a cultivar of *C. medica* L., best cultivated in the warm and moderate warm climates of Calabria (Southern Italy) along the coast of high Thyrrenium. It is a shrub or a small evergreen tree, of irregular shape, with disordered growth and branching low, slow-growing, reaching 2.5-4.5 meters in height. The fruits are oblong, oval or ellipsoid, smooth surface, or more often wrinkled, often with a more or less large conical hillock to the stalk. In the full development, the fruits are very large, with a weight that can vary from 500-600 g to 1.5-2.0 kg and an average length of 20-30 cm. The fruits have a pale or greenish-yellow flesh, not very juicy, slightly sour or sweet. The peel is very rough, tough and exceptionally thick, constituting up to 70% of the fruit. Its colour varies greatly depending on the maturity period, rising from deep green when the fruit is unripe, to the golden yellow of the ripe fruit (1). Antioxidant, anti-inflammatory, hypoglycaemic, and anticholinesterase properties of *C. medica* L. cv. Diamante have been reported (2-4).

In the developed world, about 330 million people have hypertension, as do around 640 million in the developing world. The World Health Organization (WHO) rates hypertension as one of the most important causes of premature death worldwide and the problem is growing. In 2025, it is estimated there will be 1.56 billion adults living with high blood pressure (5). The Angiotensin-Converting Enzyme (ACE) plays a key physiological role in the control of blood pressure in the renin-angiotensin system, which mediates control of the extracellular volume and arterial vasoconstriction. ACE catalyzes the conversion of the decapeptide angiotensin I to the potent vasoconstrictor angiotensin II and degrades bradykinin, leading to the systematic dilation of the arteries and decrease in arterial blood pressure (6). Because of this dual role, inhibition of ACE is a key target for the treatment of hypertension.

In the present study, *C. medica* L. cv. Diamante extracts were investigated for their potential ACE inhibitory activity. Peels, pulp and seeds were extracted by maceration with 70% aqueous alcohol (96 h × 5 times). The ACE inhibitory activity was measured through the cleavage of the chromophore-fluorophore labelled substrate dansyltriglycine by ACE preparation from rabbit lung (EC 3.4.15.1) into dansylglycine, which was quantitatively measured by HPLC (7). The most effective extracts were the peels and pulp extracts, which gave 86.73 and 80.76% of inhibition at a concentration of 330 mg/ml, respectively. The peel extract was characterized by the presence of naringin, hesperetin, quercetin and apigenin as major constituents. It is known that the active site of ACE consisted of three parts: a carboxylate binding functionality such as the guanidinium group of arginine, a pocket that accommodates a hydrophobic side chain of C-terminal amino acid residues and a zinc ion (8). It was suggested that flavonoids showed *in vitro* activity via the generation of chelate complexes within the ACE active centre (9). The obtained results underline the potential health benefits of *C. medica* L. cv. Diamante and suggest that it could be used as a potential source with functional properties.

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#### 4.2 = SURVIVAL IN DIFFERENT HABITATS: EXTREME ULTRAMAFIC AND CALCAREOUS SOILS INFLUENCE ON *STACHYS RECTA* ESSENTIAL OILS COMPOSITION

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Ultramafic soils contain large amounts of magnesium and iron; they are also frequently rich in chromium, cobalt and nickel. They represent a difficult environment for plants to grow on, as they often contain toxic amounts of magnesium and nickel, whilst lacking mineral nutrients (like silicon, phosphorus, potassium and calcium); their dark colour attracts sunlight, which leads to higher temperature and drought conditions (1). On these soils, edaphic adaptation of plants is strongly evident: plants adapted to ultramafic soils exhibit morphological traits that often differ from correlated species growing on different substrates. They are often significantly smaller and show xeromorphic foliage, with reduced leaf size as the most apparent character (2).

The aim of this study was to assess the influence of edaphic adaptation to ultramafic soils on a non-morphological level, investigating its role in the composition of the essential oil produced by plants growing on these kind of soils. We investigated the composition of the essential oil (EO) of *Stachys recta* L. subsp. *subcrenata* (Vis.) Briq. collected between Nibbiaia and Gabbro (province of Livorno, Italy), grown on an ultramafic soil mainly deriving from the alteration of serpentinites. We compared the yield and the composition of this EO to the one we hydrodistilled from *Stachys recta* L. subsp. *recta* L., which had been collected in the same phenological state in La Gruzza, near Montemarcello (province of La Spezia, Italy), on calcareous substrate.

Whilst the yields of the two species were comparable, the composition profiles were very different: the EO extracted from *S. recta* subsp. *recta* was mainly rich in terpene compounds, which accounted for 93.8%; the species grown on the ultramafic soils, instead, had a volatile profile dominated by non-terpene derivatives, which accounted for 55.7%. In the species grown on the calcareous soil, the main constituents were germacrene D (18.8%), b-caryophyllene (17.7%), 1,8-cineole (15.9%) and  $\alpha$ -pinene (14.2%). The EO from *S. recta* subsp. *subcrenata* was mainly dominated by 1-octen-3-ol (38.2%),  $\alpha$ -cadinol (6.1%), (E)-3-hexen-1-ol (5.9%) and cadinene (5.6%).

Considering the extent of the differences shown by the two EOs, it is reasonable to assume that the ultramafic soil plays a central role in the plants' secondary metabolism, leading to a very different product.

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## 4.2 = TOWARDS A COMPILATION OF AN ATLAS OF PSYCHOACTIVE PLANT SEEDS

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All plants produce secondary metabolites as defense compounds against herbivores and microorganisms and among them several poisonous substances for humans and animals are present. A sub-class of toxins affects brain and nervous system performances, often mind-altering or stimulating. For instance, alkaloids from various plants of the family Solanaceae inhibit the muscarinic acetylcholine receptor in the brain, inducing hallucinations and deep sleep with vivid dreams. This action is well known since antiquity, e.g. it is described in the Odyssey regarding the wine adulterated by Circe with plants containing atropine or scopolamine (Od. IX, 230-243). Ointments with tropane alkaloids were diffusely used by witches during medieval times in Europe, while in Central and South America, psychotropic plants have been used by shamans for more than 4000 years, as reported in the sixteenth century by Hernandez (1).

Plants or substances which influence perceptions of space and time, and emotional feelings, are known as hallucinogens, psychomimetics or entheogens, and have been used for centuries during spiritual and healing rituals. Today poisoning in adults is often the result of deliberate action, since many psychoactive plants are used as recreational drugs and hallucinogens. The last decade has seen an increase of intoxications, mainly in young people experimenting "eco-drugs" as legal alternatives to illicit ones. These products are available in a variety of formulations such as pills or tablets, smoking blends, single plant material or extracts, powders, liquids, oro-dispersible strips and chewing gum (2, 3). In addition, during police inspections and seizures, seeds of plants with psychoactive properties are often found, some of which are under law restriction (eg. *Ipomoea violacea*, *Argyrea nervosa*, etc.). Seeds can contain psychoactive compounds on their own or can be used to grow plants rich in poisonous active metabolites.

In the present work, seeds of plants belonging to different families of native and exotic species have been reported. Among these, various genera of Solanaceae are found, such as *Atropa*, *Hyosciamus*, *Datura* and *Brugmansia*, plants rich in tropane alkaloids, viz. 0.3 - 1% in dried leaves and 0.6% in seeds for *D. stramonium*; 0.04 - 0.17% in leaves and up to 0.3% in seeds for *H. niger*. Some species, such as *Banisteriopsis caapi* (Malpighiaceae) or *Mimosa hostilis* and *Sophora secundifolia* (Fabaceae) are used to prepare infusions, often mixed with other plant extracts, of traditional importance during medicinal and religious rites. The bark of *B. caapi* (ayahuasca) is used (mixed with *Psychotria viridis* and other species) to prepare an infusion called ayahuasca, caapi or yagè, in different zones of South America. Seeds of *Sophora secundifolia* (mescal bean) are added to beer by Mescalero indians to fortify it (4). The dried bark of *Mimosa hostilis* (jurema preta) has a content of DMT (dimethyltryptamine) ranging from 0.03% (stem) to 1.7% (root) and has been used since the early medieval period to prepare a psychoactive decoction, traditionally used in northeastern Brazil and called vinho de jurema (3).

Only a few data are available on the morphological characterization of seeds from these psychoactive plants, frequently belonging to exotic taxa, and therefore, their correct identification is often difficult. In addition, chemical analysis, aimed at identifying toxic compounds is time consuming, much expensive, and made difficult by the scarcity of material to be analyzed (usually a few seeds).

This work represents a preliminary report to the compilation of a an atlas of psychoactive plant seeds, to be used as a diagnostic reference for officials engaged in the control of illicit drug market and for emergency personnel operating in cases of drug poisoning.

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#### 4.2 = AN INTERESTING CASE STUDY FROM AN ETHNOBOTANICAL SURVEY IN NORTH WESTERN PIEDMONT VALLEY

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An ethnobotanical survey in Germanasca valley, a side valley of the most famous Chisone valley (North Western Turin province), have reported some interesting and current uses of medicinal and aromatic plants.

The Germanasca Valley is traditionally part of the Waldensian Valleys and, probably because of the isolation of these communities over centuries, the ethnobotanical knowledge have been handed down and some important uses of medicinal plants have been jealously preserved until today.

. The people of the Germanasca valley for centuries had not contacts with other people nor opportunity for trading, thus inducing an evolution and a deeper knowledge of herbal medicine still present in the territory. The interviewed people have demonstrated to possess complex knowledge of local wild and cultivated plants.

Among all cultivated plants, one of the most interesting is undoubtedly *Trigonella caerulea* (L) Ser., Fabaceae family (Sweet Trefoil, Blue fenugreek) a plant of West-Asian flora (Iran) (1), naturalized in Europe on waste and arable lands and cultivated in alpine zone (2).

*T.caerulea* gives off an aroma of licorice, the aerial parts are mainly used as a tea for digestive purposes, while fruits and leaves are added to the bread dough to make it more digestible.

To the best of our knowledge, the lack of botanical and phytochemical data on this species, makes this plant an interesting case study to explain its ethnobotanical uses.

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## 4.2 ANTIOXIDANT RADIOPROTECTIVE POLYPHENOLS DERIVED FROM PLANT EXTRACTS

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During long-term space missions, astronauts are exposed to a variety of potentially harmful factors, including chemical contaminants and cosmic radiation (i.e. X-rays and  $\gamma$ -rays). Epidemiological studies have shown that exposure to medium-high doses of ionizing radiation increases the risk to develop several types of cancer, such as leukemia, brain tumor, thyroid, colon and lung (1). The exposure to ionizing radiation, are often associated with an overproduction of free radicals, mainly ROS (Reactive Oxygen Species) and RNS (Reactive Nitrogen Species), that the endogenous antioxidative systems fail to neutralize. The interaction of these agents with cells showed to cause alterations in the gene expression pattern, mutations, weakening of repair mechanisms inflammation to the gut mucosa premature aging, cancer, atherosclerosis, hypertension, Parkinson's disease, Alzheimer's disease, diabetes mellitus, colitis and rheumatoid arthritis (2). Currently, the use of chemical radioprotectors clinically tested i.e. thiols, aminothiols, thiadiazoles, benzothiazoles, is limited by their toxicity and their harmful health effects. For this reason, great attention is paid to the search for radioprotective compounds derived from plants. In this study the antioxidant properties of different natural plant extracts have been evaluated. For the preparation of the formulations have been used a pool of bioactive molecules most widely studied by our research group (3). Particular interest has been focused on green bioactive total extracts or molecules by food waste matrices. The analyzed matrices were the following: seeds of *Vitis vinifera* cvs Michele Palieri and Italia; rosemary leaves, olive patè, pigmented potatoes, pomegranate fruits and vineatrol. In the preparation of final formulations, among excipients in commercially available, have been used inulin and maltodextrin (4). The total antioxidant capability has been evaluated as *in vitro* scavenging activity against ABTS•+ monocationic radical. All samples were analyzed and compared for their ROS-scavenging properties before and after the treatment with ionizing radiations (140 cSv, calculated as the amount of radiations absorbed by astronauts after an hypothetical stay of three years in the space). Matrices selected for highest antioxidant properties will be also analyzed in *ex vivo* biological essays using blood red cell membranes in order to test the *in vivo* protective effects on a biological system. The chemical profiles of the matrices selected were evaluated, before and after the treatment, by HPLC-DAD and HPLC-MS-TOF analyses. *In vitro* results obtained showed that the treatment with a high dose of radiation does not induced significant modifications in the total antioxidant capability and phenolic composition of the extracts. This study has been supported by Agenzia Spaziale Italiana (ASI) PAPARD project contract number 2014-34-RO. These results could have relevant impacts for space mission, but they also could have interesting output in biomedicine. The intake of food supplements or pharmaceutical formulations rich in antioxidant bioactive molecules could help to protect patients against damages caused by medical therapies, like as imaging and diagnostics technologies and radiotherapy, that involve exposure to ionic radiations.

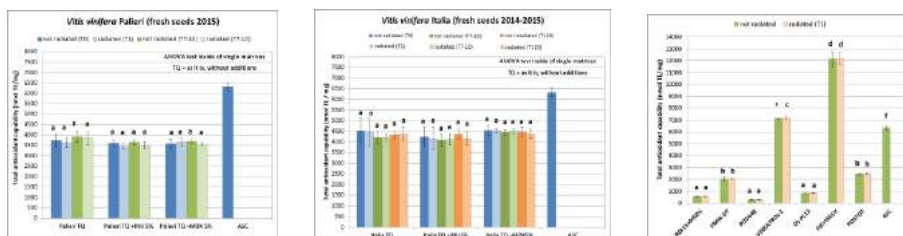


Fig. 1. Antioxidant activity of different plant extracts radiated (140 cSv) and not radiated.

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## 4.2 STILBENE PRODUCTION IN RESPONSE TO METHYL JASMONATE AND LIGHT IN CELL CULTURES OF SEVERAL *VITIS* SPECIES

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Stilbenes are secondary metabolites produced by grapevine in response to stress, which play the role of phytoalexins in the plant (1). Recently, stilbenes have received a great deal of attention both for their biological role in the plant and for their well-proven biological activities on human health (1). Plant cell cultures have been investigated for their potential as valuable system for stilbene production (2). Among different elicitors, methyl jasmonate (MeJa) was shown to be the most effective in stimulating stilbene biosynthesis in cell suspension cultures of cultivar Italia (2).

In the plant, the accumulation of stilbenes is induced by the presence of phytopathogenic fungi (3) or by abiotic stimuli as UV radiation (4). Different stilbenes produced by grapevine show a synergistic activity, furthermore the quali-quantitative composition of stilbene content determines the resistance against specific pathogens. The species *Vitis vinifera* includes several cultivars quite different from the genetic point of view, since they are exclusively agamic propagated. Consequently, each of these shows different levels of susceptibility to pathogens, such as *Botrytis cinerea*, *Aspergillus* spp. and *Plasmopara viticola* in relation to the different stilbene profile. Recently, great interest has been also addressed for species of *Vitis* popularly known as "american grapes", introduced in Europe as rootstocks due to their resistance against some important pathogens of *V. vinifera*. At present, resistance mechanisms of the american grapes are still unknown. The few available data has been obtained from samples cultivated in field, where the conditions are not fully under control.

Our study is focused on the biosynthesis of stilbenes, firstly resveratrol (the main exponent) and viniferins (resveratrol oligomers), in response to biotic stress in cell cultures of several *Vitis* species.

Stilbene content was compared in cell extracts obtained from *Vitis* spp. cell lines subjected to elicitation with MeJa, grown under continuous darkness or 16/8 h (light/dark) photoperiod. The main stilbenes involved in resistance against phytopathogenic fungi were identified and quantified by means of HPLC. Unexpectedly, the obtained results indicate that cultivars of *V. vinifera* have a higher stilbene content than the american grapes. For instance, *V. vinifera* cv. Malvasia, in response to MeJa, increases the production of total stilbenes from 2.58 to 15.58 mg/g DW while *Vitis rupestris* cv. du Lot increases from 2.80 to 8.97 mg/g DW. Moreover, cell lines grown under photoperiod accumulate higher levels of total stilbenes, both constitutively and in response to elicitation (e.g. Malvasia: from 8.69 to 37.81 mg/g DW).

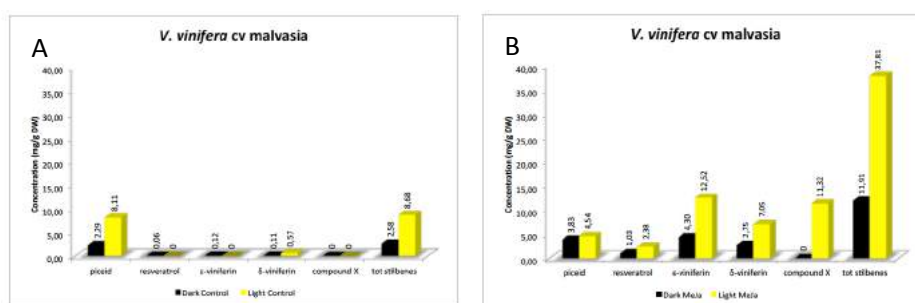


Fig.1. Combined effect of MeJa and light or dark on stilbene production in cell lines of *V. vinifera*: (A) not elicited cells; (B) elicited cells

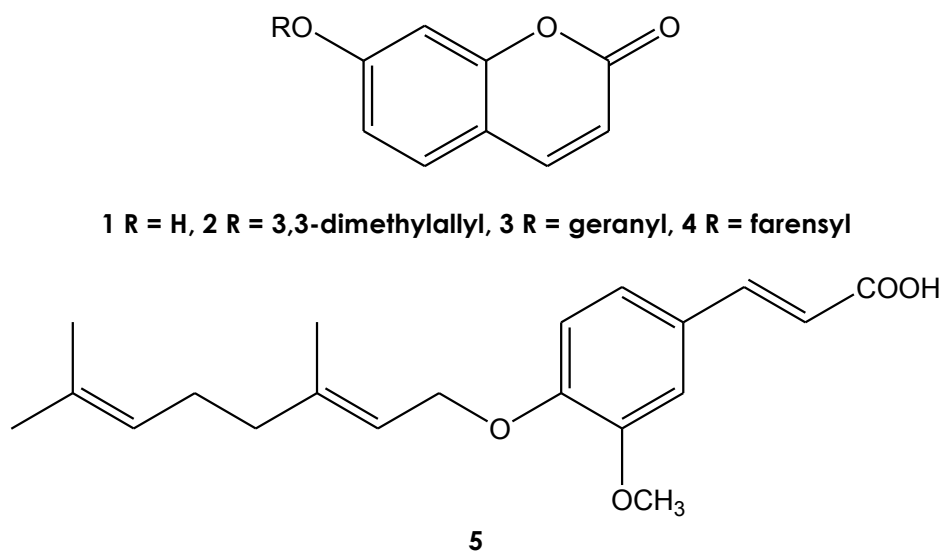
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## 4.2 = HPLC-UV-Vis QUANTIFICATION OF BIOLOGICALLY ACTIVE PHENYLPROPANOIDS IN DILL, ANISE, AND WILD CELERY

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The Apiaceae family (sin. Umbelliferae) comprise about 3700 species spread across 434 genera. Many members of this family are used for food, cosmetic, healthy, and medical purposes (1). In this context dill (*Anethum graveolens* L.), anise (*Pimpinella anisum* L.), and wild celery (*Angelica archangelica* L.) surely represent notable and explicative examples. Dill seeds are used worldwide as flavouring agents and spices, and also for medical purposes in the treatment of different diseases like jaundice, headache, boils, lack of appetite, stomach disorders, nausea, and liver syndromes (2). Anise seeds are sweet and very aromatic and have been used whole or roasted for the preparation of teas (alone or in combination with other aromatic herbs), and in a wide variety of regional and ethnic dishes. They have been also extensively employed in traditional medicine as a carminative agent and in the treatment of menstrual cramps and colic (3). Wild celery roots provide an essential oil rich in terpenes and coumarins, and extracts have been used for medical purposes as a tonic to relieve fatigue and asthenia, as a digestive, as anti-dyspepsia agent, and as an antispasmodic (4). An analytical strategy based on different extraction methodologies and HPLC with spectrophotometric (UV/Vis) detection has been developed to investigate the presence of, and to quantitate biologically active selected unprenylated and O-prenylated phenylpropanoids, namely umbelliferone **1**, 7-isopentenylcoumarin **2**, auraptene **3**, umbelliprenin **4**, and 4'-geranyloxyferulic acid **5** in dill (*Anethum graveolens* L.), anise (*Pimpinella anisum* L.), and wild celery (*Angelica archangelica* L.) (Fig. 1). Ethanol 96 % or 7:3 water / ethanol mixtures were seen to be the most powerful extraction solvents to perform "classic" maceration or ultrasound-assisted one in terms of yields in secondary metabolites. For dill and anise, umbelliprenine was found to be the most abundant prenyloxy secondary metabolite (143.98 µg/g of dry weight and 52.32 µg/g respectively), while in wild celery 4'-geranyloxyferulic acid recorded the highest concentration (1.94 mg/g of dry weight). Our experimental approach demonstrated to be efficient for the simultaneous identification and quantitation of the above mentioned prenyloxyphenylpropanoids in the title plant species that is reported herein for the first time in the literature.



**Figure 1.** Selected unprenylated and O-prenylated phenylpropanoids under study.

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#### 4.2 = NANODIAMONDS AND PLANT SECONDARY METABOLITES: NEW ENCOURAGING ANTINEOPLASTIC BIOTECHNOLOGICAL APPLICATIONS

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Detonation nanodiamonds (ND) have been recently applied, in biotechnological and medical field, as intracellular vectors for biomolecules, ions and drugs, because of their biocompatibility, reduced dimensions and high surface chemical interaction.

For the first time in literature, we coupled NDs with plant secondary metabolites (i.e. citropten) and confirmed their ability to penetrate in mammalian cells, remaining free in cytoplasm or embedded on nuclear membrane (1).

The well-known antiproliferative effects of citropten, on murine and human tumor cells, were greatly improved after its conjugation with NDs. In particular, we observed how nanomaterials could modify the bioactivity of the plant molecule, changing its pro-apoptotic and cytotoxic properties in antimitotic ones.

The antineoplastic mechanism, performed by plant drugs functionalized to ND carriers, would extraordinarily decrease the typical adverse effects that actual chemotherapies usually exercise on patients' healthy tissues. Indeed, it could limit the function of these biotechnological complexes just to cancer cells, presenting uncontrolled proliferation, excluding differentiated ones which are mitotically inactive.

All these evidences encourage and support the application of plant compounds conjugated with NDs for antitumoral therapeutical purposes (2).

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#### 4.2 = PRELIMINARY STUDY OF VARIABILITY OF ESSENTIAL OILS OBTAINED FROM SARDINIAN POPULATIONS OF *Teucrium capitatum* L. ssp. *capitatum*

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Essential oils are complex mixtures of organic compounds produced by approximately 17,500 aromatic species of plants belonging to different families, especially Myrtaceae, Lauraceae, Lamiaceae and Asteraceae. The synthesis and accumulation of EOs are associated with the presence of complex secretory structures such as glandular trichomes and resin ducts (1). A major difficulty in the studies on EOs is the chemical polymorphism, attributable to environmental (2,3) or genetic factors (4,5).

As part of a larger investigation on the *Teucrium* genus, the aim of the present study was to define the qualitative and quantitative intraspecific variability of the essential oils obtained from *T. capitatum* L. *capitatum* collected from different wild populations growing in the North-Western coast of Sardinia island (Italy).

*T. capitatum* ssp. *capitatum* is a suffruticose chamaephyte belonging to Lamiaceae family, characteristic of arid areas of scrubland and pastures, consolidated dunes, from 0 to 900 m above sea level. Flowering aerial parts have been collected from three different locations (Porto Palmas-TccPP; Capo Mannu-TccCM and Porticciolo-TccP), air-dried, and the EOs were obtained in agreement with (6) European Pharmacopoeia. Their chemical composition was analyzed by GC/FID and GC/MS. Chemical data analyses were performed by PCA and CA using IBM SPSS STATISTIC 20 software.

All essential oils were characterized by a very high percentage of hydrocarbon monoterpenes (80.4, 87.6, 62.8%, respectively) and oxygenated monoterpenes (13.5, 6.6, 11.4%), and by a lower percentage of sesquiterpenes (4.9, 5.6, 20.5%). The main compounds in the different populations are represented of  $\alpha$ -Pinene (34.0, 29.8, 20.4%), Limonene (15.6, 30.4, 20.6%),  $\beta$ -Pinene (12.2, 10.0, 7.6%) and (E)-Nerolidol (0, 0, 16.7%). This oxygenated sesquiterpene, that shows a wide range of pharmacological and biological activities (7,8), was only found in the oil obtained from TccP. There are few available data on the chemical composition of essential oil of this species (9,10), and the presence of (E)-Nerolidol is confirmed by Antunes et al., 2004 (11).

The analysis carried out with CA was performed with single linkage in order to identify statistically differences between the taxa under study. On the basis of their chemical composition the analyzed populations were grouped in two clusters, one constituted of TccPP and TccCM and a second represented only by TccP. These differences could be attributable to edaphic factors that are similar in TccPP and TccCM, whereas they are different in TccP.

This is a first step for a future broad-spectrum research on Sardinian populations of *T. capitatum capitatum* with the aim to identify chemical differences and to elucidate the genetic and ecological factors that produce them.

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#### 4.2 = STUDY OF CORRELATION BETWEEN *SEDUM HISPANICUM* L. PHENOLOGY AND ITS TOTAL FLAVONOID CONTENTS.

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*Sedum hispanicum* L. (Crassulaceae) is a succulent herb characterizing the habitat 6110\* "Rupicolous calcareous or basophilic grasslands of the *Alyso-Sedion albi*", in which it seems to get a principal role in the covering of gypsum outcrops. Besides which, *S. hispanicum* is often reported in ethnobotanical interviews for its phytoalimurgic use (1), and nevertheless this properties are poorly examined in literature. Therefore, is worth to attempt a preliminary analysis of its phytochemical characterization. The study areas were the SCI IT4050001 Gessi Bolognesi, Calanchi dell'Abbadessa, and the SCI IT4050027 Gessi di Monte Rocca, Monte Capra e Tizzano, where *S. hispanicum* grows abundantly on gypsum outcrops. Within SCI IT4050001, sampling effort was extended to a metapopulation of *S. hispanicum* spreading over four separate gypsum outcrops, and each of these sites was studied distinctly, by virtue of their physical distance. So, a fifth gypsum outcrop, included in SCI IT4050027 and separated from SCI IT4050001 by an urban landscape, was considered as outside reference point. For the phenological study, survey consisted of 20 plots randomly placed within the patches of the habitat 6110\*. For each plot, the reproductive phenophase of 20 *S. hispanicum* entity was recorded, according to the BBCH (Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie) scale (2). The surveys were carried out every 10 days, from March to June 2015. For the phytochemical analysis, was sampled the aerial part of *S. hispanicum* in phenophases 49 (=vegetatively propagated organs have reached final size), 57 (=petals visible, flower buds still closed), 65 (=full flowering: 50% of flowers open, first petals falling) and 69 (=end of flowering: all petals fallen). Samples were freeze-dried and crushed, in order to proceed with determination of their total flavonoid amount, according to Chen *et al.* (3). Results showed generally the same patterns within every sampling site. *S. hispanicum* flowering cycle in 2015 lasted approximately 34 days, from May 9 to June 12, as well as was recorded in 2013 (4). During May, *S. hispanicum* advancement seems precocious compared to 2013, in response to mean temperature that in May 2015 was 1,7°C higher than in May 2013. The maximum contents of total flavonoid corresponds to the phenophase 65, i.e. full flowering. Interpolation of phenophase 65 in Fig. 2 allows you to infer the day when *S. hispanicum* shows its full flowering and, in parallel, the maximum content of total favonoids: this was the day 150, May 31, in 2015; and the day 153, June 3, in 2013. The difference, influenced by annual temperature, is low ( $\pm 3$  days), so this study, further elaborated, might provide a instrument for the prediction of the full flowering days, during which the plant contains the greatest amount of total flavonoids.

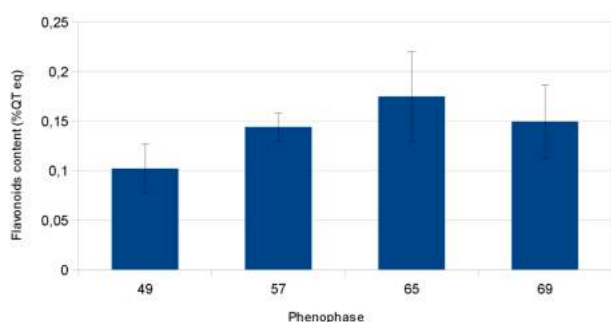


Fig. 1. Total flavonoid content as function of phenophase. The amount of flavonoids was expressed in terms of QT Equivalent: g quercetin / g extract)

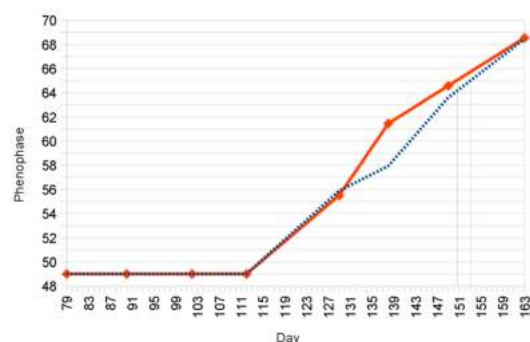


Fig. 2. Flowering development (2015, red line; The amount of flavonoids was expressed in terms of QT Equivalent: g quercetin / g extract) (Quercetin

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#### 4.2 = A PHYTOCHEMICAL APPROACH TO THE TAXONOMY OF PRICKLY JUNIPER (*JUNIPERUS OXYCEDRUS* S.L.) IN ITALY: A CRUCIAL CONTRIBUTION AT THE MEETING POINT OF TWO CRYPTOSPECIES

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The genus *Juniperus* L. consists of about 60 species, mainly occurring in the northern hemisphere, and it is divided into three sections: *J. sect. Csryocedrus* Endl., *J. sect. Juniperus*, and *J. sect. Sabina* Spach (1).

*Juniperus deltoides* R.P.Adams (2) was described as a new species of *J. sect. Juniperus* in 2004, based on a different essential oil composition compared to *J. oxycedrus* L. According to Adams (2, 3), *J. deltoides* is not clearly morphologically discernable from *J. oxycedrus* so that it was defined as a cryptospecies. However, clear differences in phytochemical composition were highlighted (2, 3), in the relative quantities of some compound shared by both species, and in some private compound.

The occurrence of both *J. oxycedrus* and *J. deltoides* was reported in Italy (4): the first one in the west portion of Liguria and Sardinia, the second one in peninsular Italy, from Tuscany to Calabria. Recently a population of *J. oxycedrus* s.str. was recorded in Tuscany (5), based on some putatively discriminant morphological characters (4). In addition to this framework, in Italy also *J. macrocarpa* Sm. (an ecotype adapted to sandy coastal dune habitats) occurs, a taxon considered as a subspecies of *J. oxycedrus* for a long time (6).

Recent studies on the essential-oil and terpene compositions of different populations of *Juniperus* have shown significant correlation with respect to the geographic distribution (6, 7). Furthermore, the significance of leaf essential oils of *Junipers* as taxonomic markers at the infraspecific level has been shown (8). In this study a phytochemical analysis, in order to clarify the taxonomy of *J. oxycedrus* s.l. in Tuscany, was performed.

The composition of the essential oils, extracted from leaves of three populations of *Juniperus deltoides/oxycedrus* collected Tuscany was determined by GC-FID and GC/MS analyses. Totally, 94 compounds were identified, representing 96.51–98.14% of the total oil composition. The oils were dominated by monoterpenes (average content of 63.22%), which are characteristic oil components of species belonging to the section *Juniperus* (9). Two monoterpenes:  $\alpha$ -pinene (on average 34.43%) and limonene (32.22%), resulted the main constituents of the essential oils in all the sampled populations. Integrating our results with data from the literature, two different matrixes (species  $\times$  percentage; species  $\times$  presence/absence of chemical compounds) were subjected to multivariate analysis (PCoA). Results revealed that the samples from Isola d'Elba and Monte Pelato (Livorno) were clearly attributable to *J. oxycedrus* s.str., whereas the third population sampled in the province of Siena, showed a chemical composition typical of *J. deltoides*. Our preliminary results suggest that in Tuscany both *J. deltoides* and *J. oxycedrus* s.str. occur. To better understand the distribution pattern of the two cryptospecies, a more complete sampling is being currently carried out, both in Tuscany and in other Italian regions.

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#### 4.2 = MODERN AND ANCIENT WHEAT (*TRITICUM* SPP.) CULTIVARS: COMPARISON REVEALS INTERESTING DIFFERENCES IN PHENOLIC COMPOUNDS PROFILES

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A project of a Germplasm Bank has been carried out at the Interdepartmental Research Centre for Agri-Food Biological Resources Improvement and Valorization (Biogest-Siteia, UNIMORE), aimed to wheat long-term conservation, valorization and exploitation. Nowadays, the Germplasm Bank keeps more than 150 accessions, including ten ancient cultivars, which have been all morphologically characterized, according to "Descriptor List for Wheat" (1).

Identification of the secondary metabolites responsible for end-use quality of wheat may be considered a key-factor for all stake-holders, i.e. breeders, producers, grain handlers, millers, bakers and final consumers (2, 3, 4). Recent studies have focused on phenolic content of whole wheat, due to their antioxidant, anti-inflammatory, anti-carcinogenic properties (3, 5, 6), highlighting furthermore that important differences in metabolic profile occur among cultivated varieties. The intense breeding programs developed since the 50s years of the last century, resulted in the selection of new wheat cultivars with high yield, less subjected to crop lodging and able to provide high strength flour, with a high aptitude to withstand mechanical processing. This resulted, in turn, in the replacement of local varieties and mostly in the decrease of the genetic variability. Notwithstanding, there has been recently a renewal of interest in the so-called "ancient grains", cultivars with a distinct regional connotation, used in the last century or even earlier.

In the present study, nine ancient varieties of wheat: *Autonomia*, *Gentil rosso*, *Grano del miracolo*, *Inallettabile*, *Leone aristato*, *Mentana*, *Poulard di Ciano*, *Risciola*, and *Terminillo* were compared with some modern cultivars. Extracts of whole meal flour were subjected to separation by LC-ESI-MS-TQ and phenolic compounds, i.e. hydroxycinnamic acids, hydroxybenzoic acids, flavones, and vanillin, were determined. Total phenolic content, expressed as gallic acid equivalents, of each sample was determined using the Folin-Ciocalteu procedure.

The ancient cultivars of wheat showed a phenolic content significantly higher in comparison with the modern cultivars. The ancient variety *Poulard di Ciano* (also known as *Turgido Reggiano*) was widely cultivated in the northern Italy and in Emilia Romagna region in the first years of XX century and can be considered the real autochthonous wheat of Reggio Emilia province. It showed the highest total phenolic content, followed by *Risciola*, *Grano del Miracolo*, *Terminillo*, and *Leone Aristato* (ranging between 0.95 and 0.70 mg/g, expressed as gallic acid equivalents). The lowest value was recorded for the modern cultivar *Zanzibar* (0.33 mg/g, as gallic acid equivalents). Many differences among cultivars emerged when quali-quantitative analysis were carried out. *Terminillo* showed statistically highest concentration of ferulic acid, followed by *Risciola*, *Grano del Miracolo*, *Gentil Rosso*, *Poulard di Ciano*, and *Leone Aristato*, while *Blasco* (taken as reference for modern cultivars) had the lowest concentration. Syringic acid presented the highest value for *Risciola*, *Leone Aristato*, and *Mentana*. *Poulard di Ciano* showed very high concentrations of sinapinic acid, while *Blasco* had the lowest content. Vanillin and vanillic acid, two biochemically correlated 4-hydroxy-3-methoxyphenyl- derivatives, are very liked flavoring agents. They were found in *Grano del Miracolo* at the highest concentrations. *Risciola*, as well, showed high content of vanillin, followed by *Inallettabile*, while vanillic acid was present in high concentration in *Gentil rosso*, *Terminillo*, and *Leone aristato*. *Blasco*, also in this case, did not show high concentration of these metabolites. A complete comparison with all the cultivars stored in the Germplasm Bank is currently in progress.

In the light of these first results the nutritional characteristics of the caryopsis, rich in micronutrients and compounds with nutraceutical activity, and the opportunity of increasing a more sustainable model of agriculture, make the ancient varieties of wheat an invaluable heritage to safeguard.

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4.2 = PHYTOCHEMICAL ANALYSIS AND ANTIOXIDANT PROPERTIES OF *EUPHORBIA DENDROIDES* L. LATEXANTONELLA SMERIGLIO<sup>1,2</sup>, SALVATORE RAGUSA<sup>3</sup>, MARIA TERESA MONFORTE<sup>1</sup>, VALERIA D'ANGELO<sup>1</sup> AND CLARA CIRCOSTA<sup>1</sup>

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The genus *Euphorbia* L. (Euphorbiaceae) is one of the largest genera of flowering plants, with approximately 2000 species. It has a worldwide distribution with great diversity of growth forms, including many xerophytic species. Despite this vast vegetative variation, the entire genus is united morphologically by the possession of a cyathium, a pseudanthial inflorescence that resembles a single flower (1,2). All *Euphorbia* species have white latex that is often rich in secondary metabolites. The presence of biologically active compounds in the latex (3, 4), contributes to the pharmacological properties of many *Euphorbia* species (5, 6). *Euphorbia dendroides* L. is a woody plant with latex, taking the form of a rounded shrub or small tree up to 3 m tall (Fig. 1). Grows naturally along the Mediterranean coasts, up to an altitude of about 700 m above sea level, mainly in Spain, France, Italy (including Sardinia, Sicily and smaller islands). There are still no phytochemical and biological studies on the *Euphorbia dendroides* latex. The aim of this study was to evaluate the antioxidant properties and phytochemical constituents of the latex collected from *Euphorbia dendroides* L. The white latex samples were drained into glass tubes by cutting the stem apices of plants growing naturally in Sicily, locality Masse (Messina). The antioxidant activity of *E. dendroides* latex have been evaluated in a series of *in vitro* tests such as DPPH, TEAC and ferric reducing/antioxidant power (FRAP) assays. Total phenolics (TPC) and total flavonoids (TF) content was also determined. From the results is evident that *E. dendroides* latex has significant antioxidative activity, as measured by DPPH assay ( $560.74 \pm 15.983$   $\mu\text{mol}$  of Trolox equivalent (TE)/100g FW). Reactivity towards ABTS radical cation and ferric-reducing antioxidant power (FRAP) values were  $428.64 \pm 12.882$   $\mu\text{mol}$  of TE/100g FW and  $1151.38 \pm 50.124$   $\mu\text{mol}$  of TE/100g FW, respectively. Latex contains a significant amount of phenolic compounds of which  $5241.49 \pm 88.241$  mg of Rutin equivalent (RE)/100 g FW of total flavonoids. Phytochemical analysis performed by GC-MS revealed that in *E. dendroides* latex the triterpenoids were found the most abundant among the identified compounds, following by ergostane-type steroids, sesquiterpenes and monoterpenes.

Fig. 1a-1b. *Euphorbia dendroides* L.

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## 4.2 = CHITOLIGOSACCHARIDES (COS) ENHANCE XANTHONE PRODUCTION IN *HYPERICUM PERFORATUM* ROOT CULTURES

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St. John's wort (*Hypericum perforatum* L.) is a medicinal plant widely used in folk medicine since ancient times, with a long list of medicinal uses. Most of the research on *H. perforatum* has been focused on the aerial organs of the plant, and only recently the root has been recognized as an attractive source of secondary metabolites of pharmacological interest [1]. The root of St. John's wort contains xanthenes, although at very low levels [2;3]. Xanthenes are a wide and structurally diverse group of polyphenol with several pharmacological properties [4]. We have recently demonstrated that *H. perforatum* root cultures constitutively produce xanthenes at higher levels than the root of the plant and that they respond to chitosan (CHIT) elicitation with a noteworthy increase in xanthone production. Among elicitors, CHIT is one of the most used to increase the biosynthesis of plant phytoalexins, including xanthenes. However, CHIT is insoluble in neutral water as well as in most organic solvents, therefore, it is commonly dissolved in water acidulated with acetic acid [5], which greatly limits its application. Furthermore we recently demonstrated that acetic acid strongly affect xanthone biosynthesis, altering the effect of CHIT [5]. To overcome these problems, in this study, water-soluble oligosaccharides obtained through enzymatic digestion and deacetylation of chitosan have been tested. Initially, chitooligosaccharides (COS) have been administered to the root cultures following the same protocols used for CHIT elicitation. Fifteen days after the addition with COS a 100% increase in the production of total xanthenes was obtained (from 4.4 to 9.8 mg/g DW). The most represented xanthenes were paxanthone and 5-O-methyl-2 deprenylrheediaxanthone B. Additional experiments were carried out to optimize the elicitation protocol with COS. In particular, it was tested the effect of different concentrations of COS (from 50 to 800 mg/l) and the residence time of the elicitor in the culture medium (from 5 to 25 days). The best results were obtained with 400 mg/l COS and with 25 days of residence time (530% xanthone increase). The antifungal activity of the methanol extracts of roots treated with COS was evaluated on several human pathogenic fungi, including *Candida albicans*, *Microsporium gypseum* and *Trichophyton mentagrophytes*. Several extracts showed interesting MIC values (16-64), suggesting a possible use as antifungal agents. Gene expression studies are in progress to evaluate the contribution of some key genes in polyphenol biosynthesis, such as phenylalanine ammonia lyase (PAL, involved in the biosynthesis of all phenols), chalcone synthase (CHS, involved in both xanthenes and flavonoids) and benzofenone synthase (BPS, specifically involved in xanthone biosynthesis). Preliminary results on COS-elicited roots shown a sub-expression of PAL and an overexpression of BPS, while the CHS expression does not shown significant alterations with respect to the control roots.

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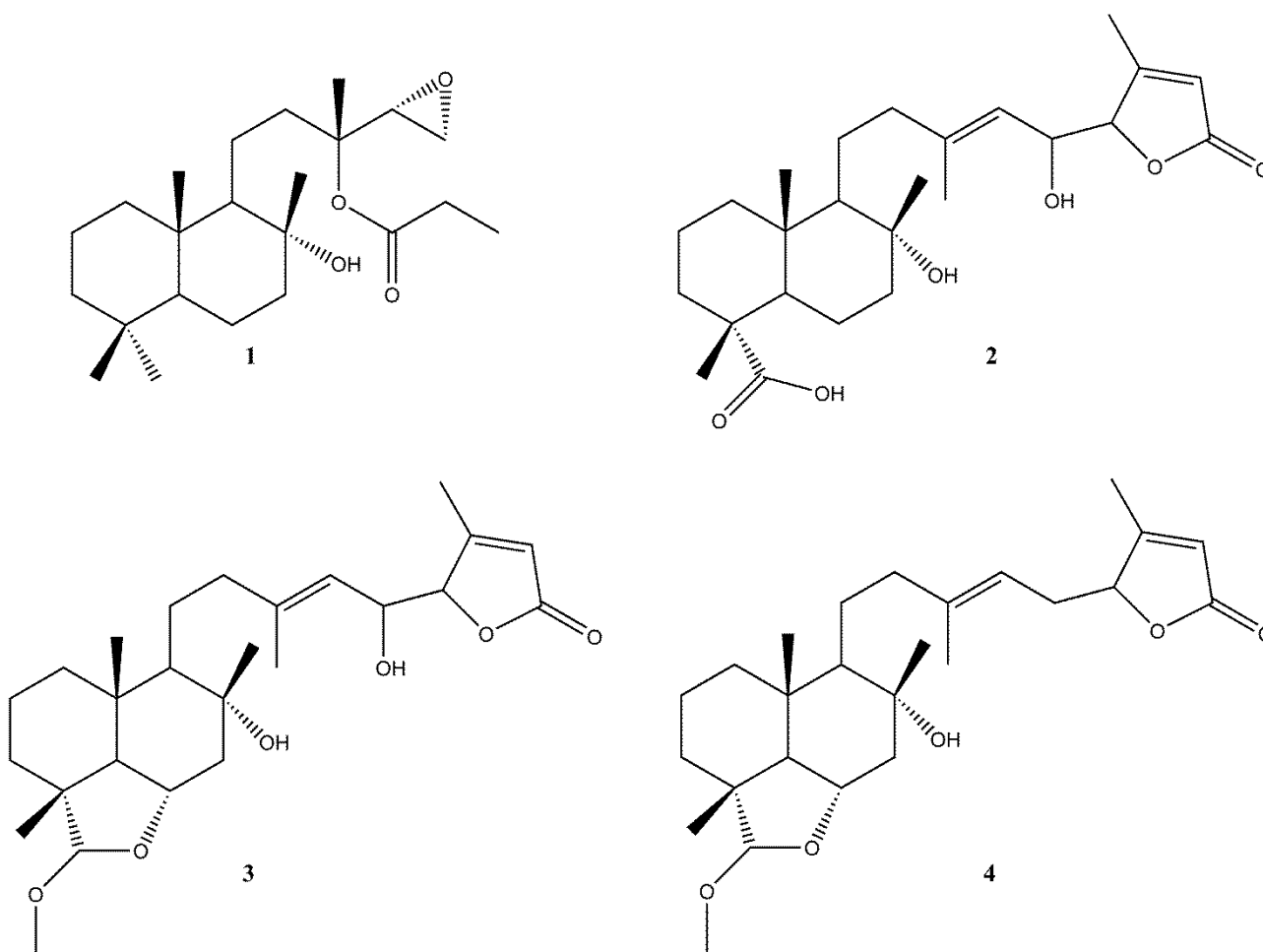
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4.3 = SECONDARY METABOLITES FROM *SALVIA TINGITANA* ETL. (LAMIACEAE).ANGELA BISIO<sup>1</sup>, ANITA PARRICCHI<sup>1</sup>, NUNZIATINA DE TOMMASI<sup>2</sup><sup>1</sup>Department of Pharmacy, University of Genova, Viale Cembrano 4, 16147 Genova, Italy; <sup>2</sup>Department of Pharmacy, University of Salerno, Via Giovanni Paolo II 132, 84084 Fisciano (SA), Italy.

In the course of our study on *Salvia* species for the identification of potential new crops with diversified value in the Mediterranean agriculture(1-3), we considered *Salvia tingitana* Etl. The species is a perennial shrub original from the Arabian region (4) and cultivated as an ornamental plant.

The surface extract, obtained by rinsing the fresh aerial parts with CH<sub>2</sub>Cl<sub>2</sub> was subjected to repeated column chromatography on Sephadex LH-20, silica gel, SPE separations and to semi-preparative RP-HPLC, and afforded four diterpenes and five known sesterterpenes, along with one new labdane diterpene (**1**) and three new sesterterpenes (**2-4**), identified by IR, NMR, including TOCSY, COSY, HSQC, HMBC and ROESY experiments, ESI-MS and HR-MS analysis.

Fig. 1. New compounds isolated from *S. tingitana*

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4.3 = COMPOSITION OF THE ESSENTIAL OILS IN *CALENDULA SUFFRUTICOSA* (ASTERACEAE) FROM SICILY

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The genus *Calendula* L. (*Calenduleae* Cass., *Asteraceae*) includes several species having medicinal properties. Pharmacological studies reveal, for instance, that *C. officinalis* L. exhibits antibacterial, antiviral, antiinflammatory, antitumor and antioxidant properties; *C. arvensis* L. has antibacterial, antiinflammatory, antimutagenic and haemolytic activities; *C. suffruticosa* Vahl has antimicrobial activity, especially against pathogenic microorganisms as *Pseudomonas syringae* Van Hall, *Pseudomonas fluorescens* (Flügge) Migula, *Xanthomonas campestris* (Pammel) Dowson and *Agrobacterium tumefaciens* Smith & Townsend. Nevertheless, despite a long tradition of use of the above species, and others, the genus has not been explored properly (1).

In this contribution, the chemical composition of the essential oils in flowers, leaves and seeds of *Calendula suffruticosa*, from Castelmola on the Peloritani Mountains (E Sicily), is described.

*C. suffruticosa* is a perennial and highly variable species growing in the Mediterranean basin: in it, a lot of subspecies and some varieties are distinguished (2). In the Mediterranean area, according to Greuter (3) nine subspecies occur on the whole, two of which also occur in the Italian Peninsula and in Sicily. Namely, these are *C. suffruticosa* subsp. *suffruticosa* and *C. suffruticosa* subsp. *fulgida* (Raf.) Guadagno. In its intraspecific variability, the species has a distribution ranging from the south-western Mediterranean basin – North Africa (Tunisia, Algeria and Morocco), southern coasts of Portugal and Spain, Italy (s.l.) and Malta – to the south east - Greece and western Turkey. In Italy besides in Tuscany and Marche, it is common in Basilicata, Calabria and Sicily, from the coast up to 500 m altitude. In Sicily, both the subsp. *suffruticosa* and *fulgida* occur. The results here reported on a chemical study of a population is referred to subsp. *fulgida*, but only temporarily, since there are some distinctive characters that suggest a taxonomic position independent on both the two subspecific taxa so far recognized for the Sicilian flora (4).

The essential oils were obtained by hydro-distillation and were analysed by GC and GC-MS.

On the whole, the essential oils are characterized by the presence of oxygenated sesquiterpenes, particularly  $\beta$ -eudesmol, and fatty acids, with hexadecanoic acid as the compound representative of this fraction and present in a very high concentration in the seeds. Along with this, essential oils from flowers and leaves also contain the diterpene neophytadiene while the flowers are the only parts of the plant in which sesquiterpene hydrocarbons are found.

Owing to the chemical composition of other subspecies of *C. suffruticosa* is not still detected, the data obtained for the Castelmola population are a basic comparison term and a support to delimit the whole intraspecific taxa of the concerned species and besides to a more deeply taxonomic evaluation of the analyzed Sicilian population.

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4.4 = CHEMODIVERSITY OF THE ESSENTIAL OIL FROM LEAVES OF *ABIES NEBRODENSIS* (LOJAC.) MATTEI

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*Abies nebrodensis* (Lojac.) Mattei is the most representative case of the Sicilian forest flora. It is an endemic relict species, at risk of extinction, and its natural population consists of thirty individuals distributed discontinuously in a small area of the territory of Polizzi Generosa, inside Madonie Natural Park (Sicily).

Besides to the small number of individuals, the risk of extinction is also due to the reduced number of plants able to produce fertile cones (1).

From the morphological point of view, the individuals of *A. nebrodensis*, according to several authors (2, 3, 4), share many features with three other Mediterranean species: *A. alba* Miller, *A. cephalonica* Loudon and *A. numidica* de Lannoy ex Carrière.

Recently Parducci & al. (5) used microsatellite markers of chloroplast DNA and isozymes (6) to investigate the genetic structure of populations of *A. alba*, *A. cephalonica*, *A. nebrodensis* and *A. numidica*. By the comparison of chloroplast haplotypes, these authors have verified that *A. nebrodensis* differs from the other three species.

As activities of the project "Preservation of *Abies nebrodensis* and restore of Geraci Siculo peatlands", the chemical composition of essential oils, obtained from leaves of Madonie fir, was compared with the ones of essential oils derived from Eurasian species studied up to now [*A. alba*, *A. borisii regis* Mattfeld, *A. bornmulleriana* Mattfeld, *A. cephalonica*, *A. cilicica* subsp. *cilicica* (Antoine & Kotschy) Carrière, *A. cilicica* subsp. *isaurica* Coode & Cullen, *A. equi-trojani* (Asch. & Sint. ex Boiss.) Coode & Cullen, *A. nordmanniana* (Steven) Spach, *A. numidica*, *A. sibirica* Ledeb] in order to obtain more information on the specificity of *A. nebrodensis* respect to these other taxa of the *Abies* genus.

The analysis shows that the oil of *A. nebrodensis* is almost completely (99.5%) constituted of nine compounds mainly monoterpene hydrocarbons, the most abundant being the  $\beta$ -pinene (48.8%), the  $\alpha$ -pinene (17.5 %) and the camphene (12.7%).

The peculiar features of the *A. nebrodensis* oil, compared to the ones obtained from other species, are both the absence of limonene always present in high concentrations in the other taxa, that the high content of  $\beta$ -pinene, the highest one of the species compared.

The results of this study provide further contribution to the diversity of *A. nebrodensis* respect to other congener taxa, also morphologically similar.

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#### 4.5 = INVESTIGATION OF CHEMICAL COMPOUNDS IN THE VOLATILE FRACTION OF FRESH LEAVES AND FLOWERS OF THREE NEW *PASSIFLORA* HYBRIDS

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The genus *Passiflora* (Tribe Passiflorae, Family Passifloraceae) comprises 4 subgenera *Astrophea*, *Decaloba*, *Deidamioides* and *Passiflora* [1] and more than five hundred species of climbing plants, distributed in a wide range of habitats. *Passiflora* species may be suitable for screening for bioactive molecules, since ethnobotanical use, chemotaxonomic information and observation of the interaction of the plants with their environment have been suggested as selection criteria for potential sources of natural molecules. Some species are known by their edible fruits, which have a distinct flavor and aroma that promote their consumption and their applicability in the food industry [2,3]. Moreover, other species are biologically active, and are well-known because of the tranquilizer and sedative effects of their leaf extract. *Passiflora* species and hybrids are very attractive both for the horticultural sector and for herbal and pharmaceutical industry. There are few reports on the chemical composition and the oil compounds responsible for the *Passiflora* properties [4]. In this work we evaluated the chemical composition of the volatile fractions of three new *Passiflora* hybrids, bred and cultivated in Italy [5], exploring fresh flowers and leaves. Several compounds were identified using gas chromatographic analysis and differences in qualitative and quantitative composition of the volatiles, from the hybrids and from the two plant organs, were highlighted. The main bulk of constituents were found to be hydrocarbons and alcohols, while terpenes and aldehydes were more abundant in leaves. The presence of eugenol in one hybrid could be related to the characteristic fragrance (MISTAKE). Comparing the data with those available from literature, many of the identified constituents have been previously found.

The remarkable health benefit and aroma exhibited by *Passiflora* essential oil can be incorporated into food, drug or cosmetic formulations to reduce skin aging and to increase flavor of foods or cosmetics. The analysis of the chemical compounds showed differences in the volatile pattern of the three *Passiflora* hybrids investigated from the vegetative and reproductive organs, revealing the presence of valuable compounds.

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#### 4.5 = THE MAIN CONSTITUENTS OF CORYLUS AVELLANA KERNEL IN WILD TYPE AND CULTIVARS GROWING IN ITALY

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*Corylus avellana* L. (hazelnut) is one of the most important tree nut crops with a yearly production of about 872,000 ton of in-shell nuts with a cultivated area of approximately 604,000 ha (average 2008–2012) (1). Among nut species, *C. avellana* plays a major role in human nutrition and health (2). It has a special composition of fats (mainly oleic acid), protein, carbohydrate, dietary fiber, vitamins (vitamin E), minerals, phytosterols (mainly  $\beta$ -sitosterol), antioxidant phenolics polyphenols, and squalene (2). In particular, 10-20% of kernel are carbohydrates (3), 1-3% cellulose and pectin (4), 1-2% organic acids (malic acid being 80% of the total organic acid content) (2) and 10-24% proteins (mainly essential aminoacids) (5). The fatty acid (60%) of *C. avellana* kernel has a profile similar to that of the olive oil (6), the oleic (C<sub>18:1</sub>)-acid and linoleic (C<sub>18:2</sub>) acid are the main fatty acids in both the oils (7). The interest in the fatty acid composition of vegetable oils is actually increasing because the most important neutral lipid in many vegetable oils included in the human diet influences the total fat and cholesterol absorption in the lumen (8). However, nutritional and chemical composition of *C. avellana* is in relation with the variety, geographical origin, climate, ecology of the species as well as cultural applications (9). In this context, the main objective of this research was to investigate the kernel composition in *C. avellana* wild plants and orchards from different geographical areas (north-west and central Italy). The research was carried out in *C. avellana* plots (wild plants and orchards) growing in the north-west of Italy (Piedmont and Lombardy, N<sub>1</sub>, N<sub>2</sub> and N<sub>3</sub>) and in central Italy (Lazio, C<sub>1</sub>, C<sub>2</sub> and C<sub>3</sub>). In particular, N<sub>1</sub> and N<sub>2</sub> were *C. avellana* cv. 'Tonda Gentile Trilobata' orchards, N<sub>1</sub> growing in Alba (CN) at 172 m a.s.l., N<sub>2</sub> in Lequio Berra (CN) at 715 m a.s.l., C<sub>1</sub> and C<sub>2</sub> growing in Nepi (VT) at 215 m a.s.l. Both N<sub>1</sub> and N<sub>2</sub> received routine horticultural care suitable for commercial production, including fertilization, weed and pest control. They were not subjected to controlled irrigation. N<sub>3</sub> was a wild *C. avellana* populations growing at Zerboldò (PV). C<sub>1</sub> and C<sub>2</sub> received routine horticultural care and were subjected to different drip irrigation systems: C<sub>1</sub> was subjected to surface drip irrigation while C<sub>2</sub> to sub-surface drip irrigation. C<sub>3</sub> was a wild population growing in Capranica (VT) at 400 m a.s.l. Immediately after nut fall (beginning of September in all the populations) randomly selected samples were hand-harvested in order to evaluate the kernel composition in all the considered plots. In particular, kernel moisture, ash, protein, fat and carbohydrate content were determined. The results showed a 89% higher kernel moisture (mean value of N<sub>1</sub>, N<sub>2</sub> and N<sub>3</sub>) in the three kernel samples from the north-west of Italy compared to the samples from the central Italy, justified by different climate conditions, mainly related to the rainfall distribution through the year and in the period between July and August. This period is crucial for kernel composition since physiological processes are related to soil water availability (10). The protein content also showed significantly variations, with the highest values in C<sub>1</sub> and C<sub>2</sub> (18.7 ± 0.854%, mean value) compared to N<sub>1</sub>, N<sub>2</sub>, N<sub>3</sub> and C<sub>3</sub>. This result can be related to the irrigation treatment having a positive effect on seed nitrogen content and overall in the biochemistry of protein biosynthesis.

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#### 4.5= METABOLITES STORAGE IN *GLYCYRRHIZA GLABRA* L. (LICORICE): MORPHO-HISTOLOGICAL INVESTIGATION

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*Glycyrrhiza glabra* is a Steno-Mediterranean and W-Asian plant (1). From ancient times, *G. glabra* is abundantly used in herbal medicine, cosmetic and agri-food sectors. This is due to the abundant presence of secondary metabolites such as: triterpenoid saponins (glycyrrhizin), flavonoids (liquiritigenin, isoliquiritigenin, pinocembrin, licoflavanone, isoquercitrin), isoflavonoids (glabridin and isoglabridin). There is little information about the distribution and localization of these elements in the organs and tissues of the species, so, the aim of our work was to localize and identify the metabolites in the different organs of the licorice plant through cyto-histological and histochemical investigation. Portions of leaf, stem, rhizome and root were collected from plants of *G. glabra* coming from Rossano Calabro (Cosenza, Italy). Sections of fresh and embedded material were stained with iodine solution, toluidine blue and osmium tetroxide. In the hand leaf sections, no particular compounds were present, while in the embedded material's sections, lipid, alkaloids and polysaccharide compounds were observed in some parenchyma and epidermal cells. From these compounds we identified the isoquercitrin (glycoside containing a phenolic component), which is the only phenolic compound present in the leaf (2) with two hydroxy group in ortho position, able to react with the osmium tetroxide, the iodine solution and the toluidine blue. In the stem, no particular deposits were observed both in the hand section or in the semi-thin sections stained with osmium. The staining with iodine solution, however, showed the presence of alkaloids in the parenchyma cells, while staining with toluidine blue localized polysaccharide substances in the vacuoles of cortical cells and in the secondary xylem vessels. In the rhizome, many starch grains were detected in the parenchyma cells of the cortical layer and of the xylem rays, and the presence of alkaloids and glycyrrhizin was evident in many cells of the central cylinder. By staining sections with toluidine blue, glycoside compounds were evident in the parenchyma cells of the cortex, xylem rays and pith; these compounds are probably attributable to glabridin, liquiritigenin, isoliquiritigenin (2). In the root we did not observe the presence of polysaccharides compounds in the parenchyma cells, but pectins were detected in the xylem vessels. The collected data showed that the metabolites are present both in the rhizome and in the root. In addition, the rhizome is the predominant proportion of the underground part of *G. glabra*, and is probably the most commercially used. In some cases, we observed in fact an inappropriate use of the term "licorice root", which also refers to rhizome parts.

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## 4.6 = VALORIZATION OF HORTICULTURAL CROPS IN SALENTO (APULIA-ITALY): THE BIODIVERSO PROJECT

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The strong agricultural vocation of Salento has led to the need to recover and safeguard the local varieties that the peasant art has been able to select and adapt to the pedo-climatic conditions of Salento.

Cultivation in dry farming on terraces near to the sea, this to harness its mitigating action on temperatures, or in the "mantagnate" (areas wind-protected by dry stone-walls) is the testimony of the highest knowledge of the environment and the sagacity of the Salento farmers.

Thanks to BiodiverSO project (acronym of "Biodiversity of horticultural crops of Apulia", financed by Apulia region with PSR 2007-2013-measure-action-subaction a) Integrated Projects for Biodiversity), the Botanic Garden of DiSTeBA has explored the Salento territory in order to assess the consistency, distribution and degree of erosion of the local varieties survived to the globalization. The project activities included: bibliographic research, recovery of the reproductive material (seeds), *in situ* and *ex situ* characterization of the retrieved varieties, data storage and exhibitions on the territory to bring the public, the stakeholders and the local guardian farmers of ancient vegetable varieties. The bibliographic research have highlighted few publications (about 110) most of them are prior to 1930 that give only a little help to identify the local varieties. The researches have involved the 70% of the geographic Salento that comprises the whole province of Lecce and the southern area of Brindisi and Taranto provinces. Were also involved 80 guardian farmers that keep alive the varieties at risk of extinction using farming protocols and product authenticity. 25 species were studied and about 50 local on a total of 122 for Apulia varieties were also analysed (1).

The biometric and agronomical characterization was performed on 15 local varieties; three of them were also characterised from a nutritional point of view. (2,3,4). The progress of Project activities and the results on BiodiverSo website ([www.biodiversitàpuglia.it](http://www.biodiversitàpuglia.it)) and on social network were disclosed with a very high resonance and liking.

The analyses of the data for BiodiverSO showed that in Salento, a large number of local horticultural crops are grown mainly by older farmers with ancient agricultural practices characterised by low environmental impact, even for their roots in folk traditions.

Their recovery can contribute to the sustainable preservation of agricultural ecosystems and rural landscape; they can also enable micro-spinnerets able to offer products at km 0 and healthy.

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#### 4.6 = "IN PIETRA" PINK APPLE: POLYPHENOLIC CONTENT AND EVALUATION OF THE ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITY OF JUICE.

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In Marche countryside there has been wide spread of apple crops since 1300, and thanks to popular tradition and culture, it has been possible to preserve many old varieties. The recovery of so-called "ancient fruits", namely fruits that have been partially or completely forgotten, has become increasingly important as they could represent a potential source of secondary metabolites and an important step for the biodiversity conservation. The "in pietra" pink apple is an ancient variety typically cultivated in the Fabriano area. Apples are one of the main sources for flavonoids and these compounds are known as natural antioxidants, very important for human health. On the basis of these remarks, in this study we considered of interest to evaluate the polyphenolic content (1), antioxidant (2,3) and anti-inflammatory activity (4) of juices obtained by whole, pulp and peel fruits. Preliminary data show the presence of a larger quantity of polyphenols and a higher antioxidant and anti-inflammatory activity in the pulp juice. Since previous studies on the other apple cultivars reported a higher polyphenolic content and a more evident antioxidant activity in the peel fruits, the results reported in this study show a peculiarity of the fruit in question.



Fig. 1. "In pietra" pink apple fruits



Fig. 2. "In pietra" pink apple leaves

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#### 4.6 = "SIGNORA" PEAR FROM THE VALLEY OF SINNI: A RECOVERED ANCIENT VARIETY AS A SOURCE OF ANTIOXIDANT COMPOUNDS

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The Convention on Biological Diversity (CBD) defines biodiversity as the variation between ecosystems and habitats, the variation between different species and the genetic variation within individual species. Biodiversity can therefore be described in terms of the diversity of ecosystems, species and genes (1,2). This work describes ecosystem diversities of fruits in the Valley of Sinni in southern Italy and their current status and management. The cultivation of fruit species is an inexhaustible genofond of extremely important varieties that yield fruit of excellent quality and high nutritional value, with a wide range of applications, including nutritional, medicinal and food production. The aim of this work was to develop an interactive and integrated long term strategy for the recovery of an ancient variety of pear native the Valley of Sinni (Fig. 1,2,3) and the aim of this work was to evaluate, in a preliminary study, the total polyphenols (3) and flavonoids (4) content, the radical scavenging activity (5) and ORAC capacity (6) of fresh juice obtained from this pear variety. Moreover, the objective of this work was to highlight the important role that the typical and traditional quality productions can have in promoting the territory both economically and culturally, and, not least, the role they play in protecting and enhancing the rich varietal heritage that has always been strongly linked to these productions.

A careful evaluation of the results obtained allows us to establish that the fruit juice of the "Signora" pear can be a good source of antioxidants with a concentration of polyphenols and flavonoids corresponding to 0,14 mg/g dry weight, and 0,018 mg/g dry weight, respectively. The fresh juice shows a good scavenging activity compared to the juice of another ancient pear ("Cocomerina" pear) with the IC<sub>50</sub> value of 28.01 and 23.74 mg dry weight, respectively (7). The fresh juice of the "Signora" pear also showed a good antioxidant capacity evaluated by ORAC assay (Oxygen Radical Absorbance Capacity) and our data are in agreement with the literature (6, 8). Based on the above considerations, the recovery, the enhancement and encouragement of the cultivation of this ancient fruit are especially important to meet the growing need to eat healthy foods free of harmful substances.

These findings are particularly significant as they allow us to consider this fruit in order to enhance the recovery for the conservation of biodiversity.



Fig. 1. "Signora" pear fruits



Fig. 2. "Signora" pear flowers



Fig. 3. "Signora" pear leaves

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## 4.6. = RED VS. GREEN LEAF: EFFECTS OF LIGHT QUALITY ON MORPHO-FUNCTIONAL TRAITS

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The light quality effects on plant pigment composition is an innovative field of research that can lead to interesting knowledge about light harvesting capability and utilization in plants. Usually the development of red leaves in green plants occurs as an occasional response mechanism to several stress conditions, and it is generally associated to an increase in additional pigments such as anthocyanins. Nevertheless, some species show red leaves throughout the life cycle, even under normal environmental conditions. Due to their antioxidant functions, anthocyanins are well known to contribute to human health and well-being. However, knowledge about red leaf plants should be improved to understand the ecological significance of such a phenomenon and the effects of environmental factors on the accumulation processes of anthocyanins in plants. Moreover, understanding plant's response to light quality especially in valuable species intended for human diet is a research issue also relevant for Space biology aiming at the improvement of food quality in the sight of long term manned missions. In this context, crop species are a source of beneficial compounds, such as antioxidants and vitamins, and plants grown under specific light spectra may increase the synthesis of these compounds. This topic is included in the activities aimed to realize bioregenerative life support systems, such as MELISSA (Micro-Ecological Life Support System Alternative), an European Space Agency (ESA) initiative aiming to develop the technologies for long term human space missions (e.g. permanence on a lunar base or a mission to Mars). The specific aim of our research activity was to compare the effects of different light qualities on green leaf vs. red leaf plants. The experiments were carried out analysing morphological, anatomical and physiological traits of two cultivated varieties of *Atriplex hortensis* L., with red or green leaves (Fig. 1), characterized by a different pigment composition and grown at different light qualities. More specifically, four different light quality regimens (Red, Red-Blue, Red-Blue-Green, White) (Fig. 2) were selected to assess the outcomes of different wavelengths on plant growth and suggest the better light spectrum to satisfy plant growth requirements. One month after sowing, our results showed that the different light qualities significantly affect the pigment composition in the two varieties, as well as the capability of photosynthetic apparatus to harvest and convert the absorbed light to photosystems. Plants with red leaves turned out as a promising biological system to be used in bioregenerative systems to support life in space.



Fig. 1. Red and green cv. of *Atriplex hortensis* L. system

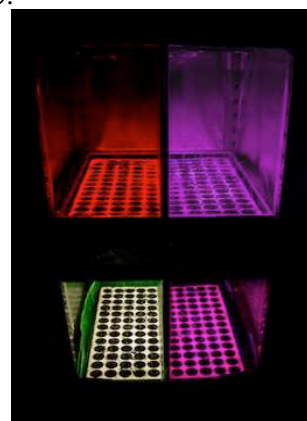


Fig. 2. Growth chamber with LED light system

#### 4.6 = PLANTS: A RICH SOURCE OF HEALTHY COMPOUNDS EFFECTS OF CURCUMIN AND CROCIN ADDITION TO CULTURE MEDIUM ON THE PROTEIN REPERTOIRE IN THE MCF-7 CELLS. PRELIMINARY ASSESSMENT.

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Curcumin and crocin are two natural compounds isolate respectively from *Curcuma longa* and *Crocus sativus*. They are a well known dietary natural pigments with anticancer activities against a variety of tumors, including human breast carcinoma.

Shotgun proteomics and in particular the label-free relative quantitation approach is a powerful technique to detect relative changes in protein amounts in complex samples.

To deepen the comprehension of the mechanisms implied in the antioxidant and anticancer activities of curcumin and crocin, we employed a classical label-free (LF) shotgun proteomics strategy to detect the modulation in protein expression of MCF-7 cells in response to treatment with sub-toxic doses of the two natural products.

Cells were cultured and treated with 50  $\mu$ M of curcumin and 30  $\mu$ M of crocin solution for 48h. Protein extraction, LF shotgun proteomics analysis and bioinformatics analysis were performed according to the protocols routinely used in our laboratories.

More than 200 proteins per condition were identified at a protein FDR < 1%. 60 and 22 proteins resulted differentially expressed (fold change < -2 and > 2) in curcumin and crocin group respectively compared to controls. Interestingly both treatments showed an increase of glyceraldehyde-3-phosphate, dehydrogenase protein disulfide-isomerase and calmodulin and a decrease of thioredoxin, suggesting the involvement of S-nitrosylation mechanism in their antioxidant and anticancer activities. IPA analysis on curcumin group revealed significant inhibition of the estrogen receptor and activation of Ion peptidase 1, mitochondrial with a significant increase of the activation state of 'cell death' disease annotation.

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#### 4.6 = QUALI-QUANTITATIVE DETERMINATION OF PHENOLIC COMPOUNDS OF CASTANEA SATIVA LEAVES AND EVALUATION OF THEIR BIOLOGICAL EFFECTS ON HUMAN KERATINOCYTES

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*Castanea sativa* Mill. (Fagaceae) is a deciduous tree growing in Southern Europe especially in the Mediterranean region and the Balkans (Fig. 1) (1). The fruit is a nut, commonly named sweet chestnut or marron, which is collected in autumn. It is a traditional basic food and can be processed into different elaborated and diversified food products. "Marrone di Roccadaspide", a PGI (Protected Geographical Indication) labelled product, represents an important economic resource for the Campania market (Fig. 2). The fruit is a marron and for the good taste it is used for the production of marron glacés.

Reports on chestnut by-products, such as leaf, shell and bur, revealed they can be considered as a good source of phenolic compounds with marked biological activity, mainly antioxidant properties (2, 3). In particular, *C. sativa* leaves, used in traditional medicine for the treatment of treat cough, diarrhea and rheumatic conditions, showed the presence of phenolic compounds responsible for the antibacterial activity and for the properties of DNA protection and prevention, and treatment of oxidative stress-mediated diseases such as photoageing (4, 5). A leaf extract from *C. sativa* exhibited in vitro scavenging activity against several reactive species that are detected in the skin after UV exposure, including O<sub>2</sub> (6). It also showed a concentration-dependent protective effect against UV-mediated DNA damage in HaCaT cells, related to a direct antioxidant effect rather than activation of the endogenous antioxidant response coordinated by NRF2 (7).

Therefore, chemical and biological investigations of the "Marrone di Roccadaspide" leaves have been carried out, aimed at achieving deeper insight into the chemical composition of this very important Campania products and at highlighting the occurrence of biologically active phytochemicals.

The MeOH extract of the leaves of *C. sativa* has been investigated affording seven phenolic compounds. In order to perform the quantitative determination of these compounds in *C. sativa* leaves, an analytical method based on liquid chromatography coupled to mass spectrometry with ESI source and triple quadrupole analyzer (LC-ESI(QqQ)MS), using the very sensitive and selective mass tandem experiment called Multiple Reaction Monitoring (MRM), was developed and validated. Moreover, on the basis of the activity reported for *C. sativa* leaves, the antioxidant capacity by TEAC assay and the ability on the UV protection of HaCaT human keratinocytes of isolated compounds were investigated.



Fig. 1 *Castanea sativa* Mill.



Fig. 2. Marrone di Roccadaspide", a PGI product.

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#### 4.6 = CORYLUS AVELLANA L. BYPRODUCTS, A SOURCE OF NEW HIGHLY HYDROXYLATED CYCLIZED DIARYLHEPTANOIDS WITH ANTIOXIDANT ACTIVITY

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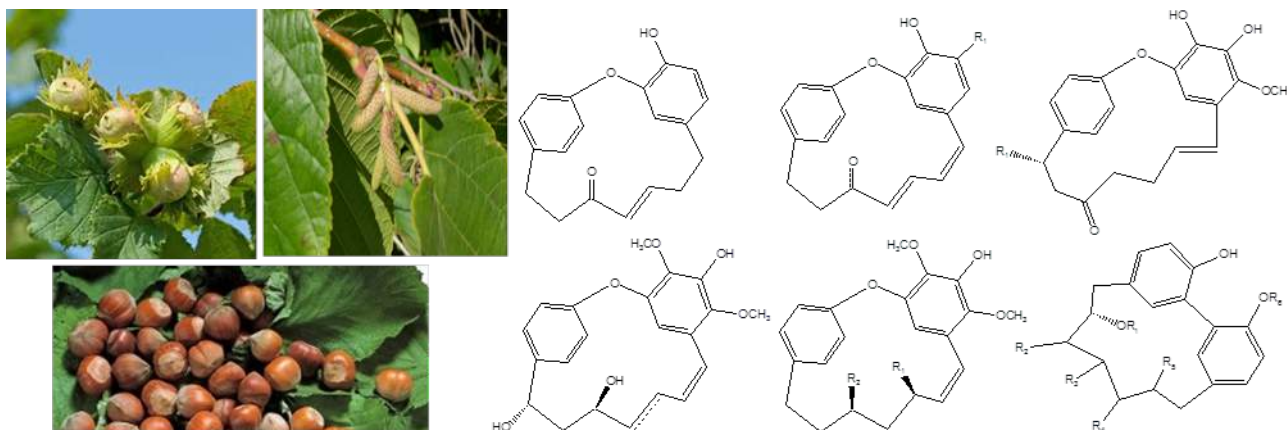
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*Corylus avellana* L. (Betulaceae) is one of the most popular nut trees on a worldwide basis. Italy is the second producer of hazelnut (13%) in the world after Turkey. In Italy, the cultivar 'Tonda di Giffoni' is much appreciated for its processing quality; its nut, known as "Nocciola di Giffoni" is a labeled PGI (protected geographical indication) product of the Campania region. Although some papers on the phenolic constituents of hazelnut (1-2) have been published, to date very few investigations have been reported on the leaves, bractes and flowers of *C. avellana* which could represent useful sources of bioactives.

The leaves are used in folk medicine for the treatment of hemorrhoids, varicose veins, phlebitis, and edema, as a consequence of their astringent, vasoprotective, and antiedema properties (2).

As a part of an ongoing effort aimed at elucidating the chemical composition of not edible parts of *C. avellana* 'Tonda di Giffoni', the phytochemical investigation of the MeOH extracts of leaves (3-4), bractes and flowers was performed to highlight the occurrence of flavonoids and diarylheptanoids as the main constituents.

In particular new highly hydroxylated cyclized diarylheptanoids named giffonins A-V have been isolated. These compounds were evaluated for their inhibitory effects on human plasma lipid peroxidation induced by H<sub>2</sub>O<sub>2</sub> and H<sub>2</sub>O<sub>2</sub>/Fe<sup>2+</sup>, by measuring the concentration of TBARS (thiobarbituric acid reactive substances). They showed, in some cases, the ability to reduce at 10 μM both H<sub>2</sub>O<sub>2</sub> and H<sub>2</sub>O<sub>2</sub>/Fe<sup>2+</sup>-induced lipid peroxidation by more than 50%, indicating higher activity than the well-known diarylheptanoid curcumin, used as reference compound.



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#### 4.6 = OFFSHOOTS OF GLOBE ARTICHOKE: PROSPECTS AS A TRADITIONAL AGRI-FOOD PRODUCT OF PUGLIA (SOUTHERN ITALY)

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Globe artichoke (*Cynara cardunculus* L. subsp. [L.] *scolymus* Hayek) is widely cultivated for its immature inflorescences, called capitula or heads<sup>1</sup>, which represent an important component of the agricultural economy of some Mediterranean countries as well as an important food product of the Mediterranean diet. Globe artichokes are generally propagated vegetatively by offshoots (Fig. 1). Nevertheless, since offshoots are continuously produced during the growing cycle of the globe artichoke plant, a part of them is removed from the field by common cultural procedures. Removed offshoots not used for the propagation are often considered as by-products, although some Authors<sup>2</sup> suggest the "functionalization" of foodstuffs by adding the extracts of these by-products as a source of nutraceutical ingredients like polyphenolic compounds and inulin.

In some areas of Puglia (Southern Italy), the offshoots of globe artichokes are traditionally used as a food ingredient (Fig. 2), like to the cultivated cardoons (*C. cardunculus* L. var. *altilis* DC) and wild cardoons (*C. cardunculus* L. var. *sylvestris* Lam.). To obtain the edible portion, fibrous parts of the offshoots are eliminated and, after boiling, cleaned offshoots are soaked in water for some hours before being used as an ingredient in several recipes; the soaking process is required for reducing their bitter taste due to the high content of polyphenolic compounds.

Several local varieties of globe artichoke have been censused in Puglia during specific actions of the 2007–2013 Rural Development Programme, thus several types of offshoots could be considered as food products. Nevertheless, to the best of our knowledge, the literature lacks of information concerning the characterization of the offshoots of globe artichoke.

Starting from these consideration, in the present study a compositional analysis of offshoots collected from two Puglia local varieties of globe artichoke was performed. The general goal was to assess some nutritional traits, such as mineral content, antioxidant activity, total phenols and inulin content, in order to furnish an overview and evaluate the potential of this foodstuff as a traditional product for human nutrition.



Fig. 1. Offshoots of globe artichoke removed from the plant.



Fig. 2. Boiled offshoots for culinary uses.

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## 4.6 = THE EFFECT OF COVER CROP ON WEED SEEDBANK IN PRUNOIDEAE ORCHARDS

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Cover crops may provide multiple benefits to soil protection, soil fertility, groundwater quality and pest management, especially in organic farming systems (1, 2) because of their potential role in reducing chemical inputs and improving soil quality. To reduce or eliminate the costs, many tree and vine crop growers prefer to use self-reseeding annual cover crops. In order to test the effects of *Trifolium subterraneum* L. cv. Clarè as cover crop on weeds, two experiments were carried out in Southern Sicily, in province of Caltanissetta (CL) in a 11-years peach orchard (37°12'55.3"N 14°05'28.3"E) and 3-years apricot orchard (37°13'36.0"N 14°05'02.4"E, 290 m a.s.l.), at a same planting density of 400 plant ha<sup>-1</sup> (5x3 m tree spacing). At the start of the experiments, the soil was sampled and analyzed on macro and micro nutrients content, organic matter, total CaCO<sub>3</sub> and pH, according to the Italian official methods (3). Therefore, the soil in peach orchard was sandy clay-loam and had a content of total N of 0,18%, organic matter 1,8% and total Ca carbonate 14%, phosphorus 82 p.p.m. and potassium 979 p.p.m; in apricot orchard the soil was silty clay-loam and had a content of total N of 1,1 g/kg, organic matter 19 g/kg, total Ca carbonate 61 g/kg, phosphorus 571 mg/kg and potassium 1,80%. For all the experiments, the study was conducted in an area of 1 hectare, four time replicated and arranged according to a randomised-block design. In order to intercept the major seed variability, every year in spring and autumn soil samples were collected in five different points, at the depth of 10 cm, using a metal probe of a 4 cm diameter. Until the beginning of the analysis, soil samples were stored for few days at 4°C in the dark. To separate soil from seeds, soil samples (four replicates of 150 g) were washed through a series of sieves (4). The extracted material was transferred into Petri dishes and was air-dried for the separation, identification and counting of the seeds with a binocular stereomicroscope (Olympus Optical Co, LTD). The two experimental field sites, despite being less than 2 km, were grown under very different conditions prior to these experiments, highlighting the importance of historical land use for the current weed seedbank. After the first year, significant differences in the qualitative and quantitative estimation of weed seedbank densities in the field experiments have been found. In peach orchard 5.200 seeds m<sup>-2</sup> allowed to seven species, where *Portulaca oleracea* L., *Amaranthus* sp. and *Setaria viridis* (L.) P. Beauv. accounted for 80% of the total soil seedbank, vs 1.200 m<sup>-2</sup> in apricot orchard allowed to only four species; in uncultivated plots, as control, seedbank was represented by 8.200 vs 3.600 respectively in peach and apricot orchards. Considering the effect of self-reseeding of *T. subterraneum*, only in the peach orchard after three years from seeding, weed seedbank was represented by 31.675 seeds m<sup>-2</sup> allowed to sixteen species, where *Amaranthus* sp., *P. oleracea*, *Lamium amplexicaule* L., *Helminthotheca echioides* (L.) Holub, *Veronica hederifolia* L., *S. viridis* and *Stellaria media* (L.) Vill. were the main representative taxa. This result was in consequence to the effect that some cover crops may revert back to natural vegetation within a few years, so it may be necessary to resow the cover crop after three or five years. Managing the weed seedbank is a long-term effort, but it can supply long-term benefits. These three years of data can only serve as an early indication as to how different cover crops might influence the weed seedbank. Cover crop can contribute to reduce the size of the weed seedbank suppressing weed emergence, growth and releasing of seeds in the soil, so it represents a valid support for a sustainable weed management.

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## 4.6 = EFFECT OF MICROBIAL TRANSGLUTAMINASE ON GLUTEN FREE FLOUR PROTEINS

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Gluten is the major factor involved in the structural properties of bakery products. Due to its ability to create aggregates, it gives dough viscosity, elasticity and cohesion, all features responsible for the baking performance (1). However, the gliadin protein fraction of gluten is also the main factor responsible for the development of celiac disease (CD) and other non-celiac gluten sensitivities (NCGS). CD is an immune-mediated systemic disorder, elicited by gluten and related prolamines, which affects approximately 1% of the European population (2). The only therapeutic option for people suffering from CD is lifelong adherence to a strict gluten-free (GF) diet (3). Cross-linking enzymes, able to organize and create protein networks, are suitable protein modifiers for the food industry. Among those enzymes, transglutaminase (TGase) can be used for protein manipulation in order to achieve higher food quality due to its ability to improve the firmness, viscosity, elasticity and water-binding capacity of food products (4).

The aim of this work was to evaluate the effect of addition of microbial TGase (mTGase), from *Streptoverticillium mobaraense*, on protein aggregation in flours as well as in baked products. In this study, the effect of mTGase treatment on different flours from wheat and GF sources (e. g. rice, corn, amaranth and lentil), were analysed. For this purpose, doughs from flour were prepared with water, and then the enzyme treatment was done by adding 1U of mTGase per 100 mg flour. The reaction was carried out for 90 min at 40°C. To investigate the enzyme effect on the formation of protein cross-linked products, three sequential protein fractions, corresponding to albumins/globulins, prolamins and glutelins, were prepared and analysed by SDS-PAGE. Results showed that mTGase has the capacity to modify GF flour proteins and to improve protein aggregates indicated by high-molecular weight proteins. In order to evaluate structural epitopes of the flour proteins upon mTGase treatment, enzyme-linked immunosorbent assay (ELISA) was performed. Therefore, sera from CD, NCGS patients and healthy donors were analysed with respect to their IgG-binding capacities. Detection of IgG-binding epitopes was conducted with an alkaline phosphatase labeled anti-human IgG antibody and signals before and after mTGase treatment were compared. First data showed that no significant difference was observed in the immunoreactivity of the wheat protein extracts after enzyme treatment, supporting others recent reports<sup>5</sup>. These preliminary results give a perspective in the GF research and suggest their possible use to create innovative products.

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## 4.6 = POTENTIAL USE OF RICE ENDOSPERM FIBERS AS REINFORCING MATERIAL IN BIOCOMPOSITES

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Biocomposites composed of biodegradable polymer, as matrix material, and natural fibers, as reinforcing material, have attracted much attention from the environmental viewpoint and their advantages such as high strength & stiffness, low cost, and biodegradable properties [1-3]. The lack of good interfacial adhesion between natural fibers and matrix, low melting point, and water sensitivity make the use of natural fiber-reinforced composites less attractive. Various green methods have been explored in order to improve the compatibility between hydrophilic natural fibers and hydrophobic polymer matrices among which enzyme digestions [4]. Enzyme catalyzed reactions are highly specific and result in the separation of fibers from their non-fiber components [5]. Rice fibers can also be considered as important potential reinforcing filler for thermoplastic composite because of its lignocellulosic characteristics and the objective of this study is to investigate the reinforcing potential of original and enzymatically modified rice endosperm fibers.

Rice endosperm fibers were biochemically characterized and enzymatically treated with Celluclast. Original and enzymatically treated rice fibers were characterized in order to know their surface morphology, thermal stability and crystallinity. Biocomposites were prepared by reinforcing poly(butylene succinate) (PBS) with original and enzymatic treated rice endosperm fibers (10, 20 and 30 wt%) by melt-mixing at 160°C. Synthesized composites were subjected for water uptake test to investigate the resistance against water absorption. Thermal and mechanical characterization of all biocomposites was carried out in order to verify the effect of the dispersion of fibers inside the polymeric matrix. Analysis shows that rice fibers and PBS degrade in a single step, at different temperatures. Moreover, enzymatically treated fibers are slightly more stable than the original ones. The thermal properties of the composites do not vary with respect to those of PBS. Therefore, the crystalline structure of the polymeric matrix is not disturbed by the presence of fibers also when present in a large amount. Finally from tensile and flexural analysis, it has been observed that reinforcement of rice endosperm fibers have a notable effect on the mechanical performances of the composites. A significant increment of tensile and flexural properties is induced by both kinds of fibers, revealing a general reinforcement of the matrix in all the samples.

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## 5. = FIRST AEROBIOLOGICAL MONITORING DATA IN PISA (ITALY) WITHIN AIS-LIFE PROJECT

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Introduction: One of the most important biological component of air is pollen, as its allergens are driver of airborne allergic diseases (1). Pollen allergy has a remarkable clinical impact over Europe. AIS LIFE ENV/IT/001107 project – Aerobiological Information Systems and allergic respiratory disease management (<http://www.ais-life.eu>), launched in 2014 (2), aims to assess the clinical impact of dissemination of pollen information. In this context, aerobiological monitoring was started in Pisa (Italy) for the first time. This contribution is the first report on aerobiological data ever collected in Pisa. The monitoring covers 21 months of operation of the pollen trap.

Materials and Methods: The sampling procedure and the count of the airborne pollen grains and fungal spores is based on UNI 11108:2004. Aerobiological samples were obtained with a “Lanzoni VPPS 2000” pollen trap installed on top of the Biology Department building in Pisa, via Derna 1, about 17 m above the road pavement (coordinates 43.718343° N, 10.395110° E). Pollen grains of eight families: Betulaceae, Cupressaceae – Taxaceae, Asteraceae, Fagaceae, Oleaceae, Urticaceae, Poaceae, Platanaceae and spores of *Alternaria* sp.pl. have been counted every day since 4 November 2014. Cycles of pollination and sporulation, the main pollen seasons (MPS), and the main spore season (MSS) were calculated according to 3, 4. Meteorological data were obtained from [www.meteopisa.it](http://www.meteopisa.it) and from a weather station placed next to the pollen trap.

Results and discussion: We recorded a cumulative value of 25377.55 pollen grains per m<sup>3</sup> and 1202.85 fungal spores per m<sup>3</sup> from January to May 2016; the same values for the whole 2015 are 38781.49 and 12846.02 respectively. As regards woody species, the highest value was contributed by Cupressaceae – Taxaceae families, with 6820.5 pollen grains in 2016 and 16032.5 in 2015; followed by Platanaceae with 2229.15 pollen grains in 2016 and 1625.03 in 2015; Coryloideae subfamily with 2794.55 pollen grains in 2016 and 866.47 in 2015.

As regards MPS, Betuloideae subfamily lasted 79 days in 2016 and 88 in 2015. The starting date for Betuloideae subfamily varied from 32 days in 2016 to 46 in 2015. As regards the ending date, Betuloideae varied with 111 days in 2016 and with 134 days in 2015.

The average temperature of the period 1 January-31 March was 10.6°C in 2016 and 9.8°C in 2015; in the same periods total rainfall was 391 mm and 205 mm respectively. Therefore our data may suggest a correlation between pollen concentration of woody species and meteorological factors, consistent with observations reported in other studies (5).

Conclusions: The aerobiological monitoring campaign started in Pisa in 2014 is providing scientific data in support of an on-going European project addressing the clinical impact of dissemination of airborne pollen information. The aerobiological monitoring represents a unique tool to supply local real data to allergologists, enabling them to calibrate the drugs therapy for their patients and providing immediate advantages both for patients' health and for better management of the national healthcare system (6). The data obtained from the aerobiological monitoring will be useful for other applications as well, from biology and medicine to agriculture. Analysis of changes in MPS and MSS may help in detecting local changes in climate factors.

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## 5. = EXPLORING PLANT – SOIL INTERACTIONS USING NEXT GENERATION SEQUENCING: ARE OOMYCETES PLAYING A ROLE IN REED DIE-BACK?

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Common reed [*Phragmites australis* (Cav.) Trin. ex Steudel] is one of the most widespread angiosperms, playing many important environmental roles: it is used in phytoremediation, and it is relevant for the maintenance of aquatic biodiversity. Since 1950s, a severe syndrome known as Reed Die-Back Syndrome (RDBS) has been detected in central Europe and recently reported also in the Mediterranean basin, where, in some cases, is leading to an irreversible retreat of reed population. In Italy, a large-scale screening is in progress to better understand the phenomenon. While some of the possible causes of the decline have been investigated, the role of microorganisms living in the soil is still poorly understood. Plants are continuously in contact with microorganisms, which can have profound effects on seedling vigor, plant growth and development, diseases, and productivity. A study conducted in Costanza Lake (Germany) using culture-dependent techniques (1), highlighted a possible involvement of *Pythium phragmitis* (Nechw.) in the reed die-back. More recently, culture-independent approaches have enriched the understanding on microbial diversity of soil and deeper knowledge on the possible RDBS causes is possible using next-generation sequencing techniques. Therefore, in this study we used barcoded 454 amplicon pyrosequencing targeting the ITS region of oomycetes, using total soil DNA extracted from five different freshwater ecosystems. The aims of this study were: 1) to understand if the oomycetes could play a key role in the reed decline, 2) to investigate the differences in the oomycetes communities' structures among different ecosystems, in two different seasons, and 3) to understand the major environmental factors that shape these communities. This study represents a step forward in the knowledge of the complexity of this important wet ecosystem.

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## 5 = DIATOM MASS CULTIVATION FOR PHOTONIC APPLICATIONS COUPLED WITH LIPID PRODUCTION

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Diatoms have been identified as a versatile biomass feedstock upon which future biorefineries can be established. Although diatoms fulfill the major prerequisites of a sustainable value added product feedstock, there are still technical challenges holding back the large scale production in an economic and environmentally-sound process. In this work we mass cultivated a set of morphologically and structurally diverse diatom strains for lipid production coupled with the use of frustule biosilica, a by-product of the lipid conversion process, for dye trapping and multiple scattering and localization of light. Indeed, we aimed at amplifying frustule photoluminescence in a random laser (RL), a special type of laser in which the optical feedback is due to light scattering in an amplifying medium instead of a conventional optical cavity.

Biosilica and lipids were extracted at the end of growth at optimized conditions in indoor photobioreactors. Frustules were structurally and microscopically characterized and their chemistry analyzed with Fourier Transform Infrared Spectroscopy. Frustule capacity of binding laser dyes was evaluated on a set of frustules/Rhodamine B solutions and with respect to silicon dioxide and diatomite by Fluorescence Spectroscopy. We observed frustule higher affinity for the organic dye and a potential ability to protect its chemical structure and to modulate its release. The effect of dye trapping property in conveying RhoB emission to frustules, with enhancement of scattering events, was analyzed by RL measurements on RhoB doped gel matrices of different nature. Emission intensity was recorded at increasing pump power indicating the onset of a RL effect in frustule filled gels.

Our past research evidenced multiple scattering and localization of light of fossil (commercial diatomite) and extant multispecies frustules material, obtaining first experimental evidence of random laser effect from RhoB in polymethylmethacrylate (1). Here we went beyond focusing on biosilica from selected diatom species intensively cultivated, providing a lipid source for food/feed and biofuel market as well as a large amount of homogeneous and predictable nanostructured material to employ for dye trapping purposes and for photonic applications in a set of composite materials. This integration process demonstrated application potential, contributing to the abatement of diatom mass culture costs and is prospect to inspire novel developments in nanotechnology, medicine and diagnostics.

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## 5= USE OF BIOTIC ELICITORS OF RESISTENCE AGAINST FLAVESCENCE DORÉE ON GRAPEVINE AND INNOVATIVE METHODS TO DETECT SYMPTOMS

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*Vitis vinifera*, one of the most typical and prestigious crops grown on Italian territory, can be strongly affected by the disease Flavescence Doreé (FD), widely occurring in the vineyards of the North of Italy, in particular in Piedmont. FD is caused by Phytoplasmas, wall-less bacteria belonging to the order *Mollicutes* and is transmitted by the vector insect *Scaphoideus titanus* Ball. The main evident symptoms on FD infected grapevine are: yellowing, leaf curling, stunting, internode shortening, fruit abortion and general deterioration of plants [1]. Nowadays the strategy to fight FD is mainly based on the control of vector insect, but every year many cases of vines suffering from FD are recorded. The aim of this work was to find innovative and sustainable solutions to improve the fight against FD through: 1) the evaluation, in field conditions, of the effectiveness of biotic elicitors of resistance, such as Arbuscular Mycorrhizal (AM) fungi and Plant Growth-Promoting Bacteria (PGPB), that have already been tested against phytoplasmas on model plants [2; 3] 2) the use of optical sensors to detect in advance the symptoms of FD, investigating if a correlation between FD and a specific reflectance occurs, 3) the development of an effective inoculation technique for bacterial endophytes for *V. vinifera*. To realize this work three vineyards were selected. Afterwards, three hundred cuttings of *V. vinifera* cv. Dolcetto were bought to replace fault plants in selected vineyards; half of them have been potted only with soil, while the other half, were also inoculated with AM fungi. The cuttings were grown in greenhouse under controlled conditions for two months. Fifteen days before their transfer in the field, the cuttings inoculated with AM fungi were also inoculated with selected PGPB.

In order to test the optical sensor, the vineyards were initially handly mapped, directly into the field and the position of each plant was recorded. Afterwards, starting in June, three reliefs to evaluate the FD and Esca disease symptoms were conducted and their positions were recorded on the maps. After these steps the diseased plants were georeferenced by an portable high precision G.P.S integrated with software G.I.S. In the same day the relief with the optical sensor on all vineyards surface (5.5 hectares) was performed. The optical sensor is part of a "mobile lab" comprising: means of transport, mobile Pc, software G.I.S. and G.P.S of precision. The functioning of the sensor is based on the reflectance, which is the ratio of reflected light and emitted light. The optical sensor emits light at known wavelength, at VIS (670 nm), RedEdge (730 nm) and in the range of NIR (780 nm) and it calculates reflectance values for each point detected. Optical sensor made a relief every fifty centimeters and, for each of these, reflectance in NIR, VIS and RedEdge spectrum and vegetation indices (NDVI and NDRE) have been calculated. These data were elaborated using statistical methods (moving averages) to filter the information and find regions with different attention levels and then thematical maps with GIS software were created and compared with the real situation, checked both by visual and molecular analyses of the symptoms.

To the purpose of developing an effective technique of inoculation of endophytic bacteria sixty cuttings of *V. vinifera* cv. Dolcetto were potted and grown in greenhouse under controlled conditions. An inoculation test on roots and a leaf spray test using three different strains of bacteria was performed.

At the end of April 2016, it was made a manual relief in the field to assess the health status of the vineyards with particular attention to the cuttings fielded the previous year. Others relieves will be made in the next months. A new test with the optical sensor will be carried out, in order to compare the new data with those already recorded. It will be also tested a new prototype equipped with more and different optical sensors. Since the development of an effective technique of bacterial inoculation was difficult, mainly for the complexity of the experimental system, the same test will be made again on *V. vinifera* and also on model plants.

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## 5 = KEY PLANT SPECIES TO MONITOR CLIMATE CHANGE: EFFECTS AT HIGH ELEVATION IN MAJELLA NATIONAL PARK.

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The global climate is rapidly warming and the causes are rooted in human activity. Greenhouse gas and aerosols emissions are the main cause of global warming and variations in natural cycles (1). Thus, in recent years, a scientific interest in understanding how climate change affects the biotic communities arose. Actually, high mountains can be considered particularly appropriate environments to detect effects of climate change on natural biocenoses in a global scale (2). In this context, to study the effect of climate change on a large scale on high altitude environment, the GLORIA project (Global Observation Research Initiative in Alpine Environments) was developed (3). The aim of this project is to collect, at global scale, quantitative information on species richness, species composition, vegetation cover, soil temperature, and length of snow cover period, for assessing the potential risks of biodiversity loss due to climate change. Since 2001, the highest peaks of the Majella National Park (central Apennines) are part of the biomonitoring network of GLORIA project (4).

In this study, we investigated the effect of altitude and aspect in the vascular plant species composition, structure (life forms) and ecology (Landolt ecological indicators) of high-mountain vegetation in Majella summits, and furthermore we analysed the dispersal traits of species, with a focus on key species (potentially invaders), which were identified according of their distribution among summits and exposures. We performed this study using the data collected in 35 plots of 1m<sup>2</sup> in 2015 in three different GLORIA summits in Majella National Park, along a vertical (elevation) and horizontal (different directions) gradients. Species composition was analysed performing a cluster analysis, followed by the analysis of similarities between summits and aspects through the ANOSIM test. Moreover, the SIMPER analysis was applied to determine which species significantly contributed to the differences along vertical and horizontal gradients. Ecological traits analysis was performed by ANOVA test, and a detailed study of dispersal traits was also realized. We found a set of twenty vascular plant species which can be considered potential invaders of highest summit and key species for monitoring the climate change effects in high mountain vegetation of central Apennines. This synchronic analysis could allow the prediction and the modelling of ecological changes before they occur. This type of study, therefore, would not be limited only to the monitoring of climate change but also to develop conservation strategies of threatened and rare species.



Fig 1a-1b Example of plots and fieldwork activity

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## 5 = IN VITRO MORPHOGENESIS OF TOMATO ROOTS AFTER TREATMENT WITH CULTURE FILTRATES OF ENDOPHYTIC FUNGI

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The root system i) anchors the plant to the substrate, ii) allows the uptake of mineral nutrients and water, iii) hosts a number of microbial communities that may result in noxious or beneficial effects on plant growth. In taproot systems of many horticultural crops, the length of primary root determines the plant ability to access deep soil resources and a more or less dense network of fine lateral roots explores topsoil and is responsible of nutrient uptake (1). Cultural filtrates of fungi can affect root length, number of lateral roots and branching of treated plants because they contain plant growth regulators and other bioactive compounds (2). In Dovana et al. (3), 19 fungal endophytes were isolated from watermint stems and roots and tested on *Arabidopsis* plants grown *in vitro*. Effects of watermint endophytes on *Arabidopsis* roots differed in a species-specific and isolate-specific manner (3). In this study, culture filtrates of three endophytes isolated from watermint roots (RL6, RT13 and RT5b) have been tested on the root growth of young tomato seedlings. Tomato seeds (*S. lycopersicon* cv. Principe Borghese) have been surface sterilized and germinated on sterile Whatman filter papers imbibed with 10 ml of 14-d old centrifuged fungal filtrates (0.2  $\mu$ m) obtained inoculating one mycelial plug of each endophyte into 200 ml ME medium. Control cultures were established adding 10 ml of sterile water or ME growth medium (WC and MEC, respectively) to the seeds. After ten days of culture, roots of seven plantlets per treatment were extracted from the petri dishes and primary root length (PRL), number of first order lateral roots (LRs) and root branching calculated as the PRL/LRs ratio were measured by imaging analysis. Results were expressed as mean $\pm$ SE and statistically analysed by ANOVA followed by Fisher's PSLD test. With the addition of ME medium, MEC roots were not statistically different from WCs. RL6 culture filtrates, on the contrary, induced about 100% increased of LRs in 10-d old tomato seedlings with respect to all other treatments and significantly increased also tomato PRL with respect to WCs. This last effect was consistent with results obtained in *Arabidopsis* roots. Fungal filtrates of RT13 and RT5b didn't affect PRL with respect to WCs. Differently from *Arabidopsis*, where PRL reduction was always significant, RT5b didn't reduce tomato primary root length. Further studies will be aimed to identify the chemical compounds involved in the effects of culture filtrates on tomato and to determine if the differences between *Arabidopsis* and tomato were a consequence of the plant species or depended on the culture system (filtrates vs. fungal mycelia).

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5 = NITROGEN METABOLISM IN ORCHID MYCORRHIZA: EXPRESSION OF FUNGAL AND PLANT GENES IN THE *TULASNELLA CALOSPORA*-*SERAPIAS VOMERACEA* ASSOCIATION

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Limited knowledge on the biological and ecological features that underpin terrestrial orchid growth, development and reproduction represents a serious obstacle for their conservation. Like the majority of terrestrial plants, orchids form in nature mycorrhizal associations with soil fungi. However, as compared to other mycorrhizal associations, orchids are very peculiar because seed germination and early plant development fully depend on the mycobionts. Orchid seeds contain little stored energy reserves, and the fungal partner provides the embryo with organic carbon (C) and other essential nutrients (Smith and Read 2008). Following seed germination, orchids form a protocorm, a heterotrophic structure that precedes seedling development and is completely fungus-dependent for C supply, a strategy known as mycoheterotrophy (Leake 2004; Hynson et al. 2013). Most experiments on nutrient exchanges in orchid mycorrhiza have focused on the acquisition and transfer of organic C by the mycobiont to the host plant (Cameron et al., 2006, 2008; Kuga et al. 2013), but far less is known about the role of orchid mycorrhizal fungi in the acquisition and transfer of other nutrients.

The aim of this work was to better understand the molecular mechanisms of N metabolism in orchid mycorrhiza. *Tulasnella calospora* isolate AL13, either as free-living mycelium or in symbiosis with the photosynthetic orchid species *Serapias vomeracea*, was used as a model system because of the complete genome sequence available for the symbiotic fungus, and of transcriptomic data obtained from experiments performed on different nitrogen sources and in symbiosis. We showed that *T. calospora* lacks a nitrate uptake system but, like other mycorrhizal fungi, possess functional ammonium transporters (AMTs). Two genes coding for AMTs were identified in the *T. calospora* genome and characterized by functional complementation in yeast. The expression pattern of these genes suggests that fungal AMT are differentially expressed during the fungal life cycle and in response to different N sources. The expression of other *T. calospora* genes potentially involved in N metabolism allowed us to draw some hypotheses on the N pathways in orchid mycorrhiza. Expression of these fungal genes was also investigated on RNA extracted from specific cell-types in symbiotic *S. vomeracea* protocorms collected by laser microdissection.

We also investigated, by RNASeq and by RT-qPCR, the expression of some plant genes in mycorrhizal and non-mycorrhizal *S. vomeracea* protocorms. Given the lack of genomic information for *S. vomeracea*, data derived from transcriptomic experiment were used. Contigs corresponding to two different ammonium transporter genes (*SvAMT1.2*, *SvAMT2*), two amino acid permeases (*SvAA3G1*, *SvAA3G2*), a lysine histidine transporter (*SvLHT1*) and a glutamine synthetase (*SvGS*) were identified. RNASeq and RT-qPCR experiments demonstrate that *SvAMT1.2* was significantly up-regulated in symbiotic protocorms, but not *SvAMT2*, and revealed significant up-regulation of *SvAA3G1*, *SvAA3G2*, *SvLHT1* and *SvGS* in mycorrhizal protocorms, as compared to asymbiotic ones. These results indicate some similarities with N metabolism in other mycorrhizal types, and support the importance of amino acids in N transfer to the orchid.

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5 = EFFECT OF PH ON GERMINATION, GROWTH AND POLLEN ALLERGENICITY OF AMBROSIA  
ARTEMISIIFOLIA L.

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By influencing nutrient bioavailability and microorganism community, the pH of soil affects plant traits. On this basis, in order to obtain information useful to the management of the noxious species *Ambrosia artemisiifolia*, the effect of soil pH on its germination, growth and pollen allergenicity was tested. To this aim, three soils with pH of 5, 6 and 7 were prepared by adding different proportions of hydrated lime ( $\text{Ca}(\text{OH})_2$ ) to a natural agriculture soil. After testing *A. artemisiifolia* germination at 15, 20, 25 °C, plantlets were grown in the three prepared soils at room temperature. Plant traits of the species (germination, plant height, leaf size, male inflorescence size, etc.) were monitored over about 100 days. During flowering time pollen from male inflorescences was collected and subjected to proteome analyses by applying slot blot and 1-D immunoblotting.

Results showed a great effect of pH on all the monitored plant traits. At 15 °C the mean percentage of germination was 28, 13 and 12 at pH 5, 6 and 7, respectively; at 25 °C the trend was opposite: the percentage were 5, 10 and 17, respectively. Concerning plant growth, in general the species exhibited the highest and the lowest growth values at pH 5 and 7, respectively. As regards pollen allergenicity, the analysis revealed a higher reactivity of pollen collected from plants grown at pH 5.

On the whole, this study provides evidence that increasing soil pH reduces the vegetative and reproductive *A. artemisiifolia* performance as well as decreases pollen allergenicity. Thus, soil amendment with  $\text{Ca}(\text{OH})_2$  may represent a management system to control *A. artemisiifolia* growth. However, further field experiments in agriculture areas must be set up to evaluate its real effectiveness.

5 = BOTANIC GARDEN OF URBINO UNIVERSITY: FLORINTESA AND ANTHOSART.

A COMMITMENT TO THE DISSEMINATION OF SCIENTIFIC KNOWLEDGE AND TO THE ENHANCEMENT OF THE ITALIAN FLORA AND TERRITORY

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In 2014 the Botanic Garden of Urbino University joined the project named "Strengthening, promotion and dissemination of Italian Botanic Gardens and Historic Gardens network: FLORINTESA". The aims of this project are consistent with those of the National Ecological Network, of the Rete Natura 2000 and of the National Strategy for Biodiversity. This project was financed by the Ministry of Education, University and Research (MIUR) and it is coordinated by ENEA (*Sustainable Management of Agricultural Ecosystems Laboratory*) in collaboration with the Italian Botanical Society and Forum Plinianum.

The main objectives are to promote the study and knowledge sharing of the Italian Flora and the enhancement of visibility of the Italian Botanic Gardens and Historical Gardens so as to enhance, through the work of dissemination and awareness-raising, the activities and the crucial roles played by University's Botanic Gardens in scientific research, in the conservation and preservation of plant biodiversity and of Italian natural heritage.

In 2016 the Botanic Garden of Urbino University joined the new project named "ANTHOSART: from the Italian flora to territory. Enhancement and promotion of Italian Botanical Gardens network for the innovative use of wild plants and for the design of green works". This project is the natural development of FLORINTESA and started with the conference "*Theatrum florum*: the potential role of Botanic Gardens, herbaria and germoplasm banks in green infrastructure designing using native flora. This project is a necessary cultural challenge that involves the Botanic Gardens as a leader of a stakeholders network (farmers, nurserymen, research planners, local government and citizens).

## 5 = THE VEGETATION OF THREE AREAS OF THE VAL CAMONICA (LOMBARDY, ITALY) AFTER ENVIRONMENTAL RESTORATION WORKS

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The environmental restoration of mountainous areas affected by instability is a topic of great importance for those involved in land management and nature conservation. The present study aims to analyze the vegetation of three areas of the Lombardy Alps where, as a result of landslides, soil stabilization (using bioengineering techniques) and environmental restoration work was performed. An analysis of the vegetation, performed according to the phytosociological method, provided information on the plant communities currently present and allowed the work conducted in these areas to be evaluated.

The study areas are located on mountain slopes of three lateral valleys of Val Camonica:

6) Scalve Valley (Azzone, BS; year in which environmental restoration work was performed: 1995; year in which vegetation was monitored: 2014) (Fig. 1).

7) Val Dorena (Monno, BS; year in which environmental restoration work was performed: 2000; year in which vegetation was monitored: 2015).

8) Val Palot (Pisogne, BS; year in which environmental restoration work was performed: 1996).

In Val Dorena, further landslides occurred (and still occur), after soil stabilization and environmental restoration work, in Azzone exotic species were sown including *Lupinus polyphyllus* (1) (currently present), while in Val Palot no exotic species were sown and no further landslides have occurred after the environmental restoration work.

Data on the vegetation of the three areas were collected performing various phytosociological relevés. These data were analyzed statistically to identify the various types of vegetation and, in each case, the value of the Ecological index of maturity (EIM) (2) was calculated in order to evaluate the level of disturbance to which the various plant communities are subject. The results of the floristic-vegetation analysis allowed the identification of the various types of vegetation in the three areas and the EIM value showed the presence of low disturbance in Val Palot (due to the absence of further landslides) and of high disturbance in Val Dorena (still affected by landslides) and in the area of Azzone (presence of exotic species introduced by man). Although the EIM has so far been applied only in three case studies, this index could be useful for the assessment of environmental restoration work.



Fig. 1. Study area in Azzone after the landslide (1992), at the time of environmental restoration (1995) and at the time of vegetation monitoring (2014).

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5 = VERTICAL DISTRIBUTION OF *TUBER MAGNATUM* MYCELIUM IN SOIL

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*Tuber magnatum* Pico is the world's most expensive truffles. Attempts to cultivate it are often failed because the ecology and biology of this ectomycorrhizal ascomycete remain largely unexplored. This gap of knowledge has resulted from the difficulties to obtain pure cultures of this fungus as well as to synthesize its ectomycorrhizas in controlled condition or to find them in the field (1, 2). Since *T. magnatum* mycelial network in soil is more widespread than its ascomata and ectomycorrhizas (3), it represents the most reliable target to investigate the distribution and dynamics of this species in natural and cultivated truffle grounds. Recently, a real-time PCR assay has been also developed to quantify *T. magnatum* mycelium in soil (4) and the spatio-temporal distribution of the mycelium has been investigated in natural truffle grounds located in Emilia Romagna, Abruzzo, Molise and Tuscany regions (Italy) (5).

In this work, we evaluated the vertical distribution of *T. magnatum* mycelium in soil of a natural truffle ground and compared the data of mycelial biomass with the soil temperature and moisture. Soil moisture and temperature were measured with DMF soil probes (SA) which measures moisture content and temperature at 4 depths in a soil profile (0, 10, 20 and 40 cm) as well as the surface temperature every 30 min. Mycelial biomass was evaluated using the real-time PCR assay after Iotti et al. (4).

The highest amounts of *T. magnatum* mycelium were registered in the first 10 cm of soil but relevant mycelial biomass was also found until 30 of depth in all plots.

An attempt to explain distribution dynamics has been performed in this analysis, by developing a soil hydro-thermodynamical model based on a soil parametrization deriving from two years plot observation by DFM probe records integrated with meteorological data. Former simulation allow to identify optimal temperature and moisture quite in agreement with previous studies, though the high difference between observations and simulations leave wide uncertainties on the real development strategy of the mycelium.

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2) M. Iotti, F. Piattoni, A. Zambonelli (2012) Edible ectomycorrhizal mushrooms. Current knowledge and future prospects, Springer Verlag, 145-161

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## 5. = WOODY FLORA DISTRIBUTION PATTERNS ALONG A LONGITUDINAL TRANSECT IN CENTRAL ITALY: A TRAIT-BASED ANALYSIS

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The analysis of floristic and vegetation data by means of trait-based approaches has assumed recently increasing interest in both theoretical and applied biodiversity science (1). By using species traits (2) instead of their identities, these approaches improve the ability to assess traits response to environmental gradients (3).

For Italy, at a medium scale, native woody flora is an important biodiversity surrogate (4); recently, the analysis of native woody genera distribution patterns added useful insight into Italy's biogeographic structure, evidencing latitudinal and longitudinal gradients (5).

In central Italy, the analysis of woody specific and subspecific taxa distribution patterns along a west-east transect at a semi-detailed scale evidenced clear gradients, mainly related to elevation and identified woody floristic types (M. Latini and collaborators, in preparation).

We present a trait-based analysis of woody flora distribution patterns along a longitudinal transect in central Italy. Starting from the dataset used by Latini et al. (6), we wanted to assess woody flora traits response to environmental variables along the same transect.

The study area is located in the central part of peninsular Italy, namely in the Lazio and Abruzzo administrative regions; the 157 km long transect ranges from Tyrrhenian to Adriatic Sea and intercepts Apennines (transect elevation range = 5-1840 m a.s.l.).

The dataset contains presence/absence data for 141 woody taxa in 153 sites (table L). Taxa are described by 14 traits (table Q): life form, spinescence, four leaf traits, three flower traits, three fruit traits, pollination and dispersal modes. Ten environmental variables have been measured in the sites (table R): elevation, distance from the sea, longitude and seven bioclimatic variables. To assess traits response to environmental gradients, RLQ analysis and fourth-corner methods were used, following the approach by Dray et al. (3): fourth-corner tests were applied on the outputs of RLQ analysis and significant associations between RLQ axis and traits and/or environmental variables were represented on the RLQ factorial map and as a table.

A global relationship between species traits and environmental variables has been detected. The first RLQ axis is significantly negatively correlated with elevation, precipitation of driest month, distance from the sea and longitude, and positively with annual mean temperature, max temperature of warmest month, min temperature of coldest month, temperature annual range and precipitation of wettest month. High elevation inland sites, located on the east Adriatic side (with high values of min precipitation of driest month and low values of max precipitation of wettest month), are characterized by reptant phanerophytes, subspinescent leaves and absence of flowers and fruits. Low elevation coastal sites (with high values of min temperature of coldest month) are characterized by evergreen taxa, sclerophylly, modified leaves, small fruits and barocorous dispersal. The second RLQ axis is significantly negatively associated with distance from the sea, temperature annual range and annual precipitation. Middle elevation inner sites (with high values of annual temperature range) are characterized by non-sclerophyllous and/or deciduous taxa.

In the highly heterogeneous analyzed area, the combined approach used was able to identify clear traits-environmental patterns. As highlighted in other studies, the use of traits could be useful also for monitoring analysis. The next step will be to apply this approach to different databases at different scales.

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## 5. = HISTORICAL AND SCIENTIFIC RELEVANCE OF THE FRANCESCO CUPANI'S HERBARIA

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Francesco Cupani (1657–1710), the Franciscan friar who founded the Garden of the Prince of Cattolica at Misilmeri near Palermo, having compiled and illustrated the first comprehensive flora of Sicily, is the most important pre-Linnaean reference for botanical studies in this territory, from the late seventeen century on. Actually all the plants occurring in the region were listed by Cupani in the *Hortus catholicus et supplementum* (1), and illustrated in the *Panphyton Siculum* (2) – probably more than 700 copper plates (almost all engraved by Cupani himself, mainly with plants but also with animals, minerals, etc...) of which several copies differing in number and succession of pages were spread, still unpublished in 2003. The whole information included in these two works was based on large collections of wild and cultivated plants that, gathered throughout Sicily by Cupani himself or his correspondents, formed the herbaria of the Prince of Cattolica and of the living collections grown in the Misilmeri garden.

After Cupani's death (3), his scientific heritage was lost or dispersed and then forgotten for a long time. In fact the garden and the copper plates were no more extant at the end of the eighteen century. Most of the herbarium is lost, nevertheless some parts are still in existence. These are:

- The *Hortus siccus* donated by the Prince of Cattolica in 1733 to Pier Antonio Micheli (1679–1737), who was curator at the Pisa and Florence Botanical Gardens. This *Hortus siccus*, consisting of 167 bound sheets (41 × 31 cm), is arranged in a single volume lacking a title and bearing 226 specimens. To these, about 45 more specimens should be added, that have been moved by Micheli and included into his own herbarium. Several specimens of this collection were attributed to Cupani, with a specific mark by Micheli himself. Baroni (4) studied the herbarium taxonomically, also taking into account the conservation status of the specimens.

- The Cupani's "*Hortus siccus Principis Catholicae*" found in the Istituto Agrario Castelnuovo library at Palermo. It consists of two volumes of 207 and 203 bound sheets bearing on the title pages "*Hortus siccus plantarum omnigenarum ex bibliotheca Principis Catholicae Anno 1713*" II and III, respectively. It was discovered by G. Inzenga probably in 1850 and then forgotten and found again in 1995 by Mazzola & Raimondo (3)–. In both volumes each sheet bears one or more specimens belonging to a single taxon. As far as conservation is concerned, both the volumes and the specimens included are in excellent conditions. It is noteworthy that in 1713 - i.e. three years after Cupani's death – this *Hortus siccus* (three volumes or more) was bound and the *Panphyton* copper plates were printed. This probably was managed under the control of the Prince.

- The pre-Linnaean *Hortus siccus* kept in the Catania University, inventory number "VII f2 Hortus Botanicus Catinensis". This herbarium was found at the end of last century (5), and has been studied recently (6). It is bound in a single volume bearing on the title page "*Plantae siccae quae omnes in Horto Catholico crescunt et multae aliae quae Siciliae nascuntur terris plerisque locis auctore Francisco Cupani sac. theol. magistro siculo a Myrtensi – Panormo*", consisting of 164 pages with 1 to 16 specimens on each sheet. As far as its conservation is concerned, despite many specimens are damaged, most of them have been identified.

Apart from their conservation state, the above outlined *horti sicci* are distinct by several features (paper, binding, characteristics of samples, nomenclature, etc.); therefore a comparison among them is advisable. This critical examination has been started taking firstly into account the herbaria conserved in Palermo and Florence. The study is aimed to specify what can be actually attributed to F. Cupani, in order to re-establish the former correspondence between the *Panphyton* and the *exsiccata*. This also for possible nomenclatural purposes. This study can also be taken as methodological model in searching other old dispersed documents.

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## 5 = DETECTING PATTERNS OF NINE ORGANISM GROUPS BETWEEN ABANDONED AND NON INTENSIVELY MANAGED MOUNTAIN FORESTS: A CASE STUDY IN THE ITALIAN ALPS

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In the last decades, a large body of literature has grown to evaluate the impact of forestry on biodiversity in European forests to provide science-based guidelines for conservation. Studies are mainly related to boreal forests (1) and are based on the comparison between intensively managed forests, subjected to industrial forestry, and forests managed for conservation. Under these circumstances, there is general agreement on the beneficial effects of forestry abandonment on biodiversity. However, the beneficial effects for biodiversity of forestry abandonment compared to non intensive forestry practices may not be so striking and consistent across organism groups. This framework could apply to several forests of the Alps that are usually non intensively managed for timber production. In this study, we compared diversity patterns of nine organism groups (dead wood and soil macrofungi, dead wood and epiphytic lichens, understory vascular plants, bark beetles, long horn beetles, soil beetles, and birds) between mountain mixed forests of two neighbouring watersheds of the Italian Alps that are subjected to forestry abandonment and to non intensive management by selective logging. The nine organism groups were selected to encompass a wide range of substrate and resource use, considering soil, dead wood and epiphytic organisms, as well as photosynthetic organisms, decomposers and predators. The study was conducted in a mountain forest district of the eastern Italian Alps: the neighbouring Tovanello and Cjada watersheds, spanning the same elevation range and being currently subjected to contrasting forest management regimes (forestry abandonment and non intensive logging), despite a similar past-history management (2). Both areas are included in the Natura 2000 network and are therefore devoted to biodiversity conservation. In each watershed, the communities of the nine organism groups were surveyed in ten randomly selected circular plots ( $r = 12.5$  m). Overall, 35 species of dead wood macrofungi, 81 of soil macrofungi, 56 of epiphytic lichens, 22 of dead wood lichens, 105 of vascular plants, 17 of bark beetles, 15 of longhorn beetles, 19 of ground beetles, and 20 of birds were found. Species richness of five organism groups, dead wood and soil macrofungi, dead wood lichens, bark beetles, and longhorn beetles, was significantly higher in abandoned than in non intensively managed forests. For three groups of organisms (epiphytic lichens, vascular plants and soil beetles), we did not detect differences in species richness between the two sites, while species richness of birds was higher in non intensively managed than in abandoned forests. Significant differences in species composition between abandoned and non intensively managed forests were found for five organism groups: soil macrofungi, epiphytic lichens, vascular plants, bark beetles and birds. Community species composition for dead wood macrofungi and lichens, as well as for longhorn and soil beetles did not differ between abandoned and non intensively managed forests.

Our results suggests that both non intensively managed and abandoned forests contribute to biodiversity conservation in mountain forests of the Alps. In this perspective, the forest landscape should be composed of a mosaic of both stand types. This hypothesis should be further tested on a network of sites across the Alps.

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## 5 = THE ROLE OF MICROTOPOGRAPHY IN A SUBALPINE GRASSLAND: NOT ONLY STRUCTURE.

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Alpine ecosystems are predicted to be severely affected by climate change. Alpine regions are ecologically sensitive with amplified responses to climate variability (1). The balance between carbon uptake through primary plant production, and carbon loss resulting from ecosystem respiration and decomposition, determines the rate at which C is accumulated or depleted (2). Climate change can impact on both sides of this balance, and it has the potential to produce a shift in the role of alpine ecosystems, from C sinks to C sources (3). Grasslands occupy more than 30% of terrestrial surface, and they store about 20% of global terrestrial carbon (4). Most of the mechanisms that are behind the decomposition process in alpine grasslands are still unknown. Therefore, there is an urgent need to investigate the role of this process in ecosystems functionality, in order to better understand links between community structure, plant distribution and ecosystem functioning.

This research was carried out in order to understand the role of litter quality and soil properties in decomposition rates of a subalpine grassland. The study area is an unmanaged grassland dominated by *Nardus stricta*, and located in Aosta Valley (Northwest Italy). The surface is characterised by a peculiar microtopography, with the alternation of hummocks and interhummocks that present different soil properties (5).

One-year litter bags experiments were performed during the seasons 2013-2014, and 2014-2015. Litter bags were placed on soil surface at the beginning of October (2013 and 2014): 32 in an area of hummock, and 32 in an interhummock. The role of environmental factors was investigated by comparing decomposition rates between these two areas. Moreover, both in the hummock and interhummock, differences in decomposition rate between forbs and grasses were analysed in order to understand the role of litter quality and community structure. Finally, a vegetation survey was performed during the growing season 2015 to describe the plant community composition separately in hummocks and interhummocks.

The decomposition process was described both quantitatively and qualitatively. Biomass, carbon and nitrogen losses were analysed in each sample, and then compared with the initial values. Moreover, lignin concentration was estimated through a chemical extraction (6) in order to evaluate quantitative differences between grasses and forbs, and to describe changes in lignin concentration during the decomposition process. Finally, an infrared spectroscopy was carried out to obtain a qualitative description of the samples.

Results showed a higher decomposition rate in hummocks and forbs. This research allowed us to describe the effect of varying microtopography on the ecosystem functioning of this subalpine grassland. We observed that hummocks are not only a structural feature, as stocks of biodiversity, but they play also an important role in the decomposition process as functional feature. In conclusion, hummocks are essential microenvironments, where ecosystem functionality (higher decomposition rates), community structure (higher percentage of forbs), and soil properties (higher nutrients content) tightly interact with each other. This interaction results in a positive feedback that enhances decomposition, and produces a suitable environment where forbs can grow, and dominate.

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## 5 = BRERA BOTANICAL GARDEN: INTERACTION BETWEEN HISTORY AND ETHNOBOTANY IN THE PLANT COLLECTION DEVELOPMENT

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The Brera Botanical Garden was founded by Maria Theresa of Austria in 1774. Today, it, nestling among the buildings of the Milan centre, preserves and demonstrates its original layout. From the outset, the Garden has included the cultivation of medicinal plants to supply the ancient Brera Apothecary and to teach botany to the students of pharmacy and medicine. The Garden, neglected for a long time, was restored and then opened to the public in 2001 by the Università degli Studi di Milano which undertakes to revive its historical memory redeveloping the collection of officinal species. Unfortunately, due to the lack of archives, it is difficult to trace a list of plant species cultivated in the past. In order to fill this gap, knowing the interest of the first curator, Vallombrosan father Fulgentius Vitman, in plants of the Italian flora used in the folk tradition, we decided to refer to ethnobotanical studies. The results of these investigations, carried out in Northern Italy (1-3), have provided information useful to select species to be introduced, chosen also on the basis of the garden ecological conditions (e.g., microclimate, light exposure, soil). In particular, 20 family collections have been enriched. Currently, new entries represent about 10% of the total herbaceous species of the Garden. Each plant is labeled with a specific identification tag in order to create a thematic trail. The realization and the promotion of this project are useful not only for the conservation of the naturalistic heritage but also for the dissemination of scientific culture. In fact, the Brera Botanical Garden, officially recognized as a museum institution, involves school classes and groups of adults in educational activities every day.

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5. = HETEROTOPY REMASTERED WITH A QUANTITATIVE TOOL: THE CASE STUDY OF EUROPEAN BEECH (*FAGUS SYLVATICA* L. SUBSP. *SYLVATICA*) IN PENINSULAR ITALY AND SICILY

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The term "heterotopic" (from the Greek roots "hetero-" meaning "other" + "topos" meaning "place" = other place) was used for the first time in biology by Haeckel (1) to define a change in germ-layer origin of reproductive organs in animals. Later, it was applied to phytogeography by Jackson (2), referring to those plant populations found on soils apparently very different from those typically occurring across their distribution range. In Italy, Negri (3) was likely the first to use this term referring to European beech (*Fagus sylvatica* L. subsp. *sylvatica*) populations occurring at low altitude. Since then, most of the further Italian authors used the term "heterotopic" mainly referring to beech or other woody species (i.e. *Ilex aquifolium* L., *Quercus ilex* L.) populations occurring out of their common altitudinal range or, in a broader sense, growing out of their typical macroclimatic context. Indeed, as regards European beech populations in Italy, those occurring below 800 m a.s.l. have been generally considered as heterotopic (4), even though in many cases stands above 800 m a.s.l. were also termed as "heterotopic" (e.g. 5, 6).

Here, we highlight the need to quantify "heterotopy" and propose a standard method to test a reliable applicability of this concept. As model species, we selected the European beech in peninsular Italy and Sicily, primarily because this species is typically dominant in mountain woods in all the considered area and, historically, a number of stands have been reported as heterotopic in literature.

We checked 18 bibliographic references reporting 108 populations as heterotopic (sometimes not explicitly, but with related terms like extra-zonal). We also randomly generated 305 points falling within the polygons of natural potential vegetation (7) with *F. sylvatica*, as provided by <http://www.va.minambiente.it/>, and considered them as controls. Both controls and putatively heterotopic populations were georeferenced by means of a GIS software. Climatic and altitudinal data associated with the occurrence sites were extracted from the Worldclim database ([www.worldclim.org](http://www.worldclim.org)). We obtained a data matrix (413 beech stands × 6 environmental variables) that was subjected to a cluster analysis applying as the distance measure the Euclidean Distance and as group linkage method the Group Average (UPGMA), following the methodology commonly used in vegetation studies.

Two main clusters were identified, with a dissimilarity index of 0.35. The first cluster is composed by all those populations (including some control points) located at an altitude ≤ 600 m a.s.l., whereas the second one includes all the remaining points. The former cluster is also characterized by those populations growing on stands with a higher mean annual temperature than the latter (t-test, difference between means = 4.09 °C,  $p < 0.01$ ).

These results led us to define as "quantitatively heterotopic" in Italy those beech populations located at an altitude ≤ 600 m a.s.l., and with a mean annual temperature generally higher than 12 °C. They mainly occur on the Tyrrhenian side, namely in Tuscany and Lazio, marked by a high oceanicity.

Many of these populations are located outside of vegetation series with European beech and, among these, some are distant more than 20 km, so that they could be interpreted not only as heterotopic, but also as biogeographical-ecological relicts (8).

Our methodology could be applied to other species in order to quantify the level of heterotopy by defining ad hoc thresholds (if any), resulting from the multivariate analysis.

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## 5. = FREE ORBICULES OF CUPRESSACEAE IN DAILY AEROBIOLOGICAL SAMPLES

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Introduction: Pollen grains of Cupressaceae are a major cause of pollinosis, especially in Mediterranean countries (1,2), and a strong risk factor for allergic asthma (2, 3). Pollen and orbicules of Cupressaceae carry a carbohydrate epitope recognised by human IgE (4). However, while airborne pollen grains of Cupressaceae are commonly observed in aerobiological monitoring, free orbicules were never reported in aerobiological samples. We report (a) the presence of orbicules in *Calocedrus decurrens* (Torr.) Florin (Cupressaceae) and (b) the observation of free orbicules belonging to Cupressaceae in daily aerobiological samples.

Materials and Methods: Dried microsporophylls of *Calocedrus decurrens* (Torr.) Florin (Cupressaceae) were fixed to aluminum stubs with double adhesive carbon tape, sputter-coated with gold, and observed and photographed with a SEM (7) (JEOL JSM 5410, Jeol Ltd, Tokyo, Japan). Daily aerobiological samples were collected in Pisa, Italy with a "Lanzoni VPPS 2000" pollen trap installed in an urban area of Pisa about 17 m from the road pavement (Google maps coordinates 43.718343° N, 10.395110° E) and equipped with pre-siliconed sampling tape Silkostrip (Lanzoni). Sections corresponding to 24-hour intervals were cut from the tape, placed on microscope slides and stained with fuchsin jelly (5). The slides were then examined with optical (Leica Diaplan) and confocal microscope (Nikon A1plus) at 400x (6).

Results and discussion: The inner side of the tapetum and the outer side of the pollen exine of *Calocedrus decurrens* are covered by free, not embedded, orbicules ranging from 0.6 to 0.7 µm. Our observations are consistent with other researches supporting the hypothesis that orbicules may become airborne as loose particles when pollen grains are released from pollen sacs (8). Under the optical microscope, daily aerobiological samples sometimes exhibit clusters of very small, well stained dots, around Cupressaceae pollen grains. When the same samples are observed with the confocal microscope, the cluster is resolved in submicronic particles, ranging from 0.5 to 0.6 µm, with the same autofluorescence emission as the exine of the pollen grains, which leads us to identify them as orbicules (7). We were able to observe a large number of orbicules both on the exine of pollen grains of Cupressaceae and as loose bodies around them.

Conclusions: For the first time, we proved that orbicules of Cupressaceae can be detected as loose particles in aerobiological samples at the same time of the "parental" pollen grains but vastly outnumbering them. On account of their smaller size, free orbicules can reach – and carry their allergenic load (4) – much deeper in respiratory systems than pollen grains. Although further cross-disciplinary research is needed, we hypothesize that free orbicules contribute to the strong correlation of airborne pollen grains of Cupressaceae with pollinosis.

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5 = REFERENCE GROWTH CHARTS FOR ASSESSING GROWTH PERFORMANCE OF *POSIDONIA OCEANICA* (L.) DELILE

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*Posidonia oceanica* is considered a key species due to its different roles as primary producer, substrate for many species, shoreline erosion protector and long-term carbon store (1). The importance of *P. oceanica* has stimulated several studies aimed at quantifying its status. In particular growth performance of rhizomes has become among the most used descriptors for monitoring changes of *P. oceanica* meadows induced by human or natural exogenous factors (2). However, ability to detect any change of growth in space or in time is often confounded by natural age-induced variations, which involves serious interpretation problems (3). A general approach adopted to overcome this problem is to build growth charts as reference tool for comparison purposes. Charts describing patterns of biometric features conditioned to age are increasingly used as comparison tools, even if almost exclusively in *Auxology* (4). Their use can be extended to other disciplines, including ecological studies, although very large data sets are required for obtaining reliable estimates and curves should be flexible enough to account for non-linear growth pattern over age (5). In this work reference growth charts involving different *P. oceanica* growth performance measures (speed of growth and primary production of rhizomes) will be presented. Curves have been built using proper statistical frameworks (GLMM, Segmented and Quantile Regressions), based on more than 13000 annual growth data recorded by lepidochronology (6) on about 1600 shoots collected at 4–32 m depth range along Sicilian coasts. Growth patterns exhibited distinct trends as regards the relationships with depth: neither speed of growth nor primary production of rhizomes depended on depth until 14 m, while at deeper stands significant linear decrease by 3.5–2.0% for 1 m increase in depth was observed, due to light and sedimentation reduction. The considerable size of the dataset allowed to estimate the accurate shapes of the percentile curves (from 5<sup>th</sup> to 95<sup>th</sup>), revealing non monotonic relationships of growth with respect to shoot age with an initial increase followed by an overall decrease of 40% during the following years of the explored lifespan. The accompanying model-based classification procedures presented, will allow to obtain comparable results also when age of shoots is largely different (up to 20 years) (7). The growth charts may represent a noteworthy tool for researchers involved in studying of different aspects of seagrass monitoring. It is hoped that the proposed framework will facilitate assessment of growth performance status and comparative analysis of growth data from different populations around the Mediterranean Sea.

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## 5 = A NEW BOTANICAL GARDEN FOR LOMBARDY REGION: THE MUSA'S BOTANICAL GARDEN (ZIBIDO SAN GIACOMO, MI, ITALY)

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A new Botanical Garden, which is part of the museum complex of MUSA, was recently inaugurated. The museum aims to promote themes linked to the Lombardy landscape, agriculture, nutrition, taste and local traditions. The MUSA is a project, co-funded by the CARIPLO Foundation, which involved 11 partners and has enabled the renovation of part of the stables of the nineteenth century Cascina Salterio. The farm, located between Milan (12 km) and Pavia (20 km) is in the Parco Agricolo Sud Milano, along the Naviglio Pavese. It is reachable both by public transportation and by bike, through the cycle path that runs parallel to the canal. The MUSA includes a multimedia museum dedicated to the Lombardy plain landscape, an exhibition hall for conferences, exhibitions and tasting sessions, a thematic library, a cooking workshop and the Botanical Garden.

The Botanical Garden occupies the areas in front and behind the museum. It was designed in order to create a strong links with the museum themes and with an educational purposes. The plants are accompanied by tags, which will be implemented with an integrated informatics system readable by smartphone. The didactic "passive" function is associated with an "active" teaching, including practical activities of the users of the Museum (e.g.: cultivation, repotting, transplant, fruits and seeds collection, pruning etc.). In the space in front of the Museum were created 10 rectangular or square shape flowerbeds, of different surfaces, reproducing the fragmentation of the surrounding farmland landscape. In the flowerbeds were planted perennial and annual herbaceous species, directly or indirectly linked to human nutrition. Every flowerbed is dedicated to a specific topic: edible species, cereal, vegetables and aromatic plants. Local varieties and crop wild relatives were also planted. Two areas are also dedicated to the theme of traditional cultivation techniques: two examples of crop rotation were setted up (three-year and four-year) (Fig. 1). In the area behind the museum there is a single large rectangular flower bed, where were planted ancient fruit cultivars (trees and shrubs).

The future aims include the recover and restoration of other areas of the complex of Cascina Salterio, in order to enlarge the Botanical Garden, with the realization of tanks for aquatic species and a small greenhouse and to extend the open area also to the historical garden of the manor, to further expand the offer to the public.

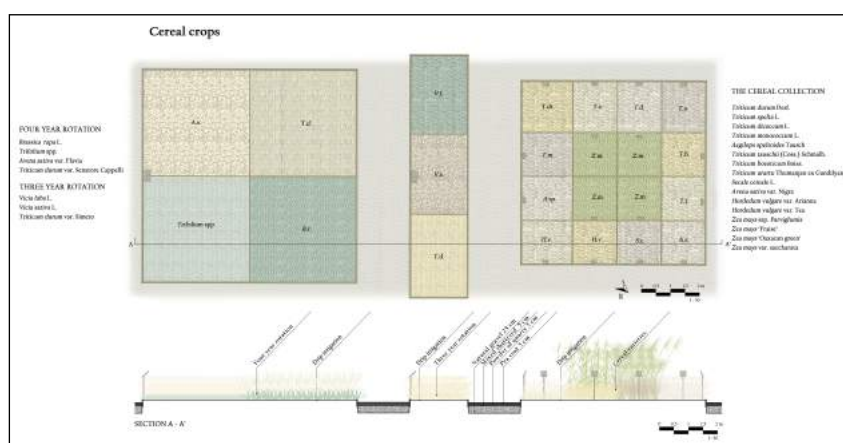


Fig. 1. Project plan of the flowerbeds cereals



Fig. 2. View of flower beds

5. = IS MORPHOLOGICAL VARIABILITY IN *PLANKTOTHRIX RUBESCENS* RELATED TO TOXIN PRODUCTION? EVIDENCES FROM THE VOLCANIC LAKE VICO (LATIUM, ITALY) OVER A FIVE YEAR PERIOD

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Human activities can substantially increase occurrence and severity of cyanobacterial harmful blooms (CHABs), and consequently the presence of cyanotoxins can reach critical concentration in water reservoirs intended for human consumption. In this paper, we address the relationship between filamentous toxic cyanobacterial succession and toxin production in Lake Vico, a volcanic lake situated north of Rome (Italy). In order to understand the seasonal succession of CHABs, 60 water samples were collected at the inlet of the water treatment plant serving the local community, and cell counts, morphometric analyses as well as quantitative toxin determination using LC/MS/MS analysis were carried out. Light microscopy analysis allowed discriminating between four toxic species: *Chrysochloris ovalisporum*, *Dolichospermum* sp., *Limnothrix redekei* and *Planktothrix rubescens*, the latter largely dominating the assemblage over the study period. This species was also the main toxin producer, showing an increasing toxicity from late summer to winter. The morphometric analysis revealed that filament elongation in *P. rubescens* anticipated toxin retrieval in water, prospecting potential for early warning of water contamination.

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