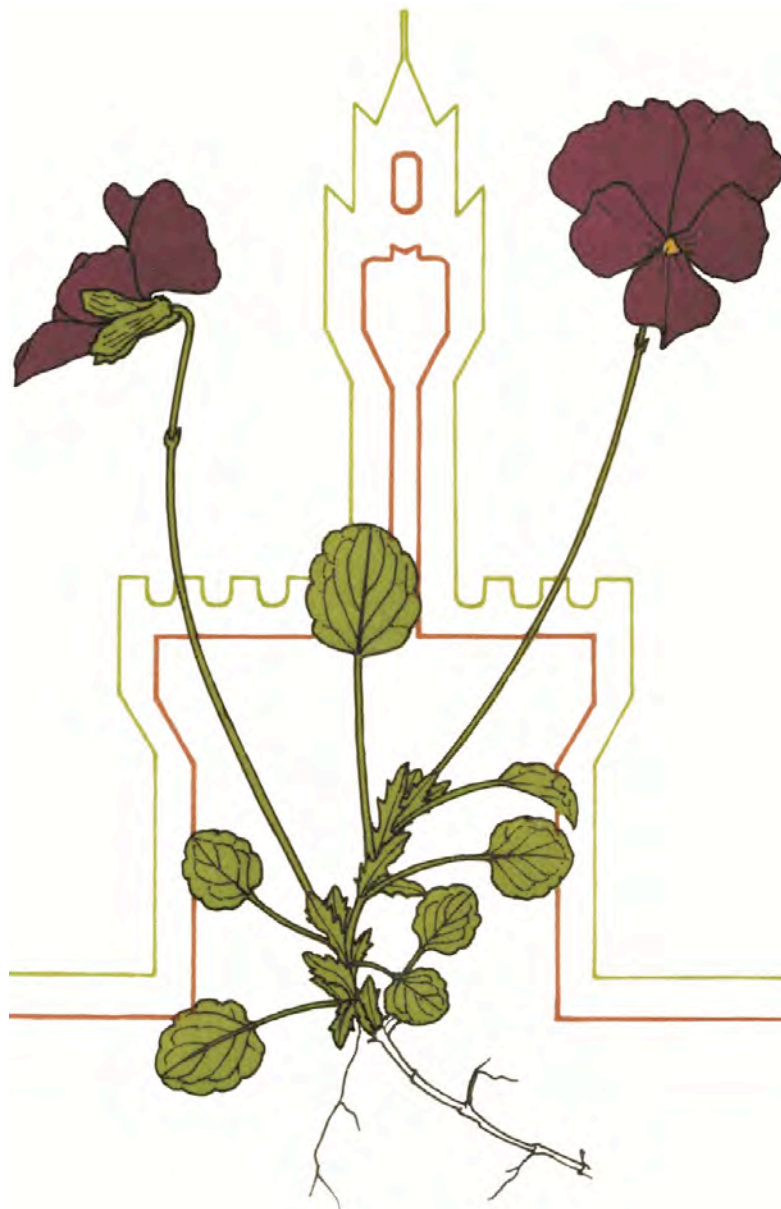


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# **Società Botanica Italiana**

## **109° Congresso**



**International Plant Science Conference (IPSC)**

**from Nature to Technological Exploitations**

Florence, 2 - 5 September 2014

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# 109° Congresso della Società Botanica Italiana

## INTERNATIONAL PLANT SCIENCE CONFERENCE

Florence, 2 - 5 September 2014

### Programme

#### Tuesday 2 September 2014

13:00-19:00 Registration

14:00-15:00 Opening Ceremony

#### Keynote lecture

15:00-15:30

- **Chiara Tonelli**, Milano IT (ERC Representative)  
(title to be announced)

#### (Topic I) Global Change, Biodiversity and Adaptation

*Plant responses to climate change*

*(Symposium 1)*

15:30-19:00

- **Giampiero Maracchi**, Firenze IT (20 + 5 min)  
Climate changes and scenarios
- **Terry Callaghan**, Sheffield UK (20 + 5 min)  
Interpreting long-term ecosystem change and stability: case studies from the Arctic and Sub-Arctic
- **F. Stuart Chapin III**, Fairbanks USA (20 + 5 min)  
Ecosystem stewardship: sustainability strategies for a rapidly changing planet

#### Communications

- **Simon Pierce**  
Understanding the response of plant biodiversity to environmental perturbation using Grime's CSR theory
- **Consolata Siniscalco**  
Phenological analysis provides insights on the spatial distribution and on response to snowmelt of plant functional types in subalpine grassland
- **Michele Dalle Fratte**  
Relationships between climate and phenology of high elevation plants. A 7-years snow and phenology monitoring in the Italian Central Alps
- **Luca Bragazza**  
Persistent extreme climatic events reduce carbon accumulation in peatlands: mechanisms and quantification
- **Andrea Mondoni**  
Effects of climate change on seed germination and recruitment success of alpine plants

- **Giovanna Aronne**  
From cold mountains to warm coastal cliffs: insights for the future of a *Primula* species
- **Francesco Malfasi**  
Long-term vegetation dynamic in relation to climate change in the Italian Central Alps

Wednesday 3 September 2014

**(Topic I) Global Change, Biodiversity and Adaptation**

*Plant responses to climate change*

*(Symposium 2)*

09:00-10:00

- **Giovanni Vendramin**, Firenze IT (20 + 5 min)  
Molecular markers, population genomics and adaptation to climate change of forest tree species
- **Sandy Knapp**, London UK (20 + 5 min)  
Botanical collections for understanding environmental change

**(Topic II) Plant and Cell Biology**

*Plant development and reproduction*

*(Symposium 1)*

10:00-13:00

- **Donato Chiatante**, Varese IT (20 + 5 min)  
Development of the hidden half: primary and secondary roots
- **Silvia Coimbra**, Oporto PT (20 + 5 min)  
AGPs cross-talk in *Arabidopsis* pollen-pistil interaction
- **Fabio Fornara**, Milano IT (20 + 5 min)  
Network controlling seasonal flowering in rice

**Communications**

- **Stefano Del Duca**  
Factors involved in pollen tube growth and their importance in allergic sensitization
- **Alessandra Manzo**  
Characterization of volatile emission of Italian populations of orchids with different reproductive strategies
- **Maria Adele Signorini**  
Investigations on *Oxalis pes-caprae* in Italy. Morphological, anatomical and ultrastructural features of stigma and style and their possible relations with self-incompatibility
- **Giampiero Cai**  
Effects of acute heat stress on the female reproductive apparatus of *Lycopersicon esculentum* cv micro-tom
- **Livio Trainotti**  
A golen-like peptide at the crossroad between auxin and ethylene during peach ripening
- **Simona Masiero**  
Transcriptional regulation of egg cell specific genes in *Arabidopsis thaliana*

13:00-14:00 Lunch

## (Topic II) Plant and Cell Biology

### Plant cell dynamics and plasticity

#### (Symposium 2)

14.00-17.00

- **Chris Hawes**, Oxford UK (20 + 5 min)  
Shaping the endoplasmic reticulum
- **Alessandro Vitale**, Milano IT (20 + 5 min)  
The endoplasmic reticulum as a protein storage compartment

#### Communications

- **Lorella Navazio**  
Integration of plastids in the plant calcium signaling network revealed by the targeting of aequorin chimeras to chloroplast subcompartments
- **Giacomo Bartoli**  
Apoptotic hallmarks support the role of PCD in aerenchyma ontogenesis of *Egeria densa* stem
- **Daniele Nocentini**  
Diversity of floral nectar composition in the tribe *Lithospermeae* (Boraginaceae)
- **Marco Mucciarelli**  
In vitro morphogenesis of *Arabidopsis* to search for novel endophytic fungi modulating plant growth
- **Andrea Andreucci**  
In vitro functional characterization of AQUA1: a new poplar (*Populus x euramericana* clone I-214) aquaporin involved in zinc stress
- **Antonio Slaviero**  
The use of ligninase enzymes to target the scarification of the seed coat: application for the propagation of the orchids *Himantoglossum adriaticum* and *Anacamptis morio*

17.00-19.00 Meeting of SBI Working Groups

Thursday 4 September 2014

## (Topic III) Biotechnology and Natural Resources

### Plant biotechnology for agriculture and environment

#### (Symposium 1)

09:00-11:00

- **Michele Morgante**, Udine IT (20 + 5 min)  
From one to the many genomes of a plant: the evolution of the grapevine pan-genome
- **Graziella Berta**, Alessandria IT (20 + 5 min)  
Reclamation of polluted or disturbed sites by revegetation and use of beneficial microorganisms
- **Robert Verpoorte**, Leiden NE (20 + 5 min)  
Metabolomic and Botany

#### Communications

- **Monica Ruffini Castiglione**  
*Vicia faba*: a model organism for plant cell biology studies assessing environmental pollution and bioremediation processes

- **Elisabetta Sgarbi**  
In vitro propagation of *Quercus robur* by plantform bioreactor

### (Topic III) Biotechnology and Natural Resources

#### Bioactive natural resources

#### (Symposium 2)

11:00-13:30

- **Nunziatina de Tommasi**, Salerno IT (20 + 5 min)  
Plant chemical diversity in target identification and drug discovery

#### Communications

- **Barbara Sgorbini**  
Aromatic plants: from the aroma to herbal teas
- **Giacomo Mele**  
Secreted material and antimicrobial activity of *Salvia cacaliaefolia*
- **Alessandra Guerrini**  
HP-TLC bioautographic assay as a preliminary research tool to match chemical and biological properties of official plant extracts
- **Cinzia Sanna**  
Preliminary study of endemic plants of Sardinia as a source of new antiviral agents
- **Filomena Conforti**  
*Origanum vulgare* subsp. *viridulum*: a phytoalimurgic plant with inhibitory against human cancer cell proliferation
- **Alessandra Braca**  
Flavonoids and phenylpropanoids from *Phlomis kurdica* (Lamiaceae) as inhibitors of lactate dehydrogenase
- **Giovanna Certo**  
*Entada africana* (Mimosaceae) as a source of skin whitening agents
- **Manuela Mandrone**  
Phytochemical profile and biological activities evaluation of three species of *Hypericum*

13:30-14:30 Lunch

14.30-18.00 General Assembly of SBI members and other related celebrations

20.00 Social Dinner (Botanical Garden “Giardino dei Semplici” - Via Lamarmora, 2)

## Friday 5 September 2014

Villa Bardini - Costa San Giorgio 2, Firenze

### Session 1

### (Topic IV) Landscape and Biogeography

#### Vegetation and Ecology

#### (Symposium 1)

9:00-11:00

- **Carlo Blasi**, Roma IT (20 + 5 min)  
Mapping and assessment of ecosystems in Italy: the contribution of modern vegetation science

- **Frank Berendse**, Wageningen NE (20 + 5 min)  
Ecosystem functions and the mechanisms that control the species diversity in plant communities

#### Communications

- **Anna Maria Mercuri**  
Palynology applied to the study of climate change and human impact: pollen from archaeological sites as tool for the assessment of long-term local impact and human induced environments
- **Lara Reale**  
Reed-beds decline: new occurrences of a dramatic threat to biodiversity in Central Italy
- **Anna Guglielmo**  
Diacronic landscape changes: a case study on S-E Sicily salt marshes
- **Sabina Burrascano**  
A dynamic key to integrate grassland and forest sustainable management in a global change perspective

### (Topic IV) Landscape and Biogeography

#### Biogeography and Taxonomy (Symposium 2)

11:00-13:00

- **Elena Conti**, Zürich CH (20+ 5 min)  
Processes and outcomes of diversification in *Primulaceae*: explorations on the functional and evolutionary roles of heterostyly
- **Federico Selvi**, Firenze IT (20 + 5 min)  
The family Boraginaceae: deep phylogeny, character evolution and still open taxonomic problems

#### Communications

- **Lorenzo Peruzzi**  
Recent advances in evolution and taxonomy of Liliaceae
- **Flavia Domizia Nardi**  
Traditional and molecular cytogenetic characterization of *Solidago* (Asteraceae) in Italy
- **Assunta Esposito**  
Analysis of *Olea europaea* subsp. *europaea* biodiversity from Cilento, Vallo di Diano and Alburni National Park

#### Session 2

### (Topic V) Historic Gardens and Vegetation

9:00-11:00

- **Luigi Zangheri**, Firenze IT (20 + 5 min)  
Trees and the cultural landscapes of World Heritage List
- **Massimo De Vico Fallani**, Roma IT (20 + 5 min)  
Trees of archaeological parks. History and types
- **Francesco Ferrini**, Firenze IT (20 + 5 min)  
“Historic gardens ecosystems”: can one provide public use and maintain the ecological balance?

### **Communications**

- **Paola Capone**, Salerno IT (10 + 5 min)  
From Salerno's Minerva Garden to the illuminated herbarium of the Circa instans: a path with no boundaries
- **Maria Chiara Pozzana**, Firenze IT  
Presentation of "Giardino Bardini" and its restoring

11:00-13:00 **Visit to "Giardino Bardini"**

14:30-18:00 **Visit to "Giardino di Boboli"** (World Heritage List)  
**Meeting point "Entrata Annalena"** (Via Romana, 37)



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- I. CALLAGHAN T. "Interpreting long-term ecosystem change and stability: case studies from the Arctic and Sub-Arctic"
- II. CHAPIN F.S. III "Ecosystem stewardship: sustainability strategies for a rapidly changing planet"
- \* VENDRAMIN G. "Molecular markers, population genomics and adaptation to climate change of forest tree species"
- \* KNAPP S. "Botanical collections for understanding environmental change"

## (Topic II) Plant and Cell Biology

### Plant Development and Reproduction

- III. CHIATANTE D., MONTAGNOLI A., TERZAGHI M., BAESSO B., FULGARO N., TRUPIANO D., SCIPPA G.S. "Development of the hidden half: primary and secondary roots"
- IV. PEREIRA A.M., DA COSTA M.L., PEREIRA L.G., COIMBRA S. "AGPs cross-talk in Arabidopsis pollen-pistil interaction"
- \* FORNARA F. "Network controlling seasonal flowering in rice"

### Plant Cell Dynamics and Plasticity

- V. HAWES C. "Shaping the endoplasmic reticulum"
- VI. VITALE A. "The endoplasmic reticulum as a protein storage compartment"

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### Plant Biotechnology for agriculture and environment

- VII. PINOSIO S., MAGRIS G., MARRONI F., DI GASPERO G., MORGANTE M. "From one to the many genomes of a plant: the evolution of the grapevine pan-genome"
- \* BERTA G. "Reclamation of polluted or disturbed sites by revegetation and use of beneficial microorganisms"
- \* VERPOORTE R. "Metabolomic and Botany"

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- VIII. DE TOMMASI N. "Plant chemical diversity in target identification and drug discovery"

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- IX. BLASI C. "Mapping and assessment of ecosystems in Italy: the contribution of modern vegetation science"
- X. BERENDSE F. "Ecosystem functions and the mechanisms that control the species diversity in plant communities"

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- XI. CONTI E. "Processes and outcomes of diversification in *Primulaceae*: explorations on the functional and evolutionary roles of heterostyly"
- XII. SELVI F., CECCHI L., COPPI A., HILGER H.H., WEIGEND M. "The family *Boraginaceae*: deep phylogeny, character evolution and still open taxonomic problems"

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- \* ZANGHERI L. "Trees and the cultural landscapes of World Heritage List"
- \* DE VICO FALLANI M. "Trees of archaeological parks. History and types"
- XIII. FERRINI F. " 'Historic gardens ecosystems': can one provide public use and maintain the ecological balance?"

\* abstract not received

# COMMUNICATIONS

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### Plant responses to climate change

- i. PIERCE S., CERABOLINI B.E.L. “Understanding the response of plant biodiversity to environmental perturbation using Grime’s CSR theory”
- ii. SINISCALCO C., JULIITA T., CREMONESE E., FILIPPA G., FREPPAZ M., ROSSINI M., GALVAGNO M., MIGLIAVACCA M., CELI L., COLOMBO R., MORRA DI CELLA U. “Phenological analysis provides insights on the spatial distribution and on response to snowmelt of plant functional types in subalpine grasslands”
- iii. CANNONE N., DALLE FRATTE M., GUGLIELMIN M. “Relationships between climate and phenology of high elevation plants. A 7-years snow and phenology monitoring in the Italian Central Alps”
- iv. BRAGAZZA L., GAVAZOV K.S., SIGNARBIEUX C., BUTTLER A. “Persistent extreme climatic events reduce carbon accumulation in peatlands: mechanisms and quantification”
- v. MONDONI A., ORSENIGO S., PROBERT R., BONOMI C., ABELI T., ROSSI G. “Effects of climate change on seed germination and recruitment success of alpine plants”
- vi. ARONNE G., BUONANNO M., DE MICCO V. “From cold mountains to warm coastal cliffs: insights for the future of a *Primula* species”
- vii. DALLE FRATTE M., CANNONE N., MALFASI F. “Long-term vegetation dynamic in relation to climate change in the Italian Central Alps”

## (Topic II) Plant and Cell Biology

### Plant development and reproduction

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- ix. GIORGI A., VAGGE I., PANSERI S., MANZO A. “Characterization of volatile emission of italian populations of orchids with different reproductive strategies”
- x. SIGNORINI M.A., TANI C., CALAMASSI R., BRUSCHI P. “Investigations on *Oxalis pes-caprae* L. in Italy. Morphological, anatomical and ultrastructural features of stigma and style and their possible relations with self-incompatibility”
- xi. MARERI L., FALERI C., MUCCIFORA S., BELLANI L.M., CRESTI M., MARIANI C., CAI G. “Effects of acute heat stress on the female reproductive apparatus of *Lycopersicon esculentum* Cv micro-tom”
- xii. TADIELLO A., BUSATTO N., ZIOSI V., NEGRI A.S., SPINELLI F., ESPEN L., VENDRAMIN E., VERDE I., COSTA G., TRAINOTTI L. “A golven-like peptide at the crossroad between auxin and ethylene during peach ripening”
- xiii. RESENTINI F., CYPARYS P., MORANDINI P., SPRUNCK S., DRESSELHAUS T., COLOMBO L., MASIERO S. “Transcriptional regulation of egg cell specific genes in *Arabidopsis thaliana*”

### Plant cell dynamics and plasticity

- xiv. SELLO S., ZANELLA F.G., MEHLMER N., CARRARETTO L., BALDAN B., SZABÒ I., VOTHKNECHT U., NAVAZIO L. “Integration of plastids in the plant calcium signalling network revealed by the targeting of aequorin chimeras to chloroplast subcompartments”
- xv. BARTOLI G., FORINO L.M.C., DURANTE M., TAGLIASACCHI A. “Apoptotic hallmarks support the role of PCD in aerenchyma ontogenesis of *Egeria densa* stem”
- xvi. NOCENTINI D., GUARNIERI M., CECCHI L., SELVI F., WEIGEND M., MACCHERINI S., NEPI M. “Diversity of floral nectar composition in the tribe Lithospermeae (Boraginaceae)”
- xvii. DOVANA F., MASCARELLO M., FUSCONI A., MUCCIARELLI M. “*In vitro* morphogenesis of *Arabidopsis* to search for novel endophytic fungi modulating plant growth”
- xviii. ARIANI A., SEBASTIANI L., ANDREUCCI A. “*In vitro* functional characterization of AQUA1: a new poplar (*Populus x euramericana* clone I-214) aquaporin involved in zinc stress”
- xix. SLAVIERO A., BUFFA G., CERIANI R.M., CERABOLINI B., SIMON P. “The use of ligninase enzymes to target the scarification of the seed coat: application for the propagation of the orchids *Himantoglossum adriaticum* and *Anacamptis morio*”

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- xx. RUFFINI CASTIGLIONE M., DI GREGORIO S., GIORGETTI L. “*Vicia faba* L.: a model organism for plant cell biology studies assessing environmental pollution and bioremediation processes”
- xxi. GATTI E., OZUDOGRU A., LAMBARDI M., SGARBI E. “*In vitro* propagation of *Quercus robur* L. by plantform bioreactor”

### Bioactive natural resources

- xxii. SGORBINI B., CAGLIERO C., BOGGIA L., COLOMBO M.L., BICCHI C., RUBIOLO P. “Aromatic plants: from the aroma to herbal teas”
- xxiii. BISIO A., SCHITO A.M., MELE G., GLASL-TAZREITER S., PARRICCHI A., ROMUSSI G., DE TOMMASI N. “Secreted material and antimicrobial activity of *Salvia cacaliaefolia* Benth.”
- xxiv. GUERRINI A., TACCHINI M., GRANDINI A., SPAGNOLETTI A., MARESCA I., ROSSI D., MAIETTI S., SACCHETTI G. “HP-TLC bioautographyc assay as a preliminary research tool to match chemical and biological properties of officinal plant extracts”
- xxv. SANNA C., BALLERO M., MAXIA A., MARENGO A., CORONA A., TRAMONTANO E., TAGLIATELLA-SCAFATI O., ESPOSITO F. “Preliminary study of endemic plants of Sardinia as a source of new antiviral agents”
- xxvi. CONFORTI F., MARRELLI M., MENICHINI F. “*Origanum vulgare* ssp. *viridulum*: a phytoalimurgic plant with inhibitory activity against human cancer cell proliferation”
- xxvii. BRACA A., DE TOMMASI N., BADER A., MINUTOLO F. “Flavonoids and phenylpropanoids from *Phlomis kurdica* Rech. F. (Lamiaceae) as inhibitors of lactate dehydrogenase”
- xxviii. CERTO G., RAPISARDA A., SANOGO R., D’ANGELO V., GERMANÒ M. “*Entada africana* Guill. & Perr. (Mimosaceae) as a source of skin whitening agents”
- xxix. MANDRONE M., LORENZI B., SCOGNAMIGLIO M., FIORENTINO A., CORNIOLI L., SANNA C., ANTOGNONI F., POLI F. “Phytochemical profile and biological activities evaluation of three species of *Hypericum*”

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- xxx. MERCURI A.M., FLORENZANO A., MONTECCHI M.C., RATTIGHIERI E., TORRI P., BANDINI MAZZANTI M. “Palynology applied to the study of climate change and human impact: pollen from archaeological sites as tool for the assessment of long-term local impact and human-induced environments”
- xxxi. REALE L., COPPI A., LASTRUCCI L., FOGGI B., VENANZONI R., FERRANTI F., GIGANTE D. “Reed-beds decline: new occurrences of a dramatic threat to biodiversity in Central Italy”
- xxxii. SPAMPINATO G., CAMERIERE P., SCIANDRELLO S., GUGLIELMO A. “Diacronic landscape changes: a case study on S-E Sicily salt marshes”
- xxxiii. BURRASCANO S., COPIZ R., DEL VICO E., FACIONI L., GIARRIZZO E., SABATINI F.M., ZANINI M., ZAVATTERO L., BLASI C. “A dynamic key to integrate grassland and forest sustainable management in a global change perspective”

### Biogeography and Taxonomy

- xxxiv. NARDI F.D., PUSTAHIJA F., SILJAK-YAKOVLEV S., PERUZZI L. “Traditional and molecular cytogenetic characterization of *Solidago* (Asteraceae) in Italy”
- xxxv. PERUZZI L. “Recent advances in evolution and taxonomy of Liliaceae”
- xxxvi. ESPOSITO A., SCOGNAMIGLIO M., DE LUCA P.F., MARINO D., CROCE A., D’ABROSCA B., FIORENTINO A. “Analysis of *Olea europaea* subsp. *europaea* biodiversity from Cilento, Vallo di Diano and Alburni National Park”

## (Topic V) Historic Gardens and Vegetation

- xxxvii. CAPONE P. “From Salerno’s Minerva Garden to the illuminated herbarium of the *Circa instans*: a path with no boundaries”

# POSTERS

## 1. Ultrastructure, Physiology, and Metabolism

### 1.1 Cell, differentiation, communication

1. ALOISI I., FALERI C., CAI G., DEL DUCA S. “Could spermine play a role during the apical growth of pollen tube?”
2. CARRARI E., AMPOORTER E., VERHEYEN K., COPPI A., SELVI F. “Investigating the role of old charcoal places for understory vegetation diversity in Mediterranean forests”
3. PAPINI A., FANI F., BELLI M., NICCOLAI C., TANI C., DI FALCO P., NUCCIO C., LAZZARA L. “Structural changes showing cannibalism phenomena in *Heterosigma akashino* (Hada) Hada ex Hara et Chihara (Raphidophyceae) cells, recovering from short or extended nutrient depletion”

### 1.2 Epigenetics and chromatin

4. GRANO A., DE TULLIO M.C. “The interactive games of the E-MOVE! Project: new tools for teaching plant diversity, evolution and development”

### 1.3 Membrane and cytoskeletal network

5. DE CAROLI M., LENUCCI M.S., DI SANSEBASTIANO G.-P., TUNNO M., MONTEFUSCO A., DALESSANDRO G., PIRO G. “The chimera AtCslA2-GFP is functionally inserted into Golgi membrane and synthesises  $\beta$ -mannans”
6. DI SANSEBASTIANO G.-P., DALESSANDRO G., PIRO G. “Direct interaction in *Arabidopsis* of snare protein SYP51 with non-snare protein NLM1”

### 1.4 Omics”: physiology and metabolism

7. FERRONI L., PANCALDI S. “Room temperature microspectrofluorimetry and photosynthetic carbon fixation in the Lycophyte *Selaginella martensii*”
8. GRECO M., SÁEZ C.A., CONTRERAS R., RAMESH K., BROWN M.T., BITONTI M.B. “Genomic and epigenomic mechanisms to cope with excess copper and cadmium levels in *Zostera marina* L. seagrass”
9. SABIA A., FERRONI L., GIOVANARDI M., BALDISSEROTTO C., PANCALDI S. “A comparison of protein content of four species of Chlorophyta microalgae”

### 1.5 Organelles

10. MOSCATIELLO R., MOSCHIN S., TEARDO E., SZABÒ I., NAVAZIO L., CENDRON L. “Preliminary structural and functional studies of a plastidial homologue of the mitochondrial calcium uniporter in *Arabidopsis thaliana*”

## 2. Growth and Reproduction

### 2.1 Control of root and shoot development

11. BAESSO B., CHIATANTE D., SCIPPA G.S., NIEMINEN K., ZHANG J., HELARIUTTA YKÄ, FULGARO N., MONTAGNOLI A., TERZAGHI M. “Identification of molecular factors controlling root system development”
12. FULGARO N., TERZAGHI M., BAESSO B., SCIPPA G.S., MONTAGNOLI A., CHIATANTE D. “Analysis of seedlings growth under different led lights”
13. GALLIANI B.M., MASIERO S., MIZZOTTI C. “Control of lateral meristem formation in *Antirrhinum majus*”
14. MARRAS T., SCHIRONE B. “Analysis of *Corylus avellana* L. growth under led lights for reforestation purposes”
15. MONTAGNOLI A., TERZAGHI M., SCIPPA G.S., BAESSO B., FULGARO N., CHIATANTE D. “Fine-root morphological and growth traits in a *Quercus ilex* L. forest”

### 2.2 Flower, fertilization, and seed development

16. BRAMBILLA V., SHRESTHRA R., GORETTI D., MARTIGNAGO D., GOMEZ-ARIZA J., GALBIATI F., MANIEZZO M., SOMSSICH M., SIMON R., FORNARA F. “Developmental reprogramming after photoperiodic induction at the shoot apical meristem of rice”
17. CUCINOTTA M., GALBIATI F., SIMONINI S., GUAZZOTTI A., MASIERO S., CAPORALI E., COLOMBO L. “Monopteros (MP) a central integrator of pathways controlling ovule primordia formation”
18. IARIA D., MUZZALUPO I., CHIAPPETTA A. “Transcriptome analysis and identification of gene related to pollen tube development in olive (*Olea europaea* L.)”
19. SAU S., PUTZOLU M.S., RODI V., CORTIS P. “Pollinators of the genus *Ophrys* in Sardinia: state of the art”

## 2.3 Germination

20. CRISTAUDO A., CATARA S., RESTUCCIA A. "Propagation protocols of native Mediterranean plant species: nursery applications, restoration projects, habitats and plants conservation"
21. FAZIO A., MUSARELLA C.M., PANUCCIO M.R., MOTA J., SPAMPINATO G. "Comparison of ex-situ germination response of *Lavandula multifida* L. in two populations of S-Italy and S-Spain"
22. FRATTAROLI A.R., DI CECCO V., DI MARTINO L., CATONI R., VARONE L., DI SANTO M., GRATANI L. "Seed germination capability of *Astragalus aquilanus* endemic species in the Central Apennines (Italy)"
23. GALIÈ M., CASAVECCHIA S., GASPARRI R., SORIANO P., ESTRELLES E., BIONDI E. "Production of native seeds for semi-natural grasslands recovery in Apennines and Pre-Apennines areas"
24. GIORGETTI L., LONGO V., GERVASI P.G., GIORGI G., BELLANI L.M. "Antioxidants content in *Brassica oleracea* var *acephala* from dry seed to plantlet"
25. PIRONDINI A., MARKS T.R., SGARBI E. "Seed storage of *Himantoglossum robertianum* (Orchidaceae): a comparison of temperature and moisture conditions"
26. PODDA L., BIAGINI L. "Germination ecophysiology and reproductive biology of invasive species that threaten environment, economic activity and human health"
27. PUGLIA G., CARTA A., GRIMALDI S., TOOROP P., PAVONE P. "*Glebionis coronaria* (L.) Spach (Asteraceae) seed germination, pericarp anatomy and water uptake in relation to seed dormancy"

## 2.4 Histology and anatomy

28. GIULIANI C., TANI C., MALECI L. "The peculiar chloroplasts of leaf parenchyma cells in *Helichrysum italicum* (Roth) G. Don."
29. MALECI L., TANI C., BINI C. "New insights on the resilience capacity of *Taraxacum officinale* Weber growing on mine soils"

# 3. Plant-environment interaction

## 3.1 Biodiversity

30. ANGELINI P., BISTOCCHI G., ARCANGELI A., RUBINI A., VENANZONI R. "Biodiversity and fungal conservation in the Collestrada forest (Umbria, Italy)"
31. ASSINI S., SUGNI M., RINALDI G., FAVARON M., CACCIANIGA M., VAGGE I., FICO G. "Promoting the use of native plants in urban areas: a project for the biodiversity conservation in Lombardy region"
32. BEDINI G., PIERINI B., ROMA-MARZIO F., CAPARELLI K., DOLCI D., GESTRI G., PERUZZI L. "Wikiplantbase #TOSCANA: breaking the dormancy of floristic data"
33. CAMARDA I., COSSU T., BRUNDU G. "The catalogue of the non-native flora of Sardinia (Italy)"
34. CAMARDA I., BRUNU A., CARTA L. "Endemic, priority and rare species in the habitats of the Nature Map of Sardinia"
35. CAMBRIA S., DOMINA G., RAIMONDO F.M., DI GREGORIO G. "*Monotropa hypophegea* Wallr., a new record for the Sicilian flora"
36. CAMPISI P., VELLA V., DIA M.G. "The Bryophytes of Gorgo Lungo, Gorgo del Drago and Coda di Riccio wetlands in the oriented nature reserve 'Bosco della Ficuzza, Rocca Busambra, Bosco del Cappelliere e Gorgo del Drago' (Palermo)"
37. CASAZZA G., GRASSI F., ZECCA G., NICOLETTI F., DE BENEDETTI L., MINUTO L. "Phylogeographical investigation on *Silene cordifolia* and *Viola argenteria* endemic to the Maritime Alps: similitude in their genetic history"
38. CECCHI L., SELVI F. "*Flora critica d'Italia*: a synopsis of *Boraginaceae* tribe *Boragineae*"
39. COMPAGNO R., GARGANO M.L., LA ROSA A., VENTURELLA G. "Fungal diversity in urban forest ecosystems"
40. COMPAGNO R., GRISAFI F., MANNINO A.M., OTTONELLO D., ALAIMO M.G., LA ROSA A., GARGANO M.L., VENTURELLA G. "Investigation on old-growth forests of Sicily: preliminary results"
41. DOMINA G., HAJ SAMI BEN, CICCARELLO S., SCAFIDI F. "Plant landscape of Ras Dimas peninsula (Governatorate of Monastir, Central Coastal Tunisia)"
42. ERCOLE S., GIACANELLI V. "Plant species protected under Habitats Directive: Italian national report for the period 2007-2012"
43. FACIONI L., BARBATI A., BURRASCANO S., DEL VICO E., SABATINI F.M., PORTOGHESI L., CORONA P., BLASI C. "Life FAGUS: a project for the enhancement of structural heterogeneity and biodiversity in Apennine beech forests (HABITAT 9210\* and 9220\*)"
44. FILIPPINO G., ZOCCHEDDU M., CORTIS P., COGONI A. "Analysis of Bryophyte's spores in Mediterranean temporary ponds"
45. GATTO R., CALÒ M.V., ALBANO A., ZUCCARELLO V., ACCOGLI R. "Plant biodiversity learning through recreative and educational experience"

46. GENTILI R., GILARDELLI F., SGORBATI S., GHIANI A., CIAPPETTA S., CITTERIO S. “Distribution range of four invasive alien species in Italy: *Ambrosia artemisiifolia* L., *Reynoutria japonica* Houtt., *Prunus serotina* Ehrh., *Senecio inaequidens* DC”
47. GIOVINO A., SCIBETTA S., BONARI G., MONTAGNANI C., TURCATO C., SAIA S. “*Chamaerops humilis* L.: Italian distribution and characterization”
48. LAGO C., BORGONOVO G., MANZO A., LANDONI M., PILU R., GIORGI A. “Preliminary characterization of an ancient colored flint maize cultivar, originating from Valcamonica (BS)-Italy”
49. MARRESE P.P., IURLARO A., PERROTTA C., DE BELLIS L., PIRO G., DALESSANDRO G., LENUCCI M.S. “Characterization of lipophilic antioxidant profile in whole kernels of durum wheat lines from Sicily”
50. MARTELLOS S., ATTORRE F. “CSMON-Life: data from the people, data for the people”
51. MICHELI C., BELMONTE A., DE CECCO L., MARTINI S., CARLI F., COGNETTI DE MARTIIS S., GNISCI V., PIERMATTEI V., MARCELLI M., BORFECCHIA F. “An integrated monitoring method to detect biodiversity of *Posidonia oceanica* (L) delile habitat”
52. POLI MARCHESE E., TURRISI R.E. “The ‘*Arboretum aetneum*’ of the Nuova Gussonea botanic garden on Mount Etna”
53. PRIVITERA M., PUGLISI M. “Bryophyte vegetation diversity for monitoring the anthropic disturbance: a study on the Eolian Islands (Sicily)”
54. RAIMONDO F.M., CASTIGLIA G., MUSACCHIA D. “Trees related to *Platanus racemosa* (Platanaceae) in the context of the city of Palermo (Sicily)”
55. ROMA-MARZIO F., BERNARDO L., PERUZZI L. “Vascular flora of Monte Sparviere (Southern Italy, Pollino Massif)”
56. SANTANGELO A., DE LUCA G., GENOVESE M., SCOTTO DI CESARE M., STRUMIA S. “Analysis of plant diversity in the Island of Vivara (NA), Southern Italy”
57. SCHETTINO A., BERNARDO L., BORGHETTI M., COLANGELO M., GARGANO D., LAPOLLA A., MARCHIANÒ V., MISANO G., PASSALACQUA N.G., RIVELLI A.R., RIPULLONE F. “Old-growth forests in the Pollino National Park: state of the art and future perspectives”
58. SCHICCHI R., AMATO F., LA PLACA G., BONOMO P. “Population trend in *Abies nebrodensis* (Lojac.) Mattei”
59. SORIANO P., ESTRELLES E., GALIÈ M., CASAVECCHIA S., BIONDI E. “Conservation of the *Halocnemum strobilaceum* and *H. cruciatum* halophytic vegetation in Mediterranean habitats through the knowledge of seed features and germination behavior”
60. SPADARO V., RAIMONDO F.M., DOMINA G. “*Verbena bonariensis* (Verbenaceae) adventive in Italy”
61. STINCA A., BONANOMI G., PERRINO E.V., MOTTI R. “Epiphytic biodiversity on *Phoenix canariensis* Chabaud in Southern Italy”
62. TERMINE R., PASTA S., LA MANTIA T. “Some remarks on the vascular flora and vegetation of the archaeological site of “Vallone Canalotto” (Calascibetta municipality, Enna province, Central Sicily)”
63. TRAVAGLINI A., DELORENZO M., CANINI A., PAOLELLA F., MURGANTE A., REDI E.L., RICCARDUCCI G., FRATARCANGELI C., MARCANTONIO G., CORLETO A., BENVENUTI V., BUCCOMINO G., BUONFIGLIO V., VINCI M. “LIFE+RI.CO.PR.I. project: from reference squares to grazing plan”
64. TROIA A., RAIMONDO F.M., GREUTER W. “On the presence, distribution and conservation status of *Lycopodium lagopus* (Lycopodiaceae) in Italy”
65. WAGENSOMMER R.P., PERRINO E.V., MEDAGLI P. “Notes on the endemic vascular plants of the Gargano promontory (Apulia, Italy)”

### 3.2 Biotic and abiotic factors

66. ARIANI A., FRANCINI A., SEBASTIANI L., ANDREUCCI A. “Characterization of the transgenic *Populus alba* plants over-expressing the aquaporin AQUA1”
67. BALDAN E., NIGRIS S., CLOCCHIATTI A., GUIDOLIN V., BORDIN N., ZOTTINI M., SQUARTINI A., BALDAN B. “Plant growth promotion and antifungal activities of the grapevine culturable microbiome”
68. BAZIHIZINA N., COLZI I., GIORNI E., MANCUSO S., GONNELLI C. “Photosynthesis under copper excess: changes in the biochemical and biophysical factors in *Silene paradoxa* L. copper tolerant and sensitive populations”
69. BILLI D., BAQUÉ M., VERSEUX C., RETTBERG P., DE VERA J.-P. “Cyanobacterial under extreme conditions on earth and beyond: contribution to human space exploration”
70. BOGGIA L., SGORBINI B., BERTEA C., CAGLIERO C., COLOMBO M.L., BICCHI C., MAFFEI M., RUBIOLO P. “Topographical dynamics of damage-related volatile emission in *Phaseolus lunatus* L.”
71. BONARI G., MOTTOLA G., AMICI V., BONINI I., ANGIOLINI C. “Macrophytes distribution pattern along a low human impact river in Mediterranean area”
72. BRUNO L., COZZA D., FERRARI M., TORELLI A., MARIESCHI M., ZANNI C., COZZA R. “A putative metallothionein from the microalga *Scenedesmus acutus* (Chlorophyceae)”
73. CICCARELLI D. “The influence of natural and anthropogenic factors on Mediterranean coastal sand dune

vegetation: a case study in Tuscany (Italy)”

74. COLZI I., VERGARI M., PIGNATTELLI S., GIORNI E., PAPINI A., GONNELLI C. “Root morphology and copper exclusion mechanisms in *Silene paradoxa* L.”
75. DE PASCALI M., APRILE A., PANNA R., GALATI C., RAMPINO P., DE BELLIS L., PERROTTA C. “Different molecular responses to drought, heat and combined stress are activated in two durum wheat cultivars”
76. DEGOLA F., PETRAGLIA A., DE BENEDICTIS M., SORBO S., BASILE A., SANITÀ DI TOPPI L. “Response to metals in the liverwort *Lunularia cruciata* and in the charophyte *Nitella mucronata*”
77. DI CORI P., FORNI C. “Effects of lead and cadmium on duckweed *Lemna minor* L.”
78. FILIPPONI P., BIONDI E., CASAVECCHIA S. “Greening education: may the geobotany be a possible approach in the primary and secondary school?”
79. GIUPPONI L., GIORGI A. “Effects of a fire on the vegetation of a mountainside of the orobic Pre-Alps (Bergamo, Italy)”
80. IURLARO A., DE CAROLI M., TUNNO M., MARRESE P.P., DE PASCALI M., RAMPINO P., DALESSANDRO G., PIRO G., FRY S. C., LENUCCI M.S. “Effect of heat and drought stresses on xet activity in different organs of durum wheat seedlings”
81. LEONARDI P., IOTTI M., PIATTONI F., LANCELLOTTI E., ZAMBONELLI A. “Effect of high temperature on mycelial growth and root colonization of *Tuber borchii* Vittad. isolates”
82. MANDOLFO A.L., CONTE L., VELLI A., FERRARI C., PEZZI G. “*Sedum hispanicum* L., a pioneer species in the gypsum outcrops (Bologna province)”
83. MANGILI F., CACCIANIGA M., PIERCE S. “Preliminary studies of the corology and autoecology of *Androsace brevis* (Hegetschw.) Ces., Primulaceae, a Lombardy endemic species”
84. BARBIERI F., MARERI L., BELLANI L.M., CAI G., FALERI C., MUCCIFORA S. “Effects of acute heat stress during anther and pollen development in *Lycopersicon esculentum* Cv micro-tom”
85. MASSARO M., SCIALABBA A., GIORGETTI L., BELLANI L.M., RIELA S. “Phytotoxicity of halloysite-supported ionic liquid-like phase (HNT-SILLP) catalyst on *Raphanus sativus* L.”
86. NOLA P., BIELLA P., ASSINI S., BRACCO F. “*Quercus robur* L. tree-ring anatomy and dendroclimatology: an image analysis approach”
87. ROCCOTIELLO E., CECCHI G., DI PIAZZA S., RIGGI A., MARIOTTI M.G., ZOTTI M. “Nickel tolerance in fungi and plants selected from metal-rich sites”
88. SAID-AL AHL H.A.H., EL GENDY A.G., OMER E.A “Effect of ascorbic acid, salicylic acid on coriander productivity and essential oil cultivated in two different locations”
89. SCIPPA G.S., LOMAGLIO T., TRUPIANO D., DE ZIO E., GROSSO A., MARRA M., DELFINE S., CHIATANTE D., ROCCO M. “Effect of short-term cadmium stress on *Populus nigra* detached leaves”
90. SCIPPA G.S., ROSSI M., TRUPIANO D., MONTAGNOLI A., TERZAGHI M., CHIATANTE D. “The response of root to bending stress: analysis at anatomical and molecular level”
91. SCOGNAMIGLIO M., D’ABROSCA B., ESPOSITO A., FIORENTINO A. “Metabolomic approach to study plant-plant interactions in Mediterranean ecosystems”
92. TAMPUCCI D., CACCIANIGA M. “Phytosociological outlines of two rock glaciers of the Ortles-Cevedale Massif (Stelvio National Park)”
93. TOMMASI F., PAGANO G., GUIDA M., ZICARI M.A., FASCIANO C., D’AQUINO L. “Rare earth elements as a double-edged effector in crop and native plants”
94. TONDELLO A., BALDAN B., FAVARO G., SQUARTINI A. “The endosphere of legumes: plant growth promotion traits of the beneficial bacterial colonizers”

### 3.3 Ecosystem processes and dynamics

95. BIELLA P., ASSINI S., BARCELLA M., OLLERTON J. “Reproduction, stability and important species of *M. Lesima* grasslands (Northern Apennine): a network analysis”
96. CHELLI S., WELLSTEIN C., CAMPETELLA G., BARTHA S., CERVELLINI M., CANULLO R. “Plant traits drive species turnovers in the herb layer of old-growth beech forests”
97. CUTINI A., CHIANUCCI F., GIANNINI T., AMORINI E. “Roe deer (*Capreolus capreolus* L.) browsing effects on mixed coppice stands in Central Italy”
98. GARGANO D., BONACCI A., DE VIVO G., MARCHIANÓ V., SCETTINO A., BERNARDO L. “Seasonal variations of biodiversity and functional biodiversity in a rocky mountain pasture under experimental warming: first data from a long-term experiment in the Pollino National Park”
99. GRIFONI F., GONNELLI V., QUILGHINI G., BOTTACCI A., ZOCCOLA A. “Impact of wild herbivores grazing on herbaceous vegetation and shrubs of the silver fir forests in the Reserve Naturali Casentinesi: removal of biomass, simplification of flora and alteration of vegetation dynamics”
100. LOSAPIO G., GOBBI M., MARANO G., COMPOSTELLA C., BORACCHI P., CACCIANIGA M. “Linking plant reproductive success and flower-visiting insects along a debris-covered glacier foreland”
101. MALFASI F., PIGNATTI S., CANNONE N. “Shrubs and trees encroachment in response to climate warming



in a high elevation alpine environment (Italian Central Alps)”

102. PERINI C., GARDIN L., SALERNI E. “Response to climate change of *Tuber borchii* fruiting bodies”
103. PONTI S., CHRISTIANSEN H.H., GUGLIELMIN M., CANNONE N. “CO<sub>2</sub> fluxes among different vegetation types during the growing season in the high arctic (Svalbard Islands)”

### 3.5 Palynology

104. BOSI G., MERCURI A.M., TORRI P., BANDINI MAZZANTI M. CON BENATTI A., FLORENZANO A., MONTECCHI M.C., RATTIGHIERI E., RINALDI R. “The LPP of Modena and archaeobotany: research in Italy over the last twenty years”
105. CARDUCCI E., BRIGHETTI M.A., TRAVAGLINI A. “Study of palynology. A database for pollen collection of monitoring centre of University of Rome Tor Vergata”
106. GHITARRINI S., ALBERTINI E., TEDESCHINI E., TIMORATO V., FRENGUELLI G. “Aerobiological monitoring of *Poaceae*: possible identification of the species through biomolecular analysis of airborne pollen DNA”
107. TEDESCHINI E., TIMORATO V., GHITARRINI S., FRENGUELLI G. “Pollen development in *Olea europaea* L. following selenium enrichment”

### 3.6 Phenology

108. PUPPI G., ZANOTTI A.L., IACOVIELLO A. “Series of phenological data in Bologna (Northern Italy)”

### 3.7 Phenotypic plasticity

109. CARTA A., PUGLIA G., SAVIO L., GIANNOTTI A., PROBERT R., BEDINI G., PERUZZI L. “Degree for seed dormancy in *Hypericum elodes* L. (Hypericaceae) is influenced by local climate and mating type”

## 4. Man and vegetation

### 4.1 Agro-ecosystems and green infrastructures

110. ALGIERI M.C., MAZZA M., STEPANCICH D. “The ecological importance of green infrastructure: the case of two experimental sites at the University of Calabria”
111. BAZAN G., BAIAMONTE G., DOMINA G., RAIMONDO F.M., SCHICCHI R., SPADARO V. “Botanical contribution to archaeological land evaluation in the FP7 Memola project”
112. BERTACCHI A., LOMBARDI T., CITTERIO G. “The forested agricultural landscape of Pisan plain: the Coltano estate”
113. CAPOTORTI G., ZAVATTERO L., FRONDONI R., MOLLO B., ANZELLOTTI I., BLASI C. “Towards the identification and sustainable management of traditional agricultural landscapes in Italy: new perspectives from vegetation science and landscape ecology”
114. FASCIANI P., MARCOZZI G., REALE S., DE ANGELIS F., PACE L. “Experimental fields of *Artemisia umbelliformis* subsp. *eriantha* (Apennines’ Genepi) in the Gran Sasso mountain”
115. PERRINO E.V., CALABRESE G., ZDRULI P., OTEKHILE A. “Plant biodiversity and soil quality in man made soils cultivated with table grapes in the Puglia region of South-Eastern Italy”
116. RAIMONDO L., SCIANNA A., RAIMONDO F.M., BAZAN G. “Detecting invasion hotspots of *Ailanthus altissima* with remote sensing”
117. SALMERI C., GUGLIELMO A., PAVONE P. “Sustainable gardens: an evaluation tool for management and planning strategies”
118. SANTO A., GRILLO O. “Origin, characterization and conservation of autochthonous grapevines of Sardinia (Italy)”
119. TERZAGHI M., MONTAGNOLI A., HERTLE B., KOMPATSCHER K., BAESSO B., FULGARO N., SCIPPA G.S., CHIATANTE D. “Use of ornamental plants in reinforced soils with anti-erosion purpose: a demanding achievement regarding root systems traits and type of shoots soil coverage”
120. TOSI S., CHINAGLIA S., RODOLFI M., DI DOMENICA M., PICCO A.M. “Bioactivity and biocontrol by the fungus *Trichoderma*: a green revolution for agro-residues”

### 4.2 Biopharmaceuticals

121. ACQUAVIVA R., GENOVESE C., DI GIACOMO C., MASTROJENI S., AMODEO A., TUNDIS R., TOMASELLO B., MALFA G., TEMPERA G., RAGUSA S. “Biological activities of *Teucrium flavum* L. and *Teucrium fruticans* L. extracts”
122. BUCCHINI A., GIAMPERI L., RICCI D., MAGGI F., PAPA F. “Antioxidant and anti-inflammatory activity of *Ferulago campestris* essential oil”
123. BUCCHINI A., RICCI D., GIAMPERI L. “Antioxidant and anti-inflammatory activity of *Pyrus communis* var. *cocomerina* extracts”
124. CIONI P., GIOVANELLI S., GIUSTI G., FLAMINI G., MINISALE P., PISTELLI L. “Volatile profile and essential

- oil composition of three samples of *Rhus coriaria* L. seeds collected in Sicily”
125. CLERICUZIO M., GROSA D., BORGHESI B., BRUNI I., RANZATO E., MARTINOTTI S., BURLANDO B., CORNARA L. “Pharmacognostic study of *Stylosanthes guianensis* revealing the occurrence of mayolene lipids with antiproliferative properties”
  126. COLOMBO M.L., FALCIOLA C., BICCHI C., BOGGIA L., CAGLIERO C., RUBIOLO P., SGORBINI B., DAVANZO F. “Toxic plants: the role of a pharmaceutical botanist as a support of the EAD (Emergency Alert Department) hospital”
  127. DEL MONTE D., MARANDINO A., DE MARTINO L., DE FEO V. “Radical scavenging and antioxidant activities of extracts from *Hypericum pefoliatum* L.”
  128. EL GENDY ABDEL NASSER, LEONARDI M., MUGNAINI L., BERTELLONI F., EBANI V.V., NARDONI S., MANCIANTI F., HENDAWY SABER, OMER ELSAYED, PISTELLI L. “Chemical composition and antimicrobial activity of essential oils of wild and cultivated *Origanum syriacum* plants grown in Sinai, Egypt”
  129. FORTINI P., DI MARZIO P., GUARRERA P.M. “Preliminary study of the plants used in the folk medicine in the Molise sector of the Abruzzo, Lazio and Molise National Park (Italy)”
  130. FRATERNALE D., RICCI D., RUDOV A., PROCOPIO A.D., VERARDO G., ALBERTINI M.C. “Anti-inflammatory property of *Vitis vinifera* L. tendrils extracts”
  131. GARIBAY INFANTE C.A., BASSOLINO L., RUFFONI B. “Hairy roots induction in different *Salvia* species”
  132. GIAMPERI L., GIOMARO G., BUCCHINI A. “ ‘Abbondanza apple’ clone with red pulp: polyphenols and anthocyanins content, antioxidant and anti-inflammatory activities”
  133. MARENGO A., SANNA C., BALLERO M., MACCIONI A., MAXIA A. “Wild edible plants or herbal medicine? Preliminary ethnobotanical investigation on Asteraceae in TBK of Sardinia island, Italy”
  134. MARRESE P.P., DE CAROLI M., IURLARO A., TUNNO M., MONTEFUSCO A., DALESSANDRO G., PIRO G., LENUCCI M.S. “Extraction of bioactive polysaccharides from cereals for the preparation of functional pasta”
  135. MASULLO M., CERULLI A., OLAS B., PIZZA C., PIACENTE S. “New diarylheptanoids with antioxidant activity from the leaves of the PGI product ‘Nocciola di Giffoni’ (*Corylus avellana* L.)”
  136. MENALE B., MUOIO R. “Ethnobotanical survey in Procida Island (Naples, Italy)”
  137. MENGHINI L., PINTORE G., TIRILLINI B., LEPORINI L. “Phytochemical and biological activity investigations on *Sideritis italica* extract”
  138. PAPINI A., GIULIANI C., BELLI M., DI FALCO P., TANI C., BILLI M., MALECI L. “The morphology of root and leaf of the tropical invasive species *Crotalaria spectabilis* (Fabaceae)”
  139. PASTORE P., PIOVAN A., CANIATO R., BADOCCO D., FILIPPINI R., MARZOCCHI M. “Metals in *Undaria pinnatifida* (Harvey) Suringar and *Sargassum muticum* (Yendo) Fensholt from Venice lagoon: an update”
  140. SANTAGOSTINI L., CAPORALI E., IRTI M., FLAMINI G., BOTTONI M., CERASA F., FICO G. “Morphological and chemical characterization of *Humulus lupulus* Cv. Saaz cultivated in Northern Italy”
  141. SOUZA L.F., INCHAUSTI DE BARROS I.B., DEL MONTE D., MANCINI E., DE MARTINO L., SCANDOLERA E., SCOGNAMIGLIO M., DE FEO V. “Chemical composition and biological activities of the essential oil from *Anredera cordifolia* grown in Brazil”
  142. SPADARO V., RAIMONDO F.M., FENNANE M., BRUNO M., SENATORE F. “Chemical composition of the essential oil of *Cladanthus scariosus* (Asteraceae) wild grown in Morocco”
  143. SPAGNOLETTI A., GRANDINI A., TACCHINI M., ROSSI D., MARESCA I., MAIETTI S., GUERRINI A., SACCHETTI G. “Chemical composition and biological activities of *Zingiber officinale* Roscoe essential oil from Amazonian and Chinese plants”
  144. TAVIANO M.F., RAGUSA S., PATERNITI MASTRAZZO G., MELCHINI A., BUONGIORNO L.P., DUGO P., CACCIOLA F., GUZMAN M.L., HSU HSIAO-TING, GALLETTI G., MICELI N. “Phytochemical characterization and cytotoxic properties of the polar extracts from the leaves of *Isatis tinctoria* L. collected in Sicily”
  145. VITALINI S., IRTI M., SIMONETTI P., TAVA A. “Volatile composition and antiradical capacity of essential oil from *Achillea moschata* Wulfen aerial parts”

### 4.3 Bioremediation

146. DI DOMENICA M., CHINAGLIA S., PICCO A.M., TOSI S. “*Trichoderma* potential into polluted soils detoxification”
147. GISMONTI A., RUGNINI L., CONGESTRI R., BRUNO L. “Study of bioremediation. Cyanobacteria and microalgae for wastewater treatment”
148. SPADA V., FRANCHI E., SERBOLISCA L., CARDACI A., IAVAZZO P., CONTE B., SCIARRILLO R., GUARINO C. “Bioremediation of an hydrocarbon polluted soil: isolation and characterization of native degrading bacteria”

#### 4.4 Biosystematics and taxonomy

149. ARDENGHI N.M.G., FOGGI B., ROSSI G. "The genus *Festuca* s.l. (Poaceae) in Italy: novelties and achievements in the new edition of 'Flora d'Italia'"
150. ASTUTI G., PETRONI G., MIRANDA V.F.O., PERUZZI L. "An integrated morphological, morphometric and molecular approach to biosystematics of carnivorous european *Utricularia* species (Lentibulariaceae)"
151. CHIESURA LORENZONI F., TOMBOLATO S., DAL COL E. "*Roberto de Visiani's Herbarium Dalmaticum*: recovery, reorder, catalogation and valorization of an historical collection"
152. CLAUSER M., SIGNORINI M., NEPI C., CIANFANELLI S., CALZOLARI C., INNOCENTI G. "Analysis of the naturalistic elements in the *Studiolo* of Francesco I in Palazzo Vecchio, Florence, Italy"
153. CORTIS P., BRUNI I., DE MATTIA F., COGONI A., LABRA M. "Integrated taxonomy to identify Sardinian plants with nutraceutical properties"
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157. SENATORE F., FORMISANO C., OLIVIERO F., RIGANO D. "Antioxidant activity and essential oil composition of three subspecies of *Sideritis libanotica*"
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164. GUGLIELMO F., BOTTI V., POGGIO L., MANDRIOLI M., VANACORE FALCO I. "A molecular approach to improve *ex situ* conservation strategies of five endangered wild plant species in the Aosta Valley (Northwest Italy)"
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# RELATIONS

## INTERPRETING LONG-TERM ECOSYSTEM CHANGE AND STABILITY: CASE STUDIES FROM THE ARCTIC AND SUB-ARCTIC

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Interest in the Arctic's changing environment has grown dramatically in the past decade because the climate there is warming at twice the rate of the global average. This warming will increase access to resources and improve shipping routes between Europe and South East Asia. In contrast, interest is also increasing because of a concern that climate change impacts in the Arctic will affect global communities, for example by increasing sea level and emissions of the greenhouse gases carbon dioxide and methane. Local communities are also affected as changes to weather and ecosystem services are likely to be significant. Understanding the responses of land-based ecosystems to climate warming is becoming urgent both to add pressure on the need to mitigate against greenhouse gas emissions at the global level and to help local communities to adapt to changes in their environment and ecosystems. However, current understanding of changes in ecosystems and their services is not as advanced as could be expected, mainly because there are more drivers of change operating than climate change alone and because ecological systems are very complex – even in the relatively low biodiversity lands of the Arctic.

Overall, observed changes in the cryosphere - snow, sea and lake ice, river ice, glaciers and permafrost are considerable, even if there is some variability around the Arctic. In contrast, satellite observations show that less than 40% of the Arctic's vegetation has shown increased productivity since 1982. Furthermore, an Arctic-wide measurement of plot-level plant responses to natural climate warming shows considerable geographical variation with the only emerging trend being an increase in shrub vegetation in warmer regions of the Arctic and an increase in graminoids in colder areas. However, even in one catchment in the sub-Arctic, all possible trends in plant growth can be recorded: increase in altitude of the treeline on one mountain, decrease on a neighbouring mountain and stability on yet another neighbouring mountain! While trends are relatively easy to document and monitor, attributing the trends to particular drivers is very difficult and the study of the "pathology" of change is in its infancy. In the treeline example above, increased growth was due to a combination of reduced reindeer herbivore numbers and plant physiology responding to warmer conditions: decreased growth was due to increased insect herbivore surviving better in warmer winters; and stability was due to inappropriate substrate at higher altitudes.

While long term trends result in heterogeneity in response of vegetation to change, there is increasing recognition of the importance of sudden events that can over-turn or moderate long-term changes. Tundra fires, rain-on-snow, mid-winter snow thaw, torrential rain and slope detachment are examples. These kill herbivores such as lemmings and their predators as well as reindeer and musk oxen. So, are the vegetation responses seen from satellite direct impacts of warming summers on plant physiology, or are they indirect effects of release from herbivory due to reductions in herbivore populations caused by extreme events in winter?

There is an increasing need for the 4M's approach to understand and respond to changes: Monitoring to detect change, Manipulation (experiments and simulations) to understand change, Modelling to predict future change and knowledge-based Management to moderate the impact of change. INTERACT ([www.eu-interact.org](http://www.eu-interact.org)) is a network of over 65 research stations that together host over 4200 researchers spending a total of 84,500 research days (=over 400 man years) in the Arctic in 2013. This network provides access to the Arctic and its data and is committed to hosting and facilitating multidisciplinary teams that can provide the understanding necessary for local peoples to adapt and for the global community to mitigate.

**ECOSYSTEM STEWARDSHIP: SUSTAINABILITY STRATEGIES FOR A RAPIDLY CHANGING PLANET****F. STUART CHAPIN III**

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Ecosystem stewardship is an action-oriented framework intended to foster social-ecological sustainability of a rapidly changing planet. Recent developments identify three strategies that make optimal use of current understanding in an environment of inevitable uncertainty and abrupt change: reducing the magnitude of, and exposure and sensitivity to, known stresses; focusing on proactive policies that shape change; and avoiding or escaping unsustainable social-ecological traps. All social-ecological systems are vulnerable to recent and projected changes but have sources of adaptive capacity and resilience that can sustain ecosystem services and human well-being through active ecosystem stewardship. There is urgent need for natural and social scientists to collaborate with practitioners and the public in developing strategies that foster stewardship at all scales. Ecologists can foster stewardship at local to global scales through education and outreach that fosters appreciation for and commitment to local and global places, monitoring threats to and progress toward sustainability, improved understanding of threshold behavior of social-ecological systems, and leadership in defining and pursuing sustainability goals. I show from collaborations with Alaska Indigenous residents, who are experiencing substantial climate change, that each of these steps is feasible.

## DEVELOPMENT OF THE HIDDEN HALF: PRIMARY AND SECONDARY ROOTS

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The study of the root system has always resulted to be more difficult than any other aerial organs of a plant because of roots' complex shapes, opacity of soil, and environmental influences. Nevertheless, during the last two decades numerous genes involved in both nutritional and anchorage functions have been discovered. In addition new technological tools have enabled a number of "omics" approaches which have shed light upon a number of complex gene networks controlling root growth and development. A new "systemic approach" combined with computer dissecting predictions (1) increases not only our overall knowledge of root biology, but also it allows us to leverage both natural and engineered variation to breed crops which are: (i) more disease resistant; (ii) less fertilizers needy; (iii) more productive even under unfavorable environmental conditions. One major challenge is in relating genotypes to phenotypes in order to move from a cellular scale to a more wide multiscale system analysis but to do that it is necessary to know mechanism/s responsible for root system architecture (RSA) definition. This particular trait of a root development kinetics remains elusive mainly for failure of any phenotyping approach attempted to date. A probable cause of these failures could be blamed on the fact that these studies rely generally on basic measurements (length, diameter, mass, area, volume, specific root length) which are often used to extrapolate system-wide traits. A further negative factor is represented by the fact that *in situ* imaging and analysis of roots remain impossible tasks. Therefore it is not surprising that while we are starting to know the principal gene machinery controlling root primordium initiation, lateral protrusion from its own parental, and root deployment in the soil, we are not sure yet whether or not a species-specific rhyzotaxy exists in analogy to specie-specific phyllotaxy of leaves which is known from centuries to be present along the stem such as to be used as a useful taxonomic discriminating factor. Another obstacle against RSA understanding is represented by the fact that lateral root origin occurs deep in internal tissues and is ascribed to 1-2 pericycle cells recruited among those facing a xylem pole. The "competence" to become initial of a new lateral is not a common property of all cells forming the pericycle tissue and decision about which cell, when, and/or where a new lateral root must be formed is an event controlled by interaction between two factors: developmental and environmental. Few root biologist suggest that a transition zone which follows closely the meristematic root zone along the root axis is in command for assigning this molecular (epigenetically controlled ??) "competence". According to them "competence" allocation resembles one of those neurologic events which regulate the life of all animal organisms (2). On this event they rest the hypothesis of occurrence of a possible form of plant intelligence. Unfortunately, all literature accumulated to date on lateral root is focused on an event occurring in primary root tissues which are peeled off and discarded (including pericycle) when a secondary root structure starts to be formed. Very little is known about lateral root emission from woody parental root even though more than half century is past after the first report was made in regard of woody roots being able to form new lateral roots as non-woody roots. This lack of knowledge is a strong bias against RSA understanding and suggests that probably lateral root emission is an event much more complex than what is thought of today. Our recent work on mechanical induction of branching in woody parental roots recalls the need to explore further this event which it seems common to all woody species tested by us to date. Furthermore, occurrence of branching in woody parental moves against the suggestion of transition zone as the command center for new lateral emission because woody roots lack i) pericycle cells and ii) their transition zone is present in a distal position which is too far away to be involved in this event. Probably if a "command center" for RSA deployment exists that could be represented by an "inheritable trait" transmitted to a limited number of cells whose progenies is firstly included in a primary tissue (pericycle) and secondly is a secondary tissue such as the vascular cambium. In this case the "competence" to form new roots could consist simply in a "inheritable" polygenic trait probably subjected to epigenetic control during the developmental stages of a root or in response to environmental factors.

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## AGPS CROSS-TALK IN ARABIDOPSIS POLLEN-PISTIL INTERACTIONS

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We have been interested in arabinogalactan proteins (AGPs) for some time, due to the fact that some of these molecules are molecular markers for gametophyte development during sexual plant reproduction. AGPs are a distinctive type of proteins, highly glycosylated that go into post-translation modifications which includes the addition of the glycosidic moieties and of a GPI anchor, both characteristics of important signalling molecules.

*AGP6* and *AGP11* are two *Arabidopsis* genes which are strongly and specifically expressed in pollen grains and pollen tubes. We have recently concluded that *AGP6* and *AGP11* are necessary for the proper pollen tube growth as well as for preventing untimely pollen grain germination.

We performed microarray experiments in the double null *agp6agp11* mutant pollen tube as well as yeast-two hybrid assays for these two proteins, in order to clarify the biological way of action of this ubiquitous class of plant proteoglycans. We ended up proposing these AGPs to be involved in several biological functions, namely signaling, vesicle trafficking, and of course, cell wall development.

After pollen tube arrival at the pistil, signal transduction cascades are initiated. The identification of key molecules involved in pollen tube guidance, possibly down-stream of Ca<sup>2+</sup> signalling for the tip-growth will help to clarify such complex and dynamic mechanisms.

Recently, we have shown the different distribution of specific AGP genes throughout the *Arabidopsis* female reproductive tissues along the pathway followed by the pollen tube during its journey to reach the embryo sac. The specific and differential presence of these proteins was observed in the stigmatic cells, the transmitting tissue, the funiculus and the integuments that surrounds the embryo sac and in the female gametophytic cells. The expression pattern of these AGPs in the female reproductive tissues brings new and important evidences for the involvement of AGPs in sexual plant reproductive processes.

## SHAPING THE ENDOPLASMIC RETICULUM

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The endoplasmic reticulum (ER) in vacuolate plant cells is mainly organised as a two dimensional network of tubules interconnecting small cisternae at the cortex of the cell. This network is dynamic with the geometrical organisation of the tubules continually changing through remodelling and new tubule growth. The ER overlies the cortical actin cytoskeleton and movement is mediated by members of the myosin XI family. In animals and yeasts the tubulation of the ER is defined by members of the reticulon family of proteins which form a wedge-like topology in the ER membrane and induce curvature of the lipid bilayer. These proteins are restricted to the curved rims of cisternae and the tubules. We have shown that the plant reticulons have a similar function (1).

More recently a family of dynamin related proteins have been identified, the atlastin GTPases, and have been implicated in the mediation of ER tubule fusion, thus putatively playing a major role in the organisation of the ER network. A long known mutant in arabidopsis, RHD3-1 (Root Hair Deficient 3-1), has recently been shown to encode for a mutant atlastin and its expression results in disruption of the geometrical structure of the cortical ER network, which forms long strands of membranes, whilst a RHD3-GFP fusion protein labels the ER network without any morphological effects. We have been using optical trapping of Golgi bodies, which are attached to the ER, to test the function of RHD3. Finally, we are starting to employ the new technique of serial block-face scanning electron microscopy to reconstruct ER networks in whole cells at high resolution (2).

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## THE ENDOPLASMIC RETICULUM AS A PROTEIN STORAGE COMPARTMENT

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In all eukaryotic cells, the endoplasmic reticulum (ER) is the protein nursery of the secretory pathway. Folding helpers present in the ER monitor and help the productive folding of newly synthesized proteins destined to vacuoles (lysosomes in animals), the cell surface, or other intermediate compartments of the endomembrane system, and promote the degradation of polypeptides that fail to reach correct folding. Like any nursery, the ER is therefore mainly a compartment of transit of the newborn, rather than a permanent residence. Consistently, high protein accumulation in the ER is associated to many human diseases. However, plants also use the ER to store proteins. The major and more spectacular example is constituted by the seed storage proteins that accumulate in the endosperm cells of cereal seeds. These proteins are unique to plants and are of great agricultural importance, being the main protein source for human nutrition. Using maize storage proteins as a model system, this lecture will illustrate recent advancements in our knowledge of the protein structural features and molecular interactions that allow high accumulation of protein in the plant ER without compromising the constitutive functions of this subcellular compartment. Supported by the FILAGRO Project of CNR-Regione Lombardia.

## FROM ONE TO THE MANY GENOMES OF A PLANT: THE EVOLUTION OF THE GRAPEVINE PAN-GENOME

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The analysis of variation in plants has revealed that their genomes are characterised by high levels of structural variation, consisting of both smaller insertion/deletions, mostly due to recent insertions of transposable elements, and of larger insertion/deletion similar to those termed in humans Copy Number Variants (CNVs). These observations indicate that a single genome sequence might not reflect the entire genomic complement of a species, and prompted us to introduce the concept of the plant pan-genome, including core genomic features common to all individuals and a Dispensable Genome (DG) composed of partially shared and/or non shared DNA sequence elements. The very active transposable element systems present in many plant genomes may account for a large fraction of the DG. The mechanisms by which the CNV-like variants are generated and the direction of the mutational events are still unknown. Uncovering the intriguing nature of the DG, i.e. its composition, origin and function, represents a step forward towards an understanding of the processes generating genetic diversity and phenotypic variation. Additionally, since the DG clearly appears to be for the most part the youngest and most dynamic component of the pan genome, it is of great interest to understand whether it is a major contributor to the creation of new genetic variation in plant evolution as well as in the artificial selection processes of plant breeding. We have resequenced to high coverage more than 50 grapevine accessions and used a variety of approaches to detect SNPs as well as structural variants of different size and origin, including de novo assembly of a selected set of genotypes. We will discuss the extent and composition of the pan genome in grapevine, the different mechanisms that generate and maintain the dispensable portion, the epigenetic and phenotypic effects of the DG and the rates and modes of creation of new genetic variation due to DG components.

## PLANT CHEMICAL DIVERSITY IN TARGET IDENTIFICATION AND DRUG DISCOVERY

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In the drug discovery project, plant small molecules are privileged structures that can explore the chemical space around a target since enzymes make them, and their shape must be complementary to that of a biological surface. This makes them privileged structures in terms of chemical space of biological relevance. Plant compounds have not only a higher chance to show biological activity and become “hits” but also have a higher chance of surviving the drug development pipeline (1,2). Drug discovery from medicinal plants has played an important role in the treatment of cancer and, indeed, most new clinical applications of plant secondary metabolites and their derivatives over the last half century have been applied towards combating cancer (3,4).

Cancer cells have intense alterations in protein homeostasis, thus adaptative mechanisms are activated in these cells to enable survival under stressful conditions. The heat-shock response is a key component of this protective process. One of the most important proteins involved in this process is Heat shock protein 90 (Hsp90). Hsp90 is a molecular chaperone that modulates cellular homeostasis by interacting with more than 200 client proteins, including proteins associated with almost all the hallmarks of cancer (5). Inhibition of Hsp90 incapacitates, simultaneously, multiple client proteins, resulting in a blockade of signaling pathways and providing a combinatorial attack to cellular oncogenic processes. Recently, Hsp90 has emerged as a target for the development of antitumor agents. Thus, we adopted a Surface Plasmon Resonance (SPR) assay to screen our natural compound libraries in order to determine Hsp90 inhibitors. Our libraries were constructed selecting plant molecules with proven pharmacological activities, in areas such as apoptosis and cell cycle inhibition. The most promising candidates then underwent an evaluation of the Hsp90 inhibitory activity by means of a panel of chemical and biological approaches, including SPR measurements, biochemical and cellular assays, limited proteolysis, and molecular docking. Our results allow to identification of several new leads (6).

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## MAPPING AND ASSESSMENT OF ECOSYSTEMS IN ITALY: THE CONTRIBUTION OF MODERN VEGETATION SCIENCE

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In recent decades, the European Commission has provided guidelines for a wide range of environmental issues. The Habitats Directive, in particular, represents the theoretical basis and the reference framework for the implementation of an effective ecological network throughout Europe (Natura2000).

Over the years, the focus of environmental research and policy has shifted from basic knowledge to the assessment of conservation status as well as from the analysis of individual animal and plant populations and habitats to an in-depth characterisation and mapping of ecosystems and their services. The output of such researches needs to be sufficiently detailed to effectively respond to scale-dependent challenges. Generic basic documents have, however, all too often been adopted in Europe to provide synthetic databases and maps at the continental scale.

The level of basic knowledge available for Italy is currently so detailed as to be considered a reference point at the European level for the description, analysis and assessment of individual ecosystem components, of comprehensive ecosystems and of their services.

Starting from a synthesis of the cultural and scientific evolution of geobotany and plant ecology, the author illustrates the most recent maps that have been produced at the national scale (regarding bioclimate, land units, vegetation series, potential natural vegetation, ecoregions) (1, 2, 3, 4, 5). Moreover, he describes the integration between these maps within the context of Italian ecosystem mapping (6, 7), highlighting the importance of the synthetic map of the Italian Potential Natural Vegetation, which was drawn up specifically for this project. All these research lines and products rely on the methodological framework of the ecological classification of land. This framework has been used to update the modern syndynamic phytosociology (from inductive to deductive integrated phytosociology) and to combine the European approach, which is prevalently typological, with the American one, which is more regionally-oriented (8).

Italy may, thanks to its updated, highly detailed maps, currently be considered one of the European member state that can most effectively address issues regarding the mapping and assessment of ecosystems at the national scale. Moreover, an original method designed to assess the conservation state of ecosystems at the level of individual polygon, individual type or homogeneous land unit (vegetation series and ecoregions) is currently being developed and tested.

The presentation concludes with an overview of research prospects in the near future and with the illustration of pilot maps of national-scale ecosystem services that have been produced in collaboration with a large group of researchers.

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**ECOSYSTEM FUNCTIONS AND THE MECHANISMS THAT CONTROL THE SPECIES DIVERSITY IN PLANT COMMUNITIES****FRANK BERENDSE**

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The Convention on Biological Diversity of Rio de Janeiro (1993) stressed the importance of biodiversity for ecosystem functions that are essential to mankind. Since then many experiments have been performed to analyse the impacts of biodiversity loss on plant production, decomposition, soil respiration, invasion resistance and ecosystem stability. Such ecosystem processes are crucial and determine – amongst others – the amount of herbivore biomass that can be sustained. However, these experiments did not yet address the impacts of diversity loss on ecosystem functions that have direct physical impacts on human societies. Examples of such functions are production of clean drinking water, erosion resistance and regulation of the temperature on the Earth's surface. Soil erosion is responsible for significant loss of soil fertility, declining food security and major off-site impacts including impeded shipping traffic in downstream watercourses and unexpected flooding disasters. In addition, soil erosion may have decisive effects on the functioning of embankments that are crucial for the safety of millions of people living in low-lying estuarine areas around the world. We show that loss of plant species diversity reduces erosion resistance of these slopes: net annual soil loss increases twofold when diversity declines fourfold. We conclude that measures to restore or protect plant species diversity can contribute to increased safety in the most densely populated areas of the world, but can also help to maintain soil fertility on sloping pastures that contribute significantly to food production in many countries.

The positive relationships between diversity and productivity and other ecosystem functions which were found in most field experiments, do not only provide insights in the consequences of diversity losses, but also help to understand the mechanisms that control plant species diversity. There is increasing evidence that an important part of such positive relationships is due to plant-pathogen interactions with the impact of species-specific pathogens declining with increasing diversity. These negative pathogen impacts that increase with increasing frequency of the plant species involved can be responsible for strong frequency-dependent regulation of the abundance of plant species and contribute to the maintenance of local plant species diversity. Another local mechanism includes competition for nutrients between plant species with different competitive abilities in different soil compartments. When soil nutrient supply increases due to increased ammonia emissions or fertilization such mechanisms are broken so that many species will disappear.

PROCESSES AND OUTCOMES OF DIVERSIFICATION IN *PRIMULACEAE*: EXPLORATIONS ON THE FUNCTIONAL AND EVOLUTIONARY ROLES OF HETEROSTYLY

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The spectacular diversity of angiosperms has often been linked to floral evolution. Most flowers are hermaphroditic, thus enabling self-fertilization, which can lead to inbreeding and its disadvantages. Several strategies have therefore evolved to avoid selfing while enforcing outcrossing, and heterostyly is one of the best studied. Occurring in 28 families, heterostyly denotes a floral polymorphism consisting of flowers that differ in the reciprocal positioning of sexual organs and in mating type, ensuring allogamy. Groups characterized by heterostyly are often, but not always, more species-rich than their non-heterostylous relatives, prompting the question of whether this floral polymorphism promotes diversification and how. Primulaceae, with their rich history of biological studies - dating back to Darwin - and wealth of data, represent a prime model to investigate both the mechanisms by which heterostyly might promote diversification and the macro-evolutionary outcomes of this trait. The main questions addressed include: How does heterostyly work within and between species? Does it promote elevated diversification? Does it affect mainly speciation or extinction rates? Are the effects of heterostyly on diversification linked with mechanisms unique to this floral syndrome or not? Do gains and losses of heterostyly have different macro-evolutionary effects at shorter vs. longer temporal scales?



THE FAMILY *BORAGINACEAE*: DEEP PHYLOGENY, CHARACTER EVOLUTION AND STILL OPEN TAXONOMIC PROBLEMS

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Both the affinities and taxonomic subdivision of Boraginaceae have been long debated and unsatisfactorily resolved until recent times. Major open issues are the placement and relationships of Boraginaceae s.str. in Boraginales and the identification of the major clades of the family. Especially the large tribes Cynoglosseae and Eritrichieae have been repeatedly retrieved as non-monophyletic groups, and recent studies suggest that several larger genera, especially *Cynoglossum* and *Omphalodes* may be paraphyletic or even polyphyletic (1, 2). These questions have been recently addressed using a set of plastid markers (trnL–trnF, rps16), and a taxon sampling including 16 outgroup taxa from related families of order Boraginales (or Boraginaceae sensu lato) and 172 ingroup species from 65 genera from all tribes of Boraginaceae s.str. (3).

The resulting phylogeny shows for the first time high statistical support for most nodes on both the backbone and the individual clades. Boraginaceae s.str. are sister to African members of Wellstediaceae, while the group of Wellstediaceae–Boraginaceae s.str. is sister to African Codonaceae (genus *Codon*). Echiochileae are retrieved as sister to the remainder of Boraginaceae s.str., which, in turn, fall into two major clades, the Boragineae–Lithospermeae (in a well-supported sister relationship) and the Cynoglosseae s.l. (including Eritrichieae). Cynoglosseae s.l. is highly resolved, with Trichodesmeae (incl. *Microcaryum* and *Lasiocaryum*) as sister to the remainder of the group. Eritrichieae s.str. (*Eritrichium*, *Hackelia*, *Lappula*) are resolved on a poorly supported polytomy together with the *Omphalodes*-clade (incl. *Myosotidium*, *Cynoglossum* p.p.), and the *Mertensia*-clade (incl. *Omphalodes scorpioides* and monotypic *Asperugo*). The Myosotideae (*Myosotis*, *Trigonotis*, *Pseudomertensia*) are retrieved in a well supported sister-relationship to the core-Cynoglosseae, the latter comprising all other genera included in the study. *Cynoglossum* is retrieved as highly para- and polyphyletic, with a large range of generic segregates nested in *Cynoglossum*, but other species of *Cynoglossum* are sister to *Microula* or to the American “Eritrichieae” (*Cryptantha* and allied genera). Representatives of the genus *Cynoglossum* in its current definition are segregated onto six independent lineages, members of *Omphalodes* onto three independent lineages. At least 11 of the genera here sampled are deeply nested in other genera. Taxonomic treatment of these genera is therefore highly problematic. The data show that individual details of nutlet morphology (e.g., winged margins, glochidia) are highly homoplasious. Conversely, a complex of nutlet characters (e.g., characters of the gynobase and cicatrix together with nutlet orientation and sculpturing) tends to circumscribe natural units. Geographical distribution of major clades suggests that the family originated in Africa and western Asia and radiated to eastern Eurasia, with several independent dispersal events into Australia and the New World.

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“HISTORIC GARDENS ECOSYSTEMS”: CAN WE PROVIDE PUBLIC USE AND MAINTAIN THE ECOLOGICAL BALANCE?

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The urbanization process, begun in the XIX century and become unstoppable after the Second War, has profoundly transformed the existing social structures, subverting the old policies, abolishing certain customs and imposing new ones.

The change between the old and the new social, economic and urban “fabric” has rarely occurred with gradual phases; in most cases, it has determined a real trauma with profound repercussions on the habits, the mind-set and the health of the single persons, as well as on the expectations of the whole community.

This has resulted in a significant environmental degradation and in a strong expansion of the suburbs around the city (urban sprawl), with the consequent disruption of the territorial organization and a strong influence of green areas planning and design, and the use of the existing ones.

The gardens and parks always belonging to noble and rich families and always attached to their villas, gradually became public. As a consequence it was assigned to them the task of correcting the imbalances in many of the industrial cities and meet the recreational, educational, hygienic needs of all those people who, thanks to changing social conditions, could enjoy all the benefits due to the presence and the active use of green areas.

The resulting degradation has therefore a multifactorial matrix that ranges from technical and agronomic reasons, such as aging and senescence of the living material, the speculative pressures for urban development, the controversial planning decisions, the loss of garden culture and of professional skill by gardeners, as well as the continuing and serious absence of public and private funds and, last but not least, to anthropogenic pressure. These spaces designed to be used by a limited number of privileged persons have actually become the destination for thousands of visitors who, although environmentally friendly, have contributed to the degradation both of the soil structure and of the plants. In addition, the historical landscape is often managed with the same techniques used for modern urban green spaces, while it requires specific interventions designed and articulated over time, as well as performed by qualified personnel.

The presentation analyses the main causes of stress related to the physical, chemical and biological characteristics of the soil (i.e. compaction, loss of organic matter, presence of microflora, links with soil sickness, etc.), to those climatic (i.e. the effects of drought and waterlogging on plant physiology), and their effects on trees, as well as possible interventions for fertility restoration and, consequently, to improve the state of health of the plants present in the numerous parks and historic gardens.

Some examples of agronomic interventions (i.e. use of mycorrhizal inoculants, the use of mulch, fertilization, etc.) will be illustrated.

The final goal of the analysis is to provide, through a critical analysis of existing information, a general theoretical framework that forms the basis for a thorough discussion and serves to understand the nature and the evolution of certain phenomena of degradation thus allowing to predict and to better understand them to plan strategies for technical and agronomic management of historical green areas.

# COMMUNICATIONS

## UNDERSTANDING THE RESPONSE OF PLANT BIODIVERSITY TO ENVIRONMENTAL PERTURBATION USING GRIME'S CSR THEORY

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Recent large-scale analyses of plant trait variation have identified two principal spectra of functional variability: i) an 'economics' spectrum ranging from acquisitive species with rapid growth and thin leaves to conservative species with tough, carbon-rich leaves and, ii) for species of intermediate economics, a size spectrum characterized in part by leaf size variation (1-4). Indeed, a triangle of viable leaf trait combinations is evident for the Italian flora, and has been used to produce a methodology (5) for the classification of woody and herbaceous angiosperms, gymnosperms and pteridophytes according to CSR (competitor, stress-tolerator, ruderal) theory (6, 7). Here we review the application of CSR classification for the interpretation of the functional characteristics and assembly of a range of plant communities in Italy and worldwide. We show how CSR classification can be applied to investigate species coexistence at the centimeter scale (8) and, by allowing a functional interpretation of macro-scale phenomena such as the relationship between biomass production and potential species richness (9), how CSR strategies can allow the prediction of ecosystem and plant community responses to environmental change.

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PHENOLOGICAL ANALYSIS PROVIDES INSIGHTS ON THE SPATIAL DISTRIBUTION AND ON RESPONSE TO SNOWMELT OF PLANT FUNCTIONAL TYPES IN SUBALPINE GRASSLANDS

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Phenological analyses provide information on the response of plant species to interannual climate variability, mainly to temperature and photoperiod and, in mountain grasslands, to the snowmelt date that drives the beginning of the growing season.

In grasslands, monitoring is carried out on single species to analyze the reproductive phenology while vegetative phenology is often studied at the ecosystem level, considering several indexes related to greenness, biomass production and Leaf Area Index (LAI) by means of repeated direct sampling and by indirect measures as nadiral or digital (webcam) photography or by remote sensing techniques.

The vegetative phenology of a subalpine grassland located in the Aosta Valley (western Alps) at 2160 m a.s.l. has been monitored from 2009 to 2013 using direct (biomass and LAI measurements) and indirect methods (nadiral repeated photography, digital camera images, eddy covariance data) (1, 2, 3, 4, 5) in the framework of the PHENOALP EU Interreg Project [www.phenoalp.eu](http://www.phenoalp.eu). The vegetation is characterized by the dominant oligotrophic grass *Nardus stricta*, a keystone species characterized by self-accumulation, and the forbs *Geum montanum*, *Arnica montana*, *Trifolium alpinum*, *Ranunculus pyrenaicus* and *Leontodon hispidus*.

The community development during the growing seasons has been described analyzing the spring trajectories of different variables tracking vegetative phenology. These trajectories are usually characterized by lower or higher slope when the snowmelt date occurred earlier or later, respectively. The comparison of the different methods/variables led to an increasing knowledge of ecosystem functioning, with in-depth analysis about the beginning of the season. Digital camera photography showed two main phenological patterns elucidating early and late greening areas and corresponding to communities with different species composition, related to convex and concave areas. The different microsite morphology, revealed by different patterns in snow accumulation and melting, resulted to affect species composition and consequently the beginning of greening. On the convex areas *Nardus stricta* is dominant (with average fractional cover of 93%) and the concave areas are characterized by forbs, the most abundant species being *Geum montanum* and *Arnica montana* while *Nardus stricta* has an average cover of about 50%. The two vegetation types determine a heterogeneous pattern related to the different micromorphological conditions which is not easily detectable in the summer.

A detailed micromorphological analysis of the soil properties in convex and concave areas has been carried out in order to show the relationships between the plant functional types and the heterogeneous environmental conditions.

The analysis highlights the different ecological requirements and plant traits of the two groups of species (*Nardus stricta* and forbs) and in particular differences in the produced biomass quality and in the consequent litter decomposition rates which can provide insights on the future dynamics of the whole ecosystem. The phenological analysis provided a detailed evaluation of the responses of the different plant functional types, but also of their spatial distribution and of their role in ecosystem functioning.

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## RELATIONSHIPS BETWEEN CLIMATE AND PHENOLOGY OF HIGH ELEVATION PLANTS. A 7-YEARS SNOW AND PHENOLOGY MONITORING IN THE ITALIAN CENTRAL ALPS

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Here we show the results of 7 years monitoring (2006-2013) focusing on the relationship between climate (with special reference to snow and air temperature) and plant phenology in a high elevation site above the tree line in the Italian Central Alps (Foscagno Valley), an area which is experiencing dramatic changes in climate and vegetation distribution (1). We selected 54 plots along an elevation range of 250 m (2360-2610 m a.s.l.) and 37 target species of the subalpine and alpine belts, also characteristic of different growth forms (2). The phenological measurements were carried out according to the ITEX protocol (3) with measurements every 2-3 days from the snowmelt to the beginning of the permanent snow cover in fall. Snow depth was measured also during the winter. The climatic data were provided by the La Foppa AWS (ArpaLombardia), located at 2700 m a.s.l. at less than 1 km far from our site.

The snow data were analyzed to identify its spatial distribution and persistence within the study area and its intra- and inter-annual variation. By using the temperature dataset, TDD (thawing degree days) and GDD (growing degree days) were calculated for each phenological phase (4). The phenological data were then analyzed through GRM (Generalized Regression Models) at different levels: a) species, b) community, c) growth form, to identify their intra-annual and inter-annual trends and assess how snow and the main climatic factors affect the development of the phenological stages.

Snow melting is a key factor influencing the vegetative development of all species (formation of the first buds and new leaves), while photoperiod is the most significant factor for flowering stages (in particular for floral anthesis) and, indirectly, for seed ripening. Leaf senescence instead occurs in a very restricted period (<15 days) for all species and all years. The 37 selected species showed two main strategies in relation to snowmelt, with implications on the following phenological stages. Half species concentrated their stages within a relatively short period, while the others showed a gap of more than one month between vegetative stages – flowering - seed ripening. Plants of different communities and growth forms differed for some phenological responses: shrubs showed a marked advance (about 10 days) in the beginning of the vegetative stages compared to the other growth forms. Whether this trend may prosecute on long-term, it may exert important consequences on plant communities promoting changes of floristic composition with further advantages for shrub communities.

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## PERSISTENT EXTREME CLIMATIC EVENTS REDUCE CARBON ACCUMULATION IN PEATLANDS: MECHANISMS AND QUANTIFICATION

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In a changing climate, extreme climatic events are expected to increase in frequency and intensity so that there is an urgent need to understand their impacts on natural systems. Extreme climatic events such as severe droughts can have profound consequences, particularly for peatlands, an ecosystem characterized by persistent waterlogged soil conditions. Indeed, increased evapotranspiration and associated greater soil oxygenation are expected to alter the capability of peatlands to continue to act as atmospheric carbon sinks, however, to what extent a persistent drought can alter the structure and functioning of peatlands is still poorly understood. Here, we present the results of a transplantation experiment of peat mesocosms from high altitude (control climatic conditions, c. 1880 m asl) to lower altitude (warmer climatic conditions, c. 550 m asl) in Switzerland. During three years, we monitored a set of biogeochemical variables in order to determine: 1) vegetation structural dynamics and ecophysiological responses of target plant species; 2) soil respiration and microbial enzymatic activity; 3) changes in quality and quantity of soil organic matter. We observed that under warmer climatic conditions, vascular plants increased their cover at expense of moss species, which showed a decreased productivity. This plant response was supported by the correspondent photosynthetic rate that, at lower altitude, was enhanced for the two dominant vascular plants (i.e., *Eriophorum vaginatum* and *Vaccinium uliginosum*), but it was reduced for *Sphagnum fallax* (the dominant moss) compared to the control mesocosms. During the study period, soil respiration was always greater at lower altitude, in accordance with a reduced water content that enhanced soil enzymatic activity. The FT-infrared spectroscopic analysis of peat samples indicated an increased mineralization of soil organic matter under increased soil oxygenation in the transplanted mesocosms. On the basis of a mass balance of the peat content in the mesocosms, we demonstrate that the observed increased productivity of vascular plants do not compensate for the reduced peat accumulation by the moss layer and the associated mineralization of soil organic matter under persistent warmer climatic conditions, ultimately resulting in a net decrease of carbon accumulation.

## EFFECTS OF CLIMATE CHANGE ON SEED GERMINATION AND RECRUITMENT SUCCESS OF ALPINE PLANTS

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Across the various mountain ranges, the species distributed at higher elevations (i.e. alpine plants) are considered at the greatest level of threat due to climate warming (1). Despite the considerable number of studies on the impacts of climate change on alpine plants, there have been few attempts to investigate its effect on regeneration (3). However, recruitment from seeds is a key event in the life-history of plants, affecting their distribution and evolution and seasonal changes in climate will inevitably affect recruitment success (8). Despite this, little is known about the effect of increased temperatures on seed germination and subsequent seedling survival of alpine plants. In this regard, existing studies focused on laboratory, greenhouse and field manipulation of temperatures and concerned species from the tree line (2), subarctic/arctic tundra (5, 7) and alpine environments (4, 6). Results of these observations are rather contradictory, with warming either increasing or decreasing seed germination and seedling establishment. The effect of warming seems therefore difficult to generalise, likely because of the different approach and/or species considered, but also depending on how local conditions (e.g. temperature and soil moisture) will change in a future climate.

To study the effects of climate change on recruitment success of alpine plants our research group is using different approaches, which combine lab and field observations and consider species from different mountain chains, such as Alps, Apennines and Himalaya. For example, using a novel approach, that considered the altitudinal variation of temperature as a surrogate for future climate scenarios, seeds of several target species from the Italian Alps were exposed to temperature cycles in the laboratory simulating monthly changes occurring at the species-growing site located at 2500m a.s.l. and 400 m lower in altitude. In both sites, subsequent experiments investigated seedling emergence and survival in the wild. First results indicate that warming will lead to a shift from mostly spring to autumn emergence in alpine plants, but that the extent of this change across species will be driven by seed dormancy status. In contrast, seed germination was less sensitive to changes in spring and/or winter temperatures/duration. Further experiments in the wild consistently showed spring emergence under the present climate, but confirmed that warming could elicit seedling emergence in the autumn as well. The survival of autumn and spring emerged seedlings is currently under investigation at the study sites. Similar experiments are on the way in the Himalayan chain, with the aims to study the effects of warming on seedling recruitment on target species growing at 4000 and 5000m a.s.l. and in the Apennines, with the aim to investigate the effects of short and extreme heat events (i.e. heat waves and drought) on seed germination on over fifty high montane and alpine species.

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FROM COLD MOUNTAINS TO WARM COASTAL CLIFFS: INSIGHTS FOR THE FUTURE OF A *PRIMULA* SPECIES

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*Primula palinuri* Petagna is the only species in the genus growing on maritime cliffs, the only from a Mediterranean habitat, and the most southerly in the whole *Auricola* section. It is an endemic species and has a small and severely fragmented geographical range confined to vertical cliffs of a narrow Tyrrhenian coastal area of southern Italy. Genetic and molecular analyses allowed to hypothesise its origin from an ancestor which moved from the western Alps by long-distance dispersal or, more likely, colonising the Apennines during a cold period of the Pleistocene ( $50,000 \pm 40,000$  yrs b.p.) (1).

Given that *P. palinuri* might have endured all past changes, from mountain to coastal cliffs, from alpine to Mediterranean environment, we considered this species a suitable comparative study system to predict possible consequences of global warming also on other *Primula* species, now surviving in mountain habitats. Specific aim of our work on *P. palinuri* was to disclose any morphological and physiological traits adaptive to a warmer environment.

Altogether, biological and ecological data suggested that local survival of this species relies on both phenotypic plasticity and selective pressure phenomena. *P. palinuri* is a fully distylous species with high reproductive success: each plant develops numerous viable seeds as a result of inter-morph pollination. Nevertheless, generation turnover is unlikely to occur because seedling establishment is rare (2). Plants of *P. palinuri* are long living due to the slow growth of rhizomes which accumulate water and starch. Most vegetative and reproductive stages occur in winter. Plants are summer deciduous: the large leaves, that develop from the beginning of autumn throughout the winter, have no anatomical traits of xeromorphy and dry up in summer (3). Reproductive features such as winter flowering, the presence of pendulous flowers avoiding rain damage and the best pollen performances at low temperatures, associate *P. palinuri* more to cold mountain habitats than to Mediterranean maritime cliffs. These features might be considered as evolving in a cooler past and persisting in the existing populations (4).

At present, the remarkable longevity of the single flowers can be a result of the interactions with a) the environmental factors affecting flower biology and b) flower pollinators. More specifically, given that flower induction and gametophyte development are under control of low winter temperatures, within a scenario of global warming, phenotypic plasticity might bring forward flowering to provide the right temperature for reproductive functions. Moreover, since the species is dependent on insect pollination, selective pressure toward late (spring) flowering can avoid pollination limitation due pollinator inactivity at winter temperatures (4).

*P. palinuri* has evolved no specific adaptations to Mediterranean environment. Under the pressure of a warming climate, it is unlikely that populations of *P. palinuri* will move towards northern latitudes and higher altitudes due to geographical limits. Phenotypic plasticity anticipating flowering and selective pressure by pollinators extending flowering could be possible strategies for local survival of this and its relative species now living in mountain habitats.

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## LONG-TERM VEGETATION DYNAMIC IN RELATION TO CLIMATE CHANGE IN THE ITALIAN CENTRAL ALPS

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The Italian central Alps are one of the areas suffering the highest warming since 1950s (1, 2). Since 1953, in a high elevation alpine site located above the treeline in the Italian Central Alps (Stelvio Pass), in response to warming, vegetation exhibited significant changes of spatial distribution, with rapid shrub expansion and upward displacement of many communities, but also with unexpected regression patterns of vegetation coverage mostly associated with degradation of permafrost (1). Moreover, significant changes of floristic composition and species richness were detected (2). Here we aim to assess: a) whether in the last decade (2003-2013) the spatial distribution of vegetation showed further displacements; b) the rate of change; and c) compare these data with the changes occurred in the period 1953-2003. For this aim, during the summer 2013 a new phytosociological map was elaborated and compared with those already existing, respectively built in 2003 (1) and 1953 (3). Vegetation changes were assessed using the same methods already adopted by Cannone et al. (2) to achieve comparable data.

During the last decade the air temperature increase was almost negligible (+0.044 °C/yr,  $p=0.5222$ ), while total liquid precipitation continued to increase (+12 mm/yr,  $p<0.05$ ). In the last decade snow cover exhibited contrasting patterns respect to the period 1953-2003. Indeed, since 2003 a delay of snow melting was detected (although not statistically significant +0.9 days/yr,  $p=0.6$ ) and a longer snow cover persistence (+1.7 days/yr,  $p=0.2$ ). Despite the lack of a significant warming in the last decade, shrub communities prosecuted their expansion and upward migration with different patterns depending on the dominant species. In particular, while *Rhododendron ferrugineum* was still expanding, *Kalmia procumbens* exhibited a regression pattern, probably due to the inter-specific competition. Snowbeds and grasslands persisted in their contraction trend. Bare ground coverage decreased promoting an increase of pioneer communities. According to our data, the areal changes of vegetation still continue despite the climatic input is now less intense.

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## FACTORS INVOLVED IN POLLEN TUBE GROWTH AND THEIR IMPORTANCE IN ALLERGIC SENSITIZATION

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The pollen tube represents a remarkable example of highly polarized cell expansion, allowed by an intricate network of proteins and factors, among which the cytoskeleton plays a crucial role. In fact, cytoskeleton proteins are involved in many aspects of pollen germination and growth, from the transport of sperm cells, to the asymmetrical distribution of organelles<sup>1</sup>. All these activities are based on the dynamics of the cytoskeleton, that must thus be regulated in minute detail by the Ca<sup>2+</sup> gradient, that on one hand fine-tunes the assembly and disassembly of actin filaments and on the other hand plays a pivotal role in signaling. However, Ca<sup>2+</sup> does not bear the burden of regulate the cytoskeleton alone and many other factors cooperate, among which the ubiquitous family of transglutaminase (TGases) enzymes. These enzymes are well known to be able to catalyze the post-translational conjugation of polyamines to different protein targets, but also the cross-linking between endo-glutamine and -lysine protein residues. We recently suggested their role in the interaction between pollen tubes and the extracellular matrix during the self-incompatibility response, when the TGases activity is enhanced but also in the regulation of the cytoskeleton activity in pollen tubes<sup>2-3-4</sup>. Many other factors are related to pollen germination and tube growth and several could be also involved in allergic sensitization. Among them the phospholipase (PL), involved in the apex growth of the elongating pollen tube, is an integrator of the tip molecular signaling. We recently described how this enzyme could participate in the activation of the allergenic inflammatory cascade, probably by generating pro-inflammatory factors and we unarguably showed that stimulation of PLA<sub>2</sub> activity occurs when it is post-translationally modified by TGase<sup>5</sup>. Also profilin is involved in the pollen tube growth as it is a key factor to control actin filament organization, but it also plays a role in allergic sensitization since it is acknowledged to be a mediator of the phenomenon of cross-reactivity that can be easily released in the oral cavity<sup>5-6</sup>. Last but not least, tip-localized ROS are nowadays known to be essential to sustain the normal rate of pollen tube growth as ROS disruption induced the arrest of pollen tube growth and leads pollen to programmed cell death. In fact, ROS are not only a by-product of normal oxygen metabolism, but evenly play an essential role in pollen tube growth since their lifespan is very short and are thus ideal molecules to act in cell signaling. This signaling role is shared both plants and animals so that pollen ROS often represent a first signal that primes and magnifies a cascade of events in the allergic response in the human mucosa<sup>7</sup>. A deeper understanding of the proteins associated with pollen germination that are also involved in allergic symptoms would enable an interesting subject for future study and could improve the clinical knowledge.

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## CHARACTERIZATION OF VOLATILE EMISSION OF ITALIAN POPULATIONS OF ORCHIDS WITH DIFFERENT REPRODUCTIVE STRATEGIES

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The family *Orchidaceae*, with about 24000 species, is the largest in the plant kingdom and the vast majority of orchid *taxa* is well known for its rarity and is threatened in several country throughout the world (1). Orchid flowers are often highly specialized to attract and fit their pollinators, and flower scent together with colour and shape are considered to be the main signal attracting pollinators. Some orchid species provide reward (e.g. nectar or pollen) to the pollinators, but other species are deceptive, so they attract pollinators in different ways, the most common strategies being mimicry of nectariferous flowers (e.g. *Orchis*, *Neotinea*, *Anacamptis*), sexual deception (e.g. *Ophrys*) and provision of shelter (e.g. *Serapias*) (2). In deceptive species attractiveness is very important to ensure reproductive success and, if *Ophrys* species have evolved a high degree of pollinator specificity, *Anacamptis* and *Neotinea* species are more generalist (3).

The aim of this study was to characterize the volatile organic compounds emitted by the flowers of four Italian populations of orchid species, with different attraction strategies, *Ophrys sphegodes* Mill. subsp. *sphogodes*, *Ophrys bertolonii* subsp. *benacensis* (Reisigl) P. Delforge, *Anacamptis morio* (L.) R.M. Bateman, Pridgeon & M.W. Caase, and *Neotinea tridentata* (Scop.) R.M. Bateman, Pridgeon & M.W. Case, using HS/SPME GC-MS. The results showed some distinctive differences in volatile metabolite composition between sexually deceptive and food-deceptive species. In particular hydrocarbons, aldehydes, alcohols and terpenes were the major constituents of both *Ophrys* species bouquet. Other author confirmed that some hydrocarbons, in particular very long-chain alkanes and alkenes and terpenes act as chemical mimicry of the sex pheromone of the virgin female pollinators in sexually deceptive species (4,5).

On the contrary the floral scent of *Anacamptis* and *Neotinea* species were represented by aldehydes, ketones and alcohols following by phenols and terpenes. In these genus colour is generally regarded as a primary cue to attract insects to food-deceptive flowers but it's not confirmed that floral odour is not of importance in pollinator attraction in food-deceptive species (6).

In conclusion HS/SPME GC-MS proved to be a suitable technique for analyzing and distinguishing the volatile fingerprint of different orchid species with different attraction strategies with potentially advantages for ecophysiological studies.

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INVESTIGATIONS ON *OXALIS PES-CAPRAE* L. IN ITALY. MORPHOLOGICAL, ANATOMICAL AND ULTRASTRUCTURAL FEATURES OF STIGMA AND STYLE AND THEIR POSSIBLE RELATIONS WITH SELF-INCOMPATIBILITY

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*Oxalis pes-caprae* L. is a South-African geophyte with trimorphic flowers. Introduced in Europe since the end of 18<sup>th</sup> century, it is now naturalized in many countries with Mediterranean or even sub-tropical climate and in several regions it is currently behaving as a troublesome invasive weed. In Italy, only short-styled populations are known for certain so far; due to self- and intramorph-incompatibility, the plant is believed to reproduce only asexually through bulbils. An investigation is currently being carried out on monomorphic (short-styled) Italian populations, with the aim of clarifying anatomical and ultrastructural features of the stigma-style complex, also in order to define their possible role in incompatibility mechanisms. Flowers collected in different phenological stages have been analyzed, both under Light Microscope and Transmission Electronic Microscope. On the ground of different anatomical and biochemical features of the transmitting tissue (TT), in each stigma-style complex three different zones could be recognized: a stigmatic zone, a sub-stigmatic zone, a stylar zone. First results suggest that TT of sub-stigmatic zone is possibly involved in auto-incompatibility mechanisms. In this zone, at anthesis TT looks quite compact compared to both stigmatic and stylar zones, being composed of tightly packed short cells with relatively thin walls. Besides soft mucopolysaccharides, cell wall matrix also contains pectins, which keep TT cells strongly tied to one another. In the two other zones, TT is made up of much more loosely arranged cells, with thicker cell walls; at anthesis, wall matrix is rich in mucopolysaccharides and it completely lacks pectins. After illegitimate self- or intramorph-pollination, pollen tubes easily grow through TT in the stigmatic zone, where wide inter-cellular spaces filled up with a soft mucopolysaccharide matrix allow their penetration towards the style. On the contrary, compact TT of sub-stigmatic zone seems to act as a mechanical barrier for illegitimate pollen tubes, which apparently arrest their path as soon as they reach this zone. Yet, in post anthesis a few tubes were observed, which succeeded in passing beyond sub-stigmatic zone. After overcoming this hurdle, they easily grow between loosely arranged TT cells in stylar zone and some of them eventually reach the ovules, where occasional fertilizations were observed.

EFFECTS OF ACUTE HEAT STRESS ON THE FEMALE REPRODUCTIVE APPARATUS OF *LYCOPERSICON ESCULENTUM* CV MICRO-TOM

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Temperatures above the normal optimum are sensed as heat stress (HS) by all living organisms, including plants. HS affects fertility, yield and production quality of crops with important consequences on food security and the well-being of human population. These negative effects can depend on the fact that all the phases of sexual reproduction in angiosperms, namely gametophyte development (from meiosis to pollination), progamic phase (from pollination to zygote formation) and embryo development (from zygote to seed), are more vulnerable to temperature stress than vegetative processes (1). Presently there are limited data about the effects of HS on the female part (pistil) and its interaction with pollen grains. The present research gives more insight into the effects of acute HS on the pistil's morphology and the progamic phase of tomato (*Lycopersicon esculentum* cv Micro-Tom). A morphological analysis of Micro-Tom pistils treated at 42°C for 3 h at different developmental stages has been performed by light microscopy using Toluidine Blue O, Periodic Acid Schiff, Alcian Blue and Calcofluor White stains. Moreover, the pattern distribution of  $\alpha$ -tubulin has been investigated in the heat-treated pistils. In addition, the carbohydrate epitope of arabinogalactan proteins (AGPs), involved in pollen tube guidance to the ovary, has been localized by JIM8 antibody. The consequences of acute HS have been also evaluated on stigmatic receptivity, pollen performance and *in vivo* pollen tube growth using epifluorescence microscopy. Results herein reported show that the general organization of the pistil tissues is substantially unaltered under HS. A significant observation is that HS seems to alter the microtubule distribution in the ovules: in control ovules, microtubules resulted well preserved in the inner integument tissue, whereas in treated ovules they were diffused in the cytoplasm. The epitope immunorecognized by JIM8 antibody seemed to disappear under HS in the transmitting tissue of the styles, thus denoting a possible alteration in the pollen tube growth pathway; on the contrary its localization was similar to the control in heat-treated ovules. Both stigma receptivity and pollen performance resulted sensitive to HS. The present work confirmed the sensitivity of sexual plant reproduction to HS: like in other species, such as peach (2) and sweet cherry (3), the receptivity of Micro-Tom stigma was reduced under HS and thus, the possibility of fertilization. Molecules involved in pollen tube guidance to the ovary (AGPs) and structural proteins (microtubules) showed an altered pattern with possible functional consequences on pistil function.

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## A GOLVEN-LIKE PEPTIDE AT THE CROSSROAD BETWEEN AUXIN AND ETHYLENE DURING PEACH RIPENING

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Peach is a climacteric fruit and many aspects of its ripening are controlled by ethylene. Ethylene synthesis depends on auxin, as the transcription of *1-aminocyclopropane-1-carboxylic acid synthase 1 (ACS1)*, coding for the key enzyme of its synthesis, is induced by auxin (1). Ethylene perception can be blocked by 1-methylcyclopropene (1-MCP) (2), a chemical that binds the receptors. In mature fruit of many species this binding affects ripening related processes as softening, but, in peach, the effect is transient; moreover the treatment leads to over-ripening disorders. Contrary to other climacteric fruit as apple and tomato, if peach fruit is treated at commercial harvest, i.e. at preclimacteric stage, 1-MCP induces ethylene synthesis. Transcriptomics investigations were used to unveil the unusual response of peach to 1-MCP. This inhibitor, besides repressing, as expected, many ethylene-responsive genes, surprisingly can induce *ACS1* and, moreover, many genes involved in auxin synthesis, transport, perception and signal transduction. The large transcriptional modulation of genes belonging to the auxin metabolism is due to the rise in auxin concentration in 1-MCP treated fruit. One of the quickest genes to be induced by 1-MCP encodes a GOLVEN-like (GLV) peptide hormone. This gene results to be strongly induced also by ripening and auxin and repressed by ethylene. In Arabidopsis, peptides of the GLV family can alter the cellular localization of PIN-FORMED (PIN) auxin efflux carrier, thus leading to localized changes in auxin gradients (3). Thus, the auxin re-localization, release from storage compartments and, possibly, new synthesis occurring upon 1-MCP treatment drive and sustain ethylene synthesis also in presence of the inhibitor. The stony hard trait impairs peach fruit ripening by repressing ethylene synthesis. Nonetheless, albeit being the ethylene synthesis machinery functional, its inactivation is probably due to decreased auxin levels. Indeed, exogenous auxin applications can rescue ethylene synthesis and thus fruit softening. Transcriptomics analyses show that genes encoding enzymes of the auxin biosynthetic pathway are at levels similar to those found in genotypes producing melting fruit. On the contrary, genes encoding GLV-like peptide hormones and their putative receptors, leucine-rich repeat receptor protein kinases, are strongly impaired in their expression levels in the stony hard genotype. The involvement of these classes of genes during the ripening syndrome has been almost completely ignored up to now. Data on the possible role of a peach GLV-like peptide acting as a mediator of the auxin-ethylene cross-talk during fruit ripening will be presented. Our findings open new possibilities to explore new loci as possible genetic determinants of traits having an impact on fruit quality and storability. Moreover, being these peptide hormones apoplasmic and hydrophilic, they might be the starting point for the rational design of environmental-friendly agrochemicals useful to improve peach storability.

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TRANSCRIPTIONAL REGULATION OF EGG CELL SPECIFIC GENES IN *ARABIDOPSIS THALIANA*

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In Angiosperms the gametophytes are composed of few haploid cells that develop within the diploid sporophytic tissues of the flower sexual organs. The female gametophyte contains seven cells among which the egg cell and the central cell (the two female gametes), which respectively give rise to the embryo and the endosperm upon fertilization. Taking advantage of the yeast one-hybrid strategy, a simple and fast protein-to-DNA interaction assay, we have searched for transcription factors driving female gamete differentiation. We have chosen as bait for our assay the EC1.1 gene, since it is exclusively expressed in the egg cell, the female gamete, therefore the comprehension of its regulation can shed light into female gamete differentiation. Our screenings indicated that EC1.1 promoter region is bound by SUF4 (SUPPRESSOR OF FRIGIDA 4), a C2H2 transcription factor. We have also been able to show that SUF4 regulates EC1.1 also in *in vivo* since we could not detect pEC1.1:GUS activity in *suf4* egg cells. Coherently with its role, SUF4 is detected in mature embryo sacs and real time PCR analyses pinpointed that all the 5 members of the EC1 family are regulated by SUF4, since their expression level is strongly reduced in *suf4* plants.

Using a bioinformatics approach we discovered that SUF4 is co-expressed with MOM1 (Morpheus's Molecule1), a gene required for transcriptional gene silencing maintenance. RT-PCR, real time PCR and promoter GUS line experiments indicate that MOM1 is also expressed during ovule and embryo development. Interestingly in *mom1* mutants, EC1.1 expression pattern is altered since it is also detected in developing carpel leaves. The interactions among MOM1, SUF4 and EC1.1 will be presented and discussed.



## INTEGRATION OF PLASTIDS IN THE PLANT CALCIUM SIGNALLING NETWORK REVEALED BY THE TARGETING OF AEQUORIN CHIMERAS TO CHLOROPLAST SUBCOMPARTMENTS

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Calcium is a universal signalling element involved in a wide range of physiological processes in all eukaryotes. Although there are common elements in Ca<sup>2+</sup>-based signal transduction networks, unique features of plant Ca<sup>2+</sup> signalling derive from differences in the lifestyle and developmental programs of plants, as well as peculiar structural features of the plant cell. In addition to the vacuole, which is commonly considered as the main accumulation site for Ca<sup>2+</sup> and an important stimulus-releasable Ca<sup>2+</sup> store, increasing evidence suggest that other intracellular compartments may act as mobilizable Ca<sup>2+</sup> stores in plant cells (1). The present work aims to elucidate how plastids, a diverse family of organelles found ubiquitously in plants and various algae, are integrated in the intracellular Ca<sup>2+</sup> signalling network in the model plant *Arabidopsis thaliana*.

*Arabidopsis* lines stably expressing the Ca<sup>2+</sup>-sensitive bioluminescent reporter aequorin targeted to different chloroplast subcompartments were generated, *i.e.* the plastid stroma, the outer envelope, the thylakoid membrane and the thylakoid lumen. All constructs included a YFP-aequorin fusion, for an easier verification of the correct subcellular localization of the different aequorin probes by laser scanning confocal microscopy. Moreover, immunocytochemical experiments are currently under way to confirm the proper targeting of all the aequorin chimeras.

[Ca<sup>2+</sup>] variations in response to different physiological stimuli were monitored in seedlings *in toto*, as well as in cell suspension cultures obtained from *in vitro* dedifferentiation of *Arabidopsis* explants (2), and compared with the corresponding cytosolic Ca<sup>2+</sup> traces. To measure Ca<sup>2+</sup> fluxes into/from the thylakoid membrane system in cultured cells, photoautotrophic cell suspension cultures were established. In all the examined subcellular locations transient elevations in [Ca<sup>2+</sup>] with differential kinetic parameters were detected, providing evidence for the ability of plastids to evoke specific Ca<sup>2+</sup> signals in response to a plethora of environmental stresses.

To get insights into the role of putative Ca<sup>2+</sup> transporters localized in plastids, a mutant plant line lacking the recently characterized glutamate receptor AtGLR3.4 (3) was transformed with the cDNA encoding the aequorin chimera targeted to the plastid stroma (4). The T1 generation is currently under screening and will be used in aequorin-based Ca<sup>2+</sup> assays. A comparative analysis of the Ca<sup>2+</sup> traces recorded in the wild-type and knock-out lines will provide insights into the role of these organellar ion channels in mediating plastidial Ca<sup>2+</sup> fluxes.

The generation of a complex toolset of differentially targeted aequorin probes allowing the parallel measurement of Ca<sup>2+</sup> changes in different chloroplast subcompartments will help to define the contribution of plastids to the plant Ca<sup>2+</sup> signalling network.

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APOPTOTIC HALLMARKS SUPPORT THE ROLE OF PCD IN AERENCHYMA ONTOGENESIS OF *EGERIA Densa* STEM

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As adaptation to chronically submersed habitats, the aquatic macrophyte *Egeria densa* differentiates in the cortical region of the stem a prominent constitutive aerenchyma, resulting from the combination of a precocious schizogenous and a late lysigenous differentiation mechanism (1). Unlike the schizogeny, usually constitutive and without death events, the lysigeny involves genetically programmed cell death (PCD), follows a specific and predictable spatial pattern (2) and shows an array of morphological and biochemical features shared also by animal apoptosis.

In this study we characterized new apoptotic hallmarks that occur during the last phase of aerenchyma differentiation in *E. densa* stem.

Our previous results, mainly based on cyto-histological observations, suggested that the aerenchyma ontogenesis is a tightly regulated process, dependent on the distance from the shoot apex: the air cavities differentiation started from a hexagonally packed pre-aerenchymatic tissue and, following a basipetal and centripetal developmental pattern, produced a schizogenous honeycomb-arranged aerenchyma (at 1-2 mm from the apex) with little air cavities delimited by few cell layers. Then, the air cavities were significantly enlarged and remodelled by a lysigenous mechanism and, at about 2 mm from the apex, the final honeycomb architecture of the cortical aerenchyma consisted in large air cavities delimited by cell monolayers. Some cytological features, such as chromatin condensation, persistence of organelles until late stages of cell death, apoptotic-like bodies, partial cell wall lyses and plasmolysis, suggested a role of PCD in aerenchyma differentiation (1).

The morphogenic role of PCD has been confirmed by some molecular and biochemical apoptotic hallmarks such as the DNA fragmentation (as evidenced by TUNEL assay and by genomic DNA electrophoresis) and the intracellular increase of H<sub>2</sub>O<sub>2</sub> and NO in the sites where lysigenous aerenchyma occurred. Interactions between these signalling molecules are considered of primary importance for the control and the production of several types of cell death, as well as in developmental processes (3, 4).

The observed PCD events have relevant morphogenetic, metabolic and functional consequences for *E. densa* plants: i) the PCD remodels and adjusts the architecture of the schizogenous air-chambers; ii) the circulation of gases utilized for photosynthetic and respiratory processes is improved by the increase of intercellular air spaces deriving from the removal of specific cells; iii) PCD allows both reduction of O<sub>2</sub>-consuming cells and a convenient recycling of substances derived from the lysigenic cell dismantling.

As a result of these events, the stem of *E. densa* can potentially enhance its metabolic efficiency and achieve optimal adaptation levels to submerged habitats, as suggested by the wide diffusion of this plant in natural and non-natural water bodies and can explain its ability to bio-remediate aquatic ecosystems.

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## DIVERSITY OF FLORAL NECTAR COMPOSITION IN THE TRIBE LITHOSPERMEAE (BORAGINACEAE)

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Nectar is a flower secretion produced by many Angiosperms to recruit and reward pollen carriers. The mixture of chemicals contained in this secretion can be shaped by several ecological, physiological or phylogenetic constraints as well as specific preferences of pollinators (1). Nectar chemistry has been reported to be a plastic character that can rapidly change in response to these constraints (2), thus elucidating the drivers can be important to understand mechanisms of plant-pollinator interactions, and, ultimately, diversity and evolution within a given taxonomic group. We studied the diversity of nectar chemistry in Boraginaceae tribe Lithospermeae, a large, monophyletic group including important nectariferous species that are still poorly known in this aspect. Sugar and amino acid profile has been determined by HPLC for 82 species (482 nectar samples) representing all the six main clades of this tribe (3). There are clear associations between specific chemical profiles: sucrose-dominant nectar contain a higher percentage of non-protein amino acids (SD profile) than hexose-dominant nectars (HD), and in particular more abundant  $\gamma$ -aminobutyric acid. On the other hand HD nectars contain a relatively higher percentage of protein aminoacids, and, among the non-protein amino acids, the most abundant is  $\beta$ -alanine (HD profile). Most of the species in all clades have SD or sucrose-rich (SR) nectar, but there are few species in clade A (8 species) and clade C (2 species) that possess an HD profile. A clear separation in clade A occur for the Macaronesian species of *Echium* with an HD nectar. Interestingly, pollination by bees and birds has been reported for some of these species (Dupont et al. 2004). In the same clade, the closely related genus *Lobostemon* shows the same dichotomy, with *L. fruticosum* having SD nectar whilst *L. montanum* an HD nectar. Pollination by birds is also reported for the latter species (4). The genus *Buglossoides* in clade C has a very heterogeneous nectar: *B. purpureocaerulea* and *B. calabra*, perennials growing in dry woods and mainly pollinated by dipterans, have ED nectar; *B. arvensis* and *B. incrassata*, annuals growing in uncultivated fields and dry pastures, have SR nectar and are pollinated by bees; *B. minima*, living in a similar habitat, has exose-rich nectar (ER). Species from all the other clades are in the range of SD-SR profile. Results suggest that a diffuse phylogenetic constraint drives nectar chemical composition towards SD profiles in Lithospermeae, although in some species other factors, such as habitat and type of interactions with pollinators, may have a stronger influence in shaping an ED profile.



Fig. 1. *Buglossoides Purpureocaerulea* visited by *Empis pennipes*

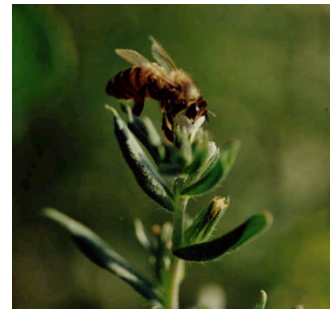


Fig. 2. *Buglossoides arvensis* visited by *Apis mellifera*

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## IN VITRO MORPHOGENESIS OF *ARABIDOPSIS* TO SEARCH FOR NOVEL ENDOPHYTIC FUNGI MODULATING PLANT GROWTH

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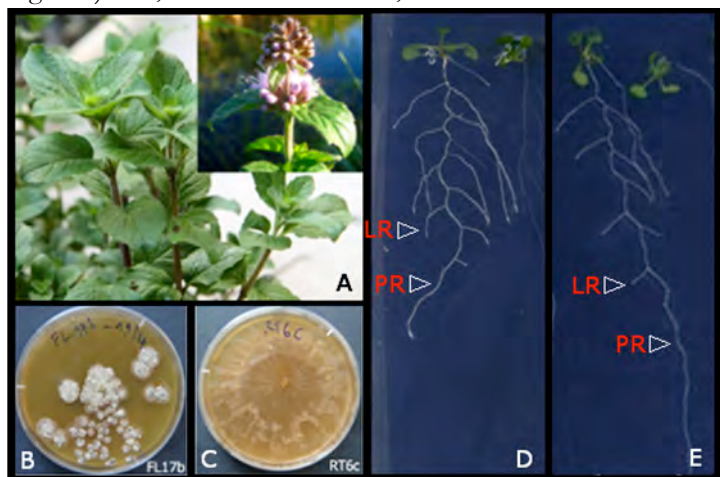
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Phytoremediation involves the use of plants to decontaminate soils, water and air. It is an emerging technology that can be applied to the uptake or degradation of both organic and inorganic pollutants (1). It has been found that root length, root surface area and root volumes are positively correlated with levels of pollutants removed from water (phyto-filtration), stored in roots (phyto-stabilization) or translocated to leaves (hyper-accumulating plants) (1). Root systems, however, are often inhibited by the stress induced by water and soil pollutants. In such circumstances, plant ability to phytoremediate declines significantly. Endophytic fungi live within roots, stems and leaves of their plant hosts forming extensive infections also in plants growing in high-stress habitats (2). Among them, class II non-systemic endophytes, which have shown to increase below- and above-ground biomass and to confer stress tolerance in some plants, may play a significant role in these environments. Therefore, exploitation of these fungi to overcome the limitations to phytoremediation brought by abiotic stress may be a promising strategy.

In this study, we evaluated the presence of endophytic fungi in submerged roots and stems of *Mentha aquatica* L. This plant has already been shown to grow in polluted waterlogged soils (3), and might be easily employed in constructed wetlands over a long range of elevations. In this study, several different fungal endophytes were isolated from this plant, and 22 of them finally selected and grown regularly on ME slants. Plugs from the young part of mature colonies were co-cultured with *A. thaliana* seedlings in Petri plates containing 0.2× MS medium, in controlled conditions. Primary and total root length, number of lateral roots and root branching were measured by imaging analysis after 13 days of incubation; total fresh and dry weights were determined after 13 and 20 days. Results were expressed as increase/decrease percentages with respect to control plants and significant differences among fungi and between stem and root isolates were estimated by analysis of variance (ANOVA, Post Hoc test at  $P \leq 0.05$ ). Species identification was based on sequence analysis from the nuclear ribosomal internal transcribed spacer region (ITS1/5.8S/ ITS2).

Morphometric analyses showed a high array of effects exerted by *M. aquatica* endophytes. Eleven fungal isolates significantly changed the total length of the root system, and twelve of them the degree of primary root branching. Frequently, greater root branching was linked to a significant decrease of the primary root length (PR, Fig. 1D) and, in some treatments, also to an increase in the number and length of lateral roots (LR, Fig 1D).



Most *Mentha* endophytes lowered fresh weights and increased dry matters in comparison with control plants. Only three isolates heavily affected *Arabidopsis* growth, showing to be pathogenic.

Fungal endophytes that showed to positively influence *A. thaliana* biomass and root branching in this study will be thoroughly tested *in vitro* and in greenhouse on *M. aquatica* plants for a possible field application.

Fig. 1. (A) *Mentha aquatica* plants in bloom at the site of sampling. (B, C) Two fungal endophytes on ME slants, isolated from stems (B) and roots (C). (D) The effect of the isolate shown in Fig. 1B on the root system of *Arabidopsis* (reduction of primary root = PR and increased branching, LR =lateral root) after 13 days of culture; (E) a control plant of the same age.

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*IN VITRO* FUNCTIONAL CHARACTERIZATION OF AQUA1: A NEW POPLAR (*POPULUS X EURAMERICANA* CLONE I-214) AQUAPORIN INVOLVED IN ZINC STRESS

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Aquaporins are water channel proteins involved in plants growth, development and response to environmental stresses (1, 2 and 3). *Populus trichocarpa* is the model tree species and has been proposed for phytoremediation application due to its high biomass production and fast growth. Despite the high number of aquaporins genes in poplar genome, few of them have been characterized at functional level in this species. Here we have focused on a putative aquaporin gene, *aqua1*, that resulted down-regulated in a microarray analysis performed in leaf of *P. x euramericana* (I-214 clone) exposed to excess Zn (4).

The cDNA of *aqua1* (GenBank: GQ918138) encode for a 257 amino acid proteins, containing the typical NPA signature motif of aquaporins, that showed 99% nucleotide identity with a putative *P. trichocarpa* TIP aquaporin. AQUA1 was down-regulated in response to Zn excess in both leaves and roots (Fig. 1) and conferred Zn tolerance when expressed in yeast Zn hypersensitive strain. AQUA1-GFP construct expressed in *Arabidopsis thaliana* protoplasts localized in all the membrane structures of the cell, and Zn excess caused a re-localization of this protein in new forming vacuoles (Fig. 2); moreover, we showed that the re-localization is regulated by phosphorylation. These findings suggest that AQUA1 is regulated at both transcriptional and post-translational level in response to Zn-excess and that could confer tolerance in response to this metal.

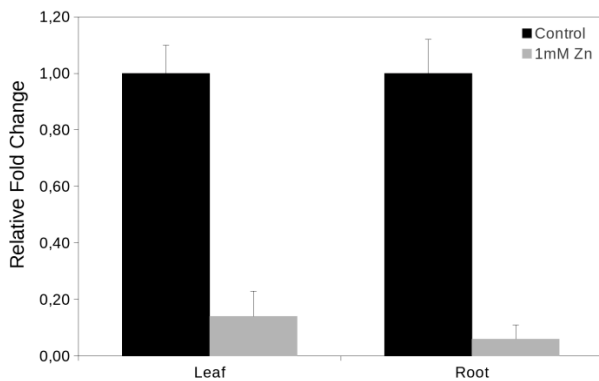


Fig. 1. *aqua1* differential expression in response to Zn excess in leaf and root tissues.

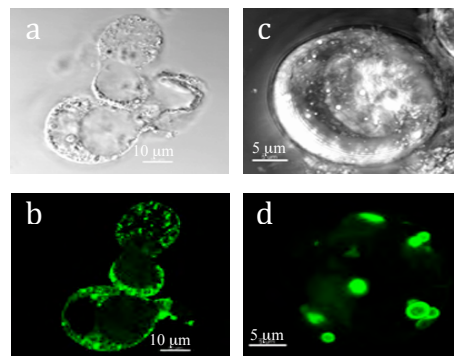


Fig. 2. Confocal AQUA1:GFP localization in control (a, b) and Zn excess (c, d) conditions, 488nm.

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THE USE OF LIGNINASE ENZYMES TO TARGET THE SCARIFICATION OF THE SEED COAT: APPLICATION FOR THE PROPAGATION OF THE ORCHIDS *HIMANTOGLOSSUM ADRIATICUM* AND *ANACAMPTIS MORIO*

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Seed dormancy may be associated with the presence of a rigid and impermeable integument that consists for the most part of lignin. Traditional methods for the scarification of the seed coat use corrosive chemicals, such as sulfuric acid or sodium hypochlorite, that can induce collateral effects on the embryo. In natural ecosystems wood/lignin is broken down by ligninase enzymes produced by fungi, and this is of particular relevance to the germination and symbiotic development of certain groups of plants such as orchids. Here we analyze the hypothesis that ligninases derived from fungi can be used to break the seed coat and stimulate germination, with particular reference to wild orchids of semi-natural grasslands. In an initial experiment the enzyme laccase was administered to seeds of *Himantoglossum adriaticum* and *Anacamptis morio* under sterile conditions *in vitro*, using two methods: 1). incorporation of the enzyme directly into the agar substrate using cool filtration, or 2). "bathing" the seeds after sowing with the addition of a sterile solution of the enzyme. In both cases a concentration of 1 unit of active enzyme per seed batch/Petri dish was used. In a second experiment germination of *A. morio* was compared on substrates containing one of three different ligninase enzymes (laccase, lignin peroxidase and manganese peroxidase) added to the substrate at a concentration of 0.04 U/Petri (the highest achievable concentration within cost constraints – while laccase is inexpensive, lignin peroxidase and manganese peroxidase cost around two hundred times the price of gold). In the first experiment, after six months the final rate of germination for both species was double that of the controls, only when laccase was added to the substrate (in the case of *A. morio* from 25 to 50 %, while for *H. adriaticum* from 2 to 5 %; statistically significant at the  $P \leq 0.05$  level). In contrast, the "bathing" treatment significantly reduced germination compared to the control and also introduced contamination. In the experiment to compare enzymes, no treatment means showed significant differences from the control. In conclusion, lignin peroxidase and manganese peroxidase are too costly to allow useful amounts of enzyme to be applied. However, as laccase is inexpensive larger amounts can be applied and were found to be effective. The germination rates obtained may appear low, but for species that produce thousands of seeds per fruit such as orchids a doubling in germination can result in a significant increase in terms of the number of plants produced. The use of ligninases, particularly laccase, in the propagation of rare species thus appears to be highly promising because the intervention is targeted at the seed coat without the risk of potential complications during the development of the embryo.



*VICIA FABEA* L.: A MODEL ORGANISM FOR PLANT CELL BIOLOGY STUDIES ASSESSING ENVIRONMENTAL POLLUTION AND BIOREMEDIATION PROCESSES

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Some higher plants present characteristics that make them excellent models to assess environmental pollutants, providing a large array of endpoints (e.g., germination rate, biomass weight, enzyme activities) pointing out matrix toxicity. Moreover they can also offer the possibility of assessing several genetic endpoints, which range from point mutations to chromosome aberrations (CA) and DNA fragmentation studies (1, 2).

The root apical meristem of *Vicia faba* has been the pioneer cytogenetic material for clastogenicity studies of physical and chemical agents since the early 1930s.

Since then there have been several proposals of *V. faba* protocol standardization for determining the genotoxicity both of liquid and solid matrices (3, 4, 5), and recently also the Italian Istituto Superiore di Sanità published guidelines for evaluation of mutagens in freshwaters and sediments with *V. faba* (6).

The aim of this research was to exploit *V. faba* system in the follow up and in the final evaluation of a bioremediation process consistent in *ex situ* biological treatment in biopile of a soil contaminated by polycyclic aromatic hydrocarbons (PAHs, 6480 ppm) and by total petroleum hydrocarbons (TPHs, 18347 ppm). At the end of the whole process the biopile treatment determined the depletion of PAHs and TPHs below 10 and 100 ppm respectively.

Different endpoints have been considered with *V. faba* system: the phytotoxicity, measured as % Germination index, that summarizes all the information on the impact of a potential toxic compound on seed germination and root elongation; the cytotoxicity, by means of the mitotic index, that is based on the measurement of the mitotic activity of the root meristem cells; the genotoxicity, by the cytological analysis of mitotic behaviour of root meristem, based on the checking of chromosomal aberrations in mitotic cells (Aberration Index), and of micronuclei formation (MCN Test), detectable in interphase cells. As additional endpoint of genotoxicity, cytohistochemical detection of *in situ* DNA fragmentation in root apices was achieved by TUNEL reaction, that allows to recognize both single and double-strand breaks at cellular and tissue level.

Considering the obtained results it was found that the toxicity of the contaminated soil was completely depleted at the end of the bio-based decontamination approach and *V. faba* represented a very good system for monitoring the biodegradation processes, essential in relation to the chemical analysis, that alone does not allow to predict the real toxicity of the matrix. Indeed it may depend on the original pollutants, but also on degradation intermediates, often unknown, and on the synergic actions of the different toxicants.

In this context, it has to be mentioned that, in order to evaluate the eventual efficacy of an applied remediation strategy, the use of bioassays with higher plants may be considered a fundamental point in risk assessment.

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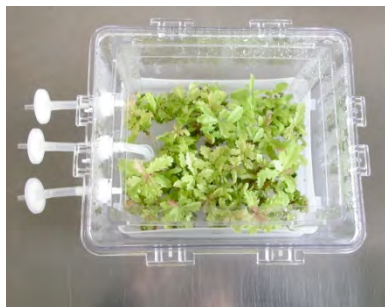
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IN VITRO PROPAGATION OF *QUERCUS ROBUR* L. BY PLANTFORM BIOREACTORENRICO GATTI<sup>1</sup>, AYLIN OZUDOGRU<sup>2</sup>, MAURIZIO LAMBARDI<sup>2</sup>, **ELISABETTA SGARBI**<sup>1</sup><sup>1</sup>Department of Life Sciences, University of Modena and Reggio Emilia, Via Amendola, 2, Padiglione Besta, 42122 Reggio Emilia, Italy; <sup>2</sup>IVALSA/Trees and Timber Institute, National Research Council (CNR), via Madonna del Piano 10, 50019 Sesto Fiorentino (Firenze), Italy

Plantform bioreactor is a new system recently developed to improve *in vitro* plant cultures. This bioreactor is based on a temporary immersion system (TIS) with a ventilation that ensures the regular air renewal inside the plastic box. This technique has been tested for the large scale micropropagation of ornamental and crop plants and demonstrated to be able to improve some growth parameters (1, 2). Pedunculate oak (*Quercus robur* L.) proved to be a recalcitrant species to micropropagation (3). Indeed, although a protocol for *in vitro* propagation of this species has been previously reported (4), the proliferation rate of shoots still remains unsatisfactory. Hence, the Plantform bioreactor was tested in this study, with the aim to evaluate its efficiency in promoting shoot proliferation of oak. The results were compared with those obtained with cultures on gelled medium.

Nodal segments (10-15 mm, on average) of *Q. robur* were taken from previously established *in vitro* shoot cultures and placed horizontally both in Microbox vessels, on Woody Plant Medium added with sucrose (20 g/l), agar (6 g/l) and 6-benzylaminopurine (0.2 mg/l), and in Plantform with the same medium without agar. Two different conditions of temporary immersion were tested in Plantform: 12 min/8 h and 8 min/16 h. Air was renewed for 15 min/4 h. Significant differences in terms of RGR (Relative Growth Rate) of shoots, based on fresh weight, were observed when the two techniques (Microbox and Plantform) were compared after 10 weeks of culture. The highest value of RGR was obtained with the Plantform bioreactor, without differences between the two conditions of temporary immersion (Fig.1, 2).

Plantform seems to be an effective, alternative method to promote a rapid and vigorous growth in *Q. robur* shoot cultures. Rooting ability, acclimatization and some morpho-physiological parameters of the leaflets will be considered afterwards, in order to evaluate the quality and the health of the micropropagated plantlets.

Fig.1 *Q. robur* shoot culture on solid medium.Fig.2. *Q. robur* shoot culture in Plantform bioreactor

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## AROMATIC PLANTS: FROM THE AROMA TO HERBAL TEAS

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Aromatic plants are worldwide known for both their attractive aroma and biological properties applied to different fields (cosmetic, food, pharmaceutical, etc.). One of the most popular use is to prepare herbal teas, as such or in blend because several components, including volatiles responsible for the aroma, present relevant biological activities (antibacterial, antiviral, antifungal, toxic...) (1). Therefore, their correct botanical identification and quality control are mandatory to guarantee their effective and, above all, safe use. The volatile fraction is therefore an important marker to characterize raw plant materials and to quantify key-aroma and/or biologically active markers (even at trace level) and dedicated analytical methods have to be developed.

Sampling of the volatile fraction entails the use of several approaches or techniques, which produce different samples that can be representative of the volatiles characterizing a vegetable matrix, e.g. headspace, essential oils and solvent extracts. Therefore, the choice of a suitable approach to analyze the composition of the volatile fraction of a plant material involves several factors including speed, ease of use, complete automation while avoiding, when possible, the use of solvents.

This lecture is a short overview to show the importance of qualitative and quantitative analysis of plants' volatile fraction as a fundamental complementary step to a correct botanical identification of plant material:

- i) to authenticate and classify aromatic plants species (i.e. sage, thyme, ... );
- ii) to define and characterize plant chemotypes (i.e. *Matricaria recutita* L.) (2);
- iii) to detect adulterations (i.e. *Mentha x piperita* L., *Citrus bergamia* Risso et Poiteau) (3,4);
- iv) to discriminate plant samples in function of their geographical origin;
- v) to monitor the influence of environmental factors on plants and, as a consequence, on their chemical composition;
- vi) to check a correct processing (drying process, storage) of plant material;
- vii) to verify the presence of marker compounds in finished products (e.g. herbal teas).

Examples will be reported to illustrate some of these points.

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SECRETED MATERIAL AND ANTIMICROBIAL ACTIVITY OF *SALVIA CACALIAEFOLIA* BENTH.

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The genus *Salvia* is known to possess bioactive constituents, commonly found in the complex secretion product of the aerial parts of these plants (1). The study of the chemical constituents of this surface exudate material (2) may thus be useful for the discovery of biologically active compounds, particularly terpenoids (3), that have shown antioxidant, anti-bacterial and anti-fungal activities against human pathogens (4). In a previous screening of antimicrobial activity of the surface exudates of various *Salvia* species, *Salvia cacaliaefolia* showed promising activity on selected multi-drug-resistant Gram-positive strains such as *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Enterococcus faecium* and *Enterococcus faecalis*. Aim of our study is to describe the morphology of the glandular trichomes, the histochemistry of the secretion products, the isolation of the isoprenoid constituents from the exudate and the evaluation of their antimicrobial activity. *S. cacaliaefolia* Benth. is a Mexican perennial herb, included into subgenus *Calosphace*, sectio *Standleyana* (5). Light and scanning electron microscopy were used to determine the morphology of the glandular hairs; histochemical procedures were used to localize the secreted substances within and on the surfaces of the hairs. The glandular hairs occurred as peltate and capitate (1). The peltate trichomes consisted of one basal epidermal cell, one stalk cell and a broad head of four secretory cells. The capitate trichomes appeared in two different types: type I, made up of a basal epidermal cell, a short stalk cell and a round head of one or two secretory cells, and type II, with a basal epidermal cell, a stalk cell, a neck cell and a secretory head of one elongated cell. In all the mature organs, the histological findings revealed a complex secretion product that appeared as an emulsion, with flavonoids and terpenoids, as reported for other *Salvia* species (6). The extraction of the secreted material afforded four abietane diterpenes (carnosol, 7-methoxyrosmanol, carnosic acid and 12-methoxycarnosic acid) identified by comparison with authentic samples, three triterpenes (betulinic acid, ursolic acid, ursolic aldehyde), and guaiol, identified by their physical and spectroscopic data which were largely consistent with those published in the literature (7-9). The GC-MS analysis of the secreted material revealed the presence of guaiol, a- and b-eudesmol, camphor, borneol, a-copaene, and caryophyllene oxide as major components. For some of these compounds the antibacterial activity was already characterized (10-13), while it was first reported here for 7-methoxyrosmanol (MR) and 12-methoxycarnosic acid (MCA). These compounds were particularly active on several methicillin resistant and susceptible *S. aureus* and *S. epidermidis* strains (MIC: 8 µg/ml for MR on both species; 16 µg/ml and 16-32 µg/ml for MCA on *S. aureus* and *S. epidermidis*, respectively). Studies aimed to determine the mechanism of action of these diterpenes on the susceptible bacterial species are ongoing. *S. cacaliaefolia* can be considered as a source of potential antibacterial compounds with activity against resistant or multi-resistant human pathogens.

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## HP-TLC BIOAUTOGRAPHIC ASSAY AS A PRELIMINARY RESEARCH TOOL TO MATCH CHEMICAL AND BIOLOGICAL PROPERTIES OF OFFICINAL PLANT EXTRACTS

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Bioautography is mainly known as a research strategy hyphenated with planar chromatography techniques aimed to detect bioactive components characterizing plant extracts, pointing out their efficacy directly on the chromatographic support used for their separation. Therefore, thin layer chromatographic (TLC) bioautography gives the opportunity to perform at the same time a semi-quantitative chemical and biological fingerprinting of the main active fractions in plant extracts (1). Our research group set up pharmaceutical biology studies developing this particular technique employing high performance thin layer chromatography plates (HP-TLC) to obtain the best separation of molecules/fractions together with the best evidence of their possible biological activity, driving further in-depth investigations toward a more focused chemical identification and quantification, and a wider biological activity profile of officinal plant extracts. We optimized HP-TLC bioautography for antimicrobial (antibacterial and antifungal) and antioxidant properties [DPPH, 1,1-diphenyl-2-picrylhydrazyl; and ABTS, 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) assays] of different plant extracts evidencing the components really involved in the bioactivity (2). Our research strategy of plant extracts is planned to have HP-TLC bioautography as preliminary data giving us the opportunity: 1. to point out the chemical compounds with antibacterial, antifungal, antioxidant capacity; 2. to focus on the chemical isolation and identification of fractions characterized by specific bioactive molecules; 3. to check biological capacities of pure single compounds, or combinations, assaying possible synergic interactions; 4. to explore other biological capacities -i.e. cytotoxic, mutagen and mutagen-preventive activity, etc. - related to those preliminarily evaluated through HP-TLC bioautography. In light of these premises, some researches in progress pointing out the role of HP-TLC bioautography in the pharmaceutical biology profile of our studies are shown below:

-Essential oils obtained from plant crude drugs of different geographical origin (e.g. *Cryptocarya massoia*, Lauraceae, from Indonesian regions; *Piper aduncum*, Piperaceae; *Croton lechleri*, Euphorbiaceae from Amazonian Ecuador). *Cryptocarya massoia* (Lauraceae) essential oil evidenced on HP-TLC plates relevant antimicrobial activity related to massoia lactones, as single pure compounds, versus Gram negative strains (*Klebsiella oxytoca*) and yeasts (*Candida albicans*). These data suggested to test pure compounds mixtures to evaluate their possible synergic potential. The result of combining benzylbenzoate and benzyl salicate was a specific efficacy against Gram positive bacteria (*Enterococcus faecalis*). The evidences will also drive the research towards in-depth investigations (cytotoxicity, mutagenicity) of the active isolated compounds and their different qualitative and quantitative combinations. The case of *Piper aduncum* (Piperaceae) essential oil, characterized by high abundance of dillapiole (45.92%), highlighted this compound as the main responsible of highly selective antibacterial activity against *Staphylococcus aureus*. Amazonian *Croton lechleri* essential oil evidenced antibacterial activity against *Escherichia coli* on HP-TLC mainly due to sesquicineole (17.29%). Caryophyllene oxide (1.24%) and 1,10-di-epi-cubenol (4.75%) showed instead the best antioxidant capacity with HP-TLC-DPPH assay.

-126 different plant extracts from industrial (agro-food) and agricultural by-products investigated through HP-TLC bioautography for achieving preliminary suggestions about their possible role as source of bioactive molecules. In this case ethanol, chloroform and acetone extracts have been assayed for matching chemical fingerprinting and biological activity, giving preliminary but clear suggestions about the most promising extracts in terms of bioactivity potential and composition. Ethanol extracts, were the most interesting among the bioactive ones obtained from agro-food by-products of *Malus domestica* and *Juglans regia*, and from agricultural wastes of *Allium sativum*.

In conclusion, HP-TLC bioautography is an important tool to quickly join on chromatographic supports preliminary chemical fingerprinting and important biological evidences of officinal plant extracts.

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## PRELIMINARY STUDY OF ENDEMIC PLANTS OF SARDINIA AS A SOURCE OF NEW ANTIVIRAL AGENTS

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Within a project aiming to find new agents inhibiting the replication of human immunodeficiency virus type 1 (HIV-1), the ethanolic extracts of six endemic plants collected in Sardinia (Italy) have been assayed on the ribonuclease H (RNase-H) activity associated of the HIV-1 reverse transcriptase (RT), a multifunctional viral enzyme which is the most important target in the antiretroviral therapy. The human immunodeficiency virus type 1 (HIV-1) epidemic is still a worldwide health issue despite the availability of more than 20 drugs currently approved for treatment (1). In particular, the selection of drug-resistant viral strains and the toxic side effects due to the chronic drug administration lead to the need of developing new inhibitors with novel mechanism of action and effective on HIV drug-resistant strains (2,3). Taking into account the enormous number and the amazing structural diversity of the currently available plant constituents, the plant kingdom should be further explored as a source of new and diverse antiviral agents.

The Sardinian flora consists of 2408 taxa (4) of which 347 are endemic (5). The geographic isolation has been caused a genetic and metabolic differentiation in these species, as shown by the high number of scientific researches that have been published until now (6,7,8,9,10). Some of Sardinian endemism have also shown very interesting biological and pharmacological activities (11,12,13).

In this preliminary work the following six endemic species have been selected: *Bituminaria morisiana* (Pignatti & Metlesics) Greuter (Fabaceae), *Helichrysum saxatile* Moris (Asteraceae), *Limonium morisianum* Arrigoni (Plumbaginaceae), *Salvia desoleana* Atzei & Picci (Lamiaceae), *Stachys corsica* Pers. (Lamiaceae) and *Tanacetum audibertii* (Req.) DC. (Asteraceae). When ethanolic extracts obtained from aerial parts of all plants were evaluated in biochemical assay on the RT-associated RNase H function, they were able to inhibit this enzymatic activity with IC<sub>50</sub> values in the 2-47 µg/mL range. Given that relevant and selective activity relates to IC<sub>50</sub> values below 100 µg/ml for extracts and below 25 µM for pure compounds (14), all our extracts have showed a significant antiviral activity. In particular, the most active extract was the one obtained from *L. morisianum* with an IC<sub>50</sub> value of 2.29 ± 0.32 µg/mL. This extract will be subjected to bioassay-guided fractionation to ascertain the bioactive compounds that could have important biological activities.

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*ORIGANUM VULGARE* SSP. *VRIDULUM*: A PHYTOALIMURGIC PLANT WITH INHIBITORY ACTIVITY AGAINST HUMAN CANCER CELL PROLIFERATION

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Wild plants have always been an important food source for people, not only in the ancient times but also more recently. A wealth of information linked to human traditions has been gained on these plants which is worth preserving, as now these experiences are primarily a prerogative of elderly in rural areas. It has been proved, time and time again, that the “quack” medical knowledge handed down by the common people constitutes sources of information useful for scientific research and that many plants utilised exclusively in popular tradition, when exposed to scientific examination, have been found to be useful for different sectors in the industry [1]. Therefore, science and tradition are strictly connected: science, actually, has often traditional origin.

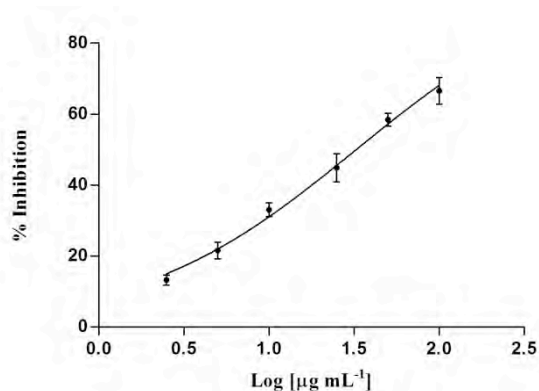
There are at least 250 000 species of vascular plants existing worldwide out of which more than 1000 plants have been found to possess anticancer properties [2]. The plant kingdom provides an enormous potential for discovery of new drugs useful for treatment and prevention of diseases. Plants have long been viewed as a potential cure for cancer, which is projected to become the major cause of death in this century. It is estimated that around 60% of antitumor and anti-infectious drugs, already on the market or under clinical trial, are of natural origin. However, most of these compounds cannot economically be synthesized and are still obtained from wild or cultivated plants [3].

Our research was directed to a preliminary screening of wild Mediterranean dietary plants to assess their antiproliferative activities on human cancer cell lines. In particular, in the present study, the ethanol extract of *Origanum vulgare* ssp. *viridulum* (Martin-Donos) Nyman aerial part was tested for its antiproliferative activity using HepG2 and MCF7 cell lines with expectation of potential benefits, not only as spices but also as a source of new anticancer agents. The plant selected for this study is very popular in Calabria (Italy). It is used as spice and food ingredient; and it is well-known as home remedy to treat different ailments.

Our previous studies showed the antiradical activity using DPPH test ( $IC_{50} = 0.013$  mg/mL) and the inhibition of nitric oxide production on RAW 267.1 macrophages ( $IC_{50} = 0.186$  mg/mL) [4]. In the present study we have also demonstrated the antiproliferative activity against human cancer cells of hydroalcoholic extract of *O. vulgare* ssp. *viridulum*. The phytochemical analysis of the extract was also evaluated.



*Origanum vulgare* L. subsp. *viridulum* (Martin-Donos) Nyman



Inhibition of human hepatocarcinoma (HepG2)

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FLAVONOIDS AND PHENYLPROPANOIDS FROM *PHLOMIS KURDICA* RECH. F. (LAMIACEAE) AS INHIBITORS OF LACTATE DEHYDROGENASE

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The genus *Phlomis* (Lamiaceae) includes about 100 species distributed mainly in the Mediterranean region, central Asia and China. Ten species of *Phlomis* are found in the flora of Jordan. Among them, *Phlomis kurdica* Rech.f. is a hairy and woody perennial herb, 30-80 cm long and many stemmed, leaves petiolate ovate-cordate, flowers up to ten arranged in circles along the verticillate (1). Many species of *Phlomis* are used in the folk medicine for the treatment of various ailments including inflammation, diabetes, ulcer, allergy, infection and cancer (2). The Jordan flora is considered one of most attractive in the Middle East due to its biodiversity (1, 3) and potentiality to discover new bioactive natural molecules (4, 5). In the last decade our research group successfully detected a good number of compounds obtained from Jordanian plants with anticancer effect, elucidating different mechanisms of action including tyrosine ligase inhibition activity and binding affinity to heat shock protein 90 (4, 5). Since some species of *Phlomis* proved to possess anti-cancer properties (2), the crude extracts of *P. kurdica* aerial parts were assayed for their lactate dehydrogenase (LDH) inhibitory activity, an enzyme that is up-regulated in tumor tissues. In fact, cancer cells depend mainly on anaerobic respiration and their glycolytic rate is up to 200 times higher than that of the normal tissue. This fermentative glycolysis is catalyzed by an overexpression of the A form of LDH. Inhibition of LDH is so considered as a promising target in cancer treatment, since it is possible to cause a starving of cancerous cells by reducing glycolysis or by inhibiting the conversion of glucose to lactate (6). Active extracts were subjected to different fractionation procedures to isolate two new flavonoids (1 and 2) and a new phenylpropanoid (3) (Fig. 1), together with eleven known phenolic compounds, including flavonoids and phenylpropanoids. All pure compounds were assayed for their LDH inhibitory activity. Luteolin 7-O-b-D-glucopyranoside showed an IC<sub>50</sub> value similar to that of reference compound galloflavin.

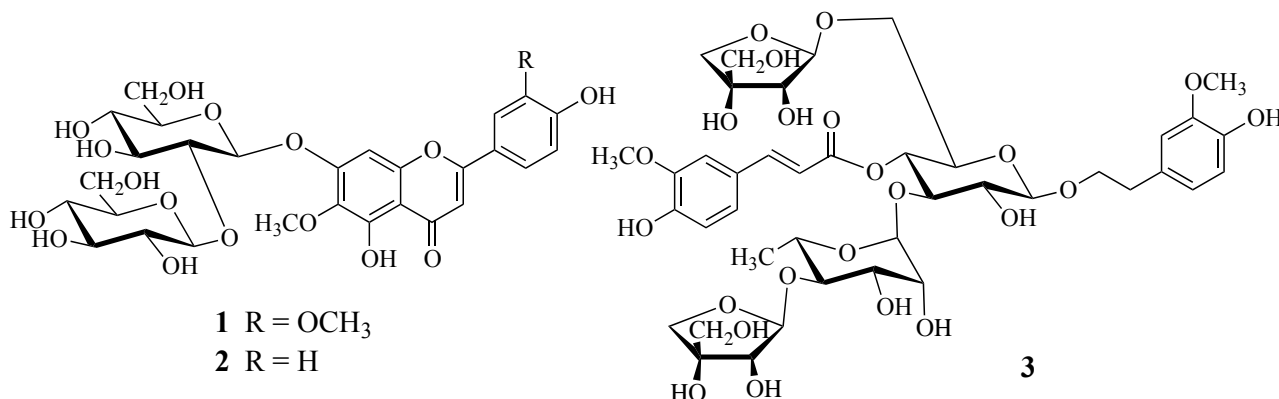


Fig. 1. Compounds 1-3 isolated from *Phlomis kurdica* aerial parts

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*ENTADA AFRICANA* GUILL. & PERR. (MIMOSACEAE) AS A SOURCE OF SKIN WHITENING AGENTS

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*Entada africana* Guill. & Perr. (Mimosaceae) is a small tree mainly growing in tropical areas. In Mali, it is found in the southern region of the country, known with the popular name of “Samanère”. Its leaves, stem bark and root are employed in Traditional Medicine for various type of illness. Based on ethnobotanical field research with healers, various uses were attributed to this species for the treatment of many diseases such as malaria fever, hepatitis, respiratory tract complaints and wound healing (1). Previous biological studies have shown anti-inflammatory, hepatoprotective, antibacterial and anti-proliferative activities (2). As a part of our investigations on plant extracts as sources of skin whitening agents (3), in this work we evaluated the inhibitory effects of *E. africana* root extracts on the activity of tyrosinase, the key enzyme for melanin biosynthesis in plants and animals. Among known melanogenesis inhibitors derived from plants, flavonoids are playing a prominent role and a previous study reported that flavonoids represent the major phytoconstituents of *E. africana* root (4).

For the study, air-dried and powdered roots were firstly defatted with *n*-hexane and then sequentially extracted with CHCl<sub>3</sub>, CHCl<sub>3</sub>/MeOH (9:1) and MeOH by exhaustive maceration to give, the respective residues, after removing the solvent under reduced pressure. Thus, all the extracts were screened for the inhibition of tyrosinase activity. Results showed that CHCl<sub>3</sub>/CH<sub>3</sub>OH extract (CM) evidenced the highest inhibitory effects on tyrosinase (51% at 200 µg/ml). The average total flavonoid content of CM was also determined as 6%. Additionally, CM showed a strong Fe<sup>2+</sup>-chelating ability (66.94% at 500 µg/ml). It is reported that many flavonoids interfere with the activity of tyrosinase through chelation of copper ions in its active site. Therefore, the tyrosinase-inhibitory activity of CM may be correlated to the presence of flavonoid compounds acting as chelating agents and *E. africana* root may be proposed as a potential source of new therapeutic whitening agents for the reduction of hyperpigmentation in cosmetic and pharmaceutical products, as well for the prevention of enzymatic browning in the food industry.

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PHYTOCHEMICAL PROFILE AND BIOLOGICAL ACTIVITIES EVALUATION OF THREE SPECIES OF *HYPERICUM*

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*Hypericum perforatum* L. (St. John's wort) is a herbaceous perennial plant native to Europe and Asia and widespread throughout Italy. Several pharmacological activities have been documented for extracts of this plant and/or its constituents including antibacterial, antiviral (1), and antidepressant effects (2). It is stated to possess sedative and astringent properties, and has been traditionally used, especially in oleolite form for topical application, in the treatment of burns, wounds, sores and arthritis (3). The aim of this study was to compare chemical composition and *in vitro* biological activities of three different species of the genus *Hypericum*, in particular: *Hypericum perforatum* (HP), *Hypericum tomentosum* L. (HT) and *Hypericum hircinum* L. (HH), with the aim of giving a scientific support to the ethno botanical use of these three species. Based on the main ethno botanical uses of all species, which is related to a vulnerary activity, treatments of burns, as well as arthritis, rheumatism and sprains (4), *in vitro* inhibitory activities against collagenase was selected as biological target (5). In fact an up-regulation of this enzyme, belonging to matrix metalloproteinases (MMPs) family, has been demonstrated in pathologies in which the *Hypericum spp.* is traditionally used as remedy. The antioxidant activities using different *in vitro* assays methods: ABTS, DPPH, FRAP-ferrozine and  $\beta$ -carotene bleaching test (BCB), were also performed. All three species were collected in late June, and three types of extracts were prepared from the flower tops: an exhaustive hydroalcoholic extract (E; EtOH:H<sub>2</sub>O, 70:30), a methanolic extract (M; MeOH: phosphate buffer pH 6, 50:50) and decoction (D). The metabolomic analysis was carried out by HPLC-DAD, HPLC-PDA and <sup>1</sup>H-NMR. Results revealed that: HH is the richest in shikimic acid (E=86,63; M=53,68; D=144,77 mg/g of extract), while HT in chlorogenic acid (E=24,78; M=8,90; D=22,75 mg/g of extract); quercetin (q) and hyperoside (hs) are present in rather high concentrations in HP (q: E=2,51; M=1,42; D=0,64 mg/g of extract – hs: E=18,36; M=12,41; D=9,47 mg/g of extract), while HH did not revealed the presence of hyperoside, while exhibiting a greater concentration of quercetin (E=1,32; M=0,55; D=0,38), that HT (E=0,23; M=not detected; D=0,15 mg/g of extract). Moreover based on the NMR signals corresponding to the anomeric hydrogen and methyl group of rhamnose in the aliphatic region and those of aglycone in the aromatic region the presence of quercetrin as main flavonoids was hypothesized in HT. In the aliphatic region pronounced signals corresponding to two phloroglucinols different from hyperforin were also detected in HT. The *Hypericum spp.* have shown interesting antioxidant properties in all test performed, linearly related to polyphenols and flavonoids content. The ethanolic extracts have been shown to be active as collagenase inhibitors (IC<sub>50</sub>: HT=0.22 mg/mL; HH=0.28 mg/mL; HP= 0.52 mg/mL); HH and HT showed an activity comparable to that of a green tea extract (*Camellia sinensis*), used as a positive control (6) (IC<sub>50</sub>=0.18 mg/mL). In conclusion, this work provide a scientific rationale for the ethno botanical use of *Hypericum spp.* and demonstrate the therapeutic potential of these three species. In particular, HH showed higher radical-scavenging and anti-collagenase activity than HP and considering the lack of hypericine and phluoroglucinols derivatives, which cause various adverse reactions *in vivo* (7), HH can thus be considered of a particular pharmacological interest and a more safe drug to be used for the treatment of diseases connected to up-regulation of MMPs.

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## PALYNOLOGY APPLIED TO THE STUDY OF CLIMATE CHANGE AND HUMAN IMPACT: POLLEN FROM ARCHAEOLOGICAL SITES AS TOOL FOR THE ASSESSMENT OF LONG-TERM LOCAL IMPACT AND HUMAN-INDUCED ENVIRONMENTS

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Most of the biological archives, including pollen, upon which past environmental reconstructions are based, are known to respond to both climate change and human impact. However, as humans lived in a region, ‘cultural transformations of natural habitats’ began, and were the inevitable consequence of human presence in lands. Consequently, changes in flora and vegetation cover may have occurred earlier near settlements and in the places that today we call ‘archaeological sites’ (1, 2, 3). The weak anthropogenic influence on the environment firstly occurred in the vicinity of the settlements, and then became a true local impact. Then, human impact became evident at a larger regional scale depending on the chronological and cultural variables, and on the distance and intensity of activity performances (4, 5).

In archaeological contexts, the preservation of pollen can be poor, humans and animals largely bring it to the site and thus cultural variables strongly influence the pollen spectrum. Far from being a problem, this taphonomical peculiarity is crucial to explore human behaviour and cultural aspects of plant exploitation (6). A set of palynological / archaeobotanical research has been carried out in the last decades by our research team. The research joins multidisciplinary archaeological study to palaeoenvironmental – ecological approach, with focus on the Italian peninsula and its impressive prehistoric and historic archaeological heritage (7, 8, 9, 10, 11).

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## REED-BEDS DECLINE: NEW OCCURRENCES OF A DRAMATIC THREAT TO BIODIVERSITY IN CENTRAL ITALY

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In Europe, where *Phragmites australis* (Cav.) Trin. ex Steud. is considered a native *taxon*, reed-beds are extremely important for biodiversity conservation, particularly in Mediterranean areas, where the hydrologic balance of wetlands may be very fragile. After the first detection of reed die-back in N- and C-Europe in the last decades, evident signs of deterioration have been detected also in freshwater habitats of the Mediterranean Basin, first in the Po Delta and then at Lakes Trasimeno, Chiusi and Montepulciano. This decline have been referred to a complex interplay of factors and their mechanism has not been exhaustingly explained yet. The overall goal of our research, carried out within a national research project (FIRB 2013), is to compare the status of five reed populations in different freshwater ecosystems in Central Italy (Colfiorito Marsh, Lake Trasimeno, Lake Chiusi, Fucecchio Marshes, Lake Vico), in order to detect the principal ecological factors and the physiological mechanisms involved in this process. Preliminary data and first outcomes will be illustrated along with the economical and environmental impact of our research.

Considering the suitable role of common reed as a good biomarker for monitoring aquatic ecosystems, our research will also supply useful tools for monitoring the conservation status of reed-dominated palustrine ecosystems, many of which are natural areas, including Natura 2000 and Ramsar sites.

## DIACRONIC LANDSCAPE CHANGES: A CASE STUDY ON S-E SICILY SALT MARSHES

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The SIMBIOTIC project, implemented within the Operative Programme Italy-Malta 2007-2013, aims to increase the ecological connectivity between the Maltese Islands and the south-eastern Sicily, carrying out studies for the management of protected areas and environmental restoration (1), as well as to disseminate knowledge and awareness on natural heritage conservation (2). Within project planned activities a pilot project of environmental restoration was realized on some biotopes as salt marshes in Sicily and Mediterranean scrub in Gozo. To this end, a diachronic analysis has been developed in order to monitor and quantify landscape changes in coastal areas of SE Sicily, mainly in Pachino territory where the SCI “Pantani della Sicilia sud-orientale” (SE Sicily salt marshes) is located.

The comparison was carried out among land use maps on the basis of available documents as aerial photos (1966), orthophotos (2008 and 1998), IGMI topographic maps (1928, 1897, 1868), on a total area of 14341 Ha and a period of 150 years. Land use according to CORINE Land Cover (3), processed by GIS, was evaluated. Aerial photos and IGMI maps were clearly georeferenced according to WGS 84 UTM Zone 33 N projection system. The CORINE land Cover easily allows a diachronic comparison in land use starting from sources with different detail and scale (4, 5).

The study highlighted that, already in the mid-1800s, the coastal landscape of south-eastern Sicily was mainly characterized by an agricultural matrix and large extensions of wild areas along the coast consisting of marshes and lagoons occupying about 7% of the territory.

Over time, the agricultural area, after a maximum increase in 1966, declined being reduced to 68% today, but at the same time it has undergone a significant transformation due to widespread cultivations in protected environment that currently occupy 10.5% of the surface. In the meantime a significant fragmentation of the agricultural areas is recorded.

The growth of urban areas in the last fifty years, from 2.7% in 1966 to 8.2% today, is caused not so much by the increase of old urban centers as by the recent extensive urbanization just along the coast.

The coastal marshes and lagoon reduced so that currently occupy 4.5%. Much more drastic is the reduction of sandy coasts extension, from 4% at the end of 19<sup>th</sup> century to 0.6% at present.

The general trend, as detected by comparison of diachronic land use maps, is that of a steady increase in environmental matrix fragmentation due to increased agricultural practices and urbanization of the coastal strip. It is this a major cause of biodiversity loss at the global level (6).

Coastal wetlands do not appear to have suffered in the last decades of a major reductions, whereas what appears definitely altered is their function in relation to the matrix they are included in: an urban-agricultural texture of very specialized crops, having a negative impact on conservation state of such a fragile habitats.

So dynamic trends of the coastal landscape clarify environmental issues and this study provides valuable elements to support decision-making processes affecting the salt marshes of south-eastern Sicily also in view of future management aimed at ensuring the protection of habitats and biodiversity.

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## A DYNAMIC KEY TO INTEGRATE GRASSLAND AND FOREST SUSTAINABLE MANAGEMENT IN A GLOBAL CHANGE PERSPECTIVE

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European grasslands are characterized by high plant diversity, from intraspecific genetic diversity to landscape diversity. The decline in the extent, connectivity and diversity of grasslands throughout Europe in recent decades has become a major conservation problem, because it affects all aspects of European biodiversity.

This process has two main drivers. On the one hand, Europe is facing dramatic land use changes, greatly in relation to the progressive abandonment of traditional agricultural practices, with a strong reduction of the total agricultural land and a marked increase of forest area. The same is true in Italy, where from 1960 to 2000 forest area increased from 18 to about 32% of the country area, while pastures were subjected to a strong decrease (from 18 to 9%). On the other hand also in the area where grazing activities are still being carried out, these occur on relatively limited areas that are subjected to a more intensive pressure.

Within this changing context the understanding of vegetation dynamics is a key tool to drive management decisions. Sound scientific methodologies are urgently needed to assess the ongoing changes in semi-natural ecosystems. In this view the long European phytosociological tradition may be effectively used to detect changes in the vascular plant species composition and diversity of semi-natural habitats through diachronic analysis of phytosociological relevés (1). Furthermore current vegetation data may be effectively used to plan grazing activities based on the evaluation of spatial and environmental patterns for the optimization of within habitat diversity (2).

However, land management policies should take into account also other environmental issues. For instance important conflicts exist between current issues relative to the conservation of semi-natural grassland areas and the Kyoto Protocol emission units, which take into account the removal of green-house gases mainly through afforestation and reforestation.

Forests and grasslands are both systems often subjected to management by humans, actions on these systems are usually focused on one system at a time. Furthermore, plenty of scientific studies focus on the effects of different management systems either on forests, or on grasslands.

Such a lack of politic and scientific integration is in clear contrast with the reality faced by land managers since forests and grasslands not only co-occur contiguously but are often linked by strong dynamic interactions.

A recent analysis based on forest inventory data demonstrated that a substantial fraction of the terrestrial carbon sink should be attributed to changes in forest management (3). Indeed through the manipulation of overstorey structure and composition forest biodiversity may be substantially enhanced (4, 5).

Our results point to the hypothesis that a less intensive management of forests would allow sinking carbon, without causing the reduction in extent of semi-natural grasslands. This management approach will not only maintain the levels of biological diversity in semi-natural grasslands but also enhance forest biodiversity through the increase of structural complexity and of the availability of microhabitats in managed forests.

Future studies and policies should be carefully evaluated through the assessment of gain and losses of carbon as well as of biodiversity, and should take into account the framework of vegetation dynamics in order to cope with the provisions of the Habitat Directive on the one hand, and of the Kyoto Protocol on the other.

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TRADITIONAL AND MOLECULAR CYTOGENETIC CHARACTERIZATION OF *SOLIDAGO* (ASTERACEAE) IN ITALY

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In Italy, only two native *Solidago* (Asteraceae) species occur (1): *S. virgaurea* L. and *S. litoralis* Savi. *Solidago virgaurea* is a herbaceous species inhabiting forest habitats and spreading across the whole Eurasia. *Solidago litoralis* is endemic to the Northern coasts of Tuscany, historically recorded also for eastern coasts of Liguria. The latter species is thought to have been recently originated from a mountain Tuscan population of *S. virgaurea*, gradually being adapted to the seashore environment (2, 3).

While there are some karyological and cytogenetic data available for *S. virgaurea* from outside Italy (see for example 4, 5, 6), *S. litoralis* has been only investigated concerning its chromosome number (7, 8), without a proper karyotype analysis.

Aim of this work is to provide deeper karyological and cytogenetic information: 1) standard karyotype analysis including asymmetry estimation; 2) investigation of number and position of 35S and 5S rDNA loci through FISH analysis; 3) genome size estimation through flow cytometry.

Four Tuscan populations were investigated, one of *S. litoralis* and three of *S. virgaurea*, including a high altitude form recognized as *S. virgaurea* subsp. *minuta* (L.) Arcang.

1) In respect to the standard karyotype analysis, we confirm the chromosome number  $2n = 2x = 18$  for all three studied taxa. Chromosome length is quite uniform among the different populations, ranging from 1.5 to 3  $\mu\text{m}$ . In all the populations, the third chromosome pair bears the Nucleolar Organizing Region (NOR) in secondary constriction (SC). Karyotypes show low intrachromosomal and interchromosomal asymmetry, albeit *S. virgaurea* seems slightly more asymmetrical than *S. litoralis*.

2) FISH analysis reveals one 35S and one 5S locus for both species. 35S localization coincides with the secondary constriction of the third pair, while 5S is located on one of the smallest chromosome pairs. The three smallest chromosome pairs are too similar in both size and shape to determine the precise position of 5S locus.

3) The genome size values are quite identical in all studied taxa and similar to those from other studies concerning *S. virgaurea* from outside Italy. According to the categories established by Leitch *et al.* (9), genome sizes estimated here are very small ( $2C < 2.8$  pg) and range from 2.31 to 2.34 pg, not showing significant differences among the investigated populations.

Given this uniformity in all considered cytogenetic parameters, the putative origin of *S. litoralis* from *S. virgaurea* seems mostly connected to gradual (ecological?) speciation processes, not involving relevant genomic rearrangements at chromosomal level. According to our results, by applying the cytogenetic criteria recently reviewed by Siljak-Yakovlev & Peruzzi (10), *S. litoralis* may be considered as a schizoendemic species.

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## RECENT ADVANCES IN EVOLUTION AND TAXONOMY OF LILIACEAE

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An overview of recent systematic and phylogenetic studies (1) carried out in the family Liliaceae is presented, allowing for a synthesis of current knowledge and a new proposal of infrafamilial taxonomic circumscription. Liliaceae are composed by 15 genera, all occurring in the Boreal hemisphere. These genera, according to their phylogenetic, karyological, embryological and morphological features can be subdivided in 6 tribes: Calochortae (*Calochortus*, 65 species), Liliae (*Cardiocrinum*, 3 species; *Fritillaria*, 130 species; *Lilium* [incl. *Nomocharis* (1, 2)], 120 species; *Notholirion*, 5 species), Medeoleae (*Clintonia*, 5 species; *Medeola*, 1 species: *M. virginiana*), Streptopeae (*Prosartes*, 6 species; *Scoliopus*, 2 species; *Streptopus*, 7 species), Tricyrtideae (*Tricyrtis*, 18 species [3]), Tulipeae (*Amana*, 5 species; *Erythronium*, 15 species [4]; *Gagea* [incl. *Lloydia*], 300 species [5]; *Tulipa*, 150 species [6]) for a total of about 900 taxa. They are herbs, bulbous (Calochortae, Liliae, Tulipeae) or rhizomatous (Medeoleae, Streptopeae, Tricyrtideae), never climbers as the sister family Smilacaceae (7, 8), hermaphroditic (rarely showing morphological gynodioecy, androdioecy and andromonoecy). The female gametophyte is monosporic, of *Polygonum*-type (Calochortae, Streptopeae, and Tricyrtideae: formerly treated as a distinct family Calochortaceae) or tetrasporic (Liliae, Medeoleae, Tulipeae). The fruit is a capsule (septicide in Calochortae, loculicide in Liliae and Tulipeae) or a berry (Medeoleae, Streptopeae, Tricyrtideae). In Italy (and in Europe) only the tribes Streptopeae (*Streptopus*), with  $x = (7)8$ , Liliae (*Fritillaria*, *Lilium*) and Tulipeae (*Erythronium*, *Gagea*, *Tulipa*), with  $x = (9)12$  occur.

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ANALYSIS OF *OLEA EUROPAEA* SUBSP. *EUROPAEA* BIODIVERSITY FROM CILENTO, VALLO DI DIANO AND ALBURNI NATIONAL PARK

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*Olea europaea* L. subsp. *europaea* is among the oldest known cultivated trees in the world. It shows multiple phenotypic expressions, usually described as cultivars. Some of them are of ancient origin and restricted to very limited local areas (endemic), whereas others are characterized by a wider distribution (1). Endemic cultivars, in particular, evolved for a very long period in a specific local area and developed adaptive traits which are well integrated with the environmental, agronomic, cultural, and traditional landscape features of the site (2). The depletion and the extensive replacement with new varieties resulted in a progressive reduction of varietal biodiversity with alarming consequences of genetic erosion and loss of biodiversity.

As a consequence, a greater sustainability of modern agriculture is required especially in protected areas where the safeguard of old local varieties is a focal point for the conservation of the traditional farming systems and landscapes (3). In addition, the beneficial health properties of olive fruits have been known for centuries, and many studies deal with their antioxidant composition as preventative superiority (4). In this context the knowledge about the potential olive biodiversity resources of a protected area represents a crucial issue to preserve it and to promote a socioeconomic development.

The aim of this study is to characterize the resource of olive cultivars of *Cilento, Vallo di Diano and Alburni National Park* by two different approach: a) census, identification and distribution; b) metabolomic analysis of fruits. Metabolomic analysis was performed by 1D and 2D NMR (5) in order to assess the different secondary metabolite content, with particular interest to phenolic composition (6).

On the basis of literature and field sampling, a list of 31 cultivars (16 endemic of the park, 9 of Campania region and 6 allochthonous) was obtained. For each cultivar a distribution map was produced by GIS analysis. 1H-NMR spectra were processed and obtained data were analyzed by multivariate data analysis (PCA). The 2D NMR techniques (HMBC, HSQC, COSY, J-resolved) allowed us to identify the main secondary metabolites. Results evidenced clear differences in metabolite composition among cultivars in relation to site location.

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FROM SALERNO'S MINERVA GARDEN TO THE ILLUMINATED HERBARIUM OF THE *CIRCA INSTANS*: A PATH WITH NO BOUNDARIES

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A finely interwoven combination of expertise is needed to be able to read the complex signs of an ancient monument. In the case of the Minerva Garden a multidisciplinary and innovative approach has greatly contributed to its restoration. Academics in the fields of history of ideas and architectural design, historians and modern art historians, botanists and curators of botanical gardens, philologists and experts in visual perception are only some of the scientists that have gravitated around the reconstruction of this Garden. They can, however, all be considered archaeologists of a past knowledge and of the reconstruction of its ancient forms: in Salerno they have had the collaboration of new explorers, specialists in computerized images, capable of blending nature with culture, texts with images, aiming to recompose and bring to light the contents of a part of the history of this city. This history, the Minerva Garden, the Arechi Castle, several illuminated botanic manuscripts from Salerno and a pharmacopoeia constitute the elements of this research, which provide a vital source for future investigations. Thanks to this gathering of knowledge, it was possible to reconstruct a Garden that, although of eighteenth-century structure, has benefited from these intertwined studies for its reconstruction which harks back to the ancient pharmacopoeias of Salerno.



# POSTERS

## 1.1 = COULD SPERMINE PLAY A ROLE DURING THE APICAL GROWTH OF POLLEN TUBE?

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Pollen tube growth is a rapid process restricted to the only tip region. 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 (1), but many other factors are involved in this process, among which polyamines (PAs), that are essential during pollen tube emergence (2) and ROS, that support the apical growth, at physiological concentration (3). We investigated the effect of the natural PA Spermine (Spm) and BD23, a synthetic aromatic derivative of Spm on the apical growth of *Pyrus communis* pollen tube and observed that both Spm inhibited the growth from 10 mM onwards. Thanks to a FITC-labelled Spm we were able to observe, that PAs enter through the pollen tube tip, then diffuse in the sub-apical region. The same region underwent drastic morphological changes, showing loss of polarity and enlarged tip when Spm and BD23 were supplied at 100 mM or higher. The effects of PAs were related, at least in part to their ability to act as ROS scavengers of both O<sub>2</sub> and H<sub>2</sub>O<sub>2</sub> in the apical zone, probably altering the Ca<sup>2+</sup> signalling network or disrupting the balance between ROS species that affect cell-wall relaxation or stiffening. Interestingly, the actin cytoskeleton was not affected by the treatment with 100 mM of both the PAs and, instead, seemed to follow the swelling of the apex. Intriguing the cell wall was enriched in both callose and cellulose exactly in the region where the swelling of the tip begins, probably to contrast the excess of ductility of the cell wall after treatment with PAs 100 mM. The viability of pollen was slightly affected by 100 mM treatment, and the degradation of nuclear DNA, as shown by DAPI labelling of pollen tube, was completely inhibited when pollen had been pre-treated with the caspase-3 inhibitor I peptide, Ac-DEVD-CHO (DEVD). Different was the scenario when the PAs were supplemented at 500 mM, concentration that is far away from the physiological concentration. In this case, the effects on the pollen were more drastic, with a rapid drop of cell viability, actin depolymerisation, stimulation of DNA-laddering after 30 minutes incubation and the complete degradation of both vegetative and generative nuclei; these degradative effects were only in part inhibited by the pre-treatment with DEVD. Thus the present data may open new research avenues to understand how the diameter of the pollen tube is regulated and which role PAs could play in the puzzling process of apical growth.

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## 1.1 = INVESTIGATING THE ROLE OF OLD CHARCOAL PLACES FOR UNDERSTOREY VEGETATION DIVERSITY IN MEDITERRANEAN FORESTS

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The production of woody charcoal in the Mediterranean forests originated in very ancient times and continued until the last century in many areas of especially southern Europe. The charcoal process production is based on the pyrolysis of wood at high temperature without oxygen; it was realized directly in the forest in special woody kilns covered by a mixture of soil and plant material, placed in small, flat areas prepared for this purpose (WSL, 2011). This traditional activity has been for long time the main form of forest use in Italian coppice woodlands, but, as many widespread traditional land use systems it almost vanished in the last decades (Scarascia-Mugnozza et al. 2000). In most regions, the activity reached the greatest importance during the industrial revolution because of the lack of other fuel sources (WSL, 2011), resulting in a very high density of charcoal sites in various types of broadleaf forests of especially the hill and montane belts. In present-day woodlands, these sites are still easily recognizable for their semicircular shape and flat surface, as well as for clear variations in soil characters and vegetation composition compared with the surrounding stands. One of the most evident is the blackish color of the upper layers of the soil and the presence of more less abundant remains of wood charcoal, as well as the lack of young trees of especially the late-successional species that are locally dominant.

On the other hand, direct observations indicate that the understory vegetation, often very sparse in dense Mediterranean woodlands, may be more abundant in these sites than in the surrounding closed stands. Therefore charcoal places may represent “ecological” islands of anthropic origin in the forest environment, contributing to its fine-scale heterogeneity (alpha- and beta-diversity) and to the dynamics of tree regeneration.

Information about the ecological role of old charcoal places in Mediterranean forest ecosystem is still completely lacking, as well data on their density and patterns of distribution in given forest areas. The aim of this work was therefore to investigate the role of these sites in shaping the understory vegetation diversity and productivity in selected forest areas, as well as the main abiotic factors (soil conditions, light intensity) affecting species diversity and composition.

Based on extensive field searches in Tuscany over one-hundred old charcoal sites were first characterized, and 61 were then selected to represent three main forest types along an altitudinal gradient: Mediterranean sclerophyll forests, thermophilous deciduous mixed forests, and mountain beech forests. Each of these sites was sampled using a square plot (3x3 m) in which data on stand structure and understory vegetation were collected according to a standard protocol. Soil samples were also collected for subsequent analyses of pH and nutrient content, and light intensity (PAR) was also measured. In addition the above-ground biomass of the understory vegetation included in a subplot of 0.5x0.5 m, located randomly within the main plot, was clipped and subsequently dried and weighted. For each site the same sampling procedure was repeated on a control site randomly located in the surrounding stands.

Preliminary results of this work suggest that old charcoal sites are characterized by a higher understory vegetation diversity and productivity, compared with the normal stands. In addition compositional changes also occurred, suggesting a significant role of some species in the colonization processes of these sites. Soil and light factors in explaining such differences are under investigation.

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1.1 = STRUCTURAL CHANGES SHOWING CANNIBALISM PHENOMENA IN *HETEROSIGMA AKASHIWO* (HADA) HADA EX HARA ET CHIHARA (RAPHIDOPHYCEAE) CELLS, RECOVERING FROM SHORT OR EXTENDED NUTRIENT DEPLETION

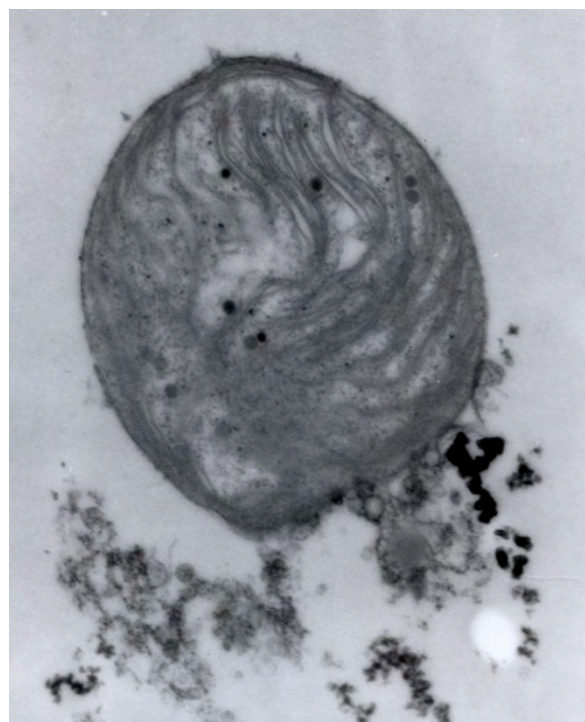
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*Heterosigma akashiwo* belongs to Raphidophyceae. All the members of Raphidophyceae are unicellular, mainly planktonic, without a wall and with two flagella: a forward hairy flagellum and a second smooth one. The members of this class show many chloroplasts, generally disposed along the plasma membrane, some dictyosomes, and mucocysts, while no eyespot has ever been observed (1).

*H. akashiwo* is frequently responsible of red tides along eutrophic coasts. We investigate structural and ultrastructural changes with Light and Electron Microscope, during the recovery from a stationary phase ('aged' culture) induced by nutrient starvation.

The *H. akashiwo* cells showed different structural types during the 'aged' culture, with the presence of irregularly shaped cells and cell fragments. Some of the cells dismantled the photosynthetic apparatus, meeting two possible dooms: i) Programmed Cell Death followed by the release of parts of the protoplast and chloroplasts in the medium; ii) cysts formation, formed by adhesion of the membranes belonging to a condensed nucleus, a condensed chloroplast and a mitochondrion. Some observed cells were apparently phagocytizing both cytoplasmic fragments and intact chloroplasts and even entire cells belonging to the same species. Such ultrastructural images may suggest a "cannibalism" behaviour by part of the *Heterosigma* cells. Caution must be taken since we interpret as dynamic phenomenon (cannibalism) static microscopy images. A possible explanation of the phenomenon may be the need of organic material recover after extended nutrient availability. If confirmed by observations of living *Heterosigma* cells, this would be the first description of cannibalism in algae and one of the few showing the ultrastructure of the phenomenon in protists.



*Heterosigma akashiwo* cells. Left: one chloroplast is entering a cell via phagocytosis. Right: a chloroplast free in the medium.

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## 1.2 = THE INTERACTIVE GAMES OF THE E-MOVE! PROJECT: NEW TOOLS FOR TEACHING PLANT DIVERSITY, EVOLUTION AND DEVELOPMENT

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The *E-MoVe!* Project (Evolution of the Plant World), funded by the Italian Ministry of Education, is an experimental exhibition for the validation and implementation of teaching strategies, including interactive games, for the training of teachers and museum operators.

Scientific Museums can have a key role in disseminating awareness on the fundamental issues of evolution (1). In the Botanical Garden Museum of our University, visitors are exposed to the importance of plant evolution by visiting an exhibition consisting of three parts: an installation of living plants placed according to a phylogenetic scheme (PhyloWall); the display of panels illustrating the main events of plant evolution and the basics of Darwin's theory; a section dedicated to the display of historical botanical books (2).

Recent research showed that novel teaching approaches (3), and namely interactive games (4), can improve the understanding of scientific concepts. Therefore, in the final part of the exhibition, visitors can play three interactive games specifically designed for teaching plant evolution. 1. The time machine (Fig.1): the player travels in time to see plants from the past ages. 2. Plant evolution and biodiversity (Fig. 2): the player associates the definition of a given photosynthetic organism with its picture and scientific name. 3. Gene expression and flower morphology (Fig. 3). The player, by regulating the expression of genes involved in the determination of flower shape (ABC model), interacts with the development of the flowers of *Arabidopsis thaliana* (L.) Heyn.

The validation of the games has been carried out by interviewing students and school teachers. The results obtained showed broad interest in such novel teaching tools. The games will be made freely available to schools and scientific institutions worldwide. The English versions of all three games are currently in preparation. For contacts and requests: [emove@uniba.it](mailto:emove@uniba.it)



Fig. 1. The time machine



Fig. 2. The interactive game on plant biodiversity



Fig. 3. The game on flower morphology

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### 1.3 = THE CHIMERA AtCslA2-GFP IS FUNCTIONALLY INSERTED INTO GOLGI MEMBRANE AND SYNTHESISES $\beta$ -MANNANS

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Cellulose, hemicelluloses and pectins are the main structural polysaccharides of the plant cell wall. Glycosyltransferases (Gts) are the enzymes involved in the synthesis of these polysaccharides and other glycans. *Cellulose synthase (CesA)* genes encode plasma membrane rosette complexes, the Gts involved in the synthesis of cellulose; whereas Gts localised in the Golgi apparatus, responsible of pectin and hemicellulose synthesis, are encoded by *Cellulose synthase-like (Csl)* genes (1). Until now, little and still unclear evidence for the functional role of *Csl* genes have been collected. Among the six groups of the *Csl* gene family identified, only few members of *CslA* and *CslC* have been functionally characterized suggesting their respective involvement in (gluco)mannan and xyloglucan syntheses. A role in the synthesis of mixed  $\beta$ -glucan has been proposed for members of *CslG* and *CslF* groups (2).

In this study we constructed a fluorescent fusion of an *Arabidopsis* *CslA* family member, AtCslA2, to identify its final localization in a heterologous system represented by tobacco epidermal cell protoplasts. Confocal observations showed that the fluorescent protein fusion AtCslA2-GFP was stable localised in the Golgi stacks, as evidenced by: i) colocalization with the Golgi marker ST52-mRFP, ii) disappearance of fluorescence from ER compartment in the presence of protein synthesis inhibitor cycloheximide, iii) redistribution of the chimera from the Golgi stacks to ER in the presence of Brefeldin A. The chimera was functionally inserted in Golgi membranes as verified by its insolubility in the non-ionic detergent Triton X-114 and biochemical tests. Membrane preparations obtained by AtCslA2-GFP transformed protoplasts carried out *in vitro* synthesis of a  $^{14}\text{C}$ -mannan in the presence of GDP-[U- $^{14}\text{C}$ ]mannose as substrate. An increase of approximately 38% was detected in the enzyme specific activity of the transformed protoplasts with respect to the wild type. Based on amino acid sequence analysis (TMHMM server v. 2.0., <http://www.cbs.dtu.dk/services/TMHMM/>), AtCslA2 was predicted to have four transmembrane (TM) domains and two hydrophilic regions of different size with the catalytic domain facing the Golgi lumen. To ascertain this hypothesis, mannan synthase activity was tested in the presence of proteinase K, with or without Triton X-100. The enzymatic activity was drastically reduced with respect to untreated AtCslA2-GFP transformed protoplasts when membranes were permeabilized with detergent Triton X-100 during proteinase K treatment. These results validate bioinformatic predictions. A hypothetical model of the polypeptide topology based on the locations of the predicted TM domains was suggested (Fig. 1) (3).

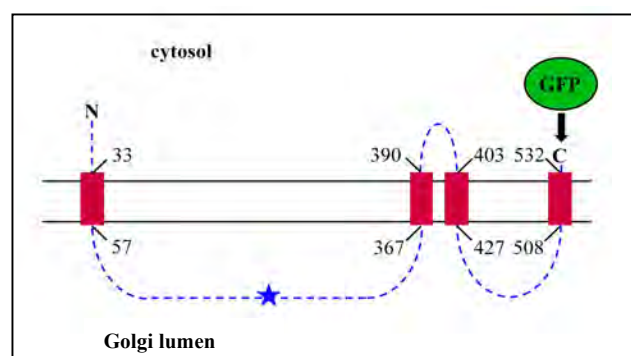


Fig.1. Hypothetical model of membrane topology of AtCslA2-GFP. TM domains are reported in red, numbers mark location of amino acids. Two hydrophilic region are predicted, the catalytic site, marked with a blue star, is present in the Golgi lumen.

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### 1.3 = DIRECT INTERACTION IN *ARABIDOPSIS* OF SNARE PROTEIN SYP51 WITH NON-SNARE PROTEIN NLM1

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SNAREs (N-ethylmaleimide-sensitive factor adaptor protein receptors) are small polypeptides characterized by a particular domain, the SNARE motif, that can form a coiled-coil structure. Via hetero-oligomeric interactions, these proteins form highly stable protein-protein interactions, the so called SNARE-complex, that allow membrane fusion. SNAREs also interact with several proteins acting as regulators of SNARE-complex formation.

Stoichiometry of these proteins reveals that they are more abundant than required for membrane traffic. Indeed their function appears to be more diversified. It was shown (1) that they may assemble to form non-fusogenic complexes acting as interfering-SNAREs or iSNARE (2) as in the case of AtSYP51 and SYP52. It was also shown that plasma membrane SNAREs can be phosphorylated as part of the signaling cascade elicited by interaction with microorganisms or hormonal stimulation and that they influence turnover of channels. In particular Grefen and co-workers (3; 4) provided direct evidence that SYP121 is part of a scaffold of proteins associated, by direct interaction with channel KAT1, with the membrane transport of K<sup>+</sup>. In fact, few SNARE proteins are known to interact with ion channels, notably mammalian Syntaxin 1A, which binds several different Ca<sup>2+</sup> and K<sup>+</sup> channels in nerves.

Here we show that AtSYP51 interacts directly with a non-SNARE protein, AtNLM1, probably regulating autophagocytosis processes.

The authors thank the contribution of the Italian project 'Reti di Laboratori Pubblici di Ricerca per la Selezione, Caratterizzazione e Conservazione di Germoplasma 2009'.

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1.4 = ROOM TEMPERATURE MICROSPECTROFLUORIMETRY AND PHOTOSYNTHETIC CARBON FIXATION IN THE LYCOPHYTE *SELAGINELLA MARTENSII*

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Lycophytes originated 415 million years ago and account today for only ca. 1% of the extant vascular species, all the other species belonging to euphyllophytes. However, starting from the early evolution of tracheophytes and up to the mid Carboniferous period, lycophytes actually dominated the Earth. Their own photosynthetic activity, which caused a progressive reduction in the atmospheric CO<sub>2</sub>, was probably one reason for their decline in favor of the broad-leaved euphyllophytes (1). In a recent study, we have shown that lycophytes and euphyllophytes are divergent with respect to the use of light energy for photosynthesis (2). In particular, lycophytes exhibit a light-dependent reversible phosphorylation of Lhcb6 antenna protein, a process never before observed in other land plants. Lhcb6, which evolved upon land conquest by plants, forms the minor Photosystem II (PSII) antenna CP24 and is involved in thermal dissipation of excess light energy (3). In lycophytes, two unprecedented observations, i.e. that Lhcb6 can bind PSI and increases its phosphorylation level under high light, suggested that these plants could take advantage of the quenching ability of PSI, which would act as a safety valve, able to dissipate excess energy as heat (2). Such a process implies that a portion of the energy harvested by the antennae is "stolen" from PSII and re-directed to PSI ("energy spillover"), and would be mediated by a PSII-LHCII-PSI interaction (4).

In order to find evidence for energy spillover in lycophytes, we performed room temperature microspectrofluorimetric analyses *in vivo* of *Selaginella martensii* Spring (Selaginellaceae, Lycopodiophyta). Plants were acclimated to three light regimes for at least one month (low, medium, high light) and occurrence of long-term acclimation was checked by photosynthetic pigment analysis. For spectrofluorimetric analyses, terminal branches of 30-min dark-adapted plants were floated on a solution of NaF (phosphatase inhibitor) and, subsequently, exposed to increasing irradiance levels (0-800  $\mu\text{mol m}^{-2} \text{s}^{-1}$ ). The fluorescence spectra, recorded from the upper epidermal cells, were elaborated by Gaussian deconvolution to obtain information on the organization of the photosynthetic membrane. In parallel experiments, light curves of CO<sub>2</sub> assimilation were obtained with an infrared gas analyzer.

Although *S. martensii* was capable of long-term acclimation to the three light regimes, in each condition its photosynthetic performance was low. The light-limited phase of photosynthesis ( $<100 \mu\text{mol m}^{-2} \text{s}^{-1}$ ) was characterized by increased emission by PSI, testifying to the occurrence of a "state 1-to-state 2" transition, as also known in angiosperms (5). The CO<sub>2</sub>-limited phase occurred for irradiance  $\geq 100 \mu\text{mol m}^{-2} \text{s}^{-1}$  and required the activation of energy dissipative mechanisms. In particular, at medium light intensities, this caused a change in emission in favor of PSII, but, when light intensity further increased, emission by PSI rose again. Such a redistribution of excitation energy from PSII to PSI under high light strongly supports the hypothesis that in *S. martensii* an energy spillover mechanism is activated in response to excess light, when the ability to consume the reducing power produced by the photosynthetic light reactions has been saturated.

In angiosperms, energy spillover has recently gained a renewed interest as a potential photoprotective mechanism, which would operate under conditions exceeding the protective capacity of the most known mechanisms of thermal dissipation (PsbS protein, xanthophyll cycle) (4). Lycophytes evolved in a CO<sub>2</sub>-rich atmosphere and probably for this reason they still maintain a low carbon fixing ability. Therefore, the effective operation of an energy spillover mechanism, possibly mediated by phosphorylated Lhcb6 (2), responds to their need to have an additional system available to limit the detrimental effects of photoinhibition.

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1.4 = GENOMIC AND EPIGENOMIC MECHANISMS TO COPE WITH EXCESS COPPER AND CADMIUM LEVELS IN *ZOSTERA MARINA* L. SEAGRASS

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Heavy metals remain among the most significant worldwide pollution factors in marine ecosystems as a result of the huge range of human activities involving the use of toxic metals and their subsequent transfer to marine environment. Aquatic plants have attracted considerable attention for their ability to maintain metal homeostasis in cellular compartments and to minimize the damaging effects of an excess of both essential and non-essential metal ions. The first defence line is active at the level of cell wall which can bind metals, thus preventing or reducing their uptake into cells (1). Once internalised, free metal level is controlled through an enhancement of metal ion efflux (2-7) and their complexation with various ligands, such as glutathione, phytochelatins and metallothioneins (2, 8,9). If these defenses are insufficient to prevent the build-up of free metal ions, plants have to cope with the oxidative stress. Therefore, an accurate regulation of defence mechanisms is crucial to respond to stress and be able to survive and reproduce.

In this context, our work was focused on investigating the effects of elevate concentrations of Cu (50 and 150 µg L<sup>-1</sup>) and Cd (0,1 and 1 mg L<sup>-1</sup>) on *Zostera marina* L., a widespread seagrass species of the northern hemisphere (10). The effects of Cu/Cd treatments, applied for 144 hours, was assessed by measuring the accumulation of these metals in the plant, some growth-related parameters (photosynthetic pigments, protein, starch, soluble sugar levels) and the changes in antioxidant molecular markers (phenols). The expression level of *ASCORBATE PEROXIDASE1* (*APX1*) and *CATALASE* (*CAT*) genes involved in the response to oxidative stress together with the activity of their respective antioxidant enzymes (*APX*) and catalase (*CAT*) were also evaluated. Other genes investigated for their expression level under Cu and Cd exposure were: *METALLOTHIONEIN2* (*MT2*), a gene involved in metal homeostasis and detoxification processes and *CHROMOMETHYLASE3* (*CMT3*) and *DOMAIN REARRANGED METHYLASE2* (*DRM2*) involved in the establishment of DNA methylation pattern. In particular *CMT3* is involved in the maintenance of non-CG methylation while *DRM2* is involved in both non-CG methylation maintenance and *de novo* methylation in all sequence contexts.

Our results show that *Z. marina* activated a pattern of epigenomic/genomics mechanisms to cope in a metal-specific way to Cu/Cd stress, including the activation of antioxidant enzymes (*CAT* and *APX*), the over-expression of genes involved in heavy metal detoxification and homeostasis (*MT2*) as well as of genes (*CMT3* and *DRM2*) involved in the regulation of gene expression through chromatin remodelling.

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#### 1.4 = A COMPARISON OF PROTEIN CONTENT OF FOUR SPECIES OF CHLOROPHYTA MICROALGAE

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The aim of this study was to investigate the protein content and a preliminary protein profile of four species of microalgae belonging to Chlorophyta. Chlorophyta include the majority of the described species of green algae (1), which are characterized by a remarkable morphological diversity and ability to grow in a wide range of environmental conditions (2). For this study, four microalgal species were selected on the basis of phylogenetic position within the Chlorophyta, in two different classes: Chlorophyceae (*Neochloris oleoabundans* and *Scenedesmus acutus*) and Trebouxiophyceae (*Chlorella vulgaris* and *Chlorella protothecoides*) (Fig. 1).

These microalgal strains were inoculated in 500 mL Erlenmeyer flasks containing BG 11 medium and cultivated under the same culture conditions ( $24 \pm 1$  °C temperature,  $80 \mu\text{mol}_{\text{photons}} \text{m}^{-2} \text{s}^{-1}$  PAR and 16:8 h of light-darkness photoperiod), with continuous shaking at 80 rpm and without external CO<sub>2</sub> supply. For morpho-physiological and biochemical analysis, aliquots of the species were collected periodically. For protein analysis, aliquots of the different species were analyzed when the microalgal cultures reached the early stationary phase of growth. In fact, previous studies reported that, in general, the percentage of proteins expressed on dry weight was higher in the early stationary phase with respect to the other growth phases (3). Moreover, samples of *N. oleoabundans*, *S. acutus*, *C. vulgaris* and *C. protothecoides* collected for protein analysis were characterized by high photosynthetic pigment content and a good photosynthetic efficiency, measured as  $F_V/F_M$  ratio. The protein content of the four microalgae was measured by the Lowry method (4), following the protein extraction of two different fractions: a soluble protein fraction and a less soluble protein fraction, which includes also transmembrane proteins. In order to compare the protein profile of *N. oleoabundans*, *S. acutus*, *C. vulgaris* and *C. protothecoides*, the protein extracts were separated by SDS-PAGE. Results of SDS-PAGE analysis showed that the number and intensity of protein bands were very different between the four microalgal species. In particular, in all samples one protein band was clearly identified as the RuBisCO large subunit, that preliminary data suggested to be differently accumulated in the strains. These results seem to indicate a very different protein content in microalgal strains all belonging to Chlorophyta.

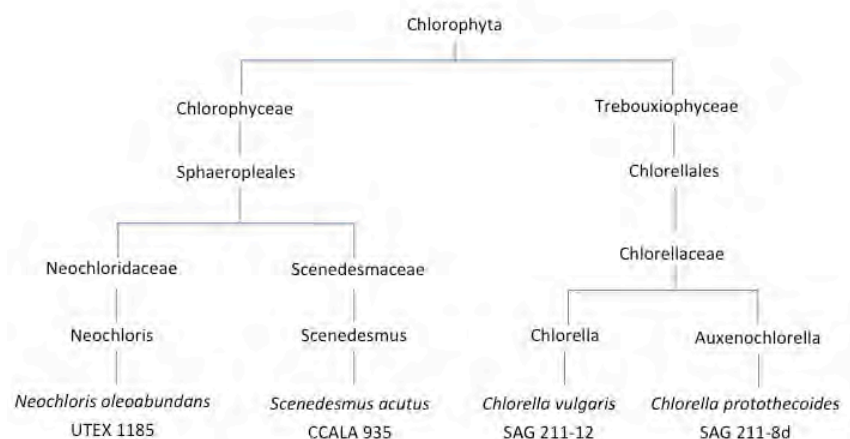


Fig. 1. The phylogenetic position of *Neochloris oleoabundans*, *Scenedesmus acutus*, *Chlorella vulgaris* and *Chlorella protothecoides* within the Chlorophyta.

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1.5 = PRELIMINARY STRUCTURAL AND FUNCTIONAL STUDIES OF A PLASTIDIAL HOMOLOGUE OF THE MITOCHONDRIAL CALCIUM UNIPORTER IN *ARABIDOPSIS THALIANA*

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Several recent studies have indicated that chloroplasts may generate specific calcium signals inside the organelle and contribute to the fine-tuning of cytoplasmic calcium signals in response to biotic and abiotic stresses (1, 2). The functional interconnection of the chloroplast-localized calcium pool with cytoplasmic signalling events is guaranteed by both integral membrane channels, active transporters and regulators, localized in different sub-organelle compartments. However, although putative members responsible for chloroplast  $\text{Ca}^{2+}$  fluxes have been proposed (3), the key actors as well as the regulatory mechanisms are far from being clarified.

Our attention has been focused on a membrane protein putatively involved in the cytoplasm-chloroplast calcium homeostasis. The gene coding for this putative 37 kDa protein has been identified in the *Arabidopsis thaliana* genome by bioinformatic analyses since it includes a chloroplast targeting peptide and shows a sequence homology with the recently identified mitochondrial calcium uniporter (MCU) (4). Evidence supporting the presence of such a component includes measurement of  $\text{Ca}^{2+}$  transport across the inner envelope membrane of pea chloroplasts possibly through a  $\text{Ca}^{2+}$  uniport-related mechanism (5). In the attempt to describe such protein in terms of structural and functional features, we cloned and expressed in *E. coli* multiple recombinant constructs of the corresponding gene, either in the mature form or as truncated versions. The most promising constructs have been purified and characterized in solution, as well as submitted to crystallization trials. Purification protocols are currently under optimization, and new constructs have been designed and cloned for crystallization purposes. Moreover, aequorin-expressing *E. coli* strains transformed with constructs encoding either the wild-type MCU plastidial homologue or a mutated version were used in  $\text{Ca}^{2+}$  measurement assays. Preliminary results suggest that the plastidial MCU homologue works as a functional  $\text{Ca}^{2+}$ -permeable channel in *E. coli*, responsible for sustained intracellular  $\text{Ca}^{2+}$  levels in response to external  $\text{Ca}^{2+}$  pulses. Determination of the structural and functional properties of the plastidial MCU homologue will contribute to decipher the still elusive calcium homeostasis in chloroplasts.

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## 2.1 = IDENTIFICATION OF MOLECULAR FACTORS CONTROLLING ROOT SYSTEM DEVELOPMENT

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One of the role of the root system is to acquire water and nutrients from the soil which are unevenly distributed. Therefore, the spatial deployment of the root system will determine the ability of a plant to exploit those resources. The primary tissue (pericycle, endodermis and xylary parenchyma) regulate root density and root distribution with branch root formation. On the other hand, in woody species, primary tissues persist only in the proximal region of each root axis, so the main factor responsible for the root development and biomass increase is the activity of the vascular cambium. At this point, without a primary tissue, how can a woody parental form new branch roots?

The present work aims to 1) investigate the similarity/differences among the factors involved in vascular cambium activity in the shoot and in the root through molecular analysis; 2) understand the formation of the new lateral root primordium from a woody parental root. The candidate tissues implicated in this fact are the vascular cambium, the medullar rays, the parenchyma cells in the secondary phloem and the phellogen.

At the beginning we started with an anatomical study of the root in samples of *Arabidopsis thaliana* where a development of a secondary growth was induced. Then the most common methods of cytology, biochemistry, and molecular biology will be used to examine some of the molecular factors known to be active in vascular cambium development like WUSCHEL-related homeobox4 (WOX4). Further investigation will be carried with *Populus* sp.

## 2.1 = ANALYSIS OF SEEDLINGS GROWTH UNDER DIFFERENT LED LIGHTS

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LED lights have a lower environmental impact than traditional lights due to a series of factors such as high energy-conversion efficiency, small volume, longer life, low thermal energy output. Concerning plant growth, the use of LED lights provide specific wavelength as well as the possibility to adjust light intensity/quality. The increasingly need to reduce energy consumption worldwide, raised the necessity to improve LED lights use. The present work aims to 1) examine the effect of different LED light spectra on seedlings growth of different species in order to define a species-specific cultivation protocol under optimal plant growth spectrum 2) compare direct measurements with non-destructive method by optical sensors. The plant species analyzed were Scots pine (*Pinus sylvestris* L.), Norway spruce (*Picea abies* L.), Holm oak (*Quercus ilex* L.) and Pomegranate (*Punica granatum* L.) Seedlings were left to grow in a growth chamber (16 h photoperiod, 120  $\mu\text{mol m}^{-2} \text{s}^{-1}$  PAR, temperature 21-22°C, humidity 80% germination - 55-70% growth) for 4 weeks under G2, AP67, AP67-3L, NS1 LED light (Valoya) and Fluorescent light (FL, control). Direct measurements of shoot height, root length, shoot and root biomass were carried. Non-destructive analysis was carried by measuring *Greenness* (percentage of shoot cover projected on tray ground) and *Plant height*. Greenness data were first obtained by a series of manually taken images (Nikon D70s digital camera) analysed with an open source software (ImageJ). Plant height was manually taken during the growth period to find a relationship with plant biomass. Furthermore, plant height was manually taken and compared with data obtained from images acquired by Optical sensors and analysed by *uEyeDualcam HeightMap* software (ACREO). Seedlings growth under AP673-L and G2 light, for Scots pine and Norway spruce, showed height values higher than values measured under control light and root length and shoot and root dry weight values similar to the values measured under control light. Seedlings growth under NS1 light, for both species, showed significantly lower total biomass and root length than seedlings growth under control light. In conclusion, seedling growth under G2 LED type shows the highest performance, representing the optimal spectrum. Similar values were found for seedling growth under AP67-3L. G2 light has the higher percentage of far-red/red (600-800 nm) wavelength ( $\lambda$ ). Hence, it could interfere with optical measurements such as greenness. Therefore, in alternative to G2 type, AP67-3L LED type could represent the best option for a standard cultivation protocol. Results about non-destructive analysis show that greenness value for Scots pine and Norway spruce species in relation to different light type, showed highest values with AP67-3L. Preliminary results showed the same pattern also for Holm oak and Pomegranate species. Highly significant positive relationships between greenness, plant height and seedlings total biomass were found for Scots pine and Norway spruce for all LED light types ( $0.89 < R^2 < 0.99$ ). For all considered species tests were carried to relate plant height data obtained by *HeightMap* Software (ACREO) with plant height manually measured, and a good relationship was found. (Scots pine:  $R^2 = 0.85$ ; Norway spruce lower relationship  $R^2 = 0.59$ ; Holm oak  $R^2 = 0.83$ ). Tests are in progress to relate Greenness data obtained by *uEyeDualcam* software with plant biomass. In conclusion tests about relationship between plant height and greenness, obtained by manual measurements and by Nikon digital camera-Image J software, highlighted that indirect analysis are good parameters for non-destructive quantification of plant biomass.

2.1 = CONTROL OF LATERAL MERISTEM FORMATION IN *ANTIRRHINUM MAJUS*

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Plant architecture is designed by shoot growth and branching developing from axillary meristem. Lateral meristem formation has been already studied in model plants, e.g. *Arabidopsis thaliana*, tomato, rice and maize (1). The characterization of plant architecture mutants pinpointed that GRAS transcription factors *LATERAL SUPPRESSOR* regulate the initiation of axillary meristem (2, 3). The *Solanum lycopersicum lateral suppressor (ls)* mutant and its orthologous in *Arabidopsis thaliana las* are unable to develop secondary meristems at the axil of leaves. Indeed, these mutants develop a single stem and do not form secondary inflorescences. In tomato *ls* flowers do not develop petals and are characterized by reduced female and male fertility.

Also in *Antirrhinum majus LAS* orthologous (named *ERAMOSA/ERA*) greatly contributes to design plant architecture. Here we show that *eramosa* mutant does not develop axillary meristem, whilst the apical meristem itself is bigger. Moreover *era* mutant forms very few flowers completely disorganized. Preliminary results suggest also that *ERA* is involved in the control of ovule number since the mutant presents less ovules respect to the wild type. Differently from its orthologous in *Arabidopsis* and tomato, *ERA* is indeed likewise expressed in the ovule primordial.

All together our data clearly indicated that *Antirrhinum ERA* participates in diverse functions and display broader, but distinct, expression pattern in comparison to the *Arabidopsis LAS* and the tomato *LS*.

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## 2.1 = ANALYSIS OF *CORYLUS AVELLANA* L. GROWTH UNDER LED LIGHTS FOR REFORESTATION PURPOSES

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Reforestation is very important in contrasting landscape degradation and desertification processes due to climate changes. In the Mediterranean area, forest restoration is also important to reestablish and to preserve the rich biodiversity of the different forest ecosystems, which has been damaged for a long time due to not appropriated silvicultural activities. The production of great amounts of high quality forest plants stocks ready to be transplanted in pots or in field at low costs is a primary challenge in this field (Astolfi *et al.*, 2012). Since 2012 the Zephyr EU-project (FP7-ENV.2012.6.3-1) is developing an automated high-density seedling production unit based on a combined action of optimal environmental conditions and LED lamps, with a noticeable reduction of emissions achieved through a low energy consumption, reduced by up to 70% in respect to the traditional nursery pre-cultivations.

After a first set of experiments on *P. granatum* L. (Marras *et al.*, 2013), which highlighted the possibility of using specific LED spectra to foster the development of roots and the production of secondary metabolites (two important elements favouring the adaption of plants in open-field), a second species was analysed: *Corylus avellana* L.; in order to avoid the gradual loss of genetic variability of populations caused by the propagation by cuttings, propagation by seeds was chosen. After 2-months of cold stratification to break dormancy, 104 seeds were sown under each light source (5 Valoya® LED spectra vs OSRAM® fluorescent tubes as control, Fig.1) in quickpots containing a peat-based substrate, in a climate growth chamber at a temperature of  $22 \pm 1^\circ\text{C}$ , at 60-70% of RH, with 12/12h of photoperiod and with  $100 \mu\text{mol m}^{-2} \text{s}^{-1}$  PAR. After 1 month morphological analysis were performed on seedlings, before transplanting them into a greenhouse. Results on *C. avellana* pointed out the best influence of LED spectra on stem growth, stem diameter (Fig.2), leaves, shoot and roots dry weights (Fig.3) in comparison with conventional fluorescent tubes. In details, higher far red percentages (AP67 spectra) best performed on shoot and root weights, while lower far red percentages (NS1 spectrum) did the same on leaves dry weight. Mean leaf area did not show statistical differences among light sources, probably because it is more linked to light quantity than quality. Once transferred into the greenhouse for outdoor adaption, the plants immediately stopped to grow and leaves started to yellow before falling down. They were subjected to an evident transplant shock reaction, differently from *P. granatum*. Reasons for transplant shock reactions of pre-cultivated forest seedlings to open land can be related to several factors, in particular the lack of protective mechanisms (as UV absorbing compounds) against higher UV-A and UV-B irradiance outdoors, that is absent/minimal during the indoor cultivation phase and the lack of protective mechanisms against high light intensity, which is much lower during indoor cultivation than outdoor. In fact, *P. granatum* seedlings were able to produce carotenoids and anthocyanins already under LED sources and these compounds led them to easily survive outdoor. Other species, as *C. avellana*, are not enough stimulated to produce secondary metabolites by LED spectra commonly used for plant growth. In this case, the exposure to UV radiation during pre-cultivation, for a short time, in order to avoid DNA damage so as the exposure to higher light intensity before transplanting, or transient phase by using shading cloths during transplantation in open-field could be suitable solutions to reduce this stress. Trials to test these solutions are subject of ongoing research.

	400-500 nm	500-600 nm	600-700 nm	700-800 nm
AP673L	11,9	19,3	60,5	8,3
AP67b	13,8	15,1	53	18,1
G2	7,7	2,4	64,4	25,5
NS1	20,2	38,9	35,7	5,2
AP67t	10,5	26,2	48,9	14,4
Fluorescence	34,8	24,1	36,7	4,4

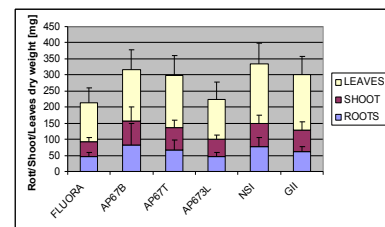
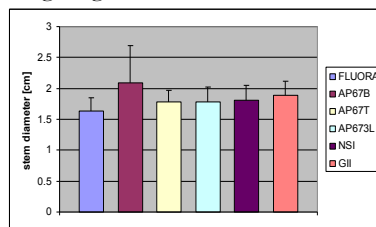


Fig.1. Spectra compositions

Fig.2. Light quality effect on stem diameter

Fig.3. Light quality effect on biomass

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2.1 = FINE-ROOT MORPHOLOGICAL AND GROWTH TRAITS IN A *QUERCUS ILEX* L. FOREST

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A substantial fraction of net primary productivity in forested ecosystems is exported belowground to produce ephemeral fine roots. Despite their relatively minor contribution to the overall root biomass (Vogt et al. 1996), fine-root turnover represents up to 33 % of the total annual net primary production in most ecosystems (McClagherty et al. 1982). Therefore, fine-root dynamics is widely recognized as an important biogeochemical process in forest soils. Within a root system, very fine ( $d < 0.5$  mm) and fine ( $0.5 < d < 2$  mm) roots (nomenclature according to Zobel and Waisel 2010) represent the most dynamic component of a root apparatus (Hendrick and Pregitzer 1992). In the present work, our objectives were to evaluate (1) the seasonal variation of live fine-root mass, length and SRL; (2) the fine-root production and turnover rate; (3) how the aforementioned fine-root traits vary in relation to two fine-root diameter classes ( $< 0.5$ ;  $0.5-2$  mm). The experimental site is located in the Orientated Natural Reserve – Bosco delle Pianelle (Puglia, Italy), at an altitude of 440 m above sea level (latitude  $40^{\circ} 38' 36''$ N, longitude  $17^{\circ} 14' 2''$ E). The climate is Mediterranean with summer drought spanning from May to September. Rainfall is usually concentrated between late autumn and early spring. The soil core sampling method (Vogt and Persson 1991) was used to quantify fine-root mass ( $< 2$  mm in diameter) during the 2013-2014 growing seasons. At the study site four plots for stand measurements were selected and within each of them a 10-m<sup>2</sup> square shaped plot was set up. At each sampling date, two soil cores (10x10-cm, 30 cm deep) were randomly collected in each plot using a motor-driven portable hammer (Makita Corporation) with a 10 cm stainless steel blade. To investigate the kinetics of biomass and necromass, we monthly collected soil samples from September 2013 to July 2014. The fine roots were examined at the microscope and were divided into two groups: oak and other understory species. Fine roots from oak trees were classified “live” “dead” depending on their color, texture and shape (Vogt and Persson 1991). Roots were scanned at a resolution of 400 dpi with a calibrated flatbed scanner coupled to a lighting system for image acquisition (Epson Expression 10000 XL). Successively, images were analyzed by WinRhizo Pro V. 2007d. Preliminary results showed a complex seasonal pattern of fine root biomass and length independently of diameter class considered. Increment of both fine-root biomass and length was observed in fall and late spring.

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## 2.2 = DEVELOPMENTAL REPROGRAMMING AFTER PHOTOPERIODIC INDUCTION AT THE SHOOT APICAL MERISTEM OF RICE

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Rice flowers when day length (or photoperiod) is shorter than a certain threshold. Photoperiod is perceived in leaves and under short days a mobile signal encoded by the Heading Date 3a (Hd3a) protein is generated and subsequently transported to the shoot apical meristem (SAM). Hd3a in the SAM elicits the vegetative-to-reproductive development reprogramming. It has been shown that Hd3a binds the bZIP transcription factor OsFD1 through a 14-3-3 protein, and this complex can activate the transcription of several target genes required for the vegetative-to-reproductive switch. Nevertheless, this is not the only mode of action of Hd3a at the SAM. We recently identified additional transcription factors that interact with Hd3a at the SAM and mediate transcriptional activation of target genes required for flowering. Plants where these factors are mutated display impaired flowering in response to photoperiod. Our findings expand the current knowledge on the mechanisms that allow flower development in rice, triggering the switch from vegetative to reproductive development at the SAM.

## 2.2 = MONOPTEROS (MP) A CENTRAL INTEGRATOR OF PATHWAYS CONTROLLING OVULE PRIMORDIA FORMATION

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Pistil contains ovules that develop into seeds after fertilization. The ovule primordia emerge as lateral organs from a meristematic tissue within the carpel referred to as placenta. It is of great importance to understand the mechanisms that control ovule numbers as they ultimately determine the final number of seeds and, thereby, the yield in seed-crop plants.

In the last decades, the fundamental role of plant hormones in ovule initiation has been discovered and few transcription factors have been identified as regulators of ovule number (1).

It has been shown that the initiation of new ovule primordia occurs where auxin accumulates (2). Auxin signaling is primarily regulated by the *AUXIN RESPONSE FACTOR (ARF)* gene family products.

*ARF5/MONOPTEROS(MP)* is a central integrator of genetic and hormonal pathways controlling ovule primordia formation. In fact MP directly activates *ANT*, *CUC1* and *CUC2* transcription factors encoding genes, that have been shown to regulate ovule primordia position, outgrowth and number (3).

In *mp* partial loss of function mutants the reproductive development is compromised. In the pistil of the *mpS319* weak allele the carpel margin meristem (CMM) does not develop, and placenta and ovules are missing (3, 4)

A more detailed morphological characterization through SEM microscopy analysis revealed that *mpS319* pistils completely lack a proper abaxial-adaxial and apical-basal patterning. Our main goal is to investigate MP downstream targets responsible of cell fate control during carpel development.

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## 2.2 = TRASCRIPTOME ANALYSIS AND IDENTIFICATION OF GENE RELATED TO POLLEN TUBE DEVELOPMENT IN OLIVE (*OLEA EUROPAEA* L.)

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Olive (*Olea europaea* L.) is a wind-pollinated, allogamous species that is generally not considered to be self-compatible (1).

Floral biology has important practical implications, in addition to its scientific relevance, given that flower characteristics and bloom affect fruit characteristics and yield.

Pollen tube elongation is a polarized cell growth process that transports the male gametes from the stigma to the ovary for fertilization. Furthermore, interaction with the pistil renders pollen tubes competent to respond to guidance cues secreted by specialized cells within the ovule. To accomplish this process, the pollen tube must have structural integrity to penetrate the cuticular layer of the stigma, to cross several layers of cells, to reach and to extend rapidly through the transmitting tissue and finally to reach the embryo sac (2). It is likely that specialized proteins in the pollen tube cell wall are required for normal pollen function, however, the molecular basis that underlie this process are still not fully characterized (3).

These topics are important both for fundamental studies on the control of fertility and reproduction in plants and represent an attractive model system to investigate the polarized tip growth, cell–cell interactions and signal transduction.

Using modern next-generation sequencing (NGS) techniques and a *de novo* transcriptome assembly strategy we show that olive pollen tubes, grown *in vitro* in the presence of its own pistil and in combination pollen/pistil from self-sterile and self-fertile cultivars, show distinct gene expression profiles and many of the differentially expressed sequences fall within gene families involved in the pollen tube development, such as lipase, carboxylesterase, pectinesterase, pectin methylesterase, callose synthase. Moreover different genes involved in signal transduction, transcription and growth are up-regulated. The analysis also allowed us to identify members in actin, actin depolymerization factor and fimbrin gene family and member of the Ca<sup>+2</sup> binding gene family, related to development and polarization of pollen apical tip.

The whole transcriptomic analysis, through the identification of differentially expressed transcripts together with an extended functional annotation of them, will lead to better understand the mechanisms involved in pollen germination and pollen tube growth.

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2.2 = POLLINATORS OF THE GENUS *OPHRYIS* IN SARDINIA: STATE OF THE ART

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Sardinia is the second largest island in the Mediterranean basin with 24,090 km<sup>2</sup>, it is located in a central position in the western Mediterranean sea between the Iberian and Italian peninsulas, and the coast of Ligurian-Provençal and northern Africa. The endemic component of the island is composed of 347 endemic species (14.4% ), of which 159 (6.6%) exclusive (1). Currently in Sardinia and , more generally, in Italy , there are few detailed studies of conservation biology aimed at understanding , conservation and management of species at risk of extinction. Only recently , in fact, studies have been initiated on this type of endemic taxa or phytogeographical interest (1) . Sardinia (with Corsica) is part of one of the " hotspots" of Mediterranean biodiversity for its endemic species and for its wealth of species (13). The geographic isolation and the high geological diversity have created a wide range of habitats with high levels of endemism, 347 plant species (1). In Sardinia there are over 60 orchids (2) that grow in the different environments, in greater numbers in the fields and in the garrigue, but also within the forests in wetlands and marshes. Mediterranean oare morphologically very similar to the tropical species, the only different characteristics are the size (very small and not very showy the first, generally large and very showy tropical ones ) and the mode of growth (soil all Mediterranean species , mostly epiphytic exotic ones).

At the "revolution" in the taxonomic classification of *Orchidaceae* (2,3) is not followed by an update of their pollinators. The family of orchids is one of the most diverse families in the Plant Kingdom, where the pollination takes place in various ways and specialized. The pollination of flowers of the genus *Ophrys* is carried out mainly by the males of some stinging wasps that are attracted selectively by labella of these *Orchidaceae*, which simulate their females and then through a kind of deception sex (sex deceptive species), guided by visual, olfactory and tactile clues are placed above the flower groped for the coupling. From this act, called pseudocopulation, follows the exchange of pollen between a flower and the other, by means of pollinia that remain attached to the head or in pollinators group fusca - lutea abdomen of these Hymenoptera during maneuvers of pseudo-copulation .

During our investigations carried out in different areas of Sardinia , in the spring of the years 2013-2014, in order to determine and confirm the pollinators of some species of *Ophrys* it was possible to observe the ritual several times to approach the flower and pollinator document photographically acts pseudocopulatori pollinators of different entities including, *O. morisii*, *O. incubacea*, *O. funerea*, *O. neglecta*. All observations were made in the hours between 12.00 and 14.30, mainly on days with clear sky, and in the absence, or nearly so, of the wind.

From what has been possible to ascertain from our observations, carried out in Sardinia pollinators are as follows: *O. morisii* male *Anthophora sicelii* (Apoidea); *O. incubacea* male *Andrena morio* (Apoidea); *O. funerea* male *Andrena wilkella* (Apoidea); of the *O. neglecta* male *Eucera clypeata* (Apoidea) of the *O. bombyli* male *Eucera longicornis* (Apoidea); *O. sphogodes ssubsp praecox* male *Andrena nigroaenea* (Apoidea); *O. annae* male *Osmia rufa* (Apoidea); *O. speculum* male *Dasycoliaciliata* (Vespoidea) .

This work aims to provide a basis for a better understanding of the genus *Ophrys* pollinators in Sardinia, the important plant - insect interaction, biology and ecology of the species studied.

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### 2.3 = PROPAGATION PROTOCOLS OF NATIVE MEDITERRANEAN PLANT SPECIES: NURSERY APPLICATIONS, RESTORATION PROJECTS, HABITATS AND PLANTS CONSERVATION

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Research on germination, seed and vegetative propagation is the first requirement for habitats and plant species conservation, habitat restorations and native plant breeding also with ornamental value (PON-SO.PRO.ME). The Sicilian territory, with about 3000 native vascular plants (1) of specific and lower ranks, is characterized by a high degree of floristic diversity, which includes 322 taxa as narrow endemics (2) and other phytogeographically important plant species. In this paper, we present the results of an investigation, carried out between 2012 and 2014, focused mainly on the propagation of important plant species of the Sicilian flora. Furthermore, evaluation of the ornamental potential of selected native plants was performed, aimed at their sustainable exploitation and cultivation in urban green spaces and in private gardens. In order to obtain propagation material of native plants (seeds and/or cuttings) a series of botanical explorations in different Sicilian territories and habitats, including the Aeolian and Aegadian Islands, were carried out. To date, more than 244 vascular plants taxa, belonging to 135 genera and 52 families, have been collected. The research has focused on seed germination ecophysiology and on seed and vegetative propagation in the nursery. In order to assess seed germinative behaviour, different assays were performed at constant and fluctuating temperature regimes, in constant darkness and in alternating light/dark regime with a 12/12h photoperiod, in thermostatically controlled growth chambers; seedling emergence experiments and plant propagations by stem cuttings were conducted in the Faro nurseries (Catania, Italy). Phenological and morphological characteristics of these species, at different growth stages, were also recorded. Highly diffused species were studied [*Arbutus unedo* L., *Coronilla valentina* L., *Emerus major* Mill., *Erica arborea* L., *Erica multiflora* L., *Lomelosia cretica* (L.) Greuter & Burdet, *Lonicera implexa* Aiton, *Myrtus communis* L., *Pistacia lentiscus* L., ecc.], as well as endemic species such as *Anthemis aetnensis* Schouw ex Spreng., *A. cupaniana* Tod. ex Nyman, *Cytisus aeolicus* Guss. and *Silene hicesiae* Brullo & Signorello, two of the most interesting plants that grow only on the Aeolian Islands, and several species of the genus *Centaurea* L., *Genista* L. and *Jacobaea* Mill. The investigation enabled identifying factors affecting seed germination, to determine the germination capacity, the emergence and rooting rates, to assess adaptability for pot cultivation and to organise a large living collection of wild plants that are currently held, cultivated and maintained in the Faro nurseries. Species-specific propagation protocols are being developed in order to integrate the *ex-situ* with the *in-situ* plant conservation, so as to ensure their possible reintroduction in natural habitats. The data obtained revealed species with rapid synchrony and high rate of germination and seedling emergence (80-100%), i.e. *Centaurea aeolica* Guss. ex Lojac., *Erysimum etnense* Jordan, *E. bonannianum* C. Presl., *Euphorbia dendroides* L., *E. rigida* M. Bieb., *Helichrysum angustifolium* (Lam.) DC., *Iberis semperflorens* L., *Ptilostemon greuteri* Raimondo & Domina, *Silene fruticosa* L., *S. hicesiae*, and plants with low germination and seedling emergence (i.e. *Capparis spinosa* L. subsp. *spinosa* var. *canescens* Cosson, etc.). Among the studied species, *Anthemis maritima* L., *Centaurea aeolica*, *C. busambarensis* Guss., *Lomelosia cretica*, *Pallenis maritima* (L.) Greuter, *Teucrium flavum* L., *T. fruticans* L. and species of the genus *Helichrysum* Mill. and *Jacobaea* showed a good aptitude to propagation by herbaceous stem cuttings (80-100% rooting capacity). In addition, a number of native plants with a highly ornamental appearance (about 60) have been identified, such as *Achillea maritima* (L.) Ehrend. & Y. P. Guo, *Alyssum nebrodense* Tineo, *Cerastium tomentosum* L., *Erysimum etnense* Jordan, *Iberis violacea* R. Br., *I. semperflorens*, *Lavandula multifida* L., *Lomelosia cretica*, *Matthiola fruticulosa* (L.) Maire subsp. *fruticulosa*, *Pallenis maritima* (L.) Greuter, *Pseudoscabiosa limonifolia* Vahl, *Ptilostemon greuteri*, *Silene fruticosa*, *Teucrium flavum*, *T. fruticans* and taxa belonging to the genus *Anthemis* L., *Centaurea*, *Dianthus* L., *Euphorbia* L., *Helichrysum* and *Jacobaea*. As a whole, the plant species analyzed are a resource both for field applications to conserve the biodiversity, integrating *in* and *ex situ*, as well as to meet the demand for new ornamental nursery products. This research therefore has a multifunctional significance, answering the needs for innovation in procedures and products and also the conservation requirements of biodiversity, aesthetic-landscape enhancement and environmental renewal.

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### 2.3 = COMPARISON OF EX-SITU GERMINATION RESPONSE OF *LAVANDULA MULTIFIDA* L. IN TWO POPULATIONS OF S-ITALY AND S-SPAIN.

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*Lavandula multifida* L. is a suffruticose chamaephyte with woody stems, but modest in size, belonging to *Lamiaceae*. In Italy is distributed only in two regions: Calabria and Sicily (1, 2). Here occupies the northernmost part of its distribution area. In fact, it can be found also in Egypt, Tunisia, Algeria, Morocco, Spain and Portugal (3). In Calabria is only present at Capo dell'Armi in the province of Reggio Calabria, and in Sicily is present at Capo S. Alessio, M. Pellegrino and Brucoli, whereas is disappeared from some historic Sicilian sites such as Capo Scaletta and Taormina (4). This species lives in the coastal garigues on marly substrates (5) and has a bloom from February to April, but often this period is more extended. *L. multifida* is considered as Critically endangered (specie CR) according to in IUCN criteria (6).

In this study, we compared plants belonging from two stations geographically very distant: Capo dell'Armi (Italy) and Almeria (Spain). We sampled any plant as herbarium specimen, leaves, seeds (from both locations) and soil (only from Capo dell'Armi).

The soil texture on which the plant spontaneously grows is sandy type (87 % of sand, 8 % of clay, 5 % of silt); it is characterized by an alkaline pH (= 8), and high electrical conductivity (85 milliSiemens/cm), and by the 27 % of total carbonates. The percentage of organic substance is 2,45.

Among the macronutrients, exchangeable potassium, ammonia and magnesium were measured: the amounts were respectively 1 mg/L, 0.4 mg/L, and 10 mg/L, while total nitrogen amounted to 1.6 mg/gr of soil.

Phenolic levels of this soil were 14 ppm. Fluorescein Diacetate hydrolysis test was performed in order to assess the general enzymatic activity of the soil: the levels of fluorescein diacetate (FDA) amounted to 1.5 µg/mL of soil sample. Among soil enzymes, dehydrogenase enzymatic activity was also measured: it was 9.7 µg/mL of soil sample.

Germination tests on *Lavandula multifida* seeds were carried out: in order to make a comparison between two different populations of the same plant species, seeds were collected from two different locations where the plant spontaneously grows, that is from Almeria (Spain) and from Capo dell'Armi (Reggio Calabria, Italy). Seeds were selected and surface-sterilized with freshly prepared 30 % solution of commercial bleach for 15 minutes, followed by washing for several times with distilled-sterilized water; the seeds were placed on filter paper in Petri dishes and then transferred at dark at 4°C for 72 hours (stratification process). After that, Petri dishes were placed in climatic chamber at different temperatures, respectively 20°C, 25°C and 30°C, with a photoperiod of 12 hours light/12 hours dark, in order to assess possible differences in germination percentage at different temperatures. Experiment at dark at room temperature will be also carried out, so as to evaluate the effect of the dark on germination process.

The preliminary results show that the optimum germination occurs at 25°C and that the seeds from Spain have a shorter germination time than Italian ones.

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### 2.3 = SEED GERMINATION CAPABILITY OF *ASTRAGALUS AQUILANUS* ENDEMIC SPECIES IN THE CENTRAL APENNINES (ITALY)

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Endemic species are a significant feature of the Mediterranean mountains (1) because of the high number of speciation events that have occurred (2). Mediterranean mountains are characterized by a high genetic diversity (3). Predictions of climate change indicate that this genetic diversity could be disturbed significantly in the future (4). Moreover, Mediterranean mountains are considered one of the most threatened ecosystems in the European Union (5). Thus, many efforts should be addressed to improve the conservation strategies for Mediterranean mountain species considering that the survival of endemic and threatened species is based on different and complementary conservation approaches and techniques (6). Germination protocols, in particular for species characterized by small populations and for which data are missing, could be an important step in this direction. In this context, the aim of this work focused on seed germination capability of *Astragalus aquilanus* Anzalone, an endemic species to the south-central Apennines (Italy) with few populations in Abruzzo and Calabria. *A. aquilanus* is included in the IUCN Red List as Data deficient (DD). Moreover, it is a priority species in Annex II of the Habitat Directive and is protected by regional laws (n.45/79 and n. 66/80, Abruzzo). The experiments were carried out in the Majella Seed Bank (Botanical Garden Michele Tenore, 42° 2' 59" N; 14° 11' 34"E; 650 m a.s.l., Italy). Freshly-matured seeds of the considered species were collected from the wild populations of *A. Aquilanus* growing on Montelucio di Roio (L'Aquila, 980 m a.s.l) in August 2013 during the fruiting period and immediately before dissemination (7). The mother plants were randomly selected (8). To characterize the ecology of germination, after morphometric measurements and tetrazolium viability test (9), in vitro tests in a sterile environment were carried out; in particular the substrate with 1% agar medium was adjusted to pH 5.7 and photoperiod 12/12 light/dark. Each of the considered treatments consisted of four replicate of 20 seeds. The results showed a physical exogenous dormancy (seed coat impermeability to water) already observed in other species of the same genus (10,11). The pretreatment which gave the best results for the interruption of dormancy was the chemical scarification with concentrated sulfuric acid at 96% for 20 minutes. A percentage of germination of  $93.8 \pm 7.5$  and a  $T_{50}$  of 3 days was obtained. The results could be used in order to define the germination protocols for reinforcement projects of the wild populations of the considered endemic species as a means of reducing their extinction risk.

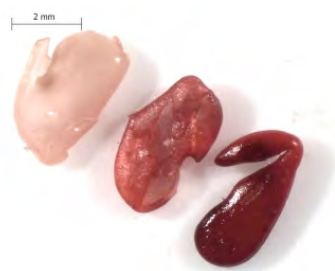


Fig. 1. Tetrazolium test



Fig. 2. Petri dishes in the germinator

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### 2.3 PRODUCTION OF NATIVE SEEDS FOR SEMI-NATURAL GRASSLANDS RECOVERY IN APENNINES AND PRE-APENNINES AREAS

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European Union Habitat Directive (92/43/EEC) purposes to protect biodiversity through habitats conservation using measures that have the aim of ensure maintenance or restoration, in an acceptable state, of habitats and wild vegetal and animal species. Among the habitats of European Union, the 6210 Habitat “Semi-natural dry grassland and scrubland facies on calcareous substrate (*Festuco-Brometalia*)” is widely represented in the grasslands of Central and Southern Apennines and in Sicily Apennines secondary. Actually, the conservation of this habitat is not easy in the area because the most of secondary grasslands are abandoned and the consequent development of vegetation series leads to their gradual disappearance. Indeed, abandonment causes a large invasion of shrub species, through spontaneous re-naturalization processes of the vegetation, abundantly documented for Central Apennines (1, 2), which leads to progressive disappearance of secondary grasslands. Habitat restoration techniques that are currently tried include shrubs removal and possible reseeding of grasses on soils that are mostly bare. Unfortunately, seeds acquirable in the market do not include the most significant species for semi-natural grasslands biodiversity and widely distributed species often have an extra-European origin, too. Therefore, the use of seeds acquirable on the market determines variations in phytocoenosis composition and causes a genetic contamination due to the introduction of clones and varieties allied to the autochthonous ones which interbreed with (3).

So, it is evident the necessity to recover autochthonous germplasm to be used in the management not only of protected areas but also of other environments such as sides of roads and ditches.

This research was aimed to recover germplasm of some Apennine semi-natural grasslands grasses to conserve and multiply *ex situ*, in order to use it in environmental restoration works. Therefore, seeds of studied species were collected in their natural sites of presence and analysis on seed morphology and germination were carried out at the *Germplasm Bank for ex-situ conservation of Amphiadriatic species* (4). Analyses were carried out on seeds from 47 different populations. Moreover, seeds were multiplied in experimental parcels at “Selva di Gallignano” Botanical Garden.

Seeds were able to germinate to high percentages in a rather wide range of temperature. Moreover, they generally did not require the removal of palea and lemma and it could allow to use a faster and less expensive extraction process. Multiplication experiments were also successful since the species adapted very well to the environmental conditions of the cultivation site and it was possible to obtain amounts of seeds that were considerably higher than those used for the seeding.

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### 2.3 = ANTIOXIDANTS CONTENT IN *BRASSICA OLERACEA* VAR. *ACEPHALA* FROM DRY SEED TO PLANTLET

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Vegetables belonging to the *Brassicaceae* family (cabbage, cauliflower, broccoli, brussel sprouts) are generally appreciated for their health benefits, since regular intake can prevent the occurrence of many human diseases like diabetes, cardiovascular and neurological diseases, and decreases the risk of many forms of cancer (1). The active components in *Brassicaceae* are mainly represented by antioxidant molecules (polyphenols, flavonoids, vitamins E and C and glucosinolates) which possess antioxidative and anti-inflammatory properties and appear to contribute to chemopreventive activity in colon, stomach and lung cancer (2). The aim of this research was to analyse the antioxidants activity of *Brassica oleracea* var *acephala* (black cabbage) considering plant portions at different developmental stages. For this purpose antioxidant determinations were carried out on seeds dry or imbibed for 1, 2, 4, 7, 10 days, immediately dissected into cotyledons and embryonic axes after removal of seed coat and on plantlets grown for 14, 23, 37 days separated into cotyledons and young leaves. All the extracts were spectrophotometrically analysed for total antioxidant activity, by the electron-transfer based assays DPPH (2,2'-diphenyl-1-picrylhydrazyl) (3) and for Vitamin C (4, 5), total phenols (6) and flavonoids (7) content. Data were analyzed considering both fresh and dry weight of the different plant samples. Mass spectrometry, by using electrospray ionization, high resolution and tandem mass spectrometry, have been used to determine the analytes present in the different samples. Results generally indicated a major antioxidant activity in cotyledons of sprouts collected 4 and 10 days from imbibition when compared to dry seeds or leaves from plantlets of 37 days. Our determinations demonstrated that black cabbage sprouts were particularly rich in antioxidant molecules, in accordance with previous works carried out on *Brassicaceae* and on other vegetables where it was reported that sprouts have higher nutritional value when compared to the mature plants (8, 9, 10). For this reason, regular consumption of sprouts from different vegetables is recommended to get the maximum nutritional value and protective effects on human health.

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### 3.1 = SEED STORAGE OF *HIMANTOGLOSSUM ROBERTIANUM* (ORCHIDACEAE): A COMPARISON OF TEMPERATURE AND MOISTURE CONDITIONS

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Seed banks maintained at -20 °C and low relative humidity are routinely used for the long-term *ex situ* conservation of orthodox seeds (1). Seed longevity, as measured by survival time during storage, is a critical aspect in the management of a seed bank because it may differ significantly between species and accessions (2). Moreover, the effectiveness of seed survival strongly depends on the correct application of standard procedures before seed storage (3). Temperature and seed moisture content are recognized as the main factors that influence the duration over which seeds can be successfully conserved (3). Orchid seeds are considered to display an orthodox behaviour as they show an extended longevity when subjected to a drying treatment (< 5% moisture content) and freezing at -20 °C, conditions commonly applied in many seed banks (4). It is also known that orchid seeds tolerate different storage conditions, but it is unclear which is the best approach for long-term storage, as differences in viability occur among different orchid species during the storage (5).

The aim of this study was to establish the optimal conditions for the conservation of seeds of *Himantoglossum robertianum* H. Baumann, an Euro-Mediterranean terrestrial orchid (Fig. 1). Investigations were addressed to compare seed storage conditions, 5 °C, -20 °C and -196 °C (storage in liquid nitrogen), after the seed had been equilibrated to a range of relative humidity (RH) conditions, *i.e.* 5%, 60% and 80% (Fig. 2; 3; 4), constructed using different concentrations of the lithium chloride solutions. Isotherms were determined to describe the relationship between seeds moisture content and RH and relate to data acquired from Differential Scanning Calorimetry. An *in vitro* germination test was carried out to verify the effectiveness of the different pre-sowing treatments and storage conditions to maintain the seed germination capability. The highest percentage of germination was obtained with the lowest RH in the cryopreserved seeds and at 5 °C storage temperature. Subsequently, we considered the growth of protocorms in order to determine if significant differences in protocorm development occurred within the following five months. The development of *H. robertianum* protocorms did not start until the third month after sowing, and showed a linear increase among all the treatments. In particular, the cryogenic treatment gave good results in combination with low RH values.



Fig. 1. *Himantoglossum robertianum*



Fig. 2. Seeds enclosed in filter paper packets



Fig. 3. Seeds equilibrated at different RH into airtight glass jars containing aqueous solutions of lithium chloride



Fig. 4. Immersion of seed samples in liquid nitrogen

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### 2.3 = GERMINATION ECOPHYSIOLOGY AND REPRODUCTIVE BIOLOGY OF INVASIVE SPECIES THAT THREATEN ENVIRONMENT, ECONOMIC ACTIVITY AND HUMAN HEALTH.

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According to the Convention on Biological Diversity (CBD), Invasive Alien Species (IAS) are those that are introduced, established, naturalized and spread outside of their home range, and whose impacts involve significant harm (1). Invasive species are considered a serious problem worldwide because of their effects on biodiversity loss and the consequences due to their impact on natural ecosystems, not only from an environmental, but also from an economic and health point of view (2).

In Sardinia, as in the rest of Italy and in every part of the world, a huge number of alien plants have been introduced over time, either voluntarily or accidentally through many different channels (3). Among these are included species of great use and interest in the field of agriculture, forestry and nursery, especially perfectly harmless, but also defined as invasive species that can cause negative impacts of various types of natural ecosystems, human activities or health (4).

Unfortunately, most invasive plants have been introduced by nurseries and botanical gardens for horticultural purpose in gardens and other public and private facilities (5). Even 80% of invasive plants in Europe is ornamental or agricultural pathways (6). For this reason it is important to adopt voluntary codes of conduct with the aim of engaging public and stakeholders and therefore reduce and control the potential introduction of invasive species into European and Mediterranean countries (7).

This project aims to study biological invasions and the resulting impacts through the most effective technologies of germination ecophysiology and reproductive biology of invasive plants, particularly to exotic species in the horticultural market. The study of germination ecology can increase the knowledge of the best conditions for seed germination in the field. The research will focus on seed ecophysiology of some invasive species in Sardinia. The aim is to characterize the abiotic factors that may favour seed germination and thus obtain more information on their potential invasiveness and thereby find efficient management methods.

The Project will last two years and will take place at Agri Ambiente Ltd of Pula (Cagliari, Italy). This company operates in nursery and green environmental field. The project is funded by "Regione Autonoma della Sardegna" through one research fellowship with "POR Sardegna FSE 2007-2013 - Obiettivo competitività regionale e occupazione, Asse IV Capitale umano, Linee di Attività 1.1.1. e 1.3.1" funds. During the two years project new collaborations will be started with research groups with proven experience in germination ecophysiology as the Sardinian Germplasm Bank (BG-SAR).

The project will be divided into four consecutive phases: 1) creation of an updated checklist of invasive species impacting on human health, economy and environment, 2) field monitoring and seed collection of selected species, 3) study of germination ecology and identification of germination protocols, 4) organization of "awareness days" for eradication of invasive species in field and dissemination of obtained results.

Results will be useful to facilitate the processes of risk analysis carried out by international organizations for prevention procedures (ex. Bern Convention, EPPO) and they can also be used by local authorities for areas' management in order to intervene quickly through actions of prevention, control and eradication.

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### 2.3 = *GLEBIONIS CORONARIA* (L.) SPACH (ASTERACEAE) SEED GERMINATION, PERICARP ANATOMY AND WATER UPTAKE IN RELATION TO SEED DORMANCY

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*Glebionis coronaria* (L.) Spach is a common Mediterranean weed producing distinctive central and peripheral dormant achenes with a hard fruit coat, which was hypothesized to exert physical dormancy (1, 2). We examined the germination behavior, the water imbibition mechanism, and the anatomical structure in both achene morphotypes. Seed dormancy was tested by measuring germination response of fresh achenes in light and constant darkness, and after different time intervals of after-ripening, cold and warm-stratification. Water-uptake was tested examining the increment of weight during imbibition, whereas anatomical analysis of pericarp tissue was carried out by using Light Microscopy and Scanning Electron Microscopy.

Fresh matured morphotypes sowed at several constant and alternating temperature regimes in both darkness and 12-h photoperiod germinated in a very limited amount. After-ripening and cold stratification did not increased germination response significantly, whereas logistic regression revealed that warm-stratification produced a highly significant improvement especially when achenes were transferred at cool temperatures. Moreover, after sowing achenes with water a significant uptake was observed in both morphotypes, although peripheral morphs showed a slower imbibition-time respect to central ones. Even-though the two achenes types show a clear distinct morphology, which confers different dispersal ability (3), the anatomical analyses of central and peripheral achene pericarp revealed in both morphs the absence of a palisade layer(s) of macrosclereids. On the contrary, a channel-like structure, which can allow water entrance, was found at the basal end of pericarp in both morphotypes.

Therefore, we reject the hypothesis of physical dormancy and, besides, we suggest that dormancy is not either physiologically imposed since both morphotypes displayed complete germination after embryo excision. These findings provide a new interpretation of dormancy behaviour in *G. coronaria*, where mechanical constraint on embryo growth exerted by the hard pericarp is the principal system of controlling seed germination and through which it establish a long-lasting soil seed bank.

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## 2.4 = THE PECULIAR CHLOROPLASTS OF LEAF PARENCHYMA CELLS IN *HELICHRYSUM ITALICUM* (ROTH) G. DON

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*Helichrysum italicum* (Roth) G. Don (Asteraceae) is a species diffused in dry, sunny places in central- southern Italy both in proximity of the sea littoral and in the internal areas. The whole plant is characterised by a green-grey colour and by a typical scent due to the essential oil secreted in the glandular trichomes; these structures are widespread on the epidermis of the whole plant, and the essential oil composition has been widely studied (1, 2). This species is used in folk medicine for several applications, in particular for its anti-inflammatory effects.

The glandular trichomes are characteristic of the Asteraceae, i.e. two lines of 4-6 cells: the two apical ones are the secreting cells bearing on the apex a well-developed subcuticular space where the secretion, mostly essential oil, is stored (3).

The leaf presents a photosynthetic parenchyma with chloroplasts containing numerous grana and intergrana tylacoids and a huge amount of starch. During our experimental trial we observed that keeping the plant in dark conditions for 8-10 hours (i.e. overnight) the starch was completely removed from the chloroplast. Conversely, exposing the plant to the natural light, even for a short time, the chloroplasts present again huge amounts of starch. Therefore a noteworthy efficiency of the plant photosynthetic activity is evident.

Moreover, the stomata, located on the adaxial side of the leaf, are in prominent position, which is contrary to what expected in plants growing in xeric sites. This position of stomata enhances gaseous exchanges and hence photosynthesis, but it is also responsible for water loosing.

The observed histo/anatomic characteristics counteract typical morpho-anatomic structure of xerophytic plants. Nevertheless it is not clear for which reason the plant quickly produces so large amounts of starch, and how it could be utilized, given the relatively slow growth rate of the plant. Our hypothesis is that photosynthesis products are related to the great variety and amount of secondary metabolites produced by the glandular trichomes.

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## 2.4 = NEW INSIGHTS ON THE RESILIENCE CAPACITY OF *TARAXACUM OFFICINALE* WEBER GROWING ON MINE SOILS

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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 (1, 2). In this work we examined the effect of heavy metals (HM) on the morphology of *T. officinale* growing on mine soils, with the following objectives: (i) to determine the fate of HM within the soil-plant system; (ii) to highlight possible damage at anatomical and cytological level; (iii) to assess the resilience capacity of *Taraxacum officinale* after three years of pot cultivation.

Wild specimens of *Taraxacum officinale* Web, with their soil clod, were gathered from four sites with different contamination levels by heavy metals (Cu, Fe, Pb, Zn) in the abandoned Imperina Valley mine (Northeast Italy). Plants were cultivated in pots at the botanical garden of the University of Florence (HBF), and appeared macroscopically not affected by toxic signals (e.g. reduced growth, leaf necrosis) possibly 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. The unpolluted samples present a well organized palisade tissue and spongy photosynthetic parenchyma. Samples from contaminated sites, instead, present a palisade parenchyma less organized, and a reduction of leaf thickness proportional to HM concentration. The poor structural organisations, and the reduced foliar thickness of the contaminated plants, are related to soil contamination. Differences in roots micromorphology concern the cortical parenchyma. Moreover, all the samples examined present mycorrhiza. Ultrastructure observations of the parenchyma cells show mitochondrial structure alteration, with lacking or reduced *crisetae* of the internal membrane at increasing metal content. Instead, chloroplast organization does not present significant differences, particularly in number and compartmentalization of thylakoids.

Although macromorphology does not present evidence of phytotoxicity, the recorded observations of the micromorphological characteristics of leaves and roots, show a suffering state of the plants, strictly related to HM content. Leaching reduced partly the HM content of the soil, therefore decreasing their phytotoxic effect. A gradual restoration of leaf 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 *Taraxacum* could be a useful species in remediation projects.

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## 3.1 = BIODIVERSITY AND FUNGAL CONSERVATION IN THE COLLESTRADA FOREST (UMBRIA, ITALY)

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The Collestrada forest (Perugia, Umbria, Italy) is located on the northern slope of a slight hill, (Colle del Monte), covering an area of approximately 68 ha (250-306 m a.s.l.). It's part of a Site of Community Importance (SCI, IT5210077) established under the Habitat Directive (92/43/EEC) for the presence of habitat 91M0 Pannonian-Balkan turkey oak-sessile oak forests. The forest ecosystem has an exceptionally rich flora and fauna, however important components of this ecosystem were only marginally studied. For example a large inventory of fungal species had not been performed and systematic studies on macro-fungi are few (1). In this paper we attempt to assess diversity and distribution of macro-fungi in seven Collestrada woodland types characterized by the prevalence of different plant species: the *Carpinus betulus* L., the *Quercus cerris* L., the *Quercus frainetto* Ten., the *Quercus petraea* (Matt.) Liebl., the *Quercus ilex* L., the plantations *Pinus pinea* L. and *Pinus pinaster* Aiton, and the plantations with deciduous species like *Quercus* spp., *Acer* ssp, *Fraxinus* ssp. The mycological field survey was carried out on macrofungi that were visible to the naked eye (greater than 1 mm in size) (2) from January 2011 through December 2013. All basidiomata and ascomata were collected weekly (during autumn and spring) or every other week (winter and summer). Species richness, abundance and relative abundance were assessed. Species accumulation curves, bootstrap estimates of total richness, and diversity were inferred using EstimateS 8.2 (Colwell, 2006; <http://purl.oclc.org/estimates>). A total of 341 species belonging to 66 families and 131 genera were identified here over the 3-yr sampling. The collected species were mainly *Basidiomycota* T.T. Moore (97%), including a high relative abundance of *Agaricomycetes* Doweld (96.6%). The *Agaricales* Underw order is the most represented. The diversity of macrofungi ranged from, 4.4-2.7 (Shannon index), 45.3-10.3 (Simpson index), and 55.4-9.48 (Fisher's) depending on woodland types. Results of this study showed that Collestrada forest in central Italy supports rich and diverse community assemblages of macrofungi. The diversity and structure of the macrofungi assemblages are mainly affected by the host tree species composition. The fungal community of *Q. frainetto* woodland, displayed the highest richness and diversity. The lowest richness and diversity was found in small area with *Pinus* spp. plantation. Among the 341 species collected, 81 fungal species were included in the Red List (or proposals for Red Lists) in several European countries because of their rarity due to natural or anthropogenic factors [i.e. *Boletus ichnusanus* (Alessio, Galli & Littini) Oolbekk., *B. permagnificus* Pöder, *B. roseoalbidus* (Alessio & Littini) G. Moreno & Heykoop, and *Sarcosypha coccinea* (Gray) Boud.] (<http://www.wsl.ch/eccf/>). Among these, it is noteworthy the presence of *Amanita eliae* included in the preliminary list of 23 species considered rare and/or threatened to Italy, given by the mycology work group for the Italian Botanical Society (3). It is also noteworthy the presence of *Sarcosphaera coronaria*, included in the list of 33 fungal species threatened at the European level included in Annex 1 of the Bern Convention, document T-PVS (2001), 34. Finally, also important is the presence of *B. ichnusanus*. It has recently been included in the Red List of the Italian Flora (2013) (with 12 other 'non policy species'), assessed against the IUCN Criteria and Categories. In conclusion we have found 81 fungal species, which appear relatively common in the Collestrada forest, that are considered at risk of endangerment or even extinction in various other European countries. For this reason it may be worth taking the Collestrada forest into consideration for the in situ conservation of these species and, therefore as an Important Fungal Area (IFA).

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2) E. Arnold (1981) *Bibl. Mycol.*, 83, 1-410

3) G. Venturella, C. Perini, C. Barluzzi, G. Pacioni, A. Bernicchia, F. Padovan, L. Quadraccia, S. Onofri (1997) *Bocconea*, 5, 867-872

### 3.1 = PROMOTING THE USE OF NATIVE PLANTS IN URBAN AREAS: A PROJECT FOR THE BIODIVERSITY CONSERVATION IN LOMBARDY REGION.

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In the fast-paced, highly connected, technology-driven 21<sup>st</sup> century it is often possible to forget just how dependent our society is on the natural world. Soil fertility, fresh water, clean air, organic waste disposal, as well as a host of food stuffs and products, are provided by the biodiversity of our planet. Conserving biodiversity only in protected areas will not be sufficient to stop the actual biodiversity loss and the ecosystem degradation. The urban areas (particularly private and public gardens) can represent an opportunity to conserve local biodiversity throughout different actions: promoting the use of native plants and discouraging the use of ornamental invasive plants. These actions constitute the core of a new project of the Network of the Lombardy Botanical Gardens that will be realized in 2014/2015. Actually in the Lombardy Region a great flurry of activities is turning around the 2015 EXPO event of Milano. The most of the stakeholders is proposing projects and actions based on an anthropogenic vision where direct benefits for man and society are provided from plants (food in particular, given the theme of the 2015 EXPO "Feeding the planet"). The Network of Lombardy Botanical Gardens intends to propose a project based on a global vision which considers the sustainability of the man actions with particular reference to the biodiversity and native plants. These plants often do not provide direct food for man and society, but play an important role in the maintenance of ecosystem balances and stability, also providing indirect benefits (such as pollination), which are essential for man and society survivor and activities.

The Network of the Lombardy Botanical Gardens includes seven gardens: Botanical Garden of Bergamo "Lorenzo Rota", Alpine Botanical Garden "Rezia" of Bormio (SO), Astronomic Museum-Botanical Garden of Brera (MI), Botanical Garden Cascina Rosa of Milano, Botanical Garden of Pavia, Botanical Garden "Giordano Emilio Ghirardi" of Toscolano Maderno (BS), Villa Carlotta – Museum and Botanical Garden (CO).

The distribution of the gardens in areas with different phytogeographical (from West to East Lombardy) and ecological characteristics (from planar to collinar to montane belt) give to this new project of the Network a great value in relation to the plant biodiversity which can be involved.

Aims of the project are: giving the Lombardy Botanical Gardens a new tool for pursue their mission and increase their visibility to the public; increasing their educational proposals based on a thematic never considered before such as the gardening with native plants; bringing their educational actions outside the Botanical gardens to reach new audiences; enlarging the message about the need to preserve plant biodiversity; developing educational proposals for multi-languages users; realizing interesting spaces linked to 2015 EXPO; realizing links with other associations and/or organizations on the Lombardy land.

The project will realize many actions. Particularly each Botanical garden of the Network will be involved in the following activities: define native plants to promote in urban areas; define invasive plants to discourage in urban areas; realize a space with the defined native plants inside the Botanical garden and outside of it in an area identified with the local Municipality.

Other activities of the project will also include: publication about the gardening with native plants; publication about the identification of native plants; workshops for the public and for specific professionals (such as architects).



## 3.1 = WIKIPLANTBASE #TOSCANA: BREAKING THE DORMANCY OF FLORISTIC DATA

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Floristic records provide baseline data for researches in plant biology. Traditionally, printed floras are the "one-stop shop" to find floristic data; unfortunately, printed floras are quickly outdated. Therefore, researchers must look for updated data in the scientific literature and herbarium specimens: the longer the time elapsed since the issue of the flora, the heavier the effort. In the case of floristically-rich Tuscany, the last regional flora dates back to the mid-XIX century (1).

Any attempt to update it must take into account the acquisition of a vast, heterogeneous, and "dormant" documentation, both published and unpublished, as well as any future exclusion / inclusion of taxa. To this end, we have started the project "Wikiplantbase #Toscana" with the following approach: i) a basic floristic record is a combination of a plant name, a locality, a date, and a source; ii) ideally some environmental and ecological data are associated to floristic records; iii) data must be entered on an online platform by registered collaborators, accessing the platform across the Internet; iv) data homogeneity and integrity must be enforced by the platform software rather than data entry operators; v) all data must be validated by a project coordinator; 6) all validated data must be freely accessible across the Internet.

Taxonomic integrity was implemented by linking the database to a working copy of the Italian checklist of vascular plants; furthermore, the database was linked to a database comprising >70.000 toponyms along with administrative boundaries and geographic coordinates. Data are entered across the Internet by registered users; following validation, the records can be displayed on a map as georeferenced points with associated data, in a dedicated interface (no registration is required for querying and viewing the database).

Wikiplantbase #Toscana went online on 15 June 2013; as of 22 April 2014, its 25 registered users have entered 46913 records, mostly from published data (77.4% of the total), then by published herbarium specimens (19%) and unpublished field data (3.2%). Unpublished herbarium records account only for 0.5% of the stored data. The count of accepted names stands at 3368, representative of 962 genera and 152 families. There is a large variation in the number of records species-wise: *Aristolochia rotunda* L. subsp. *rotunda* is represented by 219 records for 151 localities, but 652 species are still represented only by one record for a single locality. The low frequency of herbarium specimens indicates that the census is far from complete; in fact, currently the number of accepted names increases in a quasi-linear way with the number of records (Fig. 1), in agreement with Conti et al. (2), who assessed the total of the Tuscan vascular flora at 3435 units. Also the fitted Michaelis-Menten function  $f(x) = 3619.96 * x / (5556.36 + x)$  ( $S = 70.90$ ) indicates a higher figure (95% confidence interval = 3546-3698).

A better representation of the Tuscan vascular flora requires a continuing effort in data entry from users and possibly from batch operations enabled by collaborative agreements with individuals and institutions holding georeferenced floristic databases. We speculate that about 300K records are required to have a realistic picture. Such a massive dataset, maintained up-to-date with relatively little effort, might power several researches like e.g.: i) taxonomic researches especially on species and genera in Tuscany; ii) studies on the distribution of diversity across administrative or ecological boundaries; iii) evaluation of conservation status of endangered taxa; iv) static and dynamic range modelling.

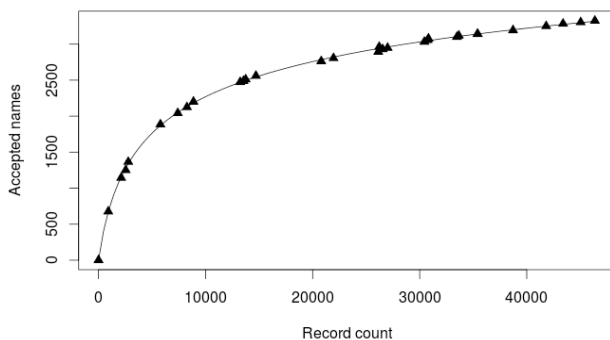


Fig. 1. Accepted names (n) plotted against record count (n). Triangles: experimental data; line: fitted inverse Harris curve

$$y = x / (0.9035 + 0.0013 * x^{0.8589}) + 1.8020 \quad (S = 16.83, df = 27)$$

1) T. Caruel (1860-1864) *Prodromo della Flora Toscana*. Firenze, Le Monnier

2) F. Conti, G. Abbate, A. Alessandrini, C. Blasi (Eds.) (2005) *An annotated checklist of the Italian vascular flora*. Palombi Ed., Roma

## 3.1 = THE CATALOGUE OF THE NON-NATIVE FLORA OF SARDINIA (ITALY)

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A complete list of all alien species ever recorded in the flora of Sardinia (Italy), including the main commonly cultivated aliens, is presented as an update of previous checklists and dedicated international, national and regional studies. New introductions and data accumulated in the last decade are here incorporated. The status of some species is reassessed based on improved knowledge on the distribution, spread, documented impacts and improved risk-analysis and impact assessment methods. Alien plants can invade natural and semi-natural environments leading to habitat changes or compete with the native species for resources causing the decrease of their populations and consequent losses in native biodiversity. Many of them can represent a problem also in anthropic environment because, e.g., allergenic or toxic to human or to domestic animals or as weeds in agro-ecosystems where they cause economical damage due to yield losses and increase in the use of plant protection products (4).

We took into consideration all the available previous studies on the Sardinian non-native flora, including the most recent ones made by Arrigoni (2006/2013), Podda et al. (2012, *Not Bot Horti Agrobo* 40.2), Celesti-Grapow et al. (2010), Bacchetta et al. (2009, *Flora Montiberica* 41) and Camarda et al. (2001) and those more dated, e.g., Gennari (1874), Barbey (1884), Terracciano (1914). This new complete list and digital database of the Island provides accepted names of the species and their synonyms, the Raunkiaer's growth form, the type of fruit, the native range, the time of introduction (archaeophyte or neophyte), the year of the first record in Sardinia and the invasive status (only planted, casual, naturalized or invasive) at species and population levels. Information on the pathway of introduction (accidental or voluntary) and main introduction purposes (e.g., crop, forage, aromatic or ornamental) are also provided. The bibliographic research was accompanied by field surveys, to assess distribution, abundance, to verify the persistence and the status of the alien species reported by literature and to detect the presence of new comers. Other existing databases were used to gain taxonomic and biological information on each species, as well as invasiveness in countries with similar climatic conditions.

This catalogue includes 918 entities and among them 888 species, 17 subspecies and one variety. The most represented families are *Fabaceae* (72 specie), *Asteraceae* (52), *Solanaceae* (40), *Poaceae* (39) and *Cactaceae* (31). Species which produce capsule (266), berry (135), achene (107), legume (71) and drupe (32) are the most numerous. The most common growth forms are the phanerophytes (43%) and the therophytes (20%). The highest number of introduced species comes from Americas (272), followed by Asia (157), Africa (132). These data, together with other datasets resulting from recent researches, will be used for a number of analyses on plant invasions in the region that will address issues such as species invasiveness, invasive traits and risk assessment. This catalogue can be seen as a work in progress as it will framework all future records of non-native species in Sardinia and will support monitoring, land management and prioritisation according to the new EU Regulation on invasive alien species.

1) P. V. Arrigoni (2006-2013). *Flora dell'Isola di Sardegna*. Vols. 1-4. Sassari: Delfino.

2) W. Barbey (1884). *Florae Sardoe Compendium*. Catalogue raisonné des végétaux observés dans l'île de Sardaigne. Lausanne: Georges Bridel Editeur.

3) I. Camarda (2001). *Ricerca sulle Specie Vegetali Aliene della Sardegna*. Rapporto Finale. Sassari: DBEV Università di Sassari, Servizio Conservazione della Natura – Ministero dell'Ambiente.

4) L. Celesti-Grapow, F. Pretto, E. Carli, C. Blasi, editors. (2010). *Flora vascolare alloctona e invasiva delle regioni d'Italia*. Roma: Casa Editrice Università La Sapienza.

5) P. Gennari (1874). *Guida dell'Orto Botanico della R. Università di Cagliari*. Cagliari: Tip. Edit. dell'Avvenire di Sardegna.

6) A. Terracciano (1914). *La "Flora Sardo" di Michele Antonio Piazza da Villafranca: redatta con i suoi manoscritti*.

### 3.1 = ENDEMIC, PRIORITY AND RARE SPECIES IN THE HABITATS OF THE NATURE MAP OF SARDINIA

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Sardinian Island presents an high number of endemic species, as well habitat like as in the large islands of Mediterranean basin, thanks to the different climatic and geopedological conditions. The Sardinian Nature Map has been realized (1:50.000 scale) (1) based on the CORINE biotopes manual (2), with the correspondence to the classification systems of EUNIS and Nature 2000 (3). The good knowledge of Sardinian endemic flora allows to link it with each habitat and estimate its abundance in the whole Island. Sardinia is completely included in the Mediterranean biogeographic region, and then only marginally affected by habitat characteristics of the Continental and even less than the Alpine Region. Only in Gennargentu mountain is possible to consider a subalpine plan, but strongly influenced by the bioclimate and typical Mediterranean flora. The mountain areas are characterized by both shrubs (e.g. *Berberis aetnensis*, *Ruta corsica*, *Astragalus genargentus*) and herbaceous species (e.g. *Trisetum gracile*, *Armeria genargentea*, *Saxifraga cervicornis*, *Plantago sarda*, *Lamium corsicum*, *Carlina macrocephala*, *Festuca sardoa*, *Arrhenatherum sardoum*, *Saponaria alsinoides*, *Euphrasia genargentea*) (4). The Gennargentu top area has the highest number of endemics, while in the sandy dunes and rocky coasts can be found the largest number of endemic thermophilous species (e.g. *Linaria sardoa*, *Silene corsica*, *Silene velutina*). Often, the endemic species are very common (e.g. *Stachys glutinosa*, *Crocus minimus*, *Genista corsica*) belonging to different habitats from the sea level to the higher mountains, while others (e.g. *Genista sardoa*, *Astragalus maritimus*, *Centaurea horrida*) are very rare living only in exclusive coastal habitats. Some others (e.g. *Ribes sardoum*, *Rubus limbarae*, *Aquilegia nuragica*) present a very restricted area. In some cases, endemic or rare species belong to different habitats, increasing their interest and value. This work draw up by the methodology proposed by ISPRA, adopted at national level, highlights the specificities of different areas and the research identifies about 250 habitats, microhabitats or complex of habitats with an original description and measuring their surface in hectares. The study take into consideration the main 93 habitats mapped, including 80 DH habitats with about 20 priority, highlighting the presence of the endemic, priority and very rare species in the Island, (e.g. *Juniperus sibirica*, *Daphne oleoides*, *Rosa serafini*, *Sorbus aria*, *Sorbus praemorsa*, *Asplenium septentrionale*, *Blechnum spicant*, *Valeriana montana*, *Epilobium angustifolium*). Their status of conservation and the issues related to reduce the impacts and measures are proposed for their protection and management in situ and ex situ.

1) Camarda I., Carta L., Brunu A. 2010. Carta della Natura della Sardegna, Regione Autonoma della Sardegna, Istituto Superiore per la Protezione e la Ricerca Ambientale, Servizio Carta della Natura

2) EUROPEAN COMMISSION, 1991. CORINE Biotopes manual, habitats of the European Community. A method to identify and describe consistently sites of major importance for nature conservation. EUR 12587/3. Office for Official publications of the European Communities. Luxembourg

3) EUROPEAN COMMUNITIES, 1992. Direttiva 92/43/CEE del Consiglio del 21 maggio 1992 relativa alla conservazione degli habitat naturali e seminaturali e della flora e della fauna selvatiche, (Direttiva Habitat). GUCE n.206 del 22 luglio 1992

4) Arrigoni P.V. et al. 1977- 2002. Le piante endemiche della Sardegna 1-202. Bull. Soc. Sarda Sci.Nat..Vol.16-28. Gallizzi, Sassari

3.1 = *MONOTROPA HYPOPHEGEA* WALLR., A NEW RECORD FOR THE SICILIAN FLORA

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During field investigations in the Mountains around Palermo, an unusual population of *Monotropa* sp. was found. After revision of the taxonomic literature and herbarium specimens, it was referred to *Monotropa hypophegea* Wallr. This species is closely related to *M. hypopitys* L., and in the past was considered only a variety (1) or a subspecies (2) of it. More recently this entity has been considered an independent taxon deserving the rank of species (3). The most important morphological differences between the two taxa are the number of flowers in the raceme and the hairiness of sepals, petals and capsule. The individuals of the observed population have short racemes, with less than 8 flowers, are glabrous in all parts, including the sepals and inside petals and have glabrous, spherical capsules. On the contrary *M. hypopitys* has denser racemes (generally with 8-11 flowers), flowers hairy in the inner part of petals and in the sepals, and ovoid capsules. According to the literature (1) *M. hypophegea* is typical of deciduous forests, especially beech woods, while *M. hypopitys* prefers coniferous forests. However, in Sicily *M. hypopitys* is known only in the beech forests of the Madonie and the Nebrodi Mountains, with the lone exception of one locality in a black pine forest of the Etna Mountain (4). *M. hypophegea*, that was unknown in the island (4, 5), was found in an artificial *Pinus halepensis*, *Pinus pinea* and *Cupressus sempervirens* plantation. In particular, the locality where the species was found is in the Casaboli Wood at 790 m a.s.l., in the municipality of Monreale, near Palermo. It is an old reforestation with a high degree of dynamism to the return of natural vegetation, represented by *Quercus ilex* L. in the form of dense shrubs that constitute an undergrowth below the conifers. This discovery represents a further enrichment for the flora of Sicily. The Mountains around Palermo are one of the richest areas in terms of plant biodiversity in Sicily. This is underlined by the recent description of punctual endemic species such as *Silene kemoniana*, *Brassica trichocarpa*, *Hieracium busambarense* and *Sorbus busambarensis* (6, 7, 8, 9).



Fig.1 : *Monotropa hypophegea* (foto di Giuseppe Di Gregorio).



Fig. 2: Dettaglio del fiore.

- 1) S. Pignatti (1982) Flora d'Italia, 1-3. Edagricole, Bologna
- 2) B. Křisa (1972) *Monotropa* P. 5 in T.G. Tutin & al. Flora europaea, 3. Cambridge University Press, Cambridge
- 3) F. Conti, G. Abbate, A. Alessandrini, C. Blasi (2005) An annotated checklist of the Italian vascular flora. Palombi, Roma
- 4) G. Giardina, F.M. Raimondo, V. Spadaro (2007) *Bocconea*, 20, 5-582
- 5) F.M. Raimondo, G. Domina, V. Spadaro (2010) *Quad. Bot. Amb. Appl.*, 21(2010), 189-252
- 6) C. Brullo, S. Brullo, G. Giusso del Galdo, V. Ilardi, S. Sciandrello (2012) *Anales Jard. Bot. Madrid*, 69(2), 209-216
- 7) C. Brullo, S. Brullo, G. Giusso del Galdo, V. Ilardi (2013) *Phytotaxa*, 122(1), 45-60
- 8) O. Caldarella, L. Gianguzzi, G. Gottschlich (2014) *Pl. Biosyst.*, 148(3), 439-443
- 9) G. Castellano, P. Marino, F.M. Raimondo, V. Spadaro (2012) *Pl. Biosyst.*, 146(suppl.), 338-344

3.1 = THE BRYOPHYTES OF GORGO LUNGO, GORGO DEL DRAGO AND CODA DI RICCIO WETLANDS IN THE ORIENTED NATURE RESERVE “BOSCO DELLA FICUZZA, ROCCA BUSAMBRA, BOSCO DEL CAPPELLIERE AND GORGO DEL DRAGO” (PALERMO)

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In the framework of a research aimed at the acquisition of knowledge of the bryophyte diversity of wetlands in Sicily a study in humid environments Gorgo Lungo, Gorgo del Drago and Coda di Riccio, falling within the Oriented Nature Reserve “Bosco della Ficuzza, Rocca Busambra, Bosco del Cappelliere e Gorgo del Drago” (Palermo), was conducted. The first two can be considered temporary ponds, the third is a small permanent stretch of water.

The bryophyte floras of the investigated wet areas increase the number of taxa known in the protect area mentioned above and in Sicilian wetlands.

They show different taxonomic, chorological, biological and ecological traits.

The bryophyte flora of Gorgo Lungo is the most unaffected and has a greater number of species, a lower degree of emerobia and a more temperate connotation.

The bryophytes of Gorgo del Drago having a more pronounced hemerobic, thermophilous, photophilous and Mediterranean character, reflect the long period of drying up of the temporary pond and show the sign of a anthropization a little longer.

The few bryophytes found in Coda di Riccio are markedly hydrophilous and indicate a significant eutrophication of this site, which is quite frequented by grazing animals.

### 3.1 = PHYLOGEOGRAPHICAL INVESTIGATION ON *SILENE CORDIFOLIA* AND *VIOLA ARGENTERIA* ENDEMIC TO THE MARITIME ALPS: SIMILITUDE IN THEIR GENETIC HISTORY

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*Silene cordifolia* and *Viola argenteria* are plant species endemic to the Argentera Massif (Maritime Alps) and they are related to the siliceous rocks that characterize it; their origin have been fixed to 17 and less than one million years respectively by recent phylogenetic studies. *V. argenteria* arouses also a lot of biogeographical interest for the existence of disjunct populations in Corse.

19 locations were sampled and analyzed, for a total of 94 individuals of *S. cordifolia*, while 21 locations, for a total of 80 individuals of *V. argenteria*. Molecular analyses were conducted with the use of seven plastidial markers in both two species. The sequences of the cpDNA were analyzed using the Bayesian spatial partition. JModelTest 2.1.1 program allowed us to define the best evolution models, while Beast v1.8.0 was used to estimate the time of coalescence (tMRCA) sequences obtained for the two species. DNASP 4:10 was used to define the number and diversity of haplotypes, the number of segregating sites and nucleotide diversity. It was also tested the phylogeographic structure, comparing the value with the value NRC GST, using the program PERMUT. The spatial analysis of molecular variance (SAMOVA) was used to identify groups of geographically homogeneous and more differentiated and were considered a progressively increasing number of groups K from 2 to 10.

In *S. cordifolia* 12 different haplotypes were identified. The difference between the populations calculated according to the polymorphisms of the sequences of chloroplast DNA ( $G_{st} = 0.565$ ;  $SE = 0.0960$ ), indicated a strong gene structure of populations within the species which is congruent with the hypothesis of its ancient origin. The difference in allele ( $N_{st} = 0.749$ ,  $SE = 0.0835$ ) was higher but not significantly different from  $G_{st}$  indicating a weak differentiation between populations. The analysis carried out with SIDIER, clearly separated the populations into two main clusters: the people of the North-West and South-East. The analysis with SAMOVA identifies three different groups of people. The tree obtained from Beast clearly shows the presence of three groups of populations, which are differentiated at different times.

In *V. argenteria* 16 different haplotypes were identified. The difference between the populations calculated according to the polymorphisms of the sequences of chloroplast DNA ( $G_{st} = 0.760$   $SE = 0.0785$ ), indicated a strong gene structure of populations within the species. The difference in allele ( $N_{st} = 0.921$   $SE = 0.0486$ ) was higher and significantly different from the  $G_{st}$ , indicating a strong differentiation between populations. Based on the distance between populations, the analysis time with SIDIER, clearly separated the populations into two distinct clusters: the populations from North-West and from South-East. The analysis with Samova identified four different groups of populations.

In *S. cordifolia* and *V. argenteria*, the results obtained from SIDIER reveal the presence of two main groups of populations, which differ in the Northwest and Southeast, along the mountain range, similarly as previously for *Saxifraga florulenta*. It is interesting to note that the three species, despite their very different historical origins (15 years M - *S. cordifolia*, 7 years old M - *S. florulenta*; <1 M years - *V. argenteria*), all showing a similar phylogeographic pattern, witnessing how historical traces that remain in genetic patterns are all linked to a relatively recent history.

Regarding *V. argenteria*, the populations from Corse show an obvious affinity with a group of Alpes-Maritimes, and they are don't have any relationship among them. To explain the origin of these populations the detachment of the Sardo - Corse plate is to be excluded, occurring before the differentiation of *V. argenteria*. On the contrary, stochastic dissemination events are eligible and they are attributable to migratory birds (passerines probably) that follow the main route Sardo - Corse used to reach N Africa and Tropical Africa.

3.1 = *FLORA CRITICA D'ITALIA*: A SYNOPSIS OF *BORAGINACEAE* TRIBE *BORAGINEAE*

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According to the last APG treatment (1), which was adopted for the general taxonomic arrangement of the *Flora Critica d'Italia* project, *Boraginaceae* s.l are intended in a broad sense to include seven to nine subfamilies within a monophyletic group with a still uncertain position in the Asterid I clade (superorder *Asteranae*). Although this is not in line with the most recent phylogenetic evidence, showing that all these groups should be treated as separate families in the order *Boraginales* (2, 3), *Boraginaceae* s.s. (= subfam. *Boraginoideae*) comprise four main tribes: *Boragineae*, *Lithospermeae*, *Cynoglosseae* and *Echiochileae*. Except for the latter, all of these groups are represented in Italy, with a total of 29 genera and 115 species (4).

Following our previous work on subfamilies *Hydrophyloideae* and *Heliotropioideae* (5), a second contribution to the treatment of the family for *Flora Critica d'Italia* focused on one of the most typical groups of the family, the *Boragineae*. Species of this tribe are characterized by non-glochidiate nutlets provided with a basal elaiosome promoting dispersal by ants.

Our synopsis was mainly based on the critical study of herbarium material and extensive literature survey, integrated with additional elements from field collections and observations on natural populations.

A list of all the accepted (49) or synonymized (44) names (basionyms) of specific and infraspecific taxa which have been correctly or erroneously reported from the National territory was compiled. The bulk of our survey was aimed to a comprehensive review of all the types of these names and allowed us to identify one hundred specimens suitable for new typifications (3 holotypes, 23 lectotypes, 2 neotypes, 1 epitype and 71 among para- and/or isotypes). Only seven out of 93 names still wait to be fixed through the designation of a type.

Furthermore, all the relevant floristic reports were examined to better define the geographic distribution of the taxa over the National territory. As a final result, 12 genera (*Brunnera*, *Pentaglottis*, *Symphytum*, *Borago*, *Pulmonaria*, *Nonea*, *Melanortocarya*, *Hormuzakia*, *Cynoglottis*, *Lycopsis*, *Anchusella* and *Anchusa*) with 37 species were recognized, of which 7 are allochthonous and 6, plus one subspecies, are endemic to Italy.

Updated analytical keys were prepared for tribal, generic and specific rank, as well as original iconographies to help with species identification.

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## 3.1 = FUNGAL DIVERSITY IN URBAN FOREST ECOSYSTEMS

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In past and recent times the anthropic pressure strongly influenced the dynamic of forest ecosystems in Europe and led to a drastic decrease of forest cover and distribution mainly in unmanaged areas (1). The term “urban and community forests” refers to the trees and forests found in urbanized settings, in the center of cities and towns, in suburbs and rural communities, and at the edge of wild lands (2). Trees in the urban environment are subjected to a number of stresses which are very different from those suffered by trees in typical rural conditions (3). Biodiversity has been reduced in urban areas through ecosystem destruction, degradation, and fragmentation of remaining ecosystems. Recent investigation reveals that urban areas can contain relatively high levels of biodiversity. Important percentages of species found in the surrounding natural habitat, including endangered species, have been found in the urban forest (4). The relationship between silvicultural activities and decline of fungal communities in forest ecosystems has been highlighted by scientists (5). This abstract refers to investigation carried out in forest ecosystems that fall into the Oriented Nature Reserve “Pizzo Manolfo, Raffo Rosso and Crocetta Trippatore” that fall on the mountains overlooking the town of Palermo. The main forest types in this area are: a) reforestation with *Eucalyptus camaldulensis* Dehnh; b) reforestation with *Pinus halepensis* Miller and *P. pinea* L. and, c) reforestation with elements of natural vegetation (6). Two plots [size 200 m<sup>2</sup> (10 m × 20 m)] were delimited in each forest types, in one of which silvicultural practices were interrupted. 83 macromycetes collected over three years were listed and recorded in the plots as follow: 1) reforestation with *E. camaldulensis* (10 taxa in plots subjected to silvicultural practices and 11 taxa in plots not subjected to silvicultural practices); 2) reforestation with *P. halepensis* and *P. pinea* (9 taxa in plots subjected to silvicultural practices and 5 taxa in plots not subjected to silvicultural practices); 3) reforestation with elements of natural vegetation (29 taxa in plots subjected to silvicultural practices and 8 taxa in plots not subjected to silvicultural practices). We also collected fungi in the surroundings of each plots and the number of species recorded was 37, 28 and, 15 taxa. On the basis of the data processing it is to be noted that the number of ectomyorrhizal fungi is low and below the threshold of 20% while more numerous are saprotrophs Sh, Pn(Sh), Sh(Pn), St, sensu Arnolds (7). Besides saprotrophs on wood are prevalent in plots not subjected to silvicultural practices. This confirms the need to prevent the removal of biomass in forest ecosystems in order to maintain wood-inhabiting fungi species which represent a highly species rich and ecologically important organism group in natural forests (8). Forest ecosystems seemingly trivial such as reforestation of conifers continue to devote significant findings. This is the case of *Mycena pseudoinclinata* A.H. Sm., recently reported for the first time in Italy (9), whose distribution was limited to France, Switzerland, China and, United States. Besides, *Ceriporia griseoviolascens* M. Pieri & B. Riv (*Polyporaceae* s.l.) was collected in decaying stumps of *P. pinea*. The stone pine wood is a new host for this species previously reported only on *Salix* sp. and *Populus nigra* L. in France and on *Arbutus unedo* L. in Sardinia.

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## 3.1 = INVESTIGATION ON OLD-GROWTH FORESTS OF SICILY: PRELIMINARY RESULTS

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Old-growth forests are natural forests that have developed over a long period of time, without experiencing severe, stand-replacing disturbance a fire, windstorm, or logging (1). According to UNEP/CBD/SBSTTA definition (2), an old-growth forest is a primary or a secondary forest which has achieved an age at which structure and species normally associated with old primary forests of that type have sufficiently accumulated to act as a forest ecosystem distinct from any younger age class. In a thematic contribution to the National Biodiversity Strategy (3, 4), the authors report the following definition of Italian old-growth forests: Forest in which human disturbance is absent or negligible, and in which natural dynamics create a mosaic of all the forest regeneration phases, including the senescing one. Such phase is characterized by large old trees, deadwood (snags logs and coarse woody debris) and a vascular plant species composition that is consistent with the biogeographical context and includes highly specialized taxa related to the small-scale disturbance and the microhabitats resulting from structural heterogeneity. In Sicily, 472 hectare were recognized as old-growth forests mainly distributed in Regional Natural Parks, Reserves, Site of Community Importance (SCI) and, Special Protected Areas (SPA). In particular, 18 forest areas, located over 1000 m of altitude and characterized by high index of woodiness, were surveyed in the provinces of Caltanissetta, Catania, Messina and Palermo. In the frame of a research project granted by the University of Palermo, an investigation was carried out in the “Bosco Pomieri”, an old-growth forest included in the Madonie Park (N.-Sicily). In 2013, a multidisciplinary research team started to analyse vascular plant and the cryptogamic diversity, and also carrying out some plant physiology tests and an environmental monitoring. The “Bosco Pomieri” is a mixed oak wood characterized by the presence of many old trees of *Quercus petraea* (Matt.) Liebl. subsp. *austrorhynrenica* Brullo, Guarino & Siracusa and a dense shrub layer with *Ilex aquifolium* L. In the forest area we also find *Fagus sylvatica* L., *Acer pseudoplatanus* L., *A. obtusatum* W. & K., *Ulmus glabra* Huds., *Sorbus torminalis* L., *Malus sylvestris* (L.) Mill., and, sporadically, *Quercus cerris* L. and *Q. pubescens* s.l. Shrubby species, such as *Daphne laureola* L., *Crataegus laciniata* Ucria, *Ruscus aculeatus* L. and, *Rhamnus catharticus* L., are also present in the investigated area (5, 6). Preliminary results of the investigation carried out in the “Bosco Pomieri” are here reported. At present, 9 benthic algal taxa of algae belonging to the Divisions *Cyanophyta*, *Rhodophyta*, *Chlorophyta*, and *Bacillariophyta* are currently listed. 74 lichens, mainly epiphytic, belonging to the genera *Caloplaca* Th. Fr., *Lecanora* Ach., *Physcia* (Schreb.) Michx. and *Ramalina* Ach. Fungi are currently represented by 62 saprotrophic, parasitic and mycorrhizal species.

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### 3.1 = PLANT LANDSCAPE OF RAS DIMAS PENINSULA (GOVERNATORATE OF MONASTIR, CENTRAL COASTAL TUNISIA)

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The Ras Dimas lagoon lies along the coast of Central Tunisia (35°37'45.84"N, 11° 2'44.61"E), in the region of Sahel, about 20 km linear distance to the south from Monastir and about 160 km from Tunis.

The lagoon is made up by a sandy peninsula with white sands rich in limestone with fine granulometry, about 3 km long, up to 200 m wide and up to 2 m high, that contains about 120 hectares of sea. Previously this peninsula was regularly isolated from the continent forming the island of Edzira, in the last years, sea storms and the different streams course, likely caused by the restructuring of the Port of Bekalta, has given the actual configuration of a peninsula.

In April 2013, as part of the Initiative PIM, a scientific mission, initially planned in the Island of Kuriat, has been moved to the coast of central Tunisia due to bad weather conditions. This shift made possible the floristic survey of areas with great naturalistic value, still not well floristically known.

During this mission, phytosociological relevés have been taken and herbarium specimens have been collected and stored in the *Herbarium Mediterraneum Panormitanum* (PAL); plant identification has been done with the help of the Flora of Italy (1) and of Tunisia (2, 3) and by direct comparison with the exsiccata housed in PAL.

The distribution of taxa and phytocoenoses resulted to be influenced by elevation and distance from the sea shore. The studied vegetation is characterized by psammophilous and halophytic communities. Flat areas near the sea are colonized by communities referable to the class *Cakiletea maritima*, followed inwards by a belt of embryo dunes that houses perennial rhizomatous *Poaceae* characterizing the class *Ammophiletea* and related *syn taxa*. The flat, periodically flooded, areas behind the dunes are dominated by *Juncus maritimus* Lam., in contact with saltmarshes dominated by *Sarcocornia perennis* (Mill.) A.J. Scott (classes *Juncetea maritimi* and *Sarcocornietea fruticosae*, respectively). Where the altitude is some decimetres a.s.l., a more mature vegetation dominated by small cushion shaped chamaephytes belonging to *Crucianelletalia maritima*. Near the inner part of the lagoon, where the elevation is a bit higher (some dm a.s.l.), there is a grassland of *Lygeum spartum* L. On the inner, on stabilized dunes, there are few remnants of a psammophilous maquis dominated by *Retama retam* subsp. *bovei* (Spach) Talavera & Gibbs, ascribed to the class *Quercetea ilicis* (Oleo-Ceratonion).

Taxa of particular biogeographic interest found in the surveyed area are: *Anagallis monelli* L., *Echiochilon fruticosum* Desf., *Helianthemum stipulatum* (Forssk.) C. Chr., *Linaria aegyptiaca* (L.) Dumort., *Silene succulenta* Forssk. and *Stachys arenaria* Vahl.

*Cuscuta palaestina* Boiss. subsp. *palaestina* and *Carduus argyrea* Biv. are here reported for the second time from Tunisia (4, 5).

The exploration of the peninsula of Ras Dimas, allowed us to collect and study many taxa and to gather an unprecedented herbarium and photographic documentation of the flora and plant communities. Further research is aimed, that will provide new data also in comparison with the previous vegetation cover of the area.

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### 3.1 = PLANT SPECIES PROTECTED UNDER HABITATS DIRECTIVE: ITALIAN NATIONAL REPORT FOR THE PERIOD 2007-2012

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The Council Directive 92/43/EEC (Habitats Directive) aims to maintain or restore natural habitats and wild species listed on the Annexes I, II, IV and V at a favourable conservation status. Article 11 of the Directive requires Member States to monitor the habitats and species listed in the Annexes, and Article 17 requires, every 6 years, a National Report on the implementation of the Directive, to be submitted to the European Commission. Assessment and reporting must follow standard methodology and format (1) also with the aim to facilitate aggregation and comparisons between Member States.

The 3<sup>rd</sup> Italian National Report (period 2007-2012), submitted in 2013, was carried out through a collaboration among the Ministry of the Environment, the Italian Institute for Environmental Protection and Research, the Regions and Autonomous Provinces and the main national scientific societies and experts. The Report includes maps, datasets and assessment of the conservation status of species and habitats referring to the whole national territory, not only to the Natura 2000 Network.

The official European checklist for Italy includes 113 plant *taxa* (lichens, bryophytes and vascular plants); 90 of these species are listed on Annex II (32 of priority interest), while 23 *taxa* (20 species and 3 genera) are exclusive of the Annexes IV and V. The reporting process for plant was based on the most up-dated information available, consisting in both published and unpublished sources, taking into account also the results of the project for the Red List of the Italian flora, carried out by the Italian Botanical Society (2). A significant contribution came from regional flora experts for a set of 44 species.

The results of the 3<sup>rd</sup> report show an "unfavourable" ("inadequate" or "bad") conservation status for half of all plant *taxa*; the percentage grows to 65% if only species on Annex II are considered (3). The deterioration of the situation of plant species compared to the previous reporting (2<sup>nd</sup> Report, period 2001-2006) may reflect both an improvement of the level of knowledge, but also for some cases a worsening of the conservation status. Despite the advances in terms of knowledge, the report still shows data gaps for a certain number of species (16% of "unknown" conservation status).

The severe level of pressures affecting our country (inadequate agricultural practices, urbanization, hydraulic and other ecosystem modifications caused by man) stresses the need of application of severe conservation measures and the importance of ensuring adequate monitoring activities. In this context it should be noted also that in Italy the high number of plant species of community interest, combined with their elevated rate of endemism (about 50%), underlines a special responsibility of our country. Then it would seem urgent to expand research and monitoring activities, also to address some specific requests of the reporting method.

Data availability: EIONET Reference Portal and ISPRA website ([http://bd.eionet.europa.eu/activities/Reporting/Article\\_17;www.sinanet.isprambiente.it/Reporting\\_Dir\\_Habitat](http://bd.eionet.europa.eu/activities/Reporting/Article_17;www.sinanet.isprambiente.it/Reporting_Dir_Habitat)).

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### 3.1 LIFE FAGUS: A PROJECT FOR THE ENHANCEMENT OF STRUCTURAL HETEROGENEITY AND BIODIVERSITY IN APENNINE BEECH FORESTS (HABITAT 9210\* AND 9220\*)

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The Life+ project (2012-2016) “FAGUS - Forests of the Apennines: Good practices to conjugate Use and Sustainability” aims at ensuring long term conservation of the Apennine beech Natura 2000 priority habitats 9210\* 9220\*, i.e. beech forests where European yew (*Taxus baccata*), European holly (*Ilex aquifolium*), and silver fir (*Abies alba*) occur. These habitats are remnants of ancient, more extensive forests, that have been extensively altered and reduced in extension. Indeed, traditional forestry practices transformed these habitats into pure beech stands, for example by repeated coppicing, with cascading consequences on the diversity of other taxonomic groups, e.g. saproxylic fungi and beetles.

Focus of the project is to test in two Italian National Parks (Cilento and Vallo di Diano and Gran Sasso Laga) experimental harvesting practices aimed at enhancing structural heterogeneity, as a way to accelerate the development of old-growth attributes and, accordingly, to increase diversity levels for focus taxa.

According to this approach, the actions of the FAGUS project are:

- i. Preparatory actions: assessment of the current habitat condition in the project area. Sampling of forest structure, composition and diversity of vascular plants, lichens, birds, saproxylic fungi and beetles;
- ii. Concrete conservation actions: implementation of experimental harvesting treatments to promote the regeneration of yew, holly and silver fir; creation of habitat trees, deadwood and gaps to enhance the diversity levels of focus taxa;
- iii. Monitoring actions: sampling of forest structure, composition and diversity of focus taxa before and after concrete conservation actions, evaluations of the outcomes of experimental treatments;
- iv. Dissemination Actions: website, promotional material meetings, courses, conferences, notice boards.

The project, that is still ongoing, will contribute to the development of Sustainable Management Strategies for the habitats 9210\* and 9220\*, and to disseminate the advantages of the experimented approach to local stakeholders as well as to a wider public.

## 3.1 = ANALYSIS OF BRYOPHYTE'S SPORES IN MEDITERRANEAN TEMPORARY PONDS

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The persistence and abundance of most of the species in an area are the result of a dynamic balance between the duration of the habitat and the ability of species to colonize such environments through the production and dispersion of spores. This study presents a preliminary analysis of bryophyte's spores from the *pauli* of the Giara of Gesturi (C Sardinia). The Giare (average altitude 550 m a.s.l.) are basaltic plateaus that rise unexpectedly from the plain of the country, as huge natural fortresses. Several authors have studied the population dynamics of some bryophytes confined to fragmented and temporary habitat (1, 2, 3) and, in particular, studies conducted by Herben & Söderström (4) show that the most important factor that regulates the survival of the species is the ability to form new colonies on substrates fragmented. The aim of this study is to determine what are the species that show a better fitness for survival and adaptation in Mediterranean temporary ponds, in order to evaluate the conservation status. In Europe, Mediterranean temporary ponds are indicated as priority natural habitats under the Habitats Directive 92/43/EEC (5): in those habitats bryophytes are recognized to have an important ecological function. Thirty soil samples were collected in November (rainy season) in three ponds until the depths of 7 cm. The samples were taken and subdivided in seven portions, which were collected for obtaining an *ex situ* germination of spores, at the Botanical Garden of University of Cagliari. The results showed the capacity of some genus and two species to germinate in *ex-situ* conditions: *Bryum* sp., *Poblia* sp., *Tortula* sp., *Fossombronia* sp., *Riccia canaliculata* Hoffm. and *Archidium alternifolium* (Hedw.) Mitt.. It is possible to observe as these bryophytes are the most recurring in the study area, showing a better fitness for survival and adaptation to these environments.

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### 3.1 = PLANT BIODIVERSITY LEARNING THROUGH RECREATIVE AND EDUCATIONAL EXPERIENCE

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The Botanic Garden of the Department of Biological and Environmental Sciences and Technologies (DiSTeBA) of the Salento University devotes special educational activities at schools of all levels. Other botanic gardens have initiated environmental education programs in Salento, thus encouraging the teaching of botanic sciences. With the aim to jointly promote educational activities replicable in any places such as parks, reserves, oases, etc., the staff of the Salento University Botanic Garden and the staff of the "Garden of secrets", with the supervision of a psychologist specialized in psychodynamic, formed a special working group. Among the different educational experiences offered to schools by each botanic garden, the dissemination of the knowledge of plant biodiversity of Salento, and its importance and value as well, was chosen. The main goal was to investigate how the concept of biological diversity is communicated, and transferred to the student personality, in full accordance with the Ministerial Decree 27/12/2012 which states "Tools intervention for students with special educational needs and territorial organization for school inclusion" (1).

Three Comprehensive Institute operating in different regional contexts and a total of 120 students of the second class, were involved in the activities. Two meetings, one in classroom and the other outdoor, were planned for each class. The psychologist's task was to track the relational dynamics emerging within the groups during the class activity. The working group members were introduced to the students as "Nature operators" needing their suggestions to learn about and improve the environment. Each student introduced himself and several working groups (indicated by the names of both plants and birds) were formed. To assess the student knowledge on biodiversity, a multiple choice questionnaire was given. Then the photo (A3-sized) of a bare lawn and numerous puzzle pieces was given to the students of each group with the aim to evaluate their ability in selecting the puzzle pieces referring to meadow and in assembling them. Of course the choice of the puzzle pieces has forced the students of each group to a mutual comparison, for the immediate communication between peers, for the internal organization to the division of puzzle pieces, and for the compliance with the rules and time. Bales' model was used to observe the group dynamics (2).

Field visits to the Salento University Botanic Garden and to the "Garden of Secrets" were performed: therefore the meadow was directly explored in all its components. Firstly each student introduced himself again and the same student groups were formed. The themes of plant biodiversity and plant value, both from the ecological and the human perspectives, were briefly discussed; therefore, to better understand how many plant species were present on the examined surface, we proceeded to the collection. Even for this activity, the groups had to respect specific rules and times: each group displayed the collected plants on a table ("Framework of the Biodiversity"), arranging them according to aesthetic criteria. In order to evaluate the work of each group, the number of collected species, and the correct plant sampling and arrangement on the table were taken into account. For each table, an expert identified the plant species and the composition of the "Framework of the Biodiversity" containing all the collected plants. Even for these activities the groups were monitored according to Bales' scheme. Finally, the same questionnaire on plant biodiversity was repeated, allowing an evaluation of the learning experience of the students. The understanding of the tasks was clear, both for the activities in classroom (70%) and outdoor (95%); the tests were easy to perform (70% in classroom, 95% outdoor); the level of participation (75% in classroom, 85% outdoor) and the integration of knowledge (90% in classroom, 90% outdoor) resulted high.

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2) [http://www.sociologia.unimib.it/DATA/Insegnamenti/14\\_3650/materiale/argomento%204%20-%20la%20comunicazione%20nei%20gruppi.pdf](http://www.sociologia.unimib.it/DATA/Insegnamenti/14_3650/materiale/argomento%204%20-%20la%20comunicazione%20nei%20gruppi.pdf)

3.1 = DISTRIBUTION RANGE OF FOUR INVASIVE ALIEN SPECIES IN ITALY: *AMBROSIA ARTEMISIIFOLIA* L., *REYNOUTRIA JAPONICA* HOUTT., *PRUNUS SEROTINA* EHRH., *SENECIO INAEQUIDENS* DC

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Invasive alien species are considered to be a major threat to native ecosystems and may have a negative impact on crop production, biodiversity and human health due to their toxicity or allergenic pollen. During the last decades in Italy, the occurrences of numerous alien species have been recorded in the Italian Flora. Four species were considered extremely invasive due to their high rate of occurrence both in ruderal and natural habitats: *Ambrosia artemisiifolia* L. (Fig. 1), *Reynoutria japonica* Houtt., *Prunus serotina* Ehrh., *Senecio inaequidens* DC.

The aim of this research was to reconstruct the spread of these invasive alien species across Italy using herbarium specimens (1,2) to represent the history of their invasion process and their habitat preferences.

Species occurrence points derived from herbarium data (Fig. 2) were georeferenced in order to reconstruct the range maps of such species. Distributional patterns of the four investigated species highlight that the core area of their invasion process is in northern Italy (Lombardia and Piemonte). As regards the habitat preference, *A. artemisiifolia* and *S. inaequidens* colonize road sides and abandoned fields, *R. japonica* river areas, while *P. serotina* prefers forest areas along river valleys. *A. artemisiifolia* was originally reported in the wild during the first years of 20th century in the area of Alba (TO). The other species seem to be of late colonization in Italy, as most herbarium specimens have been collected after 1950.

The assemblage of national databases on the distribution of invasive alien species is the first step to implement monitoring programs on their effective spreading and then to adopt proper contrasting measures.



Fig. 1. *Ambrosia artemisiifolia*



Fig. 2. Herbarium specimen of *Senecio inaequidens*

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3.1 = *CHAMAEROPS HUMILIS* L.: ITALIAN DISTRIBUTION AND CHARACTERISATION

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The number of natural populations of European fan palm (*Chamaerops humilis* L.) in Italy is continuously declining due to anthropic disturbance. Nonetheless, this species is pivotal in the thermo Mediterranean vegetation belt, where it plays an important ecological role and this induced the Italian government to envisage for its protection within Special Areas of Conservation, where genetic contaminations from cultivated plants is reduced.

However, the information about the actual distribution and size of *C. humilis* natural populations in Italy and the genetic and morphological variability of this species, are still insufficient.

Aim of the present work was collecting natural populations of European fan palm in Italy, outline its distribution and, basing on a fine scale study performed in Sicily (1), study its genetic and morphological variability, which are crucial to structure efficient strategies for its conservation.

The relations between the plants' morphological traits and climatic variables were studied to highlight which are the most important plant traits in the adaptation to the environment.

Eighteen natural populations in Italy were sampled as follows: 10 from Sicily, 5 from Sardinia, 1 from Lazio (Parco del Circeo, LT), 2 remnant populations from Tuscany (Parco della Maremma, GR) and Liguria (Parco Regionale di Portofino, GE). Each population was evaluated based on 10 morphological traits as follows: trunk height (HT); crown height (HF); trunk diameter (DT); median leaflet length (CLL); width of median leaflet (WCL); external leaflet length (ELL); hair density (HD); crown diameter to crown height ratio (DF:HT); length of lamina forking to median leaflet length ratio (LF:CLL); thorn density (TD). In addition, a leaf sample was collected to evaluate genetic distances basing on specific SSR (2). Finally, seed from each populations were collected and used to establish an ex-situ collection at Unità di ricerca per il recupero e la valorizzazione delle specie floricole mediterranee (CRA-SFM, Bagheria, PA) for further studies and conservation purposes. Preliminary results of plant morphology and its relation with the environment will be presented.

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### 3.1 = PRELIMINARY CHARACTERIZATION OF AN ANCIENT COLORED FLINT MAIZE CULTIVAR, ORIGINATING FROM VALCAMONICA (BS), ITALY

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After the discovery of the Americas, maize cultivation became diffused in Europe and elsewhere around the world [1-3], where hundreds of different landraces have been used to feed the local populations [4]. In fact historically these different corn varieties were used to make locally typical meals such as polenta, a very popular dish in the northern Italian regions [4]. However in the second half of the last century the American dent hybrids began to take the place of these less productive varieties, that almost disappeared [5]. Nowadays the characterization of traditional landraces can help breeders to discover precious alleles that could be useful for modern genetic improvement. Furthermore the detailed characterization of these open pollinated varieties (opv<sub>s</sub>) will assess their value, find out the correct conservation and be able to help further studies. In this work we characterized the ancient coloured cultivar “Spinato from Esine”, typical of the Italian Valcamonica (BS). First of all it was cultivated in the experimental field to measure some agronomic parameter and to understand its genetics. Then some trials have been performed to better characterize its kernel colour peculiarity: a preliminary spectrophotometric analysis revealed the presence of flavonols, phenolic acids and of the phlobaphenes, probably the responsible of the ear coloration. Phlobaphenes are reddish substances synthesized in maize through the flavonoids pathway with the polymerization of the flavan-4-ols [6]. The molecular analysis confirmed the presence of the *pericarp1 gene (p1)*, a transcription factor driving the accumulation of these pigments in the pericarp layers, as attested by the histological analysis. However phlobaphenes are manifold molecules, difficult to extract and to solubilize, therefore some idrolization trials have been performed to detect the basic monomers.

In the mean time HPLC analyses showed also the presence of carotenoids and probably thanks to the high amount of the whole pigments, this maize variety showed a very high antioxidant ability. Given the chronic disease prevention property of the antioxidant molecules [7], this variety could become very interesting also by a nutritional point of view. Besides this kind of characterization will allow a precise identification of this landrace and of its alleles that can help to improve the biodiversity knowledge and to enhance the mountain area.

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### 3.1 = CHARACTERIZATION OF LIPOPHILIC ANTIOXIDANT PROFILE IN WHOLE KERNELS OF DURUM WHEAT LINES FROM SICILY

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Differences among durum wheat (*Triticum durum*, Desf.) lines are well characterized from a genetic point of view, however, little is known about how these variations affect lipophilic antioxidant properties of wheat caryopsides. Carotenoids and tocochromanols are the main lipophilic antioxidants found in whole durum wheat grains, but, due to their not homogeneous distribution within the different tissues, a strong decrease of these molecules occurs during semolina production leading to a depletion in the nutritional quality and colour characteristics of the refined product. The colour of semolina is an important parameter in defining the quality of the pasta and bakery products.

In this work, the flour obtained by grinding whole kernels of 10 durum wheat lines (Anco Marzio, Cicireddo, Kamut, Madonie, Realforte bianco, Russello Pietranera, Scorsonera, Timilia Coreras, Valbelice e Vertola) from Sicily were quali-quantitatively analyzed and compared for their carotenoid and tocochromanol contents. Carotenoids and tocochromanols were simultaneously extracted and assayed by the method of Fraser et al. (1). All data were subjected to statistical analysis based on a one-way ANOVA test. Holm-Sidak Test method was applied to establish significant differences between means ( $p < 0.05$ ).

Highly significant differences were evidenced among lines in the content of total carotenoids ( $F = 9.654$ ;  $P < 0.001$ ) and tocochromanols ( $F = 5.630$ ;  $P < 0.001$ ). Madonie and Anco Marzio were the lines with the highest and lowest amounts of total carotenoids ( $50.4 \pm 9.5 \mu\text{g}/100 \text{ g dw}$  and  $20.1 \pm 3.3 \mu\text{g}/100 \text{ g dw}$ , respectively), while Vertola and Scorsonera ranked first and last for total tocochromanol content ( $2658 \pm 355 \mu\text{g}/100 \text{ g dw}$  and  $1456 \pm 228 \mu\text{g}/100 \text{ g dw}$ , respectively). In Anco Marzio, Kamut, Realforte bianco, Russello Pietranera, Scorsonera and Valbelice lines, lutein was the most abundant carotenoid in the kernel, while in the other lines (Cicireddo, Madonie, Timilia Coreras and Vertola)  $\alpha$ - and  $\beta$ -carotene predominated. Small amount of zeaxanthin ( $1.6$ - $4.0 \mu\text{g}/100 \text{ g dw}$ ) and  $\beta$ -cryptoxanthin ( $1.0$ - $2.5 \mu\text{g}/100 \text{ g dw}$ ) were also detected in all lines (Fig. 1). Tocotrienols largely exceeded tocopherols in all the assayed lines, with  $\delta$ -tocotrienol isoform contributing to 44-64% of the total tocochromanol content. These results confirm the importance of wheat whole kernels as a source of phytochemical compounds in human nutrition, pointing out a significant variability in the cultivated germplasm and in local wheat populations.

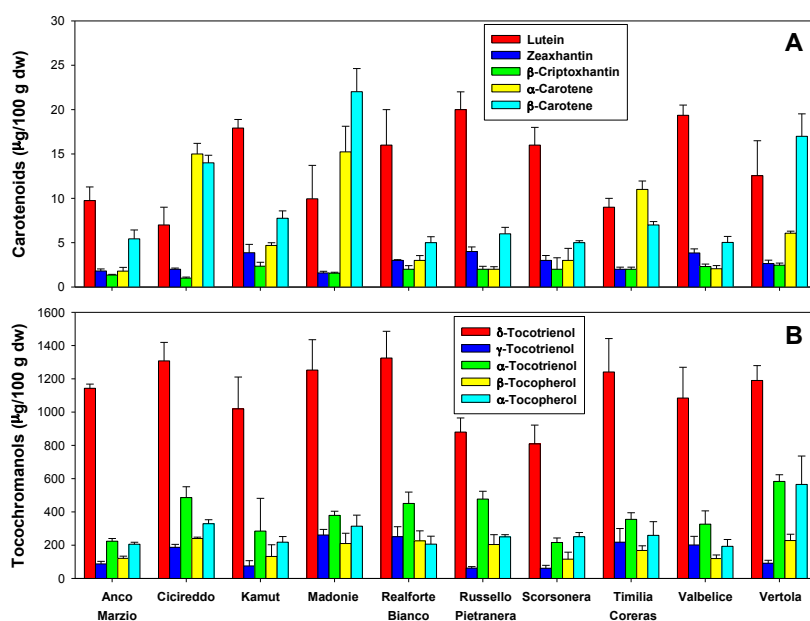


Fig. 1. Amount of carotenoids (A) and tocochromanols (B) in whole kernels of durum wheat lines from Sicily. Values, expressed as  $\mu\text{g}/100 \text{ g dw}$ , are the average  $\pm$  SD of four independent replicates ( $n=4$ ).

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## 3.1 = CSMON-LIFE: DATA FROM THE PEOPLE, DATA FOR THE PEOPLE

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The Project CSMON-LIFE was funded by the European commission in the framework of the LIFE+ programme, and it officially started in June, 2014, with the aim of contributing to the dissemination in Italy of a Citizen Science approach in collecting biodiversity data. Citizen Science approaches are well developed in Northern Europe, Northern America and Australia, and are becoming more and more common all over the world. In Europe especially, the Open Air Laboratories (OPAL) project in the British Isles demonstrated the strength of Citizen Science approaches in collecting reliable biodiversity data, and led to the formation of the European Citizen Science Association (ECSA). In Italy there exist several Citizen Science initiatives, from local to national level. CSMON-LIFE will build on previous experiences to face several environmental problems by monitoring target species, hence contributing to improve and enlarge the knowledge base for biodiversity policy in Italy. The project will promote active collaboration among scientists, public administrations and citizens in discovering, monitoring and protecting biodiversity. Citizens will be involved in data collection and validation, thus accelerating the progress towards the objectives of the European 2020 biodiversity strategy. This approach will also allow to disseminate rigorous information on the biodiversity of the country and its problems. Hence, citizens will become more aware of the importance of conservation and management of biodiversity at local and global scale, will have a better understanding of environmental policies, and will be able to ask for new strategic approaches when and where necessary. CSMON-LIFE will make extensive use of ICT, such as smartphones and tablets, to collect primary biodiversity data, which will be integrated into the databases of the Italian National Biodiversity Network. As far as botanical data are specifically concerned, CSMON-LIFE will focus on three groups of taxa: alien, invasive and rare species, in the context of global warming, biodiversity loss and conservation.

### 3.1 = AN INTEGRATED MONITORING METHOD TO DETECT BIODIVERSITY OF *POSIDONIA OCEANICA* (L.) DELILE HABITAT

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The conservation of seagrass habitat is a priority in the environmental policy programs: several integrated and innovative monitoring methods were developed and applied in particular coastal areas where seagrass loss has been recorded (1, 2).

Recently spatial ecology is mainly interested in unifying the spatial dynamics with covering topics, such as species growth and biodiversity, through the application of remote sensing techniques. Indeed it is possible monitoring the effects of shifts in species distribution, and of the species conservation within patchy and threatened habitats as well, by space innovative satellites.

Between the years 2004 and 2013, the genetic structure of *Posidonia oceanica* meadows was monitored along the coast of Lazio (Italy, Central Tyrrhenian Sea): the meadows showed an adaptive response to environmental pressure in these heterogeneous areas. In order to detect the synoptic distribution of this species within the study area, genetic, morphological and physiological data were integrated in monitoring mapping programs by means of remote sensing techniques. Measurements of several biophysical parameters (biomass, shoot density, phenology and genetic markers) of *P. oceanica* were acquired at several study sites located along the coastline between Saline di Tarquinia and S. Marinella and exploited for remotely sensed data calibration.

Moreover in the area between Civitavecchia and S. Marinella measurements of sea biophysical parameters were processed for each point with the imagery data obtained by 12 bands. Furthermore, Daedalus airborne multispectral sensor calibration we performed with the support of satellite (MERIS) derived water quality parameters.

This innovative mapping tool (3, 4) allowed us to produce an improved thematic map of the local *P. oceanica* distribution, demonstrating the usefulness of the method in this coastal area that includes rapidly changing environments and frequently turbid waters, mainly due to both anthropogenic activities (industry, urbanization and tourism) and to natural processes (hydro-dynamism and coastal erosion). Therefore it was possible to effectively assessing the ecological status of *P. oceanica* meadows on the basis of multidisciplinary techniques.

In conclusion the evaluation of the polymorphism of natural populations, detected by molecular markers, and all the tested parameters as well, are required in the studies involving changes in genetic structure of seagrass meadows.

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### 3.1 = THE “*ARBORETUM AETNEUM*” OF THE NUOVA GUSSONEA BOTANIC GARDEN ON MOUNT ETNA

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The *Nuova Gussonea* botanical garden was constituted in 1979. It is located on the south-western slope of Mt. Etna, at 1770 m a.s.l., within the Mt. Etna Natural Park. Its very wide surface (> 10 hectares) has been divided in different sectors according to the aims of the garden.

It is organized on the basis of synecological principles, since the species belonging to the Etnean flora, after their introduction in the garden, are organized in communities as in natural condition.

This work highlights the *Arboretum*, called “*Arboretum aetneum*”. The study was carried out through field relevés and personal unpublished data, some of which deriving from cartographic relevés.

The *Arboretum aetneum* includes some areas with natural woody vegetation characterized by *Pinus nigra* Arnold subsp. *calabrica* (Loud.) A. E. Murray and other areas with natural bush vegetation characterized by *Genista aetnensis* (Raf.) DC. The other areas of the *Arboretum* are constituted by different artificial phytocoenosis planted in the years 1979 and 1980. They are respectively characterized by *Betula aetnensis* Raf., *Fagus sylvatica* L., *Quercus cerris* L., *Quercus* belonging to the *Q. robur* group, *Quercus ilex* L. subsp. *ilex*, and deciduous broad leaves trees, *Quercus suber* L. In each artificial phytocoenosis, some species belonging to the respective natural community, have been planted. Some of them spontaneously spread in the phytocoenosis to which they belong (1).

The dendrological collection of the *Arboretum* started some years ago in a large area (2), where tree and bush species of the native Etnean flora, more than 100 (2, 3, 4), were planted. The presence in the garden of such a large *Arboretum* and its plant variety produced significant results about acclimatization of thermophilous species, usually living at lower altitudes (like *Quercus ilex* subsp. *ilex*, *Q. suber*, *Fraxinus ornus* L., *Ostrya carpinifolia* Scop., *Salix* sp. pl., *Populus nigra* L., etc.) and spontaneous spreading of introduced species in the typical phytocoenosis (such as *Fraxinus ornus*, *Ostrya carpinifolia*, *Acer obtusatum* Waldst. & Kit. ex Willd. and some herbaceous species). This further outlines how the garden offers very favorable habitats to species from other sites, altitude and different woody vegetation (2, 5, 6).

Moreover, the *Arboretum* plantation allowed, the *ex situ* conservation of very significant woody species of the Etnean flora like the endemic *Betula aetnensis*; rare or very rare species, such as *Salix alba* L. subsp. *alba*, *S. alba* L. subsp. *vitellina* (L.) Arcang., *S. pedicellata* Desf., *S. caprea* L., *Evonymus europaeus* L., *Erica arborea* L., *Ilex aquifolium* L., *Ostrya carpinifolia*, *Acer campestre* L., *A. platanoides* L., etc.; species threatened in the Etnean territory, such as *Quercus suber* and *Pistacia lentiscus* L.

The garden, therefore, plays an important role in the *ex situ* conservation of biodiversity, especially because it is located inside the B zone of the Natural Park of Mt. Etna. Moreover the *Arboretum aetneum* constitutes a very important institution for scientific and educational purposes too.

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### 3.1 = BRYOPHYTE VEGETATION DIVERSITY FOR MONITORING THE ANTHROPIC DISTURBANCE: A STUDY ON THE EOLIAN ISLANDS (SICILY)

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The Aeolian islands, listed by UNESCO as World Heritage sites, arouse a strong attraction that is expressed in a powerful and constant tourist call. This is very positive for improving the economy of these islands, but poses a serious risk for maintaining their natural heritage. In fact, from a naturalistic point of view, the Aeolian islands deserve a strong consideration for their historical, geological and climatic peculiarities, which allow the establishment of a typical Mediterranean flora and vegetation.

Geologically, these islands represent the emerged part of a large volcanic, mainly submarine, complex with an arcuate structure, named Aeolian Arc. This complex extends in the Tyrrhenian Sea at north of Sicily for about 200 kilometers in an area of geological instability caused by the collision of the African continental shelf toward Europe.

Despite their recent origin, dating back to about 1 million years ago, the Aeolian islands are quite well studied from the floristic point of view. Among the phanerogams we quote some endemic species, e.g. *Centaurea aeolica* Guss., *Daucus folius* Guss., *Cytisus aeolicus* Guss. ex Lindl., *Genista tyrrhena* Valsecchi, *Limonium minutiflorum* (Guss.) Kuntze, *Matthiola rupestris* (Rafin.) DC., *Dianthus rupicola* Biv., *Helichrysum litoreum* Guss., *Tolpis gussonei* Fiori, among the bryophytes some rare taxa, e.g. *Anthoceros agrestis* Paton, *Fossombronina caespitiformis* De Not. ex Rabenh. subsp. *multispira* (Schiffn.) J. R. Bray & D. C. Cargill, *Riccia crozalsii* Levier, *Tortula solmsii* (Schimp.) Limpr., *Tortella flavovirens* (Bruch) Broth. var. *papillosissima* Sérgio & Casas, *Campylopus oesterdianus* (Müll.Hal.) Mitt. (1).

As regards the bryophyte vegetation, several nitrophytic or typically urbaniphilous communities are found. They are some xerophytic or meso-xerophytic, terricolous associations belonging to the phytosociological class *Barbuletea unguiculatae* Mohan 1978, e.g. *Barbuletum convolutae* Hadac & Smarda 1944, *Lunularietum cruciatae* Giacomini 1950, *Didymodonto Vinealis-Tortuletum muralis* Privitera & Puglisi 1996, *Funarietum hygrometricae* Engel 1949 and the saxicolous *Tortuletum marginatae* v. Hübschmann 1973 of the class *Grimmietea anodontis* Hadac & Vondráček in Ježek & Vondráček 1962. However, some interesting, but not widespread communities, indicators of a minor human impact, are found; they are the coastal associations *Tortelletum papillosissimae* Puglisi 2010 of the class *Barbuletea unguiculatae* and *Gongylanthetum ericetori* Puglisi, Costa & Privitera 2012 of the class *Cladonio digitatae-Lepidozietea reptantis* Ježek & Vondráček 1962 (2). The saxicolous vegetation is represented by communities referred to the class *Grimmietea alpestris* Hadac & Vondráček in Ježek & Vondráček 1962, well represented on volcanic rocks. The natural wooded areas are rare; here it is possible occasionally to find some communities of the *Syntrichion laevipilae* Ochsner 1928, a Mediterranean alliance (class *Frullanio dilatate-Leucodontetea sciuroidis* Mohan 1978) including associations scarcely disturbed by man.

In conclusion, the wider spread of the above mentioned associations of the classes *Barbuletea unguiculatae* and *Grimmietea anodontis* show on the whole a territory subjected to intense human pressure.

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### 3.1 = TREES RELATED TO *PLATANUS RACEMOSA* (PLATANACEAE) IN THE CONTEXT OF THE CITY OF PALERMO (SICILY)

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The genus *Platanus* L. (*Platanaceae*) includes few species; among these, only *P. orientalis* L. is native to Europe. After the introduction of *P. occidentalis* L. from North America, the hybrid *P. orientalis* × *P. occidentalis* was generated in Europe (1). Today it is known as *P. ×hispanica* (= *P. ×acerifolia* (Aiton) Willd.) and is widely cultivated and sometimes naturalized in the Mediterranean region. This nothotaxon has a high phenotypic variability. This has sometimes confused Plant scientists that ascribed to this nothotaxon all the different forms belonging to the parents.

The introduction in Europe of *P. racemosa* Nutt. ex Audubon (Syn. *P. californica* Benth.), native to California (2), a region with a more similar climate to Mediterranean Europe, has never been considered. The same for hybrids between *P. orientalis* and *P. racemosa* – probably introduced but never documented in the Mediterranean – all considered belonging to *P. ×hispanica* – have sometimes led to concerns about the attribution of the same to the nothotaxon or to one of the parents.

Recent explorations of the dendroflora of urban and peri-urban areas of Palermo, succeeded in tracing, at the edge of the neighborhood Borgonuovo –area urbanized only in the last 30 years, before definitely subjected to cultivation – a centenarian tree clearly not referable to *P. ×hispanica* neither to one of the parents (*P. orientalis* and *P. occidentalis*). The taxonomic analysis of the collected samples, done in the laboratory and supported by in site observations of the tree – in particular the character of the bark, the leaves and habit of the plant – has made it possible to refer this tree to *P. racemosa* native of California and planted for ornamental purposes in other parts of the world. The presence in Sicily of this taxon, new to the flora of Europe and the Mediterranean area, opens new scenarios on the identity of the plane trees cultivated in Sicily and, at least, offers the possibility to equip the Mediterranean cities of another species to be used as urban trees, much better suited to the local climate, and probably, much more resistant to pests that until now have devastated historic plants referable to *P. ×hispanica*.

The California Sycamore, is one of the different American species of the genus (*Platanus* subgen. *Racemosae*), never recorded as cultivated in Europe. This tree grows to 15-25 meters in height with a trunk diameter of up to one meter. The bark is a patchwork of white, beige, pinkish grey and pale brown, with older bark becoming darker and peeling away. The large palmately lobed leaves may be up to 25 centimeters wide and have 3 or 5 lobes, acute to acuminate, subtire (2). Male inflorescens is made up of 1-3 spherical flower heads each around a centimeter wide; pistillate inflorescens is made up 2-7 heads; fruiting heads spherical, 2-2,5 centimeter (3).

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## 3.1 = VASCULAR FLORA OF MONTE SPARVIERE (SOUTHERN ITALY, POLLINO MASSIF)

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The vascular flora of Monte Sparviere (1713 m), the easternmost portion of the Pollino National Park, is here presented. The study area, with an extension of 7,3 Km<sup>2</sup> and an altitudinal range between 950 and 1713 meters, is located in a boundary zone between Basilicata and Calabria regions. The area became Site of Community Importance (SCI) in 2006, but despite this its floristic knowledge is very poor (only 10 taxa recorded, based on available literature).

The geological substrate is made up by a sedimentary complex with clayey-arenaceous and marl-lime in the basal part and clayey-sandstone in the superficial part. The climatic data show that the average rainfall is about 1000 mm/y and temperature is 12-14°C. Regional phytogeographic literature (1, 2) show that the area is located in a boundary zone between Eurosibiric and Mediterranean Regions. In order to clarify this question, the territory has been subdivided in 8 Operational Geographic Units (OGU) and for each of them a chorological and ecological spectrum (based on Ellenberg values) was calculated. A cluster analysis, based on presence/absence of taxa in each OGU, was carried out. To evaluate the floristic richness, we extrapolated a (locally valid) linear regression formula between the logarithm of considered areas extent (in km<sup>2</sup>) and the logarithm of the taxa density (n° taxa/km<sup>2</sup>) (3). For this aim, we considered the sub-regional floras of Calabria and Basilicata published over the last 35 years.

The floristic list includes 345 specific and subspecific taxa belonging to 64 families and 218 genera. Italian endemic taxa are 26 (7.5%) and 2 (*Plantago media* L. subsp. *brutia* (Ten.) Arcang. and *Gagea peruzzi* J.- M. Tison) are exclusive for Pollino massif. No exotic species are found except 2 conifers used for reforestation and one naturalized exotic. The observed floristic richness is lower than expected, with a ratio observed / expected of 0.79. The chorological spectrum highlights the prevalence of Eurimediterranean species, but considering Eurosibiric and Mediterranean species *sensu lato*, the former are the most abundant (52% vs. 35%). The chorological spectra for each OGU, combined with cluster analysis, finally show that the most of the area can be referred to the Eurosibiric Region, with the exception of the OGU1 located at lower altitudes, where Mediterranean species reach 45% (and Stenomediterranean reach the highest percentage compared with others OGUs) These results confirm that the study area is at the boundaries between two phytogeographic Regions. The life form spectrum show a dominance of Hemicryptophytes (47%), typical of temperate regions, followed by Terophytes (22%), Geophytes (13.9%), Phanerophytes/Nano-Phanerophytes (11.3%) and Idrophytes (0.6%). The ecological spectrum for entire area and for each OGU is agreement to the environmental and pedologic features.

The taxa included in regional (4) and/or national (5, 6) red lists are 17. Finally, 4 new records for the flora of Calabria (*Dianthus sternbergii*, *Fraxinus excelsior* subsp. *excelsior*, *Herniaria glabra* subsp. *nebrodensis*, *Plantago argentea* subsp. *argentea*) and 2 for the flora of Basilicata (*Ranunculus peltatus* subsp. *peltatus*, *Taraxacum multisinuatum*) are recorded.

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## 3.1 = ANALYSIS OF PLANT DIVERSITY IN THE ISLAND OF VIVARA (NA), SOUTHERN ITALY

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The Mediterranean Sea is reported to be a hot spot of biodiversity (1). In this context a key role is played by the small islands, because they are very rich in endemic and rare species, but at the same time these species are threatened by human exploitation (2, 3, 4). Therefore the protection actions have a crucial relevance in these areas. The island of Vivara is one of the volcanic islands of eastern Tyrrhenian Sea (Phlegrean islands, Gulf of Naples, Italy), together with Pontine archipelago. Vivara is located between Ischia and Procida and it is a small island (32 ha); in the past it was deeply exploited for agricultural purpose (mainly grapevine and olive trees), but in the middle of the XX century the island was quite completely abandoned (5). The abandonment triggered secondary dynamics processes of the vegetation which lead to the growth of sclerophyllous evergreen dominated plant communities (Mediterranean macchia and *Quercus ilex* and *Q. pubescens* woodland). In 1974 Vivara became a Fauna Protected Area (DPGR 609, 10/05/1974) and it was preserved from the urbanization and touristic over-exploitation. Currently the island is part of the Natura 2000 Network (IT803012, both SPA and CSI) and starting from 2002 it is a State Nature Reserve (6) too.

According to the Management Plan of the Reserve, the Reserve Management Committee funded several environmental requalification, involving the University of Naples Federico II (7). Concerning flora and vegetation two actions were assessed: 1) updating of the vascular plants checklist and 2) monitoring of orchids populations. Both research were done in order to verify the potential impact of the rabbit, introduced in the past for hunting purpose and currently cause of overgrazing due to its density. A deep analysis of both scientific and educational references was done (4, 8, 9, 10, 11, 12), pointing out the weakness of updated knowledges. For the updating of vascular plant checklist, floristic samplings in the whole island were carried out; for each of the main vegetation type (woodland, high and low macchia, herbaceous community and rocky shore vegetation) circular plots (10 metres of diameter) were assessed; in each of the plot floristic composition was recorded as well as cover value of each species using the cover-abundance scale according to Braun-Blanquet modified (13). The same methodology was used for the monitoring of *Orchidaceae* species, but in this case sampling plots were variable in dimension and the number of individuals was recorded. Moreover the plots were sampled in winter and spring to record seasonal variability. All sampling site were georeferenced using a GPS Garmin Dakota 20.

Gathered data has been compared with previously available ones to evaluate the changes in floristic composition; distribution maps of the more interesting species have been produced. Results underlined the effects of herbivores pressure in the current plant richness of Vivara and the potential role of other threats and pressure category in quality of the habitats of the island. Finally vegetation data will be used to check the cover values of the habitats sensu 92/43/CEE reported in the Standard Data Form of the Natura 2000 Site.

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### 3.1 = OLD-GROWTH FORESTS IN THE POLLINO NATIONAL PARK: STATE OF THE ART AND FUTURE PERSPECTIVES

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Old-growth forests are an important reference point to assess how human activity impact on woody ecosystems. Therefore, studying old-growth forest ecosystems may significantly enhance our ability to define more effective management options for enhancing biodiversity (1, 2). Although there is still no universally accepted definition, the old-growth forests are ecosystems distinguished by a low level of anthropic disturbance, high biodiversity and structural heterogeneity, presence of very old and large trees and accumulations of large dead woody material.

In 2010 the project “The Old-growth forests in the Italian National Parks” of Italian Environment Ministry allowed to identify 68 forest stands possessing old-growth features, belonging to 16 different woody ecosystem types (3).

The Pollino National Park includes the highest mountains of the southern Apennines, it is one of the largest Natural Reserve in Italy (> 1,900 square kilometers) and covers an elevation range exceeding 2,000 meters.

The complex topography of the Pollino area, along with different patterns and levels of disturbance sustains a diversified vegetation cover.

The previously national-level survey (3) listed 5 old-growth forest sites in Pollino National Park, consisting in *Fagus sylvatica* L. woods locally differentiated by the presence of *Carpinus betulus* L. or *Quercus cerris* L. or *Abies alba* Mill.

Such stands appear to be little representative of the true forest diversity occurring in the Pollino National Park. Indeed a recent work recognized 9 different woody types on a small fraction of the whole natural reserve (4). Therefore, on the behalf of Ente Parco Nazionale del Pollino, new field recognition are ongoing in order to discover new sites to be included in the old-growth forest network of the southern Apennines.

To date we are preliminary selected 8 forest sites, that may be prone to be qualified as new old-growth sites. These sites account for forests dominated by: *Quercus ilex* L., *Quercus cerris*, *Acer* sp. pl. *Fagus sylvatica* and, finally, *Pinus leucodermis*. Currently such stands are subjected to intense field researches aiming to evaluated their structural and floristic suitability as old-growth forests. We retain this work can significantly improve knowledge about the natural forest occurrence and gaps with respect to the woody diversity in the southern Apennines.

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3.1 = POPULATION TREND IN *ABIES NEBRODENSIS* (LOJAC.) MATTEI

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*Abies nebrodensis* (Lojac.) Mattei is an endemic species of Sicily seriously threatened with extinction. Its natural population consists of thirty individuals, occurring in a narrow area within the territory of the Madonie Regional Park. *A. nebrodensis* has been subject to specific conservation measures implemented by the University of Palermo, the Park Authority and the Regional Department of Forests of the Sicilian Region and other local partners for over a decade. The Life Nature project, which ended in 2005, represented a milestone in *in situ* and *ex situ* safeguard actions (1). Other conservation actions are currently being implemented as part of the new project "Conservation of *Abies nebrodensis* and restoration of peatlands of Geraci Siculo" aiming at the increase of the population of *A. nebrodensis*; actions for *in situ* conservation; maintaining the genetic integrity of this fir; the restoration of peatlands of Geraci Siculo; public awareness and dissemination of results. The activities carried out so far have allowed to monitor the thirty plants of the natural population, check their growth, their reproduction, vegetative state, and plant health. All individuals, except for the one with the inventory number 28, have good vegetative condition. Mature individuals are 25, while the spontaneous process of renewal is active only in the vicinities of 9 of them.

In particular, the total number of individuals from spontaneous regeneration increased, in the past nine years, from 80 to 155 seedlings, an increase of about 94%. The new seedlings, aged between 2 and 30 years, and varying in height from 3 to 45 cm, have the following distribution: 58 in the vicinity of the mother plant n. 18; 39 of the plant n. 22; 30 of the plant n.29; 11 of the plant n. 1; 9 around the plant n. 10; 3 the plants n. 8 and n. 27; 1 each the plants n.. 21 and n. 23.

An action project focused on the monitoring of plants of *A. nebrodensis* planted in 20 experimental plots within the scope of the Life Nature project between 2002 and 2005, in the territory of the Madonie Park. Out of the original 1426 plants only 480 have survived the crisis of transplant and are in a good vegetative condition.

The project also provides for the identification and monitoring of reforestations with *A. nebrodensis* by the Forestry Administration made in the territory of the Madonie Mountains between the 80's and the 90's of the last century, using seedlings of *A. nebrodensis* obtained in the nursery of Piano Zucchi. Six areas were established occupying a total surface of about 12 hectares in which 1715 individuals are found. The facilities of Case Prato, Vallone Prato and Quacella (Polizzi Generosa), next to the area where *A. nebrodensis* is indigenous, host in an area of about 3 hectares 562 plants between 30 and 35 years old, with individuals up to 11 m tall, several of which have born fruit abundantly for several years. The plantation in Contrada Savochella (Petralia Soprana), 1.4 ha wide, includes 130 individuals planted between 1995 and 1996, half of which shows signs of damage due to wildlife. The plant reforestation in Contrada Piano Noce (Polizzi Generosa) is located next to the homonymous forest nursery. It includes 570 plants, from 1.5 to 3.5 m tall, spreads over an area of 1.9 ha, in good vegetative and phytosanitary conditions. 5 of these have been fruiting for several years. In Contrada Marrabilici (Polizzi Generosa) 419 plants were surveyed over an area of 4.7 hectares. About 70% of these have very poor vegetative conditions due to the bites of herbivores, particularly deers, and to decortication caused by them with the rubbing of antlers. Fifteen plants, from 4 to 7 m high, have produced cones for several years.

The reforestations of Marrabilici and Savochella need for greater protection from ungulates and appropriate interventions for recovering damaged individuals.

The mature specimens of *A. nebrodensis*, surveyed in gardens and private villas in the Madonie, older than thirty years, were forty and showed good vegetative conditions.

The significant increase of the seedlings in recent years, the affirmation of the plants in the plots made within the LIFE Nature project and the sexual maturity reached by several plants for reforestation carried out between the 80's and the 90's of the last century, denote a positive trend in the population dynamics of this important taxon. The increase in population and the expansion of its genetic diversity, will help to avert more and more the extinction of this species.

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### 3.1 = CONSERVATION OF THE *HALOCNEMUM STROBILACEUM* AND *H. CRUCIATUM* HALOPHYTIC VEGETATION IN MEDITERRANEAN HABITATS THROUGH THE KNOWLEDGE OF SEED FEATURES AND GERMINATION BEHAVIOR

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European Mediterranean halophytic vegetation faces a serious risk of extinction. The relationship between vegetation and environmental factors for each single population, and the reproductive strategies that arise from it, particularly germination ecophysiology, are fundamental factors for planning management of natural habitats. To assess this relation, the environmental factors significantly involved in these processes and the determination of potential climatic predictors of these behavioral strategies for natural plant populations, the following hypotheses were posed:

- Seed features and germination response differs with the distribution of populations;
- This different response is directly correlated with the ecological factors;
- Temperature is a determinant parameter controlling germination;
- Different climatic and edaphic factors are involved in the variation in seed salt tolerance.

Seeds were collected in six populations, populations 2 and 3 correspond to *H. strobilaceum*, while the rest correspond to *H. cruciatum*. Seed dimensions and weight were determined using ImageJ software and a Cahn C-33 microbalance respectively. Seed surface was examined with a SEM Hitachi S-4100. Germination responses in a constant temperature range (5 to 35°C), salt tolerance (0 to 500 mM) at 25°C and 12/12 h photoperiod and recovery (R) were tested. Percentage and mean germination time (MGT), total germination after recovery (TG), base temperature (Tb) and thermal time (S) were determined (1, 2). Additionally, an ecological characterization of each population was carried out in order to correlate the environmental factors with the obtained results.

Seed responses seem to be particularly important for a successful management in halophytic ecosystems. Significant inter-populations differences were found in all the parameters tested. In contrast, these differences are not so evident at the inter-specific level. Seed morphological features indicated that seeds from Egypt are bigger and heavier than the rest, while seeds from El Hondo are the smallest. In the seed coat, differences in the size and distribution of the papillae, as well as in the presence/absence of secondary ornamentation were observed.

Germination responses, percentage, MGT, salt tolerance and recovery, showed an opposite behavior in seeds collected in Egypt contrasted with the population of Ravenna, the only considered as sub-Mediterranean variant of the Temperate macrobioclimate, according with (3).

In the Mediterranean basin, there are two species of the genus *Halocnemum*, it is *H. strobilaceum* and *H. cruciatum*, the first being distributed in coastal areas with sub-Mediterranean bioclimate, macroclimate temperate and the second in the warmer areas with thermo-Mediterranean. Among the priority conservation areas, salt affected environments are one of the world's most threatened and exploited ecosystems and besides they are recognized as providers of a wide range of ecosystem services (4).

The EU Habitats Directive that gave rise to Natura 2000 Network, the most important world-wide network of biodiversity conservation (4), explicitly recognizes the rarity and the reduced area of halophytic vegetation perennial habitats, among which *Halocnemum* communities are considered. The annex I includes this type of vegetation in the habitat code 1420 “Mediterranean and thermo Atlantic halophilous scrubs (*Sarcocornietea fruticosae*)”, and the Natura 2000 network designates Special Protection Areas and proposes Sites of Community Interest in the most threatened European territories. Although halophytic vegetation in the European Mediterranean is highly threatened, unfortunately these saline environments (1420) are not considered among the priority habitats despite they face a severe alteration and reduction of the distribution areas in the Mediterranean coasts, as well as a serious risk of extinction.

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2) D L. Trudgill (1995). Functional Ecology 9: 136-137

3) E. Biondi, S. Casavecchia, E. Estrelles, Soriano P. (2013). Plant Biosystems 147 (3): 536–547

4) E. Biondi, S. Casavecchia, S. Pesaresi, Zivkovic L. (2012). Biodiversity and Conservation 21, 1741-1754

5) E.B. Barbier (2012). Review of Environmental Economics & Policy 6(1), 1-19

3.1 = *VERBENA BONARIENSIS* (VERBENACEAE) ADVENTIVE IN ITALY

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Among the species of *Verbena* L. cultivated in Italy Traverso (1) reports: *V. venosa* Gilles, *V. tenera* Sprengel (= *V. pulchella* Sweet.) and the horticultural hybrid *V. hybrida* Hort. (= *V. bortensis* Hort.). No trace of *V. bonariensis* L., that is reported about 60 years later as naturalized in Tuscany (2). In the Herbarium Centrale Italcum (FI) are housed specimens collected in Piedmont and Tuscany, labelled as *V. venosa* – taxon considered a variety of *V. bonariensis*.

*V. bonariensis*, as indicated by the specific epithet, is a South American species, herbaceous, usually perennial, also cultivated as an ornamental. Several varieties and wild forms are known of this taxon. The International Plant Names Index (IPNI) records: *V. bonariensis* f. *albiflora* Moldenke, *V. bonariensis* var. *brevibracteata* Kuntze, *V. bonariensis* var. *conglomerata* Briq., *V. bonariensis* f. *gracilis* (Cham.) Voss., *V. bonariensis* var. *hispida* Moldenke, *V. bonariensis* var. *litoralis* Hook., *V. bonariensis* var. *longibracteata* Kuntze, *V. bonariensis* var. *rigida* (Spreng.) Kuntz, *V. bonariensis* f. *robustior* Chodat, *V. bonariensis* f. *venosa* (Gillies & Hook.) Voss.

This species is native of S. America (Argentina, Brasile, Paraguay and Uruguay) (3, 4) and introduced in the USA (5), Azores, Great Britain, Canary Islands, Portugal, Madeira (6) and in Italy, as reported above, in Tuscany, in Tombolo Pisano (PI) (2, 7, 8) and in Liguria (8).

Some considerations on the tendency of naturalization of the species in Sicily (9) where accidentally misinterpreted as the report of naturalization in the region (10, 11). This record was neglected in the subsequent floras and checklists (7, 8, 12, 13).

Recently we verified the spontaneous occurrence of *V. bonariensis* at the edges of a channel, in the southern outskirts of the city of Palermo (Sicily). This population, referred to *V. bonariensis* var. *hispida*, is made up of about 100 vigorous and perennial individuals with tendency to expand, actually, in the same area.

The taxonomic identity of the Tuscan populations is different. In this region, it is known from Tombolo Pisano (2) as well as from Florence as documented by specimens collected in the square in front of the rail station of S. Maria Novella (*Raimondo et Domina*, 21/06/2012, PAL), in the fenced area for works of rearrangement of the square. In comparison with the population from Tombolo Pisano, referred to the nominal type, the population from Florence looks like annual and belonging to a different variety, also in comparison with the Sicilian one.

The recent discovery in Sicily as well as increasing the quota of adventitious exotic vascular flora of the island, confirms the tendency of the species to spread further in the national territory.

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2) G. Monti, P.E. Tomei (1983) *Inform. Bot. Ital.* 13(2-3)(1981), 195

3) A.L. Cabrera (ed.) (1965-1970) *Flora de la provincia de Buenos Aires*, Buenos Aires

4) A.L. Cabrera, E.M. Zardini (1978) *Manual de la flora de los alrededores de Buenos Aires*, Buenos Aires.

5) USDA, NRCS. 2014. The PLANTS Database Published on the Internet <http://plants.usda.gov/> [accessed 05/05/2014]

6) Euro+Med (2006-) Euro+Med PlantBase - the information resource for Euro-Mediterranean plant diversity. Published on the Internet <http://ww2.bgbm.org/EuroPlusMed/> [accessed 05/05/2014]

7) F. Conti, G. Abbate, A. Alessandrini, C. Balsi (2005) *An Annotated Checklist of the Italian Vascular Flora*, Roma

8) L. Celesti, A. Alessandrini, P.V. Arrigoni, E. Banfi *et al.* (2009) *Pl. Biosyst.*, 143, 386-430

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10) L. Viegi, G. Cela Renzoni, F. Garbari (1974) *Lav. Soc. Ital. Biogeogr.* 4(1973), 125-220

11) P. Zangheri (1976) *Flora Italica*, 1-2, Padova

12) S. Pignatti (1982) *Flora d'Italia*, 1-3, Bologna

13) F.M. Raimondo, G. Domina, V. Spadaro, G. Aquila (2005) *Quad. Bot. Amb. Appl.* 15(2004), 153-164

3.1 = EPIPHYTIC BIODIVERSITY ON *PHOENIX CANARIENSIS* CHABAUD IN SOUTHERN ITALYADRIANO STINCA<sup>1\*</sup>, GIULIANO BONANOMI<sup>1</sup>, ENRICO VITO PERRINO<sup>2</sup>, RICCARDO MOTTI<sup>1</sup><sup>1</sup>Department of Agriculture, University of Naples Federico II, Via Università 100, 80055 Portici (Naples), Italy; <sup>2</sup>Museum Botanical Garden, University of Bari Aldo Moro, Via E. Orabona 4, 70126 Bari, Italy. \*e-mail: adriano.stinca@unina.it

Epiphytes are non-parasitic organisms that obtain support from host trees and derives required moisture and nutrients from air, rain, and organic debris accumulating in pockets over and inside the host plant. Vascular epiphytes are significant components of tropical forests for the high species richness and biomass (e.g. 1). Distribution patterns of vascular epiphytes are affected by phorophyte structure, substrate type, water availability, microclimatic conditions, and dispersal syndromes. In Mediterranean Basin the phorophyte favorable to epiphytic plant are few. In this area *Phoenix canariensis* Chabaud (*Arecaceae*), a palm native to the Canary Island, is widely planted and represents a potentially phorophyte. However, there are few studies concerning the epiphytic plants of *Ph. canariensis* (e.g. 2, 3). In this study we analyzed the epiphytic biodiversity on *Ph. canariensis* considering four different ontogenetic stages and in three study areas in Southern Italy: two on Tyrrhenian coast (Campania and Calabria region) and one on Adriatic coast (Apulia region). The four ontogenetic stages were: P1 (stem  $\leq$  1 m tall, all the leaf bases present); P2 (stem 1.5-4 m tall, all the leaf bases present); P3 (stem  $\geq$  4.5 m tall, just below the crown leaf bases present, the underlying falls or removed); P4 (dead plants of P3 stage, without leaves, time since death was not determined). Floristic surveys were carried out in 2012-2014 on 15 individuals for each stages randomly selected for a total of 180 samples point. The occurrence and relative abundance of all plant were visually estimated (+:  $<$  1%; 1: 1-5%; 2a: 5-10%; 2b: 10-15%; 2c: 15-25%; 3: 25-50%; 4: 50-75%; 5: 75-100%). The plant specimens were identified according to standard floras (e.g. 4, 5). The nomenclature follows Checklist of the Italian Vascular Flora (6, 7). Moreover, substrates were collected (n. 50) to perform biochemical analysis and evaluate the soil seed bank. In total 133 species growing on *Ph. canariensis* were recorded. The richest families were *Poaceae* (16.5% of all species), *Asteraceae* (15.0%), *Plantaginaceae* (6.0%) and *Fabaceae* (5.3%). Of the life forms of the 133 species observed, *Therophyta* (45.1%) are the largest component. Data analysis show a high presence of alien species (23.4%). Epiphytic total cover and species richness were highest over P2 stage plants. This likely occur because at this stage *Ph. canariensis* individuals are well developed and, at the same time, the senescent leaves are not removed creating safe sites for epiphytic plants. These results demonstrate a significance facilitative effect of *Ph. canariensis* on epiphytic plants, highlighting the importance of plant structure in relation to its ontogenetic developmental stage.

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### 3.1 = SOME REMARKS ON THE VASCULAR FLORA AND VEGETATION OF THE ARCHAEOLOGICAL SITE OF “VALLONE CANALOTTO” (CALASCIBETTA MUNICIPALITY, ENNA PROVINCE, CENTRAL SICILY)

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The main results of ongoing multidisciplinary field investigation, started in 2012 and aimed at the study of the natural heritage of the archaeological site of Vallone Canalotto, located between 550 and 650 m a.s.l. some 4 km NW of the town of Calascibetta, are here reported. Water availability induced Byzantines and Arabs to inhabit this site between VI-XI centuries A.D. Up to now more than 260 vascular plants have been observed, some of them being quite rare in Enna province.

The quite steep (40°) slopes of the valley are covered by patches of garrigue (class *Cisto-Micromerietea julianae*; most common taxa: *Ajuga reptans*, *Cistus creticus*, *Cistus salvifolius*, *Phagnalon rupestre*, *Teucrium polium*, *Thymra capitata*, etc.), xeric perennial grassland (class *Lygeo-Stipetea*: *Ampelodesmos mauritanicus*, *Asperula cynanchica*, *Bituminaria bituminosa*, *Convolvulus elegantissimus*, *Crupina crupinastrum*, *Elaeoselinum asclepium*, *Foeniculum vulgare*, *Hyparrhenia hirta*, *Kundmannia sicula*, *Pallenis spinosa*, *Phagnalon saxatile*, etc.) and annual prairie (class *Stipo-Trachynietea distachyae*: *Helianthemum ledifolium*, *Medicago minima*, *Silene conica*, *Stipa capensis*, *Trachynia distachya*, etc.). Bare gypsum sandy outcrops are characterized by a species-poor chasmophilous assemblage with *Gypsophila arrostii*, *Petrorhagia saxifraga*, *Sedum ochroleucum* subsp. *mediterraneum* and *Silene fruticosa*. The lower sectors of the valley, quite shaded and protected, host a remarkably high number of species which prefer pubescent oak woods (*Quercio-Fagetea* class and *Quercetalia pubescentis* order), such as *Achnatherum bromoides*, *Anthriscus nemorosa*, *Brachypodium sylvaticum*, *Clinopodium vulgare*, *Cornus sanguinea*, *Coronilla emerus*, *Euphorbia characias*, *Hedera helix*, *Pistacia terebinthus*, *Poa sylvicola*, *Dryopteris villarii*, *Quercus virgiliana*, *Silene sicula*, *Tamus communis*, *Teucrium flavum*, *Thalictrum calabricum*, *Viola alba* subsp. *debnhardtii*, etc. Less common are the evergreen woody species of the maquis (class *Quercetea ilicis*) like *Asparagus acutifolius*, *Cyclamen hederifolium* and *C. repandum*, *Myrtus communis*, *Olea europaea* var. *sylvestris*, *Osyris alba*, *Prasium majus*, *Rhamnus alaternus*, *Rosa sempervirens*, *Rubia peregrina*, *Ruscus aculeatus* and *Smilax aspera*. The vegetation colonising watersides is referred to the mantle communities of the class *Rhamno-Prunetea* (*Calicotome infesta*, *Pyrus spinosa*, *Rosa canina*, *Rubus ulmifolius*, *Sambucus nigra* and *Spartium junceum*), while the bottom of the thalweg is covered by some spots of riparian forest (class *Salicetea purpureae*) dominated by *Populus nigra*, with few scattered individuals of *Populus alba*, *Salix alba* and/or by tall herbs like *Carex pendula* and *Equisetum telmateja*. As concerns hygrophilous vegetation, spots of *Adiantetea* with *Adiantum capillus-veneris* and *Samolus valerandi* co-occur near springs and drippings, the drinking troughs are colonized by *Typha angustifolia* and bordered by *Agrostis stolonifera* and *Mentha rotundifolia* (cl. *Molinio-Arrhenatheretea*), while *Arundo plinii*, *Euphorbia ceratocarpa*, and *Piptatherum miliaceum* form species-poor tall grassland along stream-banks. The past presence of men and agriculture and its influence on local forest and riparian assemblages is testified by plenty of trees species which were once cultivated and now appear to be fully naturalized, such as *Corylus avellana*, *Ficus carica*, *Laurus nobilis*, *Prunus domestica*, *Pyrus communis* and *Vitis vinifera* subsp. *vinifera*. As concerns disturbance factors, wildfires appear to have affected the area biasing local progressive succession processes; on the other hand, poisonous and/or thorny geophytes and hemicryptophytes typical of overgrazed areas and typical to the classes *Onopordetea acanthii* and *Poëtea bulbosae*, such as *Asphodelus ramosus*, *Carlina gummifera*, *Carlina sicula* subsp. *sicula*, *Charybdis maritima*, *Ferula communis*, *Onopordum illyricum*, *Scolymus grandiflorus*, *Verbascum sinuatum*, etc., are quite rare and perform low cover percentages, suggesting no or low grazing impact during last decades. The herbaceous layer of the woodland is covered by some nitrophilous shade-tolerant plants such as *Acanthus mollis*, *Anthriscus nemorosa*, *Conium maculatum*, *Ranunculus bulbosus* and *Urtica dioica*. With regard to alien species, the only invasive ones are *Ailanthus altissima* and - to a lesser extent - *Agave americana* and *Arundo donax*, while the few detected individuals of *Cupressus sempervirens*, *Juglans regia*, *Opuntia ficus-indica*, *Phoenix canariensis* and *Punica granatum* are the last remnants of vanishing rural landscape. The behaviour of *Pinus halepensis* and *Eucalyptus camaldulensis* should be monitored as well because the afforestations carried out in the surroundings may induce their gradual spreading.

## 3.1 = LIFE+RI.CO.PR.I. PROJECT: FROM REFERENCE SQUARES TO GRAZING PLAN

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Project Life+ RI.CO.PR.I "Ripristino e COnservazione delle PRaterie aride dell'Italia centro-meridionale" is co-funded by the European Commission under the financial instrument for the environment LIFE+ Nature & Biodiversity 2009 (www.lifericopri.it). The main objective of RICOPRI is the restoration and conservation of two priority habitats related to dry grassland having the following codes and names: 6210 "Semi-natural dry grasslands and scrubland facies on calcareous substrates (Festuco-Brometalia) (\* important orchid sites) and 6220\* "Pseudo-steppe with grasses and annuals of the Thero-Brachypodietea". Sites studied in this project are located in Lazio: SCI IT6030037 "Monti Ruffi (versante SW)" and SCI IT6030035 "Monte Guadagnolo" and in Basilicata: SCI IT9210105 "Dolomiti di Pietrapertosa" in the district of Pietrapertosa, inside the Regional Park Gallipoli Cognato Piccole Dolomiti Lucane.

Conservation of these habitats depends on the maintenance of traditional agricultural activities with low impact, such as grazing and mowing that have historically determined and maintained these semi-natural prairies (www.lifericopri.it). Surveys are carried out to study the quality and state of pastures (1,2) and also type of livestock units, in order to collect data useful to assess the Grazing Plan, a fundamental instrument for grazing management. Its central element is the Pasture Utilization Rate, which depends on the floristic composition and the physical soil properties of a given area and which is based on the difference between the estimated and actual seasonal grazing yield (3).

One of milestones of the RICOPRI project is the application of the Grazing Plan in collaboration with local breeders. This is aimed to develop a rational grazing in order to avoid over- and under-grazing and to achieve multiple benefits for both habitats and livestock.

After the monitoring phase *ex ante*, the actions planned for the improvement of pasture, such as eradication of alien species (*Ailanthus altissima*, *Robinia pseudoacacia*), removal of shrubs (*Rubus* spp.) and restraint of *Sylibum marianum* and *Onopordum* sp., construction of fences have completed.

For a better study of the potential load of pasture that could be achieved, permanent squares have been made in the three SCIs of the project.

Year by year, these permanent squares on M. Ruffi, M. Guadagnolo and on Dolomiti di Pietrapertosa allow us to observe changing on flora composition and abundance.

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2) Pignatti S. 1982. Flora d'Italia. Bologna: Edagricole

3) Stevens P F., 2001 onwards. Angiosperm Phylogeny Website. Version 13, September 2013 - <http://www.mobot.org/MOBOT/research/APweb/>



### 3.1 = ON THE PRESENCE, DISTRIBUTION AND CONSERVATION STATUS OF *LYCOPODIUM LAGOPUS* (LYCOPODIACEAE) IN ITALY

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During our work on the *Lycopodiaceae* account for the upcoming Flora Critica d'Italia (1, 2), we assessed and accepted the presence in Italy of *Lycopodium lagopus* (Laest. ex Hartm.) Zinserl. ex Kuzen. = *L. clavatum* subsp. *monostachyon* (Grev. & Hook.) Selander (2).

Already reported by Fiori (3) as *L. clavatum* f. *monostachyum* Desv., its presence in Italy was more recently confirmed by Tribsch & Schönswetter (4) and accepted in some subsequent regional works (e.g. 5, 6), but the taxon is not recognized as distinct in the last national checklist of vascular plants (7).

*Lycopodium lagopus* has an arctic-alpine distribution in America and Eurasia (8, 9). Initially described as a variety of *L. clavatum* L., the taxon was later raised to subspecific (e.g. 10, 11) and specific rank (8, 9). In view of its largely sympatric occurrence with *L. clavatum* in the Alps, and of the apparent absence of intermediate populations or individuals, we prefer to treat *L. lagopus* as a separate species.

The main characters distinguishing *L. lagopus* from *L. clavatum* are the number of strobili (usually 1, rarely 2), and especially their being sessile or subsessile on a 0-2 cm long "peduncle".

In the Italian Alps it usually occurs at >1800 m a.s.l. As a result of our revision of specimens in several Italian herbaria, this clubmoss, formerly known only from Trentino - Alto Adige and Lombardy, is here reported for the first time for Piedmont on the basis of two specimens collected by Carestia in Valsesia in 1870 and preserved in TO. The presence in Friuli - Venezia Giulia (reported in 6 on the basis of a posthumous work of Gortani) is not confirmed: a specimen collected by Gortani in 1908 and preserved in MFU under "*L. clavatum* f. *monostachyum* Desv." is referable to *L. clavatum*. On the other hand, in view of the specie's ecology and confirmed distribution, one may reasonably expect that it is to be found, additionally, in Val d'Aosta and Veneto.

*Lycopodium* species in Europe have experienced a decline in abundance in a general way, partly due to their being collected and overexploited, and for this reason they have all been included in Annex V of the Habitats Directive 92/43/CEE. *Lycopodium clavatum* in particular, whose decline in Italy is confirmed by several authors (e.g., 6), is also included in Annex D of the Council Regulation (EC) No 338/97 on the protection of species of wild fauna and flora by regulation their trade.

It is therefore urgent to define the current distribution and conservation status of *L. lagopus* in Italy, in order to plan possible conservation measures. We hope that its inclusion (as *L. clavatum* subsp. *monostachyum*) in the most recent Red List of Italian Flora (12), even if only as DD (Data Deficient), and the contribution here presented may stimulate the study of this species in Italy.

This study is part of the "Flora Critica d'Italia" project and as such was funded by the Società Botanica Italiana onlus, the Fondazione per la Flora Italiana, and the International Foundation Pro Herbario Mediterraneo.

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## 3.1 = NOTES ON THE ENDEMIC VASCULAR PLANTS OF THE GARGANO PROMONTORY (APULIA, ITALY)

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The vascular flora of Gargano Promontory (Apulia Region) has been examined closely starting from the Sixties of the last century (1) and more recently in the last 10-15 years (2, 3). The latest study on the whole vascular flora of Gargano (3) indicates 3 taxa strictly endemic of Gargano (but it does not consider the endemic orchids): *Campanula garganica* Ten. subsp. *garganica*, *Micromeria fruticosa* (L.) Druce var. *italica* (Huter) Fen. and *Viola merxmulleri* Erben. In addition, further 5 taxa are reported (3) as possibly endemic of Gargano, because of uncertain taxonomic value: *Asperula garganica* Huter, Porta & Rigo ex Ehrend. & Krendl, *Carex extensa* Gooden. subsp. *viesina* Fen., *Cytisus decumbens* (Durande) Spach subsp. *elatus* (Ten.) Fen. var. *elatus*, *Cytisus decumbens* subsp. *elatus* var. *multiflorus* Fen. and *Lathyrus venetus* (Mill.) Wohlf. var. *latifolius* Fen.. The number of expected endemics was higher, due to the characteristics of biological insularity of the Promontory, surrounded by the Adriatic Sea and by the Tavoliere delle Puglie plain. Therefore, the author (3) hypothesize that the low number of endemic plants may be due to recent speciation processes, already started but not completed yet.

The updated state of knowledge is here presented. Most of the doubtful endemic taxa are in effect without taxonomic value. Only *Asperula garganica* has been confirmed as good species. It is a very rare species, indicated from many localities of Gargano by several authors, but often confused with other taxa of genus *Asperula*. *Aubrieta columnae* Guss. subsp. *italica* (Boiss.) Mattf., previously indicated also from southern Apulia and from Basilicata Region, is actually recognized as endemic of Gargano (4, 5), while regarding the orchids 3 taxa are actually indicated as endemic of the Promontory.

Therefore, the vascular plant species and subspecies strictly endemic of Gargano Promontory and recognized from a taxonomical point of view are the following 9 taxa: *Asperula garganica* Huter, Porta & Rigo ex Ehrend. & Krendl, *Aubrieta columnae* Guss. subsp. *italica* (Boiss.) Mattf., *Campanula garganica* Ten. subsp. *garganica*, *Iris bicapitata* Colas., *Ophrys bertolonii* Moretti subsp. *bertoloniiiformis* (O.Danesch & E.Danesch) H.Sund, *Ophrys mattinatae* Medagli, A.Rossini, Quitadamo, D'Emérico & Turco, *Ophrys oestriifera* M.Bieb. subsp. *montis-gargani* Van de Vijver & W.Looken, *Stipa oligotricha* Moraldo subsp. *kiemii* (Martinovský) Moraldo and *Viola merxmulleri* Erben.

*Alyssum diffusum* Ten. subsp. *garganicum* Španiel, Marhold, N.G.Passal. & Lihová could be added to this list, because its occurrence outside of Gargano has not been indicated without fail, but it is verisimilar that the reports at species level from the Apulian Murgia and from the Murgia of Matera have to be assigned at subspecies level to the recently described (6) subsp. *garganicum*.

Other species and subspecies have been recently described from Gargano, but they occur also outside of the Promontory: *Allium garganicum* Brullo, Pavone, Salmeri & Terrasi, collected in the province of Bari (7), *Ornithogalum etruscum* Parl. subsp. *umbratile* (Tornad. & Garbari) Peruzzi & Bartolucci, indicated in Tuscany, Latium (8) and Emilia-Romagna (9), *Ophrys cinnabarina* Romolini & Soca and *Erysimum crassistylum* C.Presl subsp. *garganicum* Peccenini & Polatschek, both occurring also in other localities in Apulia and in Basilicata (10, 11).

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### 3.2 = CHARACTERIZATION OF THE TRANSGENIC *POPULUS ALBA* PLANTS OVER-EXPRESSING THE AQUAPORIN AQUA1

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Plants aquaporins are involved in almost every aspect of plant life cycle such as cell growth and elongation, transpiration, seed germination, and response to biotic and abiotic stresses (1). In the past years several aquaporins have been related in response to different environmental stresses (1); over-expression of aquaporins in transgenic plants could confer both higher tolerance, or higher sensitivity to stresses, probably by modifying plant water relations (1). Also heavy metal toxicity could affect water absorption and transport at root level (3), but can also reduce leaf transpiration and water potential.

Previous studies on *aqua1* showed its down-regulation in both leaves and roots in response to Zn excess in I-214 hybrid poplar clone, regulation of sub-cellular localization by post-translation modifications in response to this heavy metal in *Arabidopsis* protoplast and also a protective role of this gene in yeast Zn-hypersensitive strains (2). Thanks to expression studies and the use of transgenic plants, the physiological roles of several aquaporins have been elucidated in the last years. In this study, we report the analysis of *P. alba* clone Villafranca transgenic lines over-expressing the *P. x euramericana* I-214 clone aquaporin AQUA1 (GenBank: GQ918138) in different expression vectors. Transgenic lines carrying AQUA1:HA over-expressing lines has been produced for studying the physiological role of this protein under Zn excess in *Populus* (Fig. 1). Moreover, AQUA1:GFP construct under the 35S promoter has been created for analyzing the sub-cellular localization of this aquaporin (Fig. 2). The over-expression of *aqua1* in transgenic Villafranca clones exposed to 1 mM of Zn induced a general increase of plants growth rate, in comparison to wt plants, suggesting a major role of this aquaporin in regulation of plant growth.

Since *aqua1* is down-regulated in response to Zn excess in *P. x euramericana* I-214 leaves and roots, experiments with *P. alba* clone Villafranca transgenic lines over-expressing the aquaporin AQUA1 are now in progress.

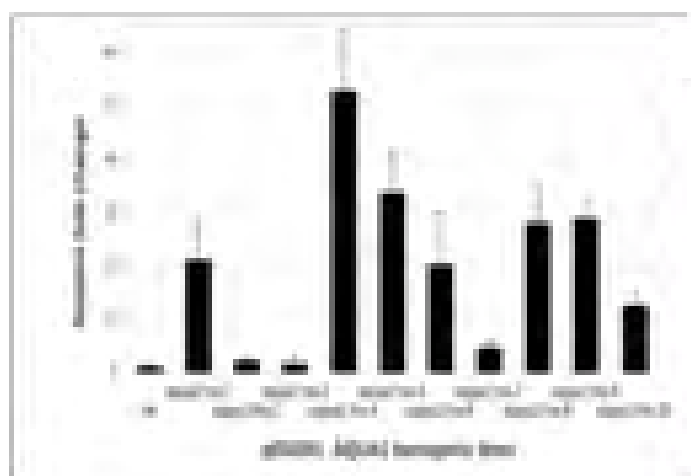


Fig. 1. *In vitro aqua1:ha* over-expressing plant and relative expression of the different lines

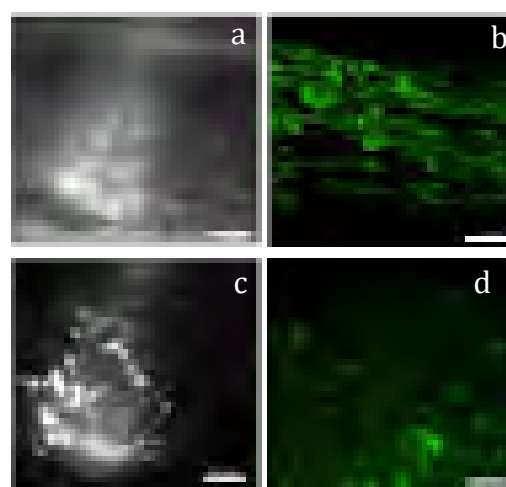


Fig. 2. Confocal imaging of AQUA1:GFP in root (a, b) and leaf (c, d), 488nm (b). Bars = 30  $\mu$ m.

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### 3.2 = PLANT GROWTH PROMOTION AND ANTIFUNGAL ACTIVITIES OF THE GRAPEVINE CULTURABLE MICROBIOME

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Endophytes are bacteria that live inside plant tissues without being pathogenic for the plants. They move into plants from soil through cracks due to emerging lateral roots; from roots bacteria spread in leaves, flowers and fruits via vascular plant system.

In this project bacterial endophytes strains, isolated from *Vitis vinifera* cv. *Glera*, were studied to characterize their plant growth promoting (PGP) properties and their ability in preventing the growth or activities of grapevine pathogenic fungi.

The PGP activities of 320 isolated strains were tested on the model plant *A. thaliana*: some strains induced an increase in the number of lateral roots and root hairs. In addition these interesting strains were demonstrated to be active: in the phosphate solubilization, nitrogen production and the synthesis of the phytohormone IAA.

To investigate the endophytic plant protection capabilities, co-cultures of *Botrytis cinerea*, *Phaeoacremonium aleophilum*, *Phaeoconiella* spp., *Botryosphaeria* sp. And endophyte strains were set up. Strains showing an inhibitory action on fungal growth were further characterized for the production of siderophores and for the presence of specific genes belonging to the fengycin synthetase and surfactin synthetase gene families, involved in non-ribosomal synthesis of cyclic lipopeptides, known for their antifungal and biosurfactant activities.

These different approaches have allowed us to select a bacterial strain, identified by 16S rDNA sequencing as *Bacillus licheniformis* (GenBank KJ889014). Its endophytic behaviour was first confirmed by its re-isolation from inoculated *in vitro* Glera plants. Moreover, in order to better understand its features and genetic traits, *B. licheniformis* was sequenced using the Ion Torrent technology. This deep analysis of this strain and of the LPs produced is the first step to unravelling possible beneficial effects and relationship dynamics of *Bacillus licheniformis* at rhizosphere level and within host plant tissues, its molecular interactions with plant host and its strain ability in shaping the endophytes/rhizosphere bacterial community.

### 3.2 = PHOTOSYNTHESIS UNDER COPPER EXCESS: CHANGES IN THE BIOCHEMICAL AND BIOPHYSICAL FACTORS IN *SILENE PARADOXA* L. COPPER TOLERANT AND SENSITIVE POPULATIONS.

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Under the selective pressure exerted by metal-enriched soils, some plant species can be amazingly able to develop tolerance to such unfavorable substrates, where the fitness takes advantage from the acquired, even though metabolically costly, tolerance mechanisms (1). As soon as a species occupies such an environment, it has to adjust itself to the excessive concentrations of the present elements through a new regulation of their homeostasis. At the cellular level, tolerance mechanisms can protect metabolically active sites through the synthesis of metal-binding ligands to sequester excess of metals in the cytosol and/or through a more efficient cellular compartmentation of the metal surplus, especially into the vacuole (2). In any case, the picture of metal tolerance mechanisms is far from being complete. For example, an almost still completely unexplored topic concerns how metallophytes cope with the excess of metals at the photosynthesis level. In the case of metal hyperaccumulators, there is some interesting information on the effect of Cd on Zn/Cd hyperaccumulating plants (even if the primary mechanism of Cd-induced inhibition of photosynthesis in such species is still a matter of controversy), whereas, in the case of metal excluder plants, almost nothing is known about how they can adapt their photosynthetic machinery to the excess of metals. The most relatively plentiful information on this topic can be retrieved on Cu, with only three reports (3, 4, 5) dealing uniquely with metal-induced effects on pigment concentration and biophysics of photosynthesis. Therefore, very little is known on this topic, whereas, on the other hand, considerable information is available on the inhibitory effect of Cu excess on photosynthesis in non-metallophytes (see for example Cambrollé et al., 2013, Mateos-Naranjo et al., 2013).

In this study, we compared two populations of the metal excluder metallophyte *Silene paradoxa* L., respectively collected from a copper-enriched soil and an uncontaminated one, to shed light on the differential effect of Cu excess on the photosynthetic machinery in copper tolerant and sensitive populations.

Plants were cultivated in hydroponics and exposed to a series of CuSO<sub>4</sub> (0, 1, 10, 20, 40 and 80 µM) concentrations for 1, 7 and 21 days. Leaf gas exchange parameters were determined simultaneously with chlorophyll fluorescence measurements using the open gas exchange system Li-6400 (LiCor Inc., Lincoln, NE, USA) with an integrated fluorescence chamber head (Li-6400-40; Li-Cor Inc.).

In the youngest fully expanded leaves of the sensitive population a significant decrease of the net photosynthesis rate occurred at lower exposure times and at lower copper concentrations used in respect to the tolerant population. Regarding the stomatal conductance, all the copper concentrations used produced a significant decrease of this parameter in the sensitive population without any difference among the treatments, whereas in the tolerant population no Cu-induced change in this parameter was recorded.

A similar trend was recorded also in chlorophyll fluorescence parameters and distribution of excitation energy absorbed in PSII antennae, the copper induced damage occurring earlier in time and concentration in the sensitive population in respect to the tolerant one.

The tolerant population seemed to have evolved copper tolerance mechanisms able to protect both the biochemical and biophysical machineries that cooperate in the photosynthesis.

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### 3.2 = CYANOBACTERIA UNDER EXTREME CONDITIONS ON EARTH AND BEYOND: CONTRIBUTION TO HUMAN SPACE EXPLORATION

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Cyanobacteria thriving in lithic habitats in cold and hot deserts, like the Dry Valleys in Antarctica and the Atacama desert in Chile, considered two Mars analogues, are extremely resistant to prolonged desiccation, ionizing and UV radiations (1, 2, 3). These features underlay their relevance when investigating the limits of life as we know it, a prerequisite to search for life beyond Earth, and when identifying bio-signatures for searching life on Mars. These tasks are addressed in two ESA projects selected for the next Expose-R2 missions (July 2014) on the International Space Station: Biofilm Organisms Surfing Space (BOSS) and BIOlogy and Mars Experiment (BIOMEX). Results on desert cyanobacteria exposed to space and Martian simulations further supported their endurance under extraterrestrial conditions (4), and contribute to identify suitable biosignatures (5, 6). While results from cyanobacterial biofilms exposed to space and Martian simulations pointed out an enhanced survivability of biofilm lifestyle under extraterrestrial conditions. Furthermore the growth capability of desert cyanobacteria mixed with lunar and Martian mineral analogues has implication in the development of in-situ resource utilization in support of human settlement on the Moon or Mars. Finally, the foreseen investigation of the effects of microgravity on these extreme-tolerant cyanobacteria will find application in the development of life-support systems for long-term travel in space.

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### 3.2 = TOPOGRAPHICAL DYNAMICS OF DAMAGE-RELATED VOLATILE EMISSION IN *PHASEOLUS LUNATUS* L.

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As sessile organisms, plants cannot avoid attacks by biotrophs that use them as a food source; therefore, through evolution they developed efficient strategies to defend themselves (1). This led plants to produce chemical compounds ecologically useful to avoid or limit damages (2) caused by herbivores and pathogens. Besides those compounds that directly act against herbivores, plants produce Volatile Organic Compounds (VOCs) that attract natural enemies, parasitoids or predators of herbivores feeding on them (1, 3, 4).

The plant response to biotic/abiotic wounding has always been studied on the whole plant emissions (5) with either head-space or disruptive methods, often by analyzing different organs or leaves separately (6). Despite the consistent amount of data, the dynamics of VOCs emission is still an open question as well as the relationship between damaged area and the biosynthesis of bioactive molecules.

In order to identify compounds actively related to plant-insect interaction, we developed a non-invasive and innovative *in vivo* high concentration-capacity sampling technique to capture volatiles from *Phaseolus lunatus* (Lima bean) leaves upon wounding of different nature. Direct Contact-Sorptive Tape Extraction (DC-STE) (7, 8, 9) is an innovative and non-disruptive technique based on the direct contact of polydimethylsiloxane (PDMS) tapes (6×10 mm) on biological surfaces. In this case, we applied the technique to *P. lunatus* fresh leaves that were sampled at fixed distances from the wounded areas in time-course experiments. The sampled fraction was then recovered from the tape by thermal desorption and on-line analysis by gas-chromatography coupled to mass spectrometry (GC-MS).

Upon herbivore wounding caused by the *larvae* of the Noctuid *Spodoptera littoralis*, mechanical damage by a pattern wheel and the combination of mechanical damage with the oral secretions of *S. littoralis*, we used DC-STE-GC-MS to analyze the topographical dynamics of VOC emission from leaves of *P. lunatus*. The statistical data treatment with multivariate analysis emphasized interesting differences depending on both the kind of wounding and the distance from wounding. The non-disruptive VOC sampling method used allowed us to run gene expression analysis of the key VOC-related enzymes on the same leaves previously analyzed. A perfect topographical correlation was found between VOC emission noticed with DC-STE-GC-MS and gene expression analyzed by Quantitative Real Time PCR, confirming the reliability of this new *in vivo* sampling technique in plant interactions studies.

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### 3.2 = MACROPHYTE DISTRIBUTION PATTERN ALONG A LOW HUMAN IMPACT RIVER IN MEDITERRANEAN AREA

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River environments are extremely interesting study areas. Profound changes, state of degradation caused by agricultural and farming practices, and intensive land-use practices within the drainage basin during the extensive dry period can have an impact on the river biota. Therefore, studying aquatic macrophytes, as a bioindicator of ecological river quality, is considered an important aim (1). Our main goal is to examine how environmental conditions affect composition and abundance of macrophyte communities in riverine ecosystems. Field survey data were collected in 2013 from the Merse river catchment (Southern Tuscany) on 34 sites identified by systematic sampling design. In each site all macrophytes (vascular plants, bryophytes and macroalgae) were surveyed and environmental parameters (geomorphological, topographical and chemical) were examined. Plant assemblages and hierarchical structure of each group of species were analyzed using hierarchical classification (Bray-Curtis) and Indicator Species Analysis (INSPAN). The relationship between macrophyte community composition and water quality was examined using direct and indirect ordinations. A total of 70 aquatic macrophyte taxa were recorded including 12 bryophytes and 2 algae. Five main groups were identified by using classification techniques. Ordinations allowed us to identify significant relationship between macrophyte distribution and some environmental parameters (distance from the spring, geology dominated by quartzites and alluvial deposits, conductivity). The results show that the distribution of aquatic macrophytes in a river located in Mediterranean area, with a low human impact, is mainly influenced by the longitudinal gradient and significantly affected by the type of environment surrounding the river. However, the environmental variables considered here explain together about 32% of the variance in species composition. This means that other processes not analyzed in this study, both biological (dispersal ability, response modality to stress) and random but more importantly, as highlighted in other recent studies (2,3), related to the physico-chemical characteristics of the water, may contribute to variability of the floristic contingents.

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### 3.2 = A PUTATIVE METALLOTHIONEIN FROM THE MICROALGA *SCENEDESMUS ACUTUS* (CHLOROPHYCEAE)

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Microalgae are predominantly aquatic organisms that should be able to discriminate between essential and non-essential heavy metal ions. In addition, they must maintain non-toxic concentrations of these ions inside their cells. In this way, two principal mechanisms have been identified, one which prevents the indiscriminate entrance of heavy metal ions into the cell (i.e., exclusion) and the other which prevents bioavailability of these toxic ions once inside the cell (i.e., the formation of complexes). The molecules responsible for the first mechanism are extra-cellular polymers, mainly carbohydrates; those responsible for the second are essentially the two type metal-chelating peptides: enzymatically synthesized phytochelatins (PCs- class III metallothioneins -MtIII), and gene-encoded metallothioneins (MTs) (1).

MTs are low molecular weight, cysteine-rich metal chelators with an ability to bind heavy metal ions through metal-thiolate bonds. In addition, the thiol(ate)s in MTs can act as powerful antioxidants, and hence MTs may have roles in protection against oxidative stress (2). Mts are widely distributed in animals, plants, fungi as well as cyanobacteria. Plant MTs (pMTs) are considerably longer than their animal counterparts owing to the exclusive presence of a 30-50 residue-long, Cys-devoid region, between the N- and C-terminal Cys-rich domains (four to eight Cys each). Specifically, the distribution of the Cys residues and the length of the spacer region have been used to further classify plant MTs into four subtypes (3, 4). Currently, pMTs have been extensively identified as a multigenic family in angiosperms (*Arabidopsis thaliana* as a model) (3), in gymnosperms (5) and in brown algae (*Fucus*) (6), constituting family 15 of the global MTs Kägi classification (7). To date, no evidences of genes encoding Mts in microalgae are reported, although the induction of cadmium-binding MT-like protein has been found in the unicellular green algae *Chlamydomonas sorokiniana* (8) and *C. vulgaris* (9). In this work, we report the first evidence of a Mts gene from the microalga *Scenedesmus acutus*. In the case of the heavy metal tolerance of *S. acutus*, previous data report the implication of reduced glutathione (GSH) and Phytochelatins-MtIII as the molecular mechanism underlying the Cd tolerance (10). By RT-PCR amplification approach using degenerate primers, we amplified a full length cDNA of 280 bp sharing high identity with plant metallothioneins. The deduced protein consisted of 91 residues (mol wt of 9,2kDa) which contain 8 Cys residues arranged in CXC and CXXC motifs and showing a high identity homology (89%-98%) with other type2 Mts of the *Silene* genus. Despite this, the primary structures of *Scenedesmus acutus* MTs ( named ScaMT) shows some differences with the canonical pMTs type 2 which relate it to an *archetypal Type 2* sequences from other pMTs members. So, the evidence that ScaMT sequences shows similarity to Types 2 is a phylogenetically important finding and supports both the existence of a common ancestral metallothionein and its diversification at a point after the evolution of Chlorophyta and Streptophyta lineage. Work is in progress in order to define the genomic feature of *ScaMT* gene and its functional role (response to different heavy metal levels and/or to stress conditions). The results will also elucidate the different implications of the two type of heavy metal binding peptides (enzyme and encoding synthesized) in *S. acutus* in order to better understand the metal tolerance and/or the bioaccumulation mechanisms in this microalga. These molecular mechanisms would be potentially engineered to improve the algal phytoremediation performance.

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### 3.2 = THE INFLUENCE OF NATURAL AND ANTHROPOGENIC FACTORS ON MEDITERRANEAN COASTAL SAND DUNE VEGETATION: A CASE STUDY IN TUSCANY (ITALY)

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Coastal dune habitats are undeniably important ecosystems in Europe, as they contain a high ecological diversity in terms of environmental heterogeneity and variability of species composition (1, 2). Because the Mediterranean plant communities of the sandy coastal systems are particularly endangered, the need to assess the conservation status of coastal dunes has become urgent in order to promote appropriate management strategies to preserve these environments (3, 4). The aim of the present work was to assess the conservation status of coastal dune systems belonging to two natural parks along the Tuscan littoral (Italy): Migliarino-San Rossore-Massaciuccoli Regional Park and Maremma Regional Park (Fig. 1). Emphasis was given to the presence and abundance of plant communities identified as habitat in accordance with the Directive 92/43/EEC. Twenty transects perpendicular to the shoreline were randomly positioned on the whole coastal area (30 km in length) in order to sample the full spectrum of plant communities that go from annual vegetation of drift lines (EC 1210), through the embryonic shifting dunes (EC 2110) and shifting dunes along the shoreline with *Ammophila arenaria* (EC 2120), as well as the *Crucianellion maritima* fixed beach dunes (EC 2210), and the *Malcolmietalia* dune grasslands (EC 2230). Vegetation zonation and relationships with the most frequent disturbance factors in the study area – beach cleaning, coastline erosion, presence of paths and roads, bathing settlements and trampling – were investigated through Principal Coordinate Analysis (PCO) and Canonical Correspondence Analysis (CCA). Natural factors, such as distance from the sea and total length, were also considered.

The analysis of coverage habitat data indicated that transects exhibit varying pattern of the typical sequence of coastal dune plant communities, ranging from the disappearance of the foredune habitats (annual vegetation of drift lines, embryonic shifting dunes and mobile dunes) to the presence of the complete sequence. The CCA analysis revealed that the most important variables in the studied habitat-environment relations were erosion, trampling and paths; which were found to be closely correlated with degradation and habitat loss. Furthermore, the overall plant species diversity of dunes was measured with  $NH_{Dunc}$ , a modified version of the Shannon index; while the incidence of invasive taxa was calculated using  $N$ , a naturalness index (5, 6). However, these diversity indices proved to be a weaker bioindicator of ecosystem integrity than habitat composition along transects.

Given the present findings, a possible strategy for the conservation and management of these coastal areas could be to protect the foredunes from erosion and limit trampling through the installation of footbridges or the use of appropriate fences.

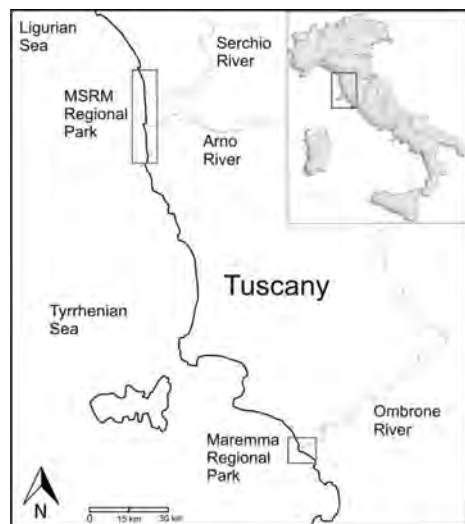


Fig. 1. Location of the Migliarino-San Rossore-Massaciuccoli (MSRM) Regional Park and Maremma Regional Park in the northern and southern parts of Tuscany, respectively. On the right a picture of Italy with Tuscany

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### 3.2 = ROOT MORPHOLOGY AND COPPER EXCLUSION MECHANISMS IN *SILENE PARADOXA* L.

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Plants need to adapt themselves to the soil in order to be able to colonize new ecological niches at the time of seed dispersal. The evolutionary pressure leading to the segregation of populations of *Silene paradoxa* in Tuscany, able to resist to metal excess in the soil have been subjected to previous studies (1). The tolerance mechanisms can increase the plants fitness, even if at the cost of an increased metabolic effort (2). The plants able to tolerate the presence of excessive metals in the soil can adopt a tolerance strategy by avoiding the entrance of ions at the root level (the so-called excluders), or may hyperaccumulate metals at high concentration in their tissues. The root is the first organ to intercept the metal flux and hence it is of particular interest in order to understand the tolerance mechanism of this plants. We studied a population growing around a mine (Fenice Capanne, Isola d'Elba, FC), compared with a “normal” population (Colle val d'Elsa, CVD), both hydroponically grown at high copper concentration. The study allowed to study quantitatively the amount of callose and lignin accumulating in the roots together with observations with Light Microscope and Transmission Electron Microscope (TEM). The sensitive population lignified the vessels more distantly from the root apex if compared to the tolerant population and showed a higher quantity of copper (TEM-EDAX analysis accoppiata al TEM) in the root walls. Moreover the tolerant population showed the precipitation of electron dense material in the walls, not copper after the EDAX analysis (Fig.1).

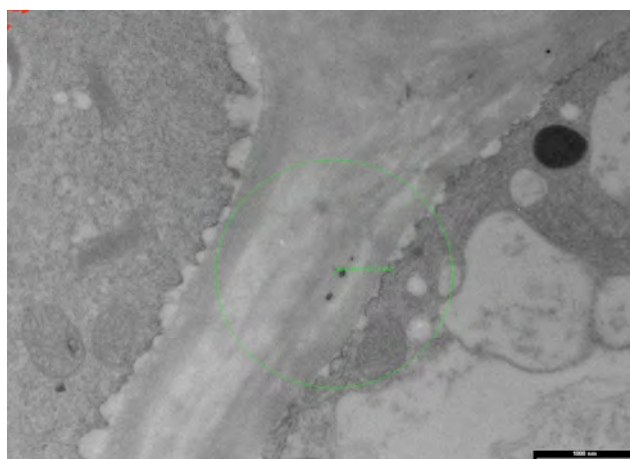


Fig. 1. The area of the EDAX analysis on the root walls (internal tangential walls of the first parenchyma layer below the epidermis of sensitive population of *Silene paradoxa*).

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### 3.2 = DIFFERENT MOLECULAR RESPONSES TO DROUGHT, HEAT AND COMBINED STRESS ARE ACTIVATED IN TWO DURUM WHEAT CULTIVARS

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Durum wheat plants often experience water scarcity and high temperatures, two stresses that usually occur simultaneously in the fields. The responses induced at transcriptional level by only drought or heat stress have been described, but much less is known about durum wheat response to the simultaneous occurring of drought and heat stress, although this is the most common event in field conditions. Several data indicate that in plants the molecular response to combined heat and drought stress activates networks different from that activated by the single stress.

The durum wheat cultivars Ofanto and Cappelli represent an ideal experimental system to investigate the stress response in this species; they are characterized by contrasting behavior in response to high temperature and drought stress.

The transcriptome profiles of the two cultivars were compared by Affymetrix microarray analysis. In Ofanto drought stress activates 707 genes, heat stress activates 3,243 genes, and drought/heat combined stress activates 5,645 genes, thus suggesting that combined stress exceeds the simple additive effects of drought and heat stress alone.

The microarray analysis identified also 1,850 stress responsive genes characterized by significantly different expression profiles in Cappelli vs Ofanto. In particular, these genes are almost not differentially expressed in response to drought in both cvs; on the contrary, in Cappelli a more pronounced molecular response, mediated by these genes, to heat stress was identified. Moreover these genes showed similar expression levels in response to combined stress. The functional category analysis on these genes describes a complex network: perception and signal transduction were characterized by the activation of genes coding for HSPs, transcription factors and RNA binding proteins suggesting both transcriptional and post-transcriptional regulation. Noteworthy, the heat response in Cappelli involved also the up-regulation of genes belonging to fatty acid  $\beta$ -oxidation pathway, glyoxylate cycle and senescence, suggesting an early activation of senescence in this cv.

By this work we have implemented the knowledge about the use of Ofanto and Cappelli as genetic experimental system as an ideal tool for the genetic dissection of the molecular response to abiotic stresses. Ofanto and Cappelli are characterized by two opposite stress-responsive strategies. In Ofanto the combination of drought and heat stress led to an increased number of modulated genes, exceeding the simple cumulative effects of the two single stresses, whereas in Cappelli the same treatment triggered a number of differentially expressed genes lower than those altered in response to heat stress alone.

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3.2 = RESPONSE TO METALS IN THE LIVERWORT *LUNULARIA CRUCIATA* AND IN THE CHAROPHYTE *NITELLA MUCRONATA*

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Our “perception” of the possible role of phytochelatin (PCs), that is metal(loid)-binding thiol peptides, is often erroneously biased by a “here and now” perspective, without considering their evolutionary debut in the distant past. We might in fact ask why the phytochelatin synthase enzyme (PCS), a gamma-glutamylcysteine dipeptidyl transpeptidase (EC 2.3.2.15) (1), is so widespread and constitutively present throughout the plant kingdom and beyond (2, 3). It is in fact not clear, for instance, how the sporadic need to sequester excess cadmium (Cd) or arsenic could have provided the selective pressure for maintaining a so ample PCS expression through a number of organisms (3). Another possibility is the involvement of PCs and/or PCS in essential functions, studies of which are only just getting underway (3). In this context, *Lunularia cruciata* (L.) Dumort. occupies a very basal position in the phylogenetic tree of liverworts (*Hepatophyta*, sin. *Marchantiophyta*), which in turn have been recognized as the basalmost clade of land plants (4). It would therefore seem appropriate to take *L. cruciata* as the starting point for deducing character evolution in the metal(loid) response of early land plants which possibly spread in the terrestrial environment from the Ordovician/Silurian periods onwards (4). Likewise, until now no information has been available regarding the PCS presence in charophytes (*Charophyta*) [i.e.: *Nitella mucronata* (A. Braun) F. Miquel], thought to be *sisters* of land plants (4). Accordingly, the hypotheses verified in this work are that: 1) the ability to synthesize PCs and the occurrence of an active PCS in *L. cruciata* gametophytes were early traits which responded to the need to regulate trace element homeostasis and to minimize the risk of exposure to toxic concentrations of certain metals; indeed, it might be of interest to note that the strongest evolutionary pressure for land colonization by plants came from potential access to much greater amounts of nutritive ions from surface rocks; 2) a PCS enzyme was also present in *N. mucronata*. Accordingly, we have demonstrated here that: 1) *L. cruciata* compartmentalizes Cd in the vacuoles of the photosynthetic parenchyma, by means of a PC-mediated detoxification strategy, and possesses a constitutive PCS activated by Cd and homeostatic concentrations of iron(II) and zinc; 2) *N. mucronata* shows a western-immunoreactive PCS signal with a molecular mass of approx. 40 kDa; moreover, in 2D-western blot, this putative enzyme shows different immunoreactive signals distributed along an acidic pH range, presumably due to post-translational modifications (i.e. phosphorylations), the role of which is currently being investigated. Overall, the knowledge advancement in the field would make it possible to construct sound-based evolutionary hypotheses.

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3.2 = EFFECTS OF LEAD AND CADMIUM ON DUCKWEED *LEMNA MINOR* L.

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The presence of heavy metals (HM) in the waters is one of the most important pollution problem, since unlike most organic compounds, metals cannot be degraded or transformed by living organisms, therefore they accumulate in water and bottom sediments as well as in biota. Aquatic plants share the great potential to take up several HM and when these not target organisms are reached by pollutants, they may also be used as indicator species in the evaluation of the quality of the aquatic systems. Among them, the small floating aquatic monocotyledon *Lemna minor* L. has been used in phytoremediation and toxicity studies on HM, phenolics and herbicides, and this species can be considered a model plant for ecotoxicological studies (1 and references therein). Most of the studies have considered the plant response to the toxicity of single metal, while data on the effects of two or more metals are very few.

The objective of this study was to determine the effect of lead and cadmium on *L. minor* plants. A laboratory screening with plants grown in medium polluted with 2.5, 5 and 10 ppm of  $Pb^{2+}$  and  $Cd^{2+}$  was set-up in order to detect the stress response of the macrophyte along seven days. The experiments were addressed to detect the effect of these HM on growth, pigments and phenolics content and on the activities of the antioxidant enzymes involved in the detoxification process, e.g. polyphenol oxidase (PPO; EC 1.14.18.1) and guaiacol peroxidase (G-POD; EC 1.11.1.7).

A reduced growth rate was detected in the HM treated plants and also the chlorophylls and carotenoids amount decreased depending on the HM concentrations. Metal stress induced an enhancement of PPO and G-POD activities as well as of phenolic compounds production.

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### 3.2 = GREENING EDUCATION: MAY THE GEOBOTANY BE A POSSIBLE APPROACH IN THE PRIMARY AND SECONDARY SCHOOL?

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The Science Education is a priority in the targets for Europe 2020 to realize a smart, intelligent and inclusive growth. In fact the scientific literacy is considered essential in the knowledge society.

Moreover, the Green Education has a very important role in the Science Education, because it teaches not only specific subjects, but also helps to develop responsible environmental behaviors.

These intelligent behaviors will be much more developed in the future citizens as early as the knowledge of the environment is included in the objectives of school. On the other hand, recent reports have found low levels of scientific knowledge and low motivation in fifteen years old students (1, 3). For all these reasons it is urgent to innovate the science curricula and to improve their teaching methods in the basic school (2)

Since Geobotany is an interdisciplinary approach to study the environment, it can be very useful also in the early years of schooling to teach the habitat biodiversity, to understand the diachronic effects of environment management and the economic opportunity in its conservation and enhancement.

Our research aims to investigate whether this approach is suitable to motivate young people to explore their territory. In the school year 2013-2014 have been involved 600 pupils from 8 to 11 years, belonging to 31 classes of ten schools. Next year will also be involved fifteen years old students.

First of all have been identified the skills to be developed and then have been prepared lessons and teaching materials for them. In the classroom the students have detected qualitative and quantitative characteristics of different soil samples. Moreover, they learned to be familiar with the biodiversity of the leaves, flowers and fruits and how to use a simple dichotomous key to identify plants (4).

Finally, they experienced the influence of different soils on vegetation at the Botanical Garden "Selva di Gallignano" of Polytechnic University of Marche, where there is a residual forest, characterized by five different plant communities and several other habitats (5, 6, 7).

Each step of the project was preceded and followed by satisfaction tests for teachers and knowledge tests for pupils, whose processing is in progress.



Fig. 1a-1b. Pupils analyzing samples of soil



Fig.2a-2b.Schoolchildren identifying plants

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### 3.2 = EFFECTS OF A FIRE ON THE VEGETATION OF A MOUNTAINSIDE OF THE OROBIC PRE-ALPS (BERGAMO, ITALY)

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Fire is one of the main factors that shape the landscape as well as a fundamental component of some ecosystems. In Italy, several studies have been conducted in order to understand the effects of fires on vegetation but most refer to the Mediterranean environment. Few data exist concerning the plant communities presenting after fires in the Alpine and pre-Alpine area. This study aims to identify the main effects of a fire on the vegetation of a mountainside of the Orobic Alps located in the municipality of San Pellegrino Terme (Brembana Valley, Bergamo, Italy) in which there was a mixed wood of *Quercus pubescens*, *Ostrya carpinifolia* and *Fraxinus ornus*, typical of the sub-montane and hilly areas of the pre-Alps of Lombardy (1, 2). The fire, caused accidentally by human intervention, spread from 2<sup>nd</sup> to 4<sup>th</sup> April 2012, and involved approximately 6 ha of forest (Fig. 1). Climate data for the area studied revealed a particularly dry period during the first three months of 2012, which could have helped to facilitate the start and the spread of the fire.

Data on the flora and vegetation were collected by performing two phytosociological relevés, one in an area of the mountainside not affected by fire (wood), the other within the burnt area (Fig. 2) adjacent to the first. Relevés were conducted from April to July 2013 (one year after the fire) according to the criteria of the sigmatist school of Zurich-Montpellier (3). Life-form and chorological spectra were elaborated for each community. The ecological indices of Landolt (4) were used to analyze the response of vegetation to the disturbance; the Index of Maturity (IM) was calculated (5) and an analysis was performed according to Grime's CSR model (6). Results showed that the post-fire plant community is de-structured and depleted in nemoral and shrub species of *Carpino-Fagetea* which are replaced by others, mostly belonging to *Elyno-Seslerietea caeruleae* and *Festuca-Brometea*, which were not present before the fire. Some of this species are rare or endemic including *Carex baldensis* which showed moderate coverage. Ecological analysis showed that the post-fire vegetation has many more heliophilous species that grown on dry soils poor in nutrients compared to the wood. The post-fire vegetation also has a lower IM value and a greater number of ruderal and stress-tolerant species which indicate the start of a regressive series.



Fig. 1. Fire of 2<sup>nd</sup> April 2012 (photo: Afric G.).



Fig. 2. Post-fire vegetation (June 2013).

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### 3.2 = EFFECT OF HEAT AND DROUGHT STRESSES ON XET ACTIVITY IN DIFFERENT ORGANS OF DURUM WHEAT SEEDLINGS

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In this work, xyloglucan endotransglycosylase (XET) activity was assayed *in vitro* on crude extracts from leaves, germinated caryopsides and roots (basal and apical segments) of 5-day old durum wheat (*Triticum durum* Desf., cv Creso) seedlings incubated under control (25°C), heat (42°C) and drought stress conditions for 2, 4 or 24h. Heat stress did not affect XET activity in leaves and germinated caryopsides, however a significant inhibition (>55%) was observed in roots regardless of incubation time. Exposition of crude extracts from control seedlings to 42°C suggested that XET isoforms expressed in roots are more sensitive to temperature than those expressed in leaves and caryopsides. On the contrary, drought stress strongly enhanced XET activity. In leaves and germinated caryopsides an increase in XET activity (>55% and >130%, respectively) was detected after 24h of drought stress, while in both basal and apical root segments XET activity increased already after 1h of stress, exceeding 70% and 200% after 24h, respectively. The application of an *in vivo* real-time assay to assess XET activity showed conflicting variations in dependence on the gradient of cell differentiation. In root cap and apical meristem of heat stressed seedlings, XET activity was stable even after 24h at 42°C. Stress caused an increase in XET activity (~65% after 24h) at the elongation zone, but a gradual decrease at the hairy region (Fig. 1a). In plants subjected to drought stress, XET activity increased in apical meristem and elongation zone (>100%) (Fig. 1b). This localized increase of XET activity may represent an adaptive response to heat and drought stresses by promoting a rapid elongation of root cells to explore deep soil. Furthermore, the increase in XET activity observed at the basal root segment under drought stress is likely due to the involvement of XET in root hair formation, to increase root water absorption surface [1]. The analysis of XET expression profile in heat or drought stressed seedlings showed a decrease in XET expression both in the basal and apical root segment, according to the decrease in the activity found in heat stressed roots, but strongly in contrast to the increase found in the same portions subjected to drought condition. This suggests that XET activity may be primarily under the control of post-transcriptional events.

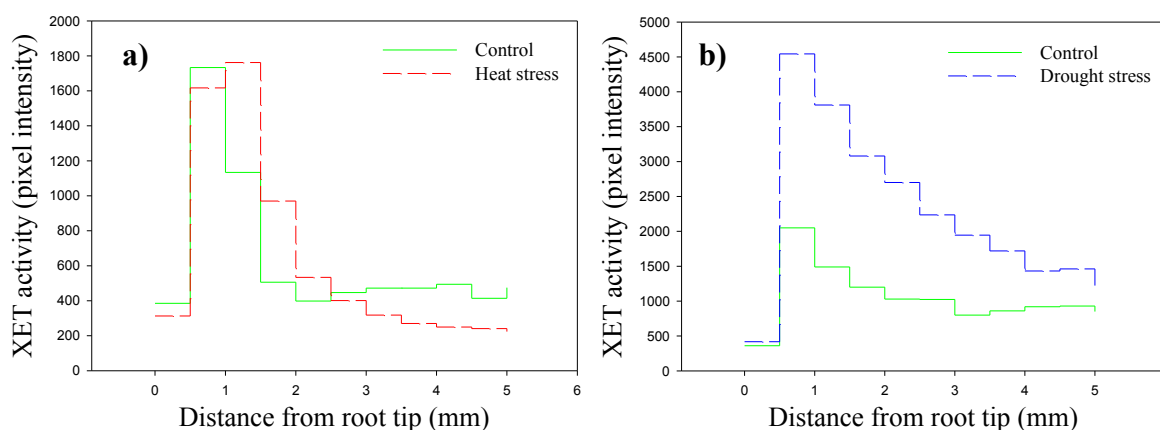


Fig. 1. *In vivo* XET activity in root segments (5 mm from the root tip) from control and 24 hours heat (42°C) (a) or drought (b) stressed durum wheat seedlings.

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### 3.2 = EFFECT OF HIGH TEMPERATURE ON MYCELIAL GROWTH AND ROOT COLONIZATION OF *TUBER BORCHII* VITTAD. ISOLATES

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*Tuber borchii* Vittad., commonly called Bianchetto truffle, is a ectomycorrhizal Ascomycete belonging to the Pezizales order. It is one of the most widespread truffle species in Europe: it can be found from Finland to Italy and from Spain to Hungary and Poland (1). This truffle species has a wide ecological magnitude and it is able to establish symbioses with a wide range of plants, including non ectomycorrhizal hosts as strawberry trees and orchids (2, 3). Unlike the other valuable truffle species, *T. borchii* is commonly found in Mediterranean semi-arid environments (4).

In this work we tested the effect of the high temperatures on *in vitro* mycelial growth of 12 *T. borchii* isolates from different geographic sites and their ability to colonize *Quercus robur* L. roots. Mycelia were grown both in agarized and liquid medium at 22, 28 and 34 °C until the staling phase. Oak seedlings inoculated with the different isolates were maintained in greenhouse conditions for 4 months at 22±2 and 28±2 °C.

In most cases, the radial growth of the colonies as well as the dry mycelial biomass decreased significantly at 28 °C. All isolates stopped to grow after a few days at 34 °C and only one regrew when transferred again to 22 °C. Greenhouse trials showed that the higher temperature had detrimental effects on ectomycorrhizas. Hence, at 28 °C the ectomycorrhizal colonization strongly decreased or disappeared.

This study represents a first step to understand the effects of the climatic changes on *T. borchii* mycelial growth and on their ability to colonize roots. Considering the burning issue of global warming, the selection and the use of more resistant strains to high temperatures have to be taken into consideration for truffle cultivation, especially in Mediterranean habitats.

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### 3.2 = *SEDUM HISPANICUM* L., PIONEER SPECIES IN THE GYPSUM OUTCROPS (BOLOGNA PROVINCE)

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In order to evaluate the colonization ability of *Sedum hispanicum* L., we analyzed the nursery effect of moss mat and the level of genetic diversity in some population growing on gypsum outcrops.

The study area was the SCI IT4050001 Gessi Bolognesi, Calanchi dell'Abbadessa where the species is found within the Habitat "6110\* Rupicolous calcareous or basophilic grasslands of the *Alyso-Sedion albi?*

Sampling design consisted of 20 plots (0.5 x 0.5 m) subdivided by an internal grid of 0.05 x 0.05 m subplots randomly placed within the patches of the Habitat. Samplings were carried out once a week from March to June 2013. For every plot the total moss and *S. hispanicum* cover was assessed. In 10 subplots randomly selected, for each *S. hispanicum* individual the crown was recorded by measuring the maximum and the minimum diameter. Reproductive phenophase, according to the BBCH (Biologische Bundesanstalt, Bundessortenamt und Chemische Industrie) scale, was also considered.

Results showed that at plot level there is only a slight positive correlation between *S. hispanicum* and bryophyte cover. At subplot level the number of individuals increases when bryophyte cover increases, although the individuals growing on mosses are less developed than in other substrates. The phenological stage is slightly more advanced when the species grows in particular on *Pleurochaete squarrosa*.

The level and distribution of genetic diversity, as estimated by ISSR markers, are typical of species with mixed mating system. In the populations of *S. hispanicum* analyzed, sexual reproduction leads to novel genotypes, while clonal regeneration supports the establishment of offspring in the extreme environments where the species grows.

### 3.2 = PRELIMINARY STUDIES OF THE COROLOGY AND AUTOECOLOGY OF *ANDROSACE BREVIS* (HEGETSCHW.) CES., PRIMULACEAE, A LOMBARDY ENDEMIC SPECIES

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*Androsace brevis* is a protected species according to Regional Law 10/2008, included in the National Red List because of its status as an endemic and as a rare species in its distribution range. It is a high altitude, light-demanding cushion plant, with an early flowering period (end of May to the start of June), present above 2000 m asl on rocky ridges exposed to strong winds, on prealpine terrigenous substrates. The distribution range of *A. brevis* is not yet well defined, and is peculiar compared to the distribution range of other Lombardy endemic species; it consists of two large discontinuous populations separated by Lake Como, on the Western Orobian Alps and the Lugano Prealps and a small portion of the Lepontine Alps (1,2), in addition to more disjointed populations in the Rhaetian Alps (3). The University of Milan has started a research project that aims to define in detail the distribution range of this species (and the causes of this distribution), its autoecology, and to verify the numerical consistency of its populations. To define the distributional range, we started in 2013 with a thorough exploration of the sites known from the literature and neighbouring areas. Data loggers recording temperature and humidity were placed at three sites, and soil and bedrock samples were taken at every site. To monitor the status of populations at each study site, we counted all individuals for each population and, for every cushion, we counted the number of live and dead rosettes, number of fruits, and the proportion of cover for nearby vegetation. The autoecology research is in progress with the Native Flora Centre (Centro Flora Autoctona, CFA; Galbiate, LC), with *ex-situ* and *in-situ* germination tests; to define the autoecological characteristics of the species the calculation of CSR strategies (4) is in progress, using the methodology proposed by Pierce et al. (5). Preliminary results of the first year of research are summarized as follows:

**DISTRIBUTION RANGE:** research in the summer of 2013 confirmed that M. Fioraro (2431 m) is the eastern range limit in the western Orobian Alps, and M. Camoghè (2228 m, Switzerland), in the Lugano Prealps is the western range limit: in the next season we will investigate the northern range limit, currently suggested as M. Sasso Canale (2411 m) in the Lepontine Alps, and the eastern distributional range limit in the Rhaetian Alps, where the species is currently confirmed only for the Codera Valley (6) on Pizzo Prata (2727 m): the sites reported in (3) for Passo dell'Oro and Passo del Muretto appear very unlikely, because in these areas *Androsace alpina* Lam. is very common. The data-loggers were placed at the eastern range limit, at the same altitude, on M. Ponteranica (2373 m, rich populations), M. Fioraro (poor populations, eastern limit) and on Pizzo Rotondo (2224 m), close to M. Fioraro but outside the distribution range. Calcium content and pH analysis of soil and rock samples are in progress.

**AUTOECOLOGY:** seed germination tests are in progress at the CFA, on a sample of 1500 seeds collected in 2013: preliminary results show that *in vitro* germination is favoured by absence of light and by hormone presence (gibberellins): germination rate in these conditions, after 2 months, is close to 70%, with only 10% in the control treatment. To estimate reproductive capacity *in situ* we put, in September 2013, 150 seeds in three plots on the ridges of M. Ponteranica in the western Orobian Alps.

The calculation of the CSR strategy reveals a stress-tolerant strategy, with a poor ruderal component and absence of competitive traits: a limited degree of plasticity in the values of S and R is apparent between different populations.

**CENSUS:** in the 2013 season we identified and counted 25 populations (11 in the Lugano Prealps and 14 in the western Orobian Alps) for a total of 610 individuals counted: preliminary observations show that the number of fruits is low in comparison to the number of flowers.

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### 3.2 = EFFECTS OF ACUTE HEAT STRESS DURING ANTHHER AND POLLEN DEVELOPMENT IN *LYCOPERSICON ESCULENTUM* CV MICRO-TOM

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The exposure of plants to extreme temperatures affects their geographic distribution and their yield quality and quantity (1). One of the most sensitive process to high temperature is plant reproduction, particularly the male apparatus (2). Thanks to its small size, rapid growth and easy transformation, *Lycopersicon esculentum* cv Micro-Tom has been proposed as one of the preferred variety of tomato plants to carry out molecular research (3). Although it is widely used as model organism, its use is quite recent and little is known about its response to heat stress. Plants at three flower bud stages: meiotic stage (2 mm long), microspore stage (4 mm long) and mature stage (6 mm long) were stressed with high temperature (42 °C for 3 hours) to study the effects on mature anthers. Investigations by light microscopy were carried out with three different stains: Alcian Blue, Toluidine Blue O and Periodic Acid Schiff. The only remarkable difference in morphological features was the absence of starch in cells of anthers treated at microspore stage. Pressman (4) reported that in tomato high temperature inhibited starch deposition in developing pollen, either through a decrease in the availability of assimilates or through the impairment of the activities of enzymes involved in starch biosynthesis or in sucrose hydrolysis, namely invertase and sucrose synthase (SuS). The immunolocalization evidenced the absence of the SuS enzyme in mature anthers treated at microspore stage, confirming that probably the HS interfered with the presence of SuS and starch. Scanning electron microscope observations revealed several alterations in shape and size of pollen grains. Pollen grains from anthers treated at the meiotic stage were reduced to collapsed exine coat; the majority of pollen grains from anthers treated at the microspore stage was crushed, whereas most of pollen grains treated at the mature stage was round in shape. Pollen viability and germinability tests showed that only pollen grains of mature treated anthers had a viability percentage similar to the control; the germination percentage of pollen grains of anthers treated at all developmental stages was extremely low. Molecular analysis on two heat shock factors (HSFA2 and HSFB3a) highlighted an attempt of HS response by treated anthers, especially for HSFA2, which showed a significant increase of expression after HS exposure. The severe physiological alterations caused by HS irreversibly affected the male apparatus of Micro-Tom plants, thus impairing its reproductive performance.

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### 3.2 = PHYTOTOXICITY OF HALLOYSITE-SUPPORTED IONIC LIQUID-LIKE PHASE (HNT-SILLP) CATALYST ON *RAPHANUS SATIVUS* L.

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Nanotechnologies and nanomaterials are increasingly involved in the production of fillers, opacifiers, catalysts, semiconductors, cosmetics, microelectronic components and drug carriers with improved properties. Nevertheless, the production, use and disposal of nanomaterials, will inevitably lead to their release into the soil, with potential phytotoxicity on plants and negative impacts on economy, society and environment (1). In the last years halloysite nanotubes (HNT) emerged as promising materials with appealing perspective for technological applications. We have recently reported the generation of HNT derivatives carrying octylimidazolium moieties on the external surface (HNT supported ionic liquid-like phase, HNT-SILLP) and employed them as supports for palladium catalyst (HNT-SILLP/Pd) (2, 3). These studies have shown that these materials are good catalysts and encourage their potential application in large-scale industrial processes.

The present research aimed to investigate the potential phytotoxicity of HNT, HNT-SILLP and HNT-SILLP/Pd, by considering different endpoints as seed germination physiology and cytogenetic analyses (4).

*Raphanus sativus* L. seeds were imbibed in distilled water (control) or in HNT, HNT-SILLP and HNT-SILLP/Pd, incubated at 25°C in the dark, under continuous agitation up to 72 h. Germination percentage and mean germination time, together with fresh and dry weights were evaluated in control and HNTs-treated seeds. To further rule out possible impacts of these nanomaterials on genetic stability, the accumulation of nanotubes during seedling development by means of thermogravimetric analysis, IR spectroscopy and cytogenetical analysis on radical meristems were performed.

The preliminary results here presented show that these nanomaterials do not affect the germinative process and the development of the seedling. Thermogravimetric analysis and IR spectroscopy studies additionally showed that exposure to the investigated nanomaterials does not lead to an accumulation into the seedling organs since the plant cell wall might act as a barrier, efficiently preventing entry of these nanoparticles into the cell.

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### 3.2 = *QUERCUS ROBUR* L. TREE-RING ANATOMY AND DENDROCLIMATOLOGY: AN IMAGE ANALYSIS APPROACH

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In trees, changes in environmental conditions cause metabolic processes variations that result in vessel density, width and general wood structure of annual rings (1). By means of tree ring-anatomy, an approach based on dendrochronology and quantitative wood anatomy, it is possible to assess cell anatomical characteristics (such as vessel size, density and tissues percentage) along a series of dated tree-rings and to analyse them through time in order to characterize the relationships between tree growth and various environmental factors, such as climate (2).

Herein we present the preliminary results of a tree-ring anatomy investigation, based on image analysis, conducted in the "Siro Negri" Forest Reserve (Pavia, N-Italy), a natural broadleaves mixed forest dominated by oak (*Quercus robur* L.).

Within the Reserve, we sampled ten dominant oaks by a 5-mm increment borer (1 or 2 cores per tree). After dendrochronological preparation, tree-ring measuring and cross-dating, a core from each tree was scanned at 1,500 dpi resolution and 48-bit colour depth using an Epson Expression 10000 XL scanner. Digital images were processed by means of ROXAS, automated image analysis system, which allows the extraction of xylem vessels according to morphometric criteria and the detection of annual rings by pattern algorithms that analyse the local anatomical context of each vessel (3). Among the variables furnished by ROXAS, the following were chosen: within earlywood the number of vessels (VNo), mean vessel diameter (MVD) and total area of all counted vessels (VTA), linked to water conduction; total ring area (TRA) and net wood (NW), roughly estimating tree productivity (fig.1). Variables autocorrelations were eliminated using ARSTAN (4). Correlation analyses were performed between tree-ring variables and climatic variables obtained from HISTALP data-base (monthly precipitation and monthly temperature, 5).

Significant relations are detected mostly between tree-ring variables and temperature. In fact monthly precipitations are scarcely correlated with all the variables, expressing both water conduction capacity and tree productivity. This may be expected, being the Reserve sited in the Po river alluvial plain where water supply is guaranteed regardless of precipitations. Temperatures result to be constraining mainly conduction variables. In fact, the number of vessels (VNo) and vessels total area (VTA) are positively correlated with temperatures of the previous autumn and negatively correlated with winter temperatures. In addition, higher spring temperatures positively influence vessel dimensions (MVD). Thus it is likely that temperatures are crucial for plant metabolism: mild autumn temperatures lead to a higher vessel-cells production. Their development is favoured by higher spring temperatures, while colder winters block the carbohydrates consumption of the reserve that would be used for the vegetative growth.

Surprisingly, a positive relation is obtained between vessels total area (VTA) and net wood of the previous year ( $NW_{t-1}$ ), suggesting that processes governing the big earlywood vessels production were influenced not only by environmental conditions at the moment of their formation, but also by anatomical features of wood formed in the preceding season. This may be linked to water transport capacity of small conduits widespread within latewood. In the future it will be interesting to model and eliminate this previous-year wood signal from vessels chronologies in order to enhance their response to climate and better understand the role played by temperature on earlywood vessels formation.



Fig. 1 - Example of earlywood vessel extraction and tree-ring detection by image analysis on oak wood

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## 3.2 = NICKEL TOLERANCE IN FUNGI AND PLANTS SELECTED FROM METAL-RICH SITES

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Metal contamination is one of the world's major environmental problems, posing significant risks to ecosystems. The physicochemical properties of the contaminated environment tend to inhibit soil-forming processes and, consequently, plant growth, affecting the area's biodiversity and exerting a strong selective pressure on the local flora and mycobiota (1, 2, 3, 4). Therefore soil communities are strongly affected by environmental changes; in particular, microorganism communities can provide important information about soil metal bioavailability (5). The importance of below and aboveground biodiversity is increasingly considered for the cleanup of the metal-contaminated ecosystems. Care should be taken in choosing the right species for the application of bioremediation techniques, because the introduction of alien fungi and plants may alter and disrupt indigenous ecosystems (6), and because the species may be unsuitable for local climate conditions (7). This subject represents a key area of research thanks to its ecological and commercial significance in the contemporary field of green technology.

In the present study we evaluated the plant and fungal diversity in Ni-rich soils under natural and altered conditions (co-contaminations with other metals e.g. Cu, Zn, Cr, Co, etc.) in order to identify and select tolerant and hyperaccumulating plants and fungal strains suitable for Ni-rich soil remediation.

To test the growth responses of isolated strains in nickel enriched media and to evaluate their potential use in mycoremediation, native soil microfungi were screened for Ni tolerance: *Trichoderma barzianum* Rifai, *Clonostachys rosea* (Link) Schroers, Samuels, Seifert & W. Gams, *Aspergillus alliaceus* Thom & Church and *Eurotium amstelodami* L. Mangin. All microfungi strains were screened for Ni tolerance at Ni 0, 200, 400 and 800 mg L<sup>-1</sup>. The tests revealed *Trichoderma barzianum* as the species with the best Ni-tolerance capability with high growing rate also at Ni 800 mg L<sup>-1</sup>.

These preliminary analyses prove that several fungal and plant species are able to grow in Ni-contaminated media, underlying the importance to select new tolerant strains and test their potential metal uptake capability for application in bioremediation protocols.

A screening test with dimethylglyoxime (DMG) and ICP-MS analyses highlighted *Alysioides utriculata* (L.) Medik. as a new Ni hyperaccumulator (8). Plant efficiency test under Ni-rich soils (total Ni=2448.7±841.4 mg kg<sup>-1</sup>, bioavailable Ni=155.46±75.89 mg kg<sup>-1</sup>) and "normal" soils (total Ni=149.15±62.41 mg kg<sup>-1</sup>, bioavailable Ni=11.09±8.02 mg kg<sup>-1</sup>) were carried out to evaluate the growing ability of this promising species, considering any possible toxic effect, i.e. low germination index, leaf necrosis occurrence, biomass and photosynthetic decrease.

The results suggest the use of these plants and fungi for developing experimental protocols of bioremediation and habitat restoration avoiding ecosystem disruption and minimising interventions and costs in a Mediterranean habitat.

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### 3.2 = EFFECT OF ASCORBIC ACID, SALICYLIC ACID ON CORIANDER PRODUCTIVITY AND ESSENTIAL OIL CULTIVATED IN TWO DIFFERENT LOCATIONS

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Coriander is recognized as one of the most important spices in the world and is of great significance in international trade (1). The dried fruits are used for different purposes, such as food ingredients, cosmetics, perfumery and drugs. As a medicinal plant, it has been recommended for dyspeptic complaints, loss of appetite, convulsion, insomnia, anxiety, hypolipidemic, indigestion, carminative, diuretic, tonic, stomachic, and against worms and rheumatism (2, 3). As a result of higher demand on Coriander plant as raw material and its products and maximizing the use of coriander straw as a new source of essential oil instead of neglecting this byproduct. For this, it is better to study the behavior of this plant and its cultivation under the conditions of soil salinity in El-Tina plain area as a step towards the development of Sinai Peninsula that represents severe soil salinity (4) since no reports were traced on coriander productivity cultivated in El-Tina Plain, North Sinai, Egypt. The objective of this work was to evaluate the effect of salicylic acid, ascorbic acid or combination on productivity, essential oil of coriander plant cultivated in two different locations.

For this, a field experiment in 2010/2011 and 2011/2012, was conducted in Egypt to determine the effect of vitamin C (0 and 400 ppm), salicylic acid (0 and 400 ppm) and region (Nile Valley and Delta, Giza governorate) and (Sinai Peninsula, North Sinai governorate) on coriander productivity, oil content and composition. Generally found that the cultivation of coriander in Giza gave the best results from cultivation in the North Sinai. For transactions spraying found that spraying vitamin C + salicylic acid was superior at a positive impact compared to vitamin C or salicylic acid alone or the control at most of the studied traits. As for the transactions of interaction was observed that the treatment by spraying vitamin C + salicylic acid under the conditions of the Giza region gave the best results for all traits with the exception of the percentage of oil in both the seed and straw where given a treatment spray with vitamin C gave the highest percentage of seed and straw volatile oil in both Giza and Sinai, respectively. In view of the components of the volatile oil found that compounds Linalool,  $\gamma$ -terpinene and  $\alpha$ -pinene in the seed and compounds linalool,  $\gamma$ -terpinene, *p*-cymene, decanal and limonene in straw is the main compounds.

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3.2 = EFFECT OF SHORT-TERM CADMIUM STRESS ON *POPULUS NIGRA* DETACHED LEAVES

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Soil pollution by toxic metals from human activities is a worldwide major concern which can be faced by phytoremediation, a low cost and low impact green technology to clean contaminated environments. Plants exhibit different degrees of tolerance to heavy metals, as a consequence of their ability to exclude or accumulate them in particular tissues, organs or sub-cellular compartments. Different pieces of evidence indicate that poplar trees (*Populus* sp.) possess a high tolerance to most of toxic heavy metals, thereby representing a very promising tool in phytoremediation. To investigate poplar (*Populus nigra*) short-term response to cadmium stress, we analyzed PSII quantum yield, ROS generation, hormone levels variation, as well as proteome profile alteration of 50  $\mu$ M CdSO<sub>4</sub> vacuum-infiltrated leaves. Cadmium treatment brought about an early and sustained production of hydrogen peroxide, an increase abscisic acid, ethylene and gibberellins content as well as decrease in cytokinins and auxin levels, whereas photosynthetic electron transport was unaffected. Proteomic analysis revealed that twenty-one proteins were differentially induced in cadmium-treated leaves. Identification of fifteen polypeptides allowed to ascertain that most of them were involved in stress response, photosynthesis and energy production.

### 3.2 = THE RESPONSE OF ROOT TO BENDING STRESS: ANALYSIS AT ANATOMICAL AND MOLECULAR LEVEL

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Despite their great importance for productivity and survival, plant biology investigations have poorly characterized the perennials root growth cycle and its response to environmental stresses. Indeed while the molecular mechanisms implicated in root response to mechanical stress have been extensively investigated in the model plant *Arabidopsis thaliana*, (1,2) the response of woody plant roots to mechanical stresses have been little investigated. By using a simple experimental system to mimic mechanical stress in poplar woody roots, we previously found that the intensity of tension and compression forces and the direction of gravity in bent woody roots can elicit specific responses such as lateral root emission and reaction wood formation (3, 4). In addition we found that that poplar woody taproot: a) uses different temporal and spatial mechanisms to respond to mechanical stress; b) these mechanisms are finely regulated by hormones; c) the long-term treatment reinforce the defence machinery, thereby enabling the taproot to better overcome winter and to be ready to resume growth earlier than controls (4, 5, 6,7). To further understand how different intensity of tension and compression forces can elicit specific responses in the three bent root sectors (Above Bending Stress, Bending Stress and Below Bending stress) each bent sector was divided in a left and right side (Fig. 1) and subjected to anatomical investigation. Preliminary results indicated the occurrence of significant differences between the left and right side of each sector especially evident in terms of number of cambial cells and differentiated cells, fibers and vessels wall, phloem and xylem thickness.

Moreover, in order to identify key factors controlling poplar woody root responses to mechanical stress we analysed the expression pattern of mechanically induced miRNAs Ptc-miR408, Ptc-miR172, Ptc-miR164, Ptc-miR162 and Ptc-miR473, previously identified by Lu et al.(8).

Quantitative RT-PCR analysis revealed that amplification products had nucleotide sequences aligned with the mechanically-responsive miRNAs identified by Lu et al. (8) and that their expression undergo to spatial and temporal variations. Overlapping miRNAs expression patterns and target gene functions analysis with our previous morphological and proteomic data, we highlight how in the bent poplar root, miRNAs play a pivotal role in the formation of reaction woody and lateral root development.

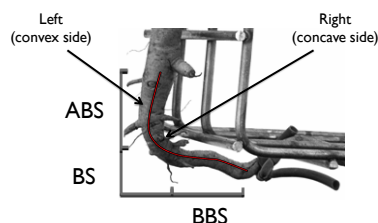


Fig. 1. Bent poplar root divided according with different intensities of tension and compression forces.

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### 3.2 = METABOLOMIC APPROACH TO STUDY PLANT-PLANT INTERACTIONS IN MEDITERRANEAN ECOSYSTEMS

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Metabolomics has shown to be a useful tool for studying plant responses to biotic and abiotic stresses (1) and it seems a promising approach also for the study of allelopathic interactions (2), the chemical mediated plant-plant communication system (3). Such interactions play probably a central role in structuring ecosystems like the Mediterranean one, characterized by high plant diversity (4).

An NMR-based metabolomic approach has been proposed to study these interactions (5).

In order to explore the potential of a metabolomic approach in allelopathy studies, selected Mediterranean plants were chosen and analysed for their plant growth regulation potential.

Donor plant extracts were analysed by NMR-based metabolomics and used for the bioassays on the receiving plant. The receiving plants were analysed and compared with controls by NMR-based metabolomics using chemometrics.

The experimental design allowed the chemical analysis of donor plant extracts as well as the assessment of the effects of the potential allelochemicals on receiving plant metabolism. Many donor plants were found to be active and, among them, different groups could be observed. The activity was correlated to the donor plant metabolome (hence, putative allelochemicals or active "phytochemicals" were identified).

Concerning the receiving plants, the metabolic pathways affected by the allelochemicals were identified. Furthermore, the fate of allelochemicals in the receiving plants was studied and many of them seem to be taken up by the receiving plants.

As secondary metabolite production could vary with seasons and phenological stages, the presence of active compounds was also monthly determined over a two years period. Finally, for some active compounds, their presence in the soil was proven.

Although the full elucidation of allelopathic interactions requires further studies, the NMR based metabolomic approach proved to be a useful tool and gave important new insights in the interactions occurring in Mediterranean ecosystems.

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### 3.2 = PHYTOSOCIOLOGICAL OUTLINES OF TWO ROCK GLACIERS OF THE ORTLES-CEVEDALE MASSIF (STELVIO NATIONAL PARK)

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Rock glaciers are periglacial landforms composed by debris and ice, that are drawing the attention of plant ecologists as harsh habitats and potential refugia in the global change context (1, 2, 4, 5). We have analyzed the vegetational features of two alpine rock glaciers, comparing them with surrounding environments: alpine grasslands as climax vegetations and scree slopes as iceless debris substrates. The study has been carried out in two areas of the Ortles-Cevedale Massif, in Stelvio National Park: one on silicate acid rocks (Val d'Ultimo, BZ) and the other on carbonate substrate (Valle del Braulio, SO). Plant communities have been detected by phytosociological method; data have been analyzed by cluster analysis based on the presence or absence of species, using the UPGMA method with Jaccard Similarity Index (fig. 1). The cluster analysis identified first of all the two areas, highlighting the substrate as main discriminating factor. The alpine climax vegetation observed in Val d'Ultimo was an acidophilous grassland belonging to *Caricetum curvulae* (Kerner 1863) Brockm.-Jer. 1907; plant assemblage of the rock glacier is ascribable to *Luzuletum spadiceae* (Brockm.-Jer. 1907) Br.-Bl. 1926, while the neighboring scree slope is characterized by *Oxyrietum digynae* (Lüdi 1921) Br.-Bl. 1926 communities (3). The alpine climax vegetation observed in Valle del Braulio was a basiphilous grassland belonging to *Caricetum firmae* (Kerner 1863) Br.-Bl. 1926; the communities of rock glacier and scree slopes are undiscerned and both afferent to *Thlaspectum rotundifolii* Br.-Bl. (1918) 1926 (3), but rock glacier's flora stands out for the presence of two cold-adapted species: *Arabis caerulea* All. and *Saxifraga oppositifolia* L.. In summary, the analyzed rock glaciers differ more or less evidently from the adjacent scree slopes and drive an articulate landscape framework that allows the permanence of cold-adapted entities at alpine grassland's elevations.

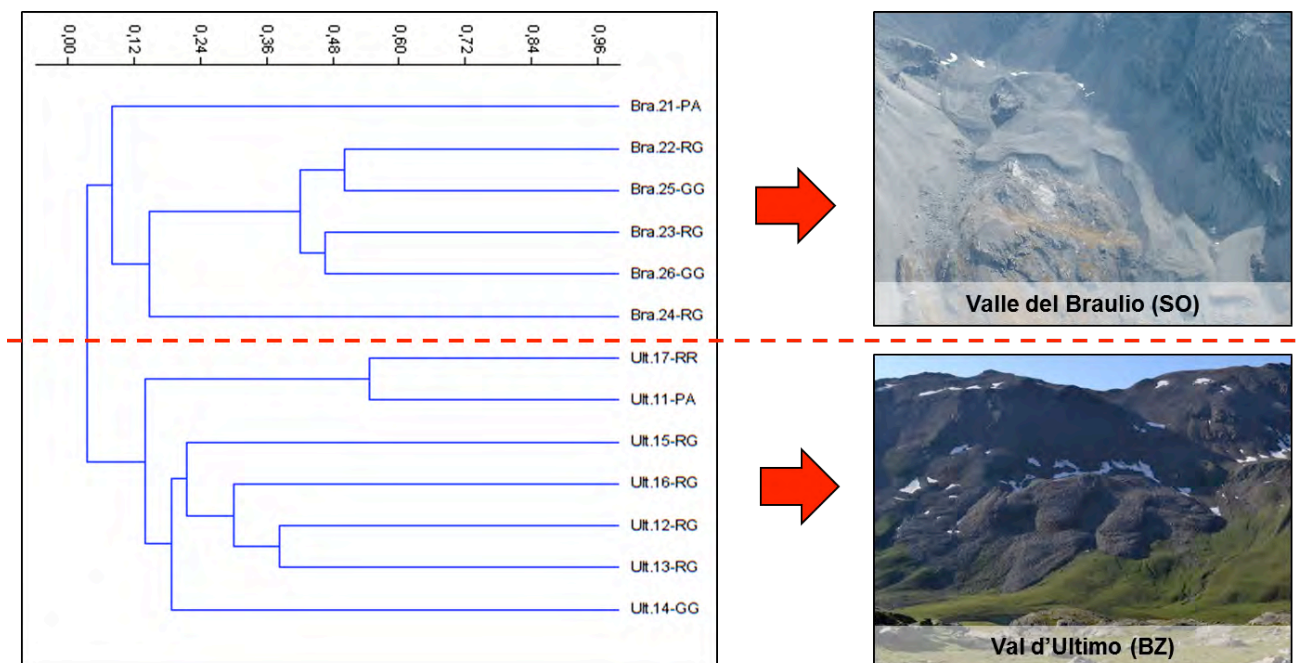


Fig. 1. Dendrogram resulting from the cluster analysis and images of the two analyzed sites.

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## 3.2 = RARE EARTH ELEMENTS AS A DOUBLE-EDGED EFFECTOR IN CROP AND NATIVE PLANTS

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Rare earth elements (REE) are largely used for high technology applications worldwide but also as fertilizers and animal feeding additives in the Far East. The increasing use of REE in agricultural systems raises concerns related to a possible alteration of their natural biogeochemical cycle, with effects on food chain and human health. The effects of REE on plants are poorly understood and somewhat controversial, since growing evidence points both to the use of REE enriched fertilizers in Chinese agriculture and to REE-associated toxicity in plants and animals in either *in vivo* and *in vitro* systems. A number of adverse effects have been so far reported in REE-exposed plants or plant cells, including growth inhibition, decreased photosynthetic activity, mitochondrial dysfunction, oxidative stress, and cytogenetic anomalies (1-3), while accumulation of REE has been reported in some soil-borne fungi (4). REE accumulation in the soil system and REE uptake and accumulation in plants and microorganisms should be properly elucidated in view of a decision to banning or extending REE use as fertilizers. Another relevant co-effector in REE-associated toxicity consists of pH decrease in soil or in growth medium associated to the coating REE nanoparticles by organic acids, that results in an enhancement of REE-associated toxicity. The association between acid and REE pollution, especially in areas close to mining sites and to REE manufacturing facilities makes the low pH-associated REE toxicity a major environmental threat to environmental health (5-6). Beyond the thriving literature on REE-related health effects, outstanding differences remain between the number of published reports on the effects of a restricted number of REE, mainly La, Ce and Gd, on plants and other biological systems, on one side, and the scarce, if any, studies so far reported on other REE, such as Sm and other high atomic number REE (7). It should be recognized that the extensive and growing technological applications of Sm and other heavy REE imply massive environmental spread of these elements and, therefore, an increasing exposure of human beings to the those elements, especially following metallurgical processes. Under this point of view, the lack of toxicological information should be regarded as a potential concern to environmental and human health. Thus, suitable investigations are needed for both elucidating REE-associated toxicity and focusing on the health effects of heavy REE. Further studies are needed to elucidate the REE-induced effects on the health of plants and other organisms, in order to establish concentration-related trends of any favorable or harmful effects of REE on Biota. In the present study, the effect of Ce supply on growth performance and antioxidant metabolism in *Lemna minor* plants is reported. The metabolic alterations observed support the hypothesis that Ce exerts a potential toxic effect although under a hormetic trend, since a shift from stimulating to inhibitory effects were observed.

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### 3.2 = THE ENDOSPHERE OF LEGUMES: PLANT GROWTH PROMOTION TRAITS OF THE BENEFICIAL BACTERIAL COLONIZERS

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Plants host distinct microorganism communities on and inside various organs; the diversity of microorganisms associated with healthy plants is enormous, and a critical importance is attributed to the remarkable richness of beneficial bacteria that can promote plant growth and yield. It has been reported that the life cycles of some endophytes are not restricted to plant but can cross different biological kingdoms including strategies within the animal and human hosts. The presence of bacteria belonging to the class Gammaproteobacteria, including *Pseudomonas* and *Enterobacter* in the root nodules of some Mediterranean wild legume species has been reported (1). In the present work two wild legume endophytes, *Pseudomonas* sp Hs1 (GenBank AY531218) and *Enterobacter agglomerans* Hs6 (GenBank AY531217), were investigated for their growth promoting activities. Direct PGP abilities of the strains were screened using biochemical tests; nitrogen fixation (endophytic diazotrophy), ammonia production from peptones, phosphate solubilization and siderophores production were evaluated. By *in vitro* screening on *Arabidopsis thaliana* plantlets, growth promoting effects of the strains were assessed analyzing roots architecture and shoots development. Roots grown in the presence of bacterial endophyte were shorter than the control roots and showed an increased number of lateral roots and hairs. In addition, a search for interesting molecules produced by *Pseudomonas* sp Hs1 and *Enterobacter agglomerans* Hs6 was performed. By mass spectrometry techniques the identification of valuable metabolites potentially related to plant growth and yield was carried out. In this work different approaches were used to select endophytic bacteria useful to improve and manage, in an environmental friendly way, the healthy growth of Leguminous plants that have a recognized role in agriculture, animal and human diets and forestry.

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### 3.3 = REPRODUCTION, STABILITY AND IMPORTANT SPECIES OF M. LESIMA GRASSLANDS (NORTHERN APENNINE): A NETWORK ANALYSIS

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Northern Apennine grasslands have never been studied from a pollination ecology perspective. Nevertheless this area hosts a high amount of biodiversity and protected species and habitats. Therefore, it is important to understand the reproductive strategies of the vegetation, the structure of plant-pollinator interactions, the key taxa that keep stable the structure and connect otherwise isolated sub-networks, and the influence of cattle on plant reproduction. In our work, we investigated two montane grasslands, sited on Mt. Lesima (at about 1650 m a.s.l., Pavia Province), which were previously analyzed according to the phytosociological method (1): *Brachypodium rupestre* and *Festuca laevigata* community and *Laserpitium siler* and *Sesleria picchiana* community (*Bromion erecti*, *Brometalia erecti*, *Festuco-Brometea*). The *Brachypodium rupestre* and *Festuca laevigata* community is subjected to cattle grazing. In 2013 quantitative data were collected to describe their plant-pollinator networks. To record insects foraging on flowers, a sampling scheme based on three plots of 2.5x2.5 m was established in each plant community, sampled weekly for 20 minutes, twice a day. All insects in contact with reproductive structures of plants were considered pollinators and thus they were captured. At each date, the total number of inflorescences for each species in flower was counted. To identify the prevailing pollination strategy we computed the percentage of entomophilous plants and anemophilous plants, with both cover-abundance data and number of species. Seasonal modules of plants that flower together (pheno-modules) were identified treating the number of per-species inflorescences as variables of reproductive effort. On such seasonal units, we performed a network analysis of plant-pollinator interactions by means of several metrics computed in the Bipartite R package v.2.03 (2): realized links (connectance), number of pollinator species/number of plant species (web asymmetry), generalism (H2'), nestedness (NODF) and species-level weighted betweenness. In addition, clusters of intensely connected species (sub-networks) were detected by means of the weighted Newman's modularity measure (2). The main results show the prevalence of entomophilous taxa in comparison to the anemophilous ones, for both abundance and number of species, underlying the importance of insects in pollination (3) and the key role of species-rich grasslands in diversity maintenance. *Laserpitium siler* and *Sesleria picchiana* community (not grazed, 3 pheno-modules, 34 pollinated plant species, 145 pollinator species, 1677 interactions) resulted more complex and richer than *Brachypodium rupestre* and *Festuca laevigata* community (grazed, 2 pheno-modules, 29 pollinated species, 76 pollinator species, 323 interactions). Regarding plant-pollinator networks of montane grasslands, low levels of realized links (connectance) were detected, suggesting a probable bias towards specialization in plants. Nevertheless, network metrics changed over the season: as the season progressed to the final stages, both plants and insects became more generalized, possibly due to an increase in the pollinator/plant ratio. The presence of intense cattle grazing mainly in the last part of the flowering season largely accounts for the reduction in flower availability to insects and cattle also impacts on plant reproduction (eating both the flowers and the developing ovules). Nestedness values (NODF) were generally low. Thus, the nested structures are based on few species as pivot clusters, probably as result of altitude effect on insects. Because it is theoretically believed that networks with higher nestedness values are more cohesive and with higher possibility to rare species to survive (4), nestedness can be also interpreted as a stability measure. In the first phase of the season, NODF values of both plant associations were the lowest and also not significantly different to the null models suggesting a low stability in the starting phase (when the weather is also particularly unstable). Any additional disturbance may be critical in this part of the season. The pivot species (that links sub-networks of intensely connected species) are highlighted by high values of weighted betweenness. The presence of such important species establishes a cohesive structure in these plant-insect interactions. Such taxa were: *Ranunculus* spp., *Tulipa australis*, *Knautia drymeia*, *Hypericum richeri*, *Serratula tinctoria* and *Phyteuma ovatum*. Their protection is important for reproduction and stability of the entire grasslands.

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### 3.3 = PLANT TRAITS DRIVE SPECIES TURNOVERS IN THE HERB LAYER OF OLD-GROWTH BEECH FORESTS

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Studies of fine-scale species dynamics (i.e. local species turnover and species mobility) have great importance for understanding of patterns of species coexistence and relative ecological mechanisms, but few of them are related to forest ecosystems and are supported by long term observations. It represents a noticeable lack because the herb layer in forests seems to be particularly sensitive to disturbance, including sudden changes of environmental condition (1). In this study, we assess if the species turnovers in herb layer of old-growth forests is driven by stochastic processes or if some specific plant traits can explain mechanisms of species extinction and species persistence.

We selected four Permanent Monitoring Plots (PMPs, 50 x 50 m) of the Italian forest ecosystem monitoring network (CONECOFOR) located in old growth (>100 years) beech forests distributed along a latitudinal (span of Latitude: 38°25'– 46°03') and climatic gradient (span of the mean annual precipitation: 1250–1900 mm). The four PMPs are located in the following Italian Regions: Veneto, Abruzzo, Campania, Calabria.

Within each PMP, we established a systematic grid of 100 quadrates (0.5 x 0.5 m each) to monitor the coverage of all plant species of the herb layer with eight surveys during a period of twelve years (1999–2011). In order to improve the consistency and data sets comparability, a Quality Control (QC) programme has been applied (2).

We selected 19 above-ground and below-ground easy-to-measure plant traits related to key processes such as resource acquisition, regeneration, reproduction, and stress tolerance, and compiled a complete species x trait matrix for all 93 species found. Distributions of traits within the entire community were compared to the trait distributions in subsets of species that became extinct or remained persistent during the surveys. We used Chi-Square statistics to test if there were significant differences between the expected versus observed trait probability distributions.

Considerable temporal species turnover was found but without successional trends. Within stands spatial heterogeneity was also significant. In all PMPs, *therophyte* species, plants with *non-clonal stem* and plants with low *seed mass* became extinct more often than expected. In the three southern PMPs, the extinction was also more probable for species not equipped with consistent below ground *bud bank* and having *hygromorphic* leaves, while these patterns did not appear as prominent in the northernmost PMP (Veneto) characterized with the highest annual rainfall (1900 mm). Intermediate *SLA* values and *long dispersion* distances were characteristic for species with high persistence. We conclude that a rich pool of plant traits plays an important role in determining the fine-scale temporal dynamics of species in understories of old-growth beech forest. The slight variability found along the climatic gradient may point upon a context-dependent role of the functional traits.

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### 3.3 = ROE DEER (*CAPREOLUS CAPREOLUS* L.) BROWSING EFFECTS ON MIXED COPPICE STANDS IN CENTRAL ITALY

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The coexistence of ungulates and forest ecosystems has been gaining increasing attention, mainly because of the relevant shift in wild ungulate populations observed in the last few decades in most European countries, where, ungulates have exhibited geographic and demographic expansion (1, 2). This is a particularly relevant issue for roe deer (*Capreolus capreolus* L.), i.e., the most widespread European deer species, with an estimated 10 million individuals occurring in the continent (1). Roe deer population densities have been growing notably during the last few decades due to the abandonment of rural areas, changes in human land use and lack of predators (1). In addition, changes in silvicultural systems have entailed a general decline in wood exploitation, improving productivity and structural complexity of forest ecosystems. Among the consequences of these relevant ecological changes was the development of favourable conditions for the natural diffusion of roe deer (3).

The problems of roe deer browsing have long been recognized in conifer species (4, 5). Recently, the problem of roe deer impact has also been observed in coppice woods, a silvicultural system which is widespread over Mediterranean countries. In Italy, coppice is the most frequently adopted silvicultural systems in private forests, and it amount to about 56% of the total forested areas in Italy. Despite such a large diffusion and importance, poor studies have focused on roe deer impact on species composition, structure and growth of coppice woods (3).

The aim of this study was to asses the effects of roe deer browsing on vegetative regeneration of mixed coppiced stands of Turkey oak (*Quercus cerris*) and chestnut (*Castanea sativa*), two of the most important broadleaved species in Italy. The impact was monitored through a 12 -year period following the coppicing, in order to analyse differences in tree species palatability, the evolution of the degree of browsing through time and to compare the recovering capacity of the two broadleaved species. In “Alpe di Catenaia” study area (43°48’N, 11°49’E), six experimental stands were selected, where, after coppicing, fenced (ungulate access excluded, P) and non-fenced (ungulate influence present, NP) plots were established. From 2002 to 2013, we monitored the impact of roe deer on number, collar diameter, shoot height and biomass growth in both fenced and non-fenced plots.

Results indicated that roe deer feed selectively the two species sampled, with chestnut showing any browsing-related impact on shoot development just after one year from coppicing. Conversely, roe deer browsing produced heavy and prolonged impacts on Turkey oak, with a reduction in growth of -80% after four years, -57% after seven years and -41% after 11 years from coppicing. Our results outlined the high and selective impact of roe deer on oak coppice woods, in agreement with previous reports. Analysis of browsing impact in coppiced areas under different roe deer density confirmed that the current high density of ungulates occurring in many areas of Apennines is enough to produce lasting impact to the coppice species composition and development.

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### 3.3 = SEASONAL VARIATIONS OF BIODIVERSITY AND FUNCTIONAL DIVERSITY IN A ROCKY MOUNTAIN PASTURE UNDER EXPERIMENTAL WARMING: FIRST DATA FROM A LONG-TERM EXPERIMENT IN THE POLLINO NATIONAL PARK

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Global surface temperatures rapidly increased over the last 30 years, with a major emphasis for the Northern Hemisphere (1). Because these changes may induce latitudinal and altitudinal shifts of plant ranges (2, 3), they may have significant effects on biodiversity patterns. Large-scale studies across European mountains (4) revealed significant climate-related changes in mountain vegetation. Warmer scenarios are likely to promote a decline of cold-adapted species, that may be replaced by thermophilous ones. The consequence of such rearrangement is a thermophilization of mountain floras (4). Mediterranean-latitude ecosystems are also expected to undergo substantial variations due to global warming. In such contexts the variation of abiotic conditions promoted by warmer temperatures may further reduce the ecological space available for rare cold-adapted plants, and favour their replacement by mesophilous taxa (5). Nonetheless, at Mediterranean latitudes, the vegetation responses to increasing temperatures are still little known. On behalf of Ente Parco Nazionale del Pollino, in the autumn 2011 three 10 x 10 m permanent research areas were established at different elevation (from 1400 to more than 2000 m a.s.l.) and in different plant communities in the Pollino National Park (6). Each study area, fenced to exclude grazing, includes 8 sample units having surface more than 3.0 m<sup>2</sup>. Four sample units per site are subjected to experimental warming by using hexagonal open top chambers (OTCs) (7). Temperatures internal and external to OTCs are monitored by means of data loggers set on 1-hour of measure interval. To evaluate variations in the community status under warmed and control conditions, during the 2012 and 2013 floristic and vegetation surveys were carried out weekly in each sample unit, from early spring to late summer. Here, we evaluate the effects of two years of experimental warming on seasonal patterns of biodiversity and functional diversity in the study area located at Mt. Serra (1400 m a.s.l.). This site shows mountain rocky pastures dominated by *Bromopsis erecta* and *Festuca circummediterranea*, a vegetation type very widespread on Apennines (8). Levels of Species Richness (*SR*), Shannon diversity index (*H*), and Rao's quadratic entropy (*Q*) were obtained from 152 releves, and then related to the average temperature of the decade preceding each field survey. Overall, the experimental warming increased soil temperature by 0.8 C°. While soil temperature was little related to *SR* and *H*, it showed a significant positive correlation with Rao's *Q*, that was more pronounced in OTC's. This suggests that warming may alter the functional bases which determine patterns of species assembling in the study community.

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### 3.3 = IMPACT OF WILD HERBIVORES GRAZING ON HERBACEOUS VEGETATION AND SHRUBS OF THE SILVER FIR FORESTS IN THE RESERVE NATURALI CASENTINESI: REMOVAL OF BIOMASS, SIMPLIFICATION OF FLORA AND ALTERATION OF VEGETATION DYNAMICS

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The results of ten years of measurements and observations about the impact of wild herbivores in the Foreste Casentinesi are presented here. This research has been carried out in cooperation with the "Ufficio Territoriale per la Biodiversità (CFS)" of Pratovecchio (Arezzo province). The work dealt with flora consistency and consumption of herbaceous and shrub portion (blackberry and raspberry) living in the openings occurring following collapse of trees in the fir plantation forests. Measurements were taken at six different locations and at different elevations and aspects. Open and fenced plots for the protection of vegetation by wild herbivores were established in each location.

At each site, a pair of contiguous permanent micro-plots (2x2m), opposite to the fenced perimeter (i.e. open and close to grazing) were established. Surveys were carried inside micro-plots. The specific composition of flora was determined. Grass and shrub aboveground production was cut and divided into three fractions: grass, blackberry, raspberry. The three components of fresh biomass were weighed and then dried to get the dry matter. In this way, the animal consumption in the grazed plots was estimated.

Findings were as follows: (i) dry matter reveals a remarkable variability between micro-plots and between open and fenced plots; (ii) the amount of herbaceous portion removed by grazing reaches up to 70% of total production; (iii) the consumption of blackberry and raspberry is usually close to 100%. The quite total removal of blackberry determines an obvious simplification in the structure of vegetation cover, too; (iv) the number of plant species consumed is reduced, even though the early animal consumption affects both the completion of the reproduction cycle and their potential diffusion. This phenomenon too, results in a simplification of local flora and breaks the evolutionary dynamics of vegetation, which on the contrary continues inside the fenced plots.

Natural establishment of tree species is quite lacking into the open areas because regeneration does not survive the seedling stage. Silver fir regeneration, historical component of these forests, is especially consumed. Furthermore, this evergreen species is consumable also in winter time. Further investigations are needed on the humus form and pedogenesis process; the grazing overload being a driving factor on these basic bio-ecological patterns, too. Findings underline the evidence of wild herbivores grazing load management in this forest.

### 3.3 = LINKING PLANT REPRODUCTIVE SUCCESS AND FLOWER-VISITING INSECTS ALONG A DEBRIS-COVERED GLACIER FORELAND

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Knowledge about how plants and plant-related insects interact along a primary succession is still scarce (1, 2). We investigated how the changes in plant communities explain the variations in flower-visiting insects assemblages in conjunction with the assess of the reproductive success of flowering plants *Leucanthemopsis alpina* and *Saxifraga bryoides*, two focus species in plant communities colonizing Vedretta d'Amola debris-covered glacier foreland (Central Italian Alps).

Sampling design consisted of five sites representing the main deglaciation stages. Breeding experiments were carried out in each site during 2013 flowering season through the exclusion of pollinators from flowers and the successive analysis of the seed set of both visited and non-visited flowers.

*L. alpina* is mainly visited by ubiquitous pollinators, whose abundance increased along the glacier foreland following vegetation development. *S. bryoides* pollinator abundance was positively influenced by entomophilous plant density and saxifrage cover, showing a decrease in late successional stage.

*L. alpina* resulted self-incompatible while *S. bryoides* was self-compatible. The reproductive success of both species varied among deglaciation stages. Surprisingly, *L. alpina* fitness decreased along glacier foreland and showed an independent trend from pollinator abundance (Fig. 1a). *S. bryoides* showed that out-crossing took priority over selfing as its visited flowers seed set was positively influenced by pollinator availability.

In summary, our results illustrate that plant-pollinator relationship responded to glacier retreat dynamic and therefore emphasizes the consideration of multiple biotic interactions when preserving rare species and habitats.

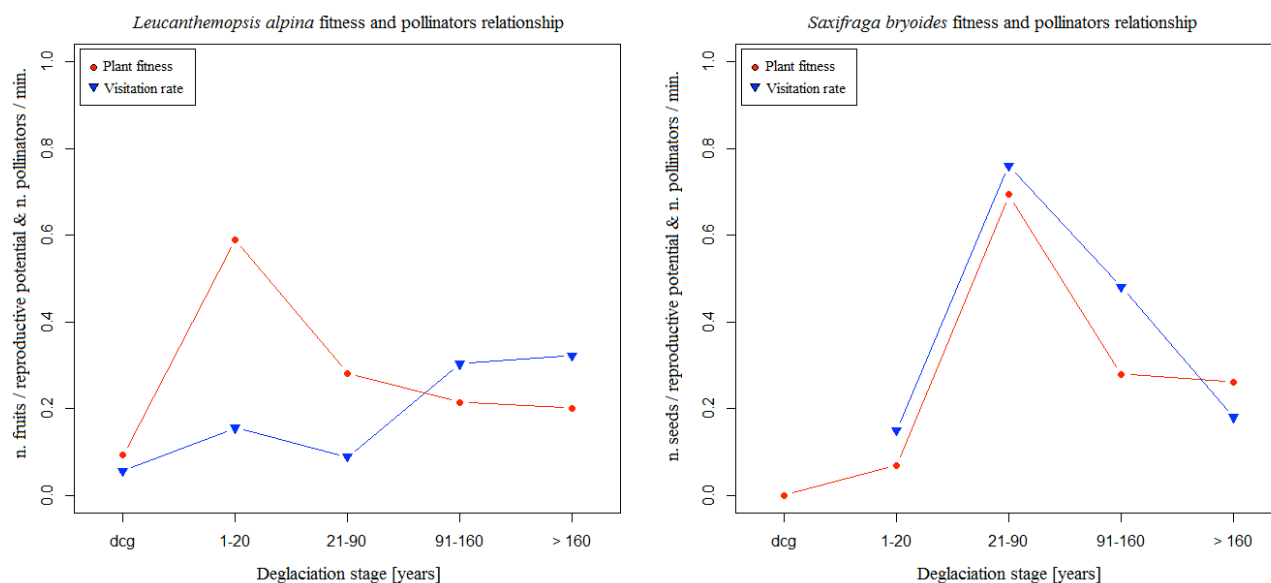


Fig. 1a-1b. Fitness and insects visitation rate of *L. alpina* and *S. bryoides* along the Amola chronosequence.

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### 3.3 = SHRUBS AND TREES ENCROACHMENT IN RESPONSE TO CLIMATE WARMING IN A HIGH ELEVATION ALPINE ENVIRONMENT (ITALIAN CENTRAL ALPS)

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One of the most worldwide evidences of climate change impacts are range expansion and upward migration of shrubs (1). Further, an upward shift of forest tree species is predicted by the 21st century also as a result of climatic changes (2, 3, 4). However, it is still not clear whether shrubs may facilitate or reduce tree recruitment (5,6). In a high elevation alpine area above the treeline (close to Stelvio Pass, in the Central Italian Alps) a recent expansion and upward migration of shrub vegetation was documented since 1950 in a site without any appreciable land use change (7). Moreover, this area is characterized by an important tree colonization (*Larix decidua*, *Pinus mugo*) between 2269 and 2484 m a.s.l. Here we aim to: a) assess when shrub expansion (in particular of *Rhododendron ferrugineum*) started, its expansion mechanisms and whether it was promoted by climate warming, b) assess when tree colonization started in this area, analyze the relationship with *R. ferrugineum* and predict potential feedbacks for the alpine environment.

During the summer 2012 and 2013, we mapped the distribution of *R. ferrugineum* and of the two dominant tree species individuals (including all seedlings and saplings) and, for the dendrochronological analyses (LINTAB 6, Rinntech), we sampled 32 individuals of *R. ferrugineum*, 51 of *L. decidua* and 37 of *P. mugo*.

Totally 471 tree seedlings and samplings were mapped: 342 of *L. decidua* (2296-2484 m asl), 123 *P. mugo* (2269-2480 m asl). More than half (52.7%) of the tree-species individuals were located within shrub communities, half associated with *R. ferrugineum* (27.2%) and half with *Kalmia procumbens* (25.5%).

*R. ferrugineum* showed an age spanning from 12 to 134 years, with the first colonization of the area occurring at the end of the Little Ice Age (LIA) (1878 A.D.) and 75% of the population being  $\leq 80$  years old. Shrub encroachment was relatively slow until the second half of 1960s, when the recruitment of *R. ferrugineum* exhibited a peak involving half of the population in response to a strong increase of air temperature. The expansion mechanisms adopted by *R. ferrugineum* involved: a) lateral growth from existing shrubs, b) recruitment from neighbour patches (infilling), c) clonal growth.

Both *L. decidua* and *P. mugo* exhibited ages significantly younger than *R. ferrugineum*, with half of the population of both species ranging between 5-20 years, meaning that their colonization mainly occurred since the second half of the 1990s, during the most intense air temperature warming since the LIA. Concerning the expansion mechanisms, they were assessed only for *P. mugo*. *L. decidua* was not used for this task due to the low regression coefficients. *P. mugo* followed the same strategy of *R. ferrugineum* for its expansion, except for the clonal growth.

Our data allow to demonstrate that in our study area shrub encroachment and tree colonization started in response to climate warming and that shrub occurrence likely facilitated colonization and development of tree species, probably through a shelter effect, as shown by the high overlap of tree and shrub distribution. If these trends will prosecute, a habitat homogenization may occur, with feedbacks involving potential losses of biodiversity, alpine landscapes, ecosystem processes and services.

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3.3 = RESPONSE TO CLIMATE CHANGE OF *TUBER BORCHII* FRUITING BODIES

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In recent years the issue of climate change has steadily increased in importance both within the scientific world and the political world within civil society. This has set a new awareness: an understanding that climate change is not distant, but rather is already in place and that its effects could be very large.

Several studies have underlined that changes in climate bring changes in fruiting patterns of fungal species. An analysis made in Austria, Norway, Switzerland, and the United Kingdom revealed a widening of the annual fruiting season in all countries during the period 1970–2007 (1).

Climatic changes also influence the optimum growth and distribution of several truffle species (2; 3). *Tuber borchii* Vittad. is an ectomycorrhizal edible truffle, commonly called “bianchetto” (whitish truffle) to distinguish it from the more valuable white truffle found in Italy (*T. magnatum*). Although *T.r borchii* also has a fairly high commercial value, information on its ecology, especially its optimum rainfall and temperature, is lacking.

This contribution aims to illustrate the fluctuation of *T. borchii* fruiting body production in different timescales and show how these alterations are driven by rainfall and temperature. The research, carried out in five different natural *T. borchii* production areas, reveals that the production of truffles is significantly greater after autumn months characterized by abundant rainfall and cold temperatures.

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### 3.3 = CO<sub>2</sub> FLUXES AMONG DIFFERENT VEGETATION TYPES DURING THE GROWING SEASON IN THE HIGH ARCTIC (SVALBARD ISLANDS)

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Arctic ecosystems are highly susceptible to climate change and they play a key role for soil carbon-storage, being a potential CO<sub>2</sub> source in response to climate warming and permafrost thawing.

Here we aim to: 1) analyze the spatial variation of soil CO<sub>2</sub> emission in terrestrial ecosystems measured under selected target vegetation communities in different plot scale; 2) assess which abiotic (soil temperature, soil moisture, PAR, time, active layer thickness) and biotic (vegetation type, dominant species phenology) factors influence CO<sub>2</sub> emissions. For these aims we selected different vegetation types on 8 different plots (dwarf shrub tundra dominated by *Cassiope tetragona*, dwarf shrub tundra dominated by *Salix polaris*, graminoid tundra, moss dominated tundra) along 2 transects of moisture gradient at Adventdalen (Svalbard Islands) where we measured the net ecosystem exchange (NEE) and the ecosystem respiration (ER) during the whole growing season (June-October) in 2013.

Both NEE and ER change depending on vegetation type, while, among the environmental factors, soil temperature is the most important driver for almost all vegetation types, affecting NEE as well as ER. NEE is influenced by soil temperature, soil moisture and dominant species phenology for the graminoid tundra plots dominated by *Calamagrostis stricta*, *Dupontia pelligera* and for the dwarf shrub tundra dominated by *Cassiope tetragona*. Only phenology and ground moisture are the main drivers of NEE for the graminoid tundra dominated by *Poa arctica* and *Calamagrostis stricta*. Concerning ER, ground temperature is the most important driver for all plots except for the graminoid tundra dominated by *Calamagrostis stricta*, *Dupontia pelligera* and *Poa arctica*, where also phenology exhibited a significant effect.

For what concerns the balance of the CO<sub>2</sub> fluxes across the entire growing season, the dwarf shrub tundra dominated by *Salix polaris* acts as a CO<sub>2</sub> source, while the graminoid tundra communities mainly act as a sink.



## 3.5 = THE LPP OF MODENA AND ARCHAEOBOTANY: RESEARCH IN ITALY OVER THE LAST TWENTY YEARS

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 WITH ALESSANDRA BENATTI, ASSUNTA FLORENZANO, MARIA CHIARA MONTECCHI, ELEONORA RATTIGHIERI, ROSSELLA RINALDI  
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The study of pollen, NPPs, seeds and fruits, wood and charcoal from archaeological contexts is essential to investigate the history of man and the environmental changes connected to the anthropic pressure in a territory. The archaeobotanical record is highly influenced by human activity; this feature has to be considered in order to correctly interpret the data, and also it is valuable to obtain detailed informations on relationship between humans and plants from prehistoric to recent times.

Over the last twenty years, archaeobotanical materials from about eighty Italian sites have been studied by our Laboratory (1); the investigation has focused on regions of Northern (especially Emilia Romagna), Central (mainly Tuscany) and Southern (mainly Basilicata and Sicily) Italy. The sites range in dates from the Bronze Age (2), Roman Period (3), Middle Ages (4, 5), Renaissance (6), up to the modern age. These investigations have become more multidisciplinary using the paleo-ecological (7) and ethnobotanical (8) approaches. The data have been interpreted as a possible source of information for the understanding of the biodiversity of the past (9), even for purposes of conservation (10). A new field is represented by the analysis of ancient DNA (aDNA), which can be a source of new and interesting information, especially on crops (11).

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### 3.5 = STUDY OF PALYNOLOGY. A DATABASE FOR POLLEN COLLECTION OF THE MONITORING CENTRE OF THE UNIVERSITY OF ROME TOR VERGATA

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The morphological characterization of pollen grains is very important in Aerobiology and related sciences but in many cases the assignment of a pollen grain to the relevant taxon may be difficult.

The reference pollen collection can be a useful tool to obtain a good morphological identification of pollen grains in different fields of application: aerobiology, melissopalynology, forensic palynology, ecology (1,2,3). For several years, samples were collected from flowers of trees, shrubs, weeds and grasses. A new brand *palinoteca* was realized at the Aerobiological Monitoring Center of University of Rome Tor Vergata: it includes pollen samples from 242 taxa, belonging to 79 families and 191 genera.

The preparation of slides was done according to official procedures (4,5) using pollen extracted from fresh specimens collected in the Italian territory and identified with the aid of dichotomous keys (6,7,8).

To catalogue and quickly find a slide stored in boxes, we created a database using FileMaker pro software.

All samples were then analyzed with the optical microscope Nikon Eclipse 200 and inserted in the database.

Each record is labeled with family, genus and species name, and morphologically described by referring to N.P.C. code and size. Moreover, collection date and site, availability of pollen grains in eppendorf and other useful notes are reported. The input of pollen pictures is in progress.

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### 3.5 = AEROBIOLOGICAL MONITORING OF *POACEAE*: POSSIBLE IDENTIFICATION OF THE SPECIES THROUGH BIOMOLECULAR ANALYSIS OF AIRBORNE POLLEN DNA

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Poaceae are very common plants which are extremely diffused in all environments and urban areas. Although their economical importance, they can represent a problem to humans due to their abundant production of allergenic pollen. Detailed information about the pollen season for these species is needed in order to plan adequate therapies and to warn allergic people about the risks they take in certain areas at certain times. Moreover, precise identification of the causative species and their allergens is necessary when the patient is treated with allergen-specific immunotherapy (SIT).

While classic aerobiological monitoring (that employs optical microscopy techniques) works good for most of the species that produce allergenic airborne pollen, the morphological similarity of grasses pollen grains makes it impossible to distinguish which particular species are present in the atmosphere in a given moment.

In this study we investigate the possibility to extract pollen DNA from the same support used for the traditional monitoring - an adhesive plastic tape exposed to the atmosphere day by day - and then analyse it with grass-specific primers directed to three different genes: the *waxy* gene (1), which encodes for the grass-enzyme granule-bound starch synthase, and the two plastidial genes *matK* (Maturase K) and *rbcL* (Rubisco large subunit), already used in grass-barcoding works (2).

Primers were previously tested on leaf-extracted genomic DNA of five of the most allergenic grass species (*Poa pratensis*, *Dactylis glomerata*, *Phleum pratense*, *Lolium perenne* and *Festuca arundinaceae*), and the PCR protocols were set. Contemporarily, different commercial kits and techniques were tested to efficiently extract pollen DNA from the monitoring tape. The best results have been obtained combining the Qiagen DNeasy Plant Kit<sup>®</sup> with some material pre-treatments.

In the next stages of the work, the amplified fragments will be sequenced, and specific primers will be drawn and tested on the genetic material obtained from the monitoring tape. Once suitable primers for each species will be obtained, it will be possible to monitor which *Poaceae* are releasing their pollen over a given period.

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3.5 = POLLEN DEVELOPMENT IN *OLEA EUROPAEA* L. FOLLOWING SELENIUM ENRICHMENTEMMA TEDESCHINI<sup>1</sup>, VERONICA TIMORATO<sup>1</sup>, SOFIA GHITARRINI<sup>1</sup>, GIUSEPPE FRENGUELLI<sup>1</sup><sup>1</sup>Department of Agricultural, Food and Environmental Sciences, University of Perugia, Borgo XX Giugno 74, 06121 Perugia, Italy

Selenium (Se) is a trace element with antioxidant power. Recent studies have shown that it increases the tolerance of plants to biotic and abiotic stress (1, 2). The olive (*Olea europaea* L.) is one of the most representative and economically important fruit crops cultivated in the Mediterranean basin for its oil production; thus it has been the subject of numerous researches in various fields (agronomic, biological, physiological, biochemical, ecology, etc.) and is still the target of modern investigations. It is known that the olive tree is characterized by considerable variability in the production of fruits. This variability is demonstrated by the different ratio between leaves and flowers, hermaphrodite flowers and male flowers, a variability that exists among different years, among specimens and also among branches of the same plant. This variability, which has been studied for a long time, is part of a reproductive strategy that regulates the maternal investment and the sexual expression in response to available resources and the environmental conditions in which the plant lives. The olive is an anemophilous plant, with pollination occurring by wind, a very aspecific vector. This means that a large quantity of pollen is necessary to achieve satisfactory fertilization and adequate fruit yields. For this reason, the correct development, an adequate quantity and overall, a correct functionality of the pollen, can drastically influence the plant's production and have serious economic impacts (3). Based on these assumptions, this study aims to investigate the development of olive pollen as a result of enrichment with Se in water stressed and non-stressed conditions. The results obtained showed that Selenium enrichment have different effects on pollen development under stress and no-stress conditions.

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## 3.6 = SERIES OF PHENOLOGICAL DATA IN BOLOGNA (NORTHERN ITALY)

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Global climatic changes affect the phenology of plant species, particularly the flowering and the leaf unfolding. This study presents an analysis of the phenological behaviour of several woody species in relation to climatic data (temperature). Phenological data were recorded over approximately 3 decades in the city centre (University Botanical Garden) of Bologna (Italy) (1, 2). In the last thirty years the temperature of the first months of the year in Bologna has shown an increasing trend of about 0,4-0,5°C per decade. The flowerings of *Corylus avellana* (hazel), *Fraxinus ornus* (ash tree), *Ostrya carpinifolia* (hop-hornbeam), *Sambucus nigra* (black elder) and other species, show significant shifts towards earlier dates and high correlations with the temperature of the preceding months (3-5 days of earlier start per degree of increasing temperature); their leafing times display a similar behaviour too. If the current warming trend were to continue, an earlier occurrence of the spring phenophases can be forecasted for the future.

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2) Puppi G., Zanotti A.L., 2010 - Phenological series in Bologna (Northern Italy): temporal trends and spatial pattern of greening – Geophysical Research Abstracts, Vol 12 EGU2010-4369 -Phenology session at EGU General Assembly 2010 (Wien, 2-7 May 2010)

### 3.7 = DEGREE FOR SEED DORMANCY IN *HYPERICUM ELODES* L. (HYPERICACEAE) IS INFLUENCED BY LOCAL CLIMATE AND MATING TYPE

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Knowledge of seed dormancy and germination variation within the same species is crucial to understand plant reproductive systems evolution and adaptation to environmental changes. We examined the correlation of climate and population genetic differentiation (ISSR) with primary dormancy and germination behaviour in the Atlantic–European soft-water pools specialist *Hypericum elodes* (1). We also examined the relation between seed dormancy and type of mating, obtaining seeds through hand pollination treatments performed in a natural population during three consecutive years (2). Primary dormancy was measured by analysing seed germination response (final proportion and progress curves) of fresh seeds and after various periods of cold-stratification (3, 4).

Laboratory germination experiments revealed that seed germination requirements were similar among populations; on the contrary the degree for primary dormancy varied considerably and was not associated with the population genetic differentiation. Primary dormancy was instead associated to local climate: higher temperature in summer and rainfall in winter predicted for weak and rapid loss of dormancy suggesting that seed maturation environment may be responsible for this variation (5).

Primary dormancy can be modulated according to local climate (through seed maturation environment), but is also mediated by the type of mating. Weak primary dormancy was associated to seeds recovered from self-pollinated flowers while strong dormancy was found in seeds obtained from cross-pollination. Seeds obtained from unmanipulated flowers showed an intermediate degree of dormancy. Consequently, despite the climatic influences, there is phenotypic variation for primary dormancy within populations proportional to self- and cross-pollinations. The association between the degree for primary dormancy and seasonal germination response should be verified to demonstrate the presence of polymorphism for regeneration strategies within populations, classically interpreted as bet hedging in an unpredictable environment (6, 7).

This study highlights the importance of evaluating both pregermination (dormancy) and germination processes to explore the evolution of mating systems in plants and we conclude that seed germination is not an appropriate fitness measure for inbreeding depression estimations, unless dormancy is removed.

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3) E. Fernández-Pascual, B. Jiménez–Alfaro, J. Caujapé–Castells, R. Jaén–Molina, T.E. Díaz (2013) *Ann. Bot.*, 112, 937-945

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#### 4.1 = THE ECOLOGICAL IMPORTANCE OF GREEN INFRASTRUCTURE: THE CASE OF TWO EXPERIMENTAL SITES AT THE UNIVERSITY OF CALABRIA

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Two different types of green infrastructures were installed on the campus of the University of Calabria, a green roof and a filter strip, with the aim to mitigate the effect of heat islands, to improve water quality and air and to increase plant and animal biodiversity, thus creating new links between natural areas and the urban and suburban ones (ecological corridors, areas of refuge and rest).

On the green roof (extensive type), located on one of the pre-existing buildings (cube) of the University of Calabria, the plant species used were *Cerastium tomentosum*, *Dianthus grantianopolitanus* and *Carpobrotus edulis* (3). These plants possess a high adaptability to both the climatic conditions of the site and to the reduced thickness of the substrate (8 cm), besides a great ability to establish and reproduce. In this perspective, the plants were chosen among the Mediterranean native species and the exotic species naturalized in the campus and neighbouring areas, further selecting the plants with specific adaptations to the local climatic conditions.

The range filter was installed in the area adjacent to a draining pavement where the stormwater flows from a parking lot. The plant species, selected among native plants with potential capacity for phytoremediation (1), were *Mirabilis jalapa* and *Alyssum maritimum* (3).

In the selection of the plant species for the two experimental sites, the mutualistic interactions between the plants and the insect visitors were taken into consideration. Additionally special attention was paid in the colonization of specific ecological niches in peri-urban environment by different animal species (arthropods, gastropods, reptiles, birds).

In this perspective, a weekly ecological monitoring of the two sites has started from October 2012; in the vegetation analysis the total plant cover, the growth of the epigeous apparatus, the infestations status and the general plant health were detected. A faunal monitoring on the two experimental sites was also performed to record the presence of arthropods, gastropods, reptiles, and birds by means of different trapping methods.

As regards to the vegetation monitoring, the plants better adapted to the environmental conditions of the green roof were: *Carpobrotus edulis* and *Dianthus grantianopolitanus*; *Cerastium tomentosum* showed signs related to water stress only during the summer period. In the filter strip, *Alyssum maritimum* was well established and showed a typical physiological development, tolerating various factors specific to the experimental site (soil, presence of pollutants, climatic conditions).

Currently it is not possible to evaluate the performance of *Mirabilis jalapa*, due to both the diversified sowing time and to the unfavourable environmental conditions for the germination of seeds.

In the green roof experimental site, *Dianthus grantianopolitanus* was the plant most visited by insects (Diptera, Hymenoptera, Lepidoptera, Coleoptera); the same insects were recorded as constant visitors of *Alyssum maritimum* in the site of the filter strip; presumably the strong scent emitted by the flowers of both plant species plays an important role in the insect attraction.

At present, the ecological surveys are continuing with the aim to qualitatively and quantitatively evaluate the biodiversity related to the presence of green infrastructure in urban and peri-urban areas.

The study was co-funding from the Italian National Operative Project (PON) – Research and Competitiveness for the convergence regions 2007/2013 – I Axis “Support to structural changes” operative objective 4.1.1.1. “Scientific-technological generators of transformation processes of the productive system and creation of new sectors” Action II: “Interventions to support industrial research”.

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#### 4.1 = BOTANICAL CONTRIBUTION TO ARCHAEOLOGICAL LAND EVALUATION IN THE FP7 MEMOLA PROJECT

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The Memola Project (FP7-SSH-2013-2), “MEDiterranean MOntainous LAndscapes: an historical approach to cultural heritage”, aims to analyse cultural landscapes basing on interdisciplinary approach to cultural landscapes of Mediterranean mountainous areas, taking as a central axis the historical study of two natural resources essential to generate agro-systems: water and soil.

The different exploitation strategies have resulted in different landscapes and forms of cultural expression throughout Europe and the Mediterranean, but have also produced very important common areas. Agro-systems represent one of their greatest expressions.

The knowledge of the different ways in which the natural resources are exploited and managed over time is crucial for landscape conservation and its adaptation to current global changes: globalization, agrarian industrialization, climate change, loss of peasants knowledge and rural population.

The project is focused on Sierra Nevada (Spain), Monti di Trapani (Italy), Colli Euganei (Italy) and Vjosa Valley (Albania).

The main objectives of the Project are:

- Investigate the logic that rules the process of historical landscapes formation in relation to natural resources within a diachronic framework. Introduce the historical perspective (4th dimension), which we consider to be a powerful interpretation key, in landscape studies.
- Draw context-tailored strategies of preservation, diffusion and valorisation of the cultural heritage (both tangible and intangible) and of the environment. Stimulate sustainable development in rural areas.
- Analyse the efficiency of these systems and the current problems of survival within the context of global climate change and the framework of European policies.
- Develop new methodologies for the study of cultural landscapes, through the creation of scalable working protocols, able to take advantage of the solid background of technologies and analysis methods available to the research group.
- Use a multidisciplinary approach, thus widening the range of specialists involved in cultural heritage study to agronomist, hydrologists, botanists, hydro-geologists, geologists and architects. Promote skills hybridization among researches (humanistic and scientific sides), prompting new forms of job creation.

Diachronic analysis of landscape is carried out using the Land evaluation to reach the objectives of the project. In particular for reconstruction of ancient landscape and for evaluation of the actual landscape structure (e.g. is the vineyard in the mountains around Trapani (Sicily) the best use of the territory in terms of environmental and economic sustainability?)

The techniques of land evaluation refer mainly to “Framework for Land Evaluation” of the Food and Agriculture Organization (1) and this approach has been generally well received and has been used for many surveys. A theoretical framework for Land Evaluation is given by Rossiter (2).

The same approach is used for the reconstruction of ancient landscapes. Van Joelen defined the archaeological land evaluation discipline (3).

The method applied is based on matching and comparing of historical Land Use and Ecological Land Unit taking into account the landscape ecology approaches (4). The natural potential vegetation is used to represent territorial areas ecologically homogeneous useful for evaluating the fitness of a type of land for a specific kind of land use. The phytosociological analysis of semi-natural and natural vegetation is the starting point of ecological characterization. Archaeological, Archaeopedological, Archaeobotanical data, Written sources, Toponymy, Ethnographical data, Monumental Trees, are needed to historical landscape reconstruction.

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3) E. Van Joelen (2003) PhD thesis, Rijksuniversiteit Groningen

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## 4.1 = THE FORESTED AGRICULTURAL LANDSCAPE OF PISAN PLAIN: THE COLTANO ESTATE

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The Pisan plain, if we exclude the coastal sector covered by forests of the Regional Park of Migliarino S.Rossore Massaciuccoli (Selva Pisana), is characterized by a vast agricultural landscape. To the southwest of the urban area of Pisa meets a weak relief of Pleistocene deposits (max. 8.70 m asl, 700 ha) called Coltano. The area, which was, at least until late antiquity, a true island in a vast lagoon system, was the object, together with the entire surrounding area, of repeated attempts to reclaim from the Medici era (XVI century). The final reclamation (1920-1930) has radically changed the whole area and the great marshlands, in the low lands and covered with sedges and straws gave way to arable crops. The wooded area, ascribable before the reclamation, about 250 ha, was partially reduced but remained basically the initial design as well as providing fuel and hunting, and it continued to play an important role in protection of the agricultural crops from the sea winds (Fig. 1). Nowadays, where the forest survives (about 140 ha), to the thinness of the nemoral strips in the agricultural matrix, is associated, in contrast, a high peculiarity of vegetation. If you exclude some batches represented by plants of *Pinus pinea*, the seminatural wooded areas, no deeper than a few hundred meters, are largely represented by the woods on hydric soils hydromorphic (*Fraxino oxycarpae - Quercetum roboris*) of hygrophile peninsular geosigmetum of riparian vegetation, in catenal contact with more xeric woods of *Quercion ilicis* (habitat 91F0 and 9340). These forest habitats, rather common in the Pisan of the neighboring Park (1), however, constitute a relict woodland element with regard to the vast countryside outside Pisa Park. Of particular naturalistic relevance, are also worth mentioning the extensive populations of *Laurus nobilis*, which here should be to characterize the codominant tree layer of *Fraxino oxycarpae - Quercetum roboris* (Fig. 2). These phytocoenoses are not easily interpretable by phytosociological point of view, since their floristic cortege reveals in some cases elements of *Populetalia albae*, in other of *Quercetalia ilicis*, however seems can be related with reasonableness to the priority habitat 5230\* (arborescent matorral of *Laurus nobilis*). The frequency and coverage of individuals of *L. nobilis* has reached values over time such as to induce the scientific community and local governments to establish in the northern sector, a nature reserve of about 9 ha called "Forest of laurels", considered by some authors, one of the few wild and spontaneous stations of *Laurus nobilis* of peninsular and insular Italy (2).



Fig. 1 The woods of Coltano Estate



Fig. 2. The wooded strips

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#### 4.1 = TOWARDS THE IDENTIFICATION AND SUSTAINABLE MANAGEMENT OF TRADITIONAL AGRICULTURAL LANDSCAPES IN ITALY: NEW PERSPECTIVES FROM VEGETATION SCIENCE AND LANDSCAPE ECOLOGY

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Traditional agricultural landscapes are ecosystems that derive from the long lasting interaction between man and the environment and that are relatively stable over time. These systems are generally associated with the use of low-impact agricultural practices, significant habitat diversity and presence of semi natural vegetation (1,2). All these characteristics have a positive influence on biodiversity at different levels. Therefore, conservation and sustainable management of traditional agricultural landscapes have been taken into account in several strategies and conventions, such as the European Biodiversity Strategy for 2020, the European Landscape Convention, the Common Agricultural Policy 2014-2020, and the Green Infrastructure Strategy. In Italy the new National Strategic Plan for agricultural and rural development and the National Biodiversity Strategy represent comprehensive policies embracing both agriculture and biodiversity.

In this context, this work aims to illustrate how the integration of land cover (3), vegetation series (4, 5), and ecoregion information (6, 7) can significantly contribute to the identification of traditional agricultural landscapes at the national level, and more generally to the characterization of agricultural landscapes. In particular, the specific objectives are: i) to analyze the consistency between ecological potential and agricultural land use, as a basis for assessing coevolution over time; ii) to assess stability of agricultural landscapes by analyzing temporal and spatial changes in composition and configuration of land use and vegetation cover; and iii) to select priority areas among agricultural landscapes for enhancing green infrastructures.

Landscape structure and land cover change are analyzed within ecological land units at different scales, to account for the spatial heterogeneity of patterns and their underlying drivers.

The adopted approach integrates vegetation science and landscape ecology principles, and can be combined with social and economic aspects for improving sustainable land planning and management at multiple levels.

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#### 4.1 = EXPERIMENTAL FIELDS OF *ARTEMISIA UMBELLIFORMIS* SUBSP. *ERIANTHA* (APENNINES' GENEPÌ) IN THE GRAN SASSO MOUNTAIN

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The experience gained over the last decade has highlighted the importance of projects aimed at identifying alternative and eco-sustainable economies. Mountain areas are characterized by steep slopes, shallow soils and low temperatures, whereby agricultural activities are often no longer profitable. For these reasons, it becomes essential to find economic strategies both eco-compatible and economically advantageous. The study of the characteristics of the medicinal plants together with the appreciation of their benefits could promote new economic activities in high altitude areas, by cultivating these plants and exploiting them for the manufacture of various products intended for the market. The mountain habitat is known to affect the physical and organoleptic characteristics of the products, especially the unprocessed ones, owing to the altitude, the soil quality, the poor use of chemical fertilizers, and the absence of industrial activities (1). The aim of the project "*Nuove Piante*" (PSR 2007-2013 Misura 124) concerning the preservation and valorisation of protected plants consists in the development of economy models compatible with areas dedicated to environment protection. In particular, our attention has been focused on *Artemisia umbelliformis* subsp. *eriantha*. This plant lives in high altitude areas and it has been collected by the local Abruzzo population to obtain, after drying and infusion in alcohol, the namesake aromatic and invigorating liqueur. Our previous studies (2-4), in some cases with the collaboration of Gran Sasso and Laga Park, allowed to lay the basis for the applicative purposes of "*Nuove Piante*" project. The technique exploited in the project is the *in vitro* propagation, which allowed us to produce a high number of clones intended for the preparation of experimental fields. These fields were identified taking into account the climatic and soil requirements of *A. umbelliformis* subsp. *eriantha*. The altitude of the fields is greater than 1100 m above sea level. Preliminary analyses performed on some soils showed a pH value greater than 8 and a content of nitrogen, phosphorus, and potassium of 1.65 g/kg, 0.86 g/kg and 6.00 g/kg, respectively. We provided specifications for field cultivation and crop care, concerning the choice of mulching, irrigation systems and soil preparation. Chemical analysis performed on micropropagated plants confirm the data previously obtained (5) showing thujones quantities (metabolites characteristic of this species) in about 60%.

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#### 4.1 = PLANT BIODIVERSITY AND SOIL QUALITY IN MAN MADE SOILS CULTIVATED WITH TABLE GRAPES IN THE PUGLIA REGION OF SOUTH-EASTERN ITALY

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A unique soil management technique, locally known in Apulia as "frantumazione", consists of breaking and grinding calcareous rocky sub-layers and rock outcrops and mixing them with the remaining soil. Over the last two decades such a process has expanded to cover about 20,000 ha. Much of the land within these manmade soils is used for table grape production and less for cereals or forage crops. We studied and sampled 11 fields for biodiversity and soil profiles, 9 in manmade soil and 2 in natural habitats, to assess the impacts of frantumazione on biodiversity and soil quality. Comparison of flora and of soil characteristics showed a general decline in the quality of the environment due to these fields where soils are manmade.

The floristic analysis was worked out by three different methodologies: (A) surveys in the fields were made according to the Raunkiaer method (1); (B) surveys in the ecological infrastructures were performed applying the method of Braun-Blanquet (2); (C) visual and oriented surveys, based on the experience of the botanist, were made to detect species that could escape with A and B methodologies. Objectives of this research work are also to produce at the end: a) list species; b) species of conservation interest; c) plant communities.

The flora species diversity of the fields of the natural areas and those of the cultivated and abandoned fields were compared (3). The diversity was highest in the natural areas, followed by the abandoned and cultivated fields. The same situation also prevailed in the corresponding ecological infrastructure.

Testing all the sites together showed the overall structure of correlation of the whole fields and the ecological infrastructure and describes the general pattern in species distribution along the gradients of species composition. The results show that that cultivated fields and natural areas present a very different floristic composition. From a preliminary analysis coming after 4 years of survey time of cultivation influence the differences between the fields and the related ecological infrastructures. This suggests that the impact of human intervention and agricultural activities has taken its course, particularly in the cultivated fields.

From a first analysis of preliminary data it might be inferred that the abandonment of such vineyards is tending towards natural area due to the effects of secondary succession. Looks like that many or all species which are present in the cultivated fields can be even present in the abandoned ones, but not all species present in the abandoned areas can be present in the cultivated fields. Each typology of area (natural, cultivated and abandoned) have unique species composition. This means that once we loose the natural areas we loose the species living there.

In the course of the research the analysis of the physical and chemical parameters were performed for each field. The most significant changes were a 57% decrease in soil organic matter (SOM) and 32% reduction in total N content over a 15 year period immediately following change land cover. Soil structure was rendered structureless in cultivated soils, but no significant changes were found in soil texture composition compared to soils of the natural areas. Correlation between soil parameters and presence of species was investigated.

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4.1 = DETECTING INVASION HOTSPOTS OF *AILANTHUS ALTISSIMA* WITH REMOTE SENSING

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Named as “tree-of-heaven”, *Ailanthus altissima* is an invasive pioneer species and rapidly spreads onto disturbed sites. The *taxon*, native of Moluccas Island, North Vietnam and China, was first introduced into England and France in 1751 by a missionary who mistook it for a Japan varnish tree (*Toxicodendron vernicifluum*) (1).

It was introduced into Italy in 1760 in the Botanical Garden of Padoa (2), and between 1784 and 1786 it was present into botanical gardens of Tuscany (3). *Ailanthus altissima* was reported in Sicily for the first time in the floristic list of Palermo Botanical Garden edited by Giuseppe Tineo (4).

For the past 250 years, *Ailanthus altissima* has been rapidly spreading throughout Europe and North America, cultivated as ornamental plant (5).

This plant is so invasive because of its biological and ecological characters. *Ailanthus* produces large numbers of wind-borne seeds, grows quickly, tolerates stress, and can also reproduce asexually via root sprouts (6).

*Ailanthus altissima* today is only invasive alien tree still present in all Italian regions (7).

The effect of its invasiveness are really visible also in Sicily (8), in particular in Palermo, where it colonizes ruderal environments like road borders, ruins in the historical centre. *Ailanthus altissima* is a serious threat to historical gardens and city parks.

The distribution of the species in the urban areas and the hotspots of plant spread need to be evaluated in order to set action of invasiveness control.

Remote-sensing technology allows as to define with good approximation the present of the species in the environment wise the most compromised urban areas

For this purpose we decided to utilize the *MIVIS airborne multispectral sensor* imaging because, compared to other satellite sensors they have a band range of 102.

Furthermore MIVIS images allow, through a specific noise screening, a more precise tree-of-heaven spotting and the extrapolation of specific spectral signatures using the NDVI (*Normalized Different Vegetation Index*). The result of this process is a raster map of hotspots of *Ailanthus altissima* in Palermo.

This ground-breaking methodology allows a fast and accurate data collection for mapping of invasive species in urban areas.

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#### 4.1 = SUSTAINABLE GARDENS: AN EVALUATION TOOL FOR MANAGEMENT AND PLANNING STRATEGIES

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Gardens, especially if public accessible, are important resources in the sustainable development and contribute to improve the quality of life in urban and suburban areas in many ways. In addition to the usual social functions, like citizen recreation, welfare, gathering, and ecological education, gardens also provide many so-called ecosystem services, e.g. pollutant mitigation, noise reduction, microclimate improvement, biodiversity conservation and enhancement, landscape connectivity. Often gardens are also key elements of cultural heritage, reflecting local history, customs, and traditions and including valued artistic and natural features (1).

Integration between management needs and socio-ecological benefits of gardens within environmental policies and local development strategies is often very limited. Urban gardens and related issues still arouse minor interest, especially in the southern Mediterranean cities. Gardens in the public domain, run by civic municipalities, government entities or educational institutions, suffer from the lack of financial support and of integrated management planning; thus they are viewed as a cost to society rather than a resource. At the same time, both historical or botanical gardens and public ones have opposite missions that range from conservation and scientific aims to civic amenity, so requiring different approaches in their management policy. Therefore, we need to identify the key criteria for the best management strategies ensuring long-term maintenance and improvement of gardens, their collections and structural features, as well as to develop integrated tools for evaluating/monitoring garden state and sustainability (2, 3). Main scope is to make both garden operators and managers aware of garden strength/ weakness and management priorities in order to better plan and organize their efforts. The tool should also be able to guide them to consider items beyond their expertise that may nonetheless be critical to achieve sustainability (4).

With the support of GARDMED The Network of Mediterranean Gardens (project implemented within EU funds ERDF Operative Programme Italy-Malta 2007-2013), a first evaluation tool for the sustainability of Mediterranean gardens was developed and practically applied to different gardens (public, private and botanical gardens) in Sicily and Malta. This tool is based on a Sustainability Framework including assessment for almost 100 criteria arranged in six dimensions of sustainability and three levels of achievement (basic, advanced and reference). Scores for each criterion and each dimension provide a Garden Sustainability Index (GSI) that indicates the overall sustainability rating of the garden. Scores are weighted with reference to the different garden types having different sustainability goals. Current garden status is checked and visualized using a table and a web diagram (Fig. 1) that clearly target required actions to achieve and monitor progress in the medium to long term. Future development of this tool is expected in improving quantitative indicators and weighted dynamic scoring in order to easily assess environmental and socio-economical benefits of green practices and eco-sustainable actions applied to the garden management.

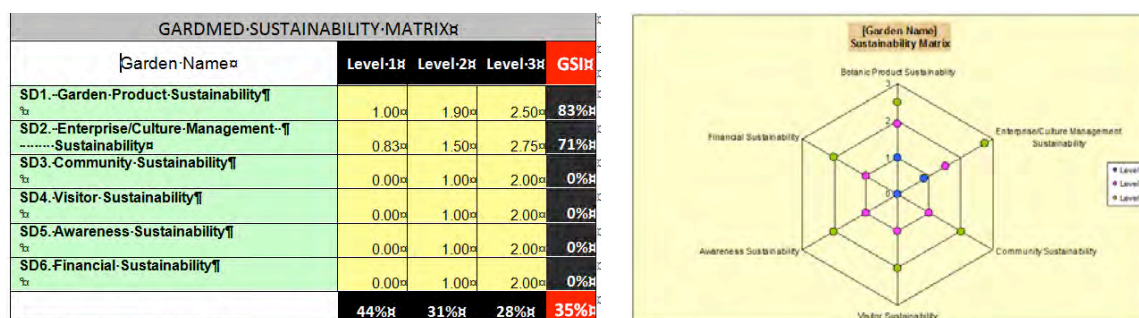


Fig. 1 Sustainability table and diagram from Gardmed Project outputs

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#### 4.1 = ORIGIN, CHARACTERIZATION AND CONSERVATION OF AUTOCHTHONOUS GRAPEVINES OF SARDINIA (ITALY)

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The wine heritage in Sardinia (Italy) consists of 151 cultivars. Considered as local varieties, they are the product of different events, such as direct domestication of wild grape, crosses between local varieties and the adoption of agricultural techniques and cultivars from different ethnic groups that colonized the Island over the centuries. The remaining varieties can be considered as false attributions (synonyms and/or homonyms) due to the existence of different dialects within the same insular region. As is known, the grapevine seeds are highly polymorphic and play a crucial role both in taxonomic studies of the genus *Vitis* L. (1), both in the understanding of the processes of domestication and distribution of wild grapes, as suggested by many studies of archaeobotanical character (2). In archaeobotanical studies, the taxonomic classification of diasporas has usually been done by simple morphological observation and visual comparison with *ex situ* collections of seeds (3), although the use of biometric indices has often proved to be a powerful approach in the taxonomic studies of the genus *Vitis* as well as for the species attribution of archaeological remains (1, 4). As the measurement of seed biometric traits can provide useful information for the correct taxonomic recognition, allowing an objective comparison among the diasporas, the study of the germination ecology can allow to increase the knowledge of the best conditions that lead to the seed germination in field (5).

The aim of the project “*Origin, characterization and conservation of autochthonous grapevines of Sardinia*”, funded by “*Regione Autonoma della Sardegna*” through two research fellowships financed with funds drawn on *POR Sardegna FSE 2007-2013*, is to use some of seed morpho-colorimetric parameters and the Fourier Elliptic Descriptors, measured with computer-aided image analysis techniques, to characterize the studied cultivars and implement statistical classifiers able to discriminate among them, and comparing them with archaeological seed lots founded in Sardinia, in order to attempt an historical reconstruction of the origins of viticulture in Sardinia and the processes of domestication of wild grape. Moreover, the project aims to expand the view of knowledge concerning the germination of the genus *Vitis* L., developing germination protocols and obtaining relevant ecological information for the conservation of natural populations and for eventual restoration and populations reinforcement in the study area.

Under this two-year project, specific collaboration agreements will be signed with the Sardinian Germplasm Bank of University of Cagliari, where all the seed lots collected during the project will be stored for *ex situ* conservation; and with the Soprintendenza per i Beni Archeologici di Cagliari & Oristano and Sassari & Nuoro, which will provide the archaeological seed lots.

The project involves a network of private companies operating in C-N of Sardinia, in particular: Azienda Gostolai S.A.S. of Giovanni Antonio Arcadu and C., from Oliena (NU); Azienda Vitivinicola Ledda of Luciano Ledda, from Oliena (NU); and Azienda Vitivinicola Canudu of Pietrino Canudu from Oliena (NU), all producers of wines from autochthonous grapes, as *Cannonau di Sardegna* and *Nepente di Oliena*; Azienda Fradiles Vitivinicola s.n.c. of Flore and Savoldo, from Atzara (NU), winemaker of *Mandrolisai*, valuing one of the most ancient red Sardinian varieties, *Muristellu*; and Azienda Agricola Mulas of Francesca Cabras, from Bono (SS), winemaker of another one of the most ancient Sardinian autochthonous varieties, *Arvesiniadu*.

The contribution of these companies would impact not only on the scientific achievements, but also on the studied territory and desirably on the same companies profits, spreading their brands beyond the regional or national boundaries.

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#### 4.1 = USE OF ORNAMENTAL PLANTS IN REINFORCED SOILS WITH ANTI-EROSION PURPOSE: A DEMANDING ACHIEVEMENT REGARDING ROOT SYSTEMS TRAITS AND TYPE OF SHOOTS SOIL COVERAGE

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Reinforced soils are usually constructed to stabilize unstable slopes, retain the soil on steep slopes and under crest loads. The reinforcement materials is placed in horizontal layers throughout the height of the wall. Different kind of geosynthetics can be used. Modified soil slope profiles (beyond their natural angle of repose) require a vegetation cover that minimizes superficial soil erosion. Usually reinforced soils are covered by a grass mat that provide a total ground cover and an intense root colonization. The use of ornamental species will provide a more pleasant sight but may lack in ground covering and in root soil colonization, with consequent significant soil erosion. Aims of this study are: 1) selection of the optimal species for soil erosion mitigation in reinforced soil, based on root morphology and shoot coverage characterization; 2) test different materials to use on slope surface in order to enhance growth of ornamental species on steep slope.

Preliminary data on root apparatus were obtained from plants grown in pots. For each species, root system was carefully washed to remove growing substrate, scanned at 800 dpi and images were analyzed categorizing roots in diameter classes of 0.05 mm. Type of shoot and its development in time was obtained by taking picture of plant. Moreover, a 3-dimensional aliphatic polyamides mat was chosen to be tested as permanent erosion prevention system. In future tasks, root morphology and shoot coverage of selected species growing on steep reinforced soils will be analysed.



#### 4.1 = BIOACTIVITY AND BIOCONTROL BY THE FUNGUS *TRICHODERMA*: A GREEN REVOLUTION FOR AGRO-RESIDUES

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Large amount of agricultural wastes are produced every year and, among them, rice straw is one of the most abundant lignocellulosic crop residues in the world (1). Rice straw contains approximately 35-40% cellulose, 25-30% hemicellulose and 10-15% lignin. Native cellulose and lignin are highly resistant to enzymatic hydrolysis but fungal biological pretreatment, or biodegradation, serves as an attractive option that is both energy-saving and environmentally friendly (2, 3). Despite these agricultural residues contribute significantly to the waste disposal problem, their organic nature and nutrient content make them an interesting raw material that can be reintroduced in the agricultural sector as a fertilizer.

An innovative approach consists in the use of these agro-waste residues for the development of “Bioactive Agro-matrices”, obtained by the integration of matrices derived from the agricultural and agri-food cycle (for example rice straw, corn cob, digestate,...) with viable microorganisms, opportunely selected, agronomically useful and safe for human health.

The aim of the present work was to select microfungi able to grow on rice straw, to degrade it and, simultaneously, able to antagonize the phytopathogenic *Fusarium* spp., very harmful on rice crop. The work focused on *Trichoderma* spp., which are generally known as antagonists of several plant pathogenic fungi and as plant growth promoters. Their economic importance includes their roles as primary decomposers, producers of antibiotics and enzymes as well as biocontrol agents against a wide range of plant pathogens (4).

In this study, the survival and growth ability of several *Trichoderma* isolates on rice straw were evaluated. Furthermore, their antagonistic behavior against different *Fusarium* strains were tested *in vitro* throughout plate assays. One of these, *Trichoderma asperellum* EVT4, was the most active towards *Fusarium* spp., in particular towards *F. fujikuroi* EVF3 and its antagonistic potential was also assessed *in vivo* (*i.e.* a microcosm test consisting of soil and rice straw). The fungal load of *F. fujikuroi* EVF3, expressed as CFU/gr, was significantly lower in the tests carried out in the presence of *T. asperellum* EVT4 compared to the control, in which this strain was alone. The load of *T. asperellum* EVT4, however, did not change significantly in the presence of *F. fujikuroi* EVF3.

These first results indicated *T. asperellum* EVT4 as an interesting fungal strain, that can be added to rice straw in order to create a “Bioactive Agro-matrix” useful in field both for biological control of plant pathogens and for rice straw degradation.

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4.2 = BIOLOGICAL ACTIVITIES OF *TEUCRIUM FLAVUM* L. AND *TEUCRIUM FRUTICANS* L. EXTRACTS

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The genus *Teucrium* (Lamiaceae) includes 300 species widespread all around the world which are perennial herbs or shrubs commonly named germanders (1). *Teucrium flavum* L. is an evergreen perennial shrub possessing pubescent stems up to 60 cm, triangular-ovate leaves and yellow flowers, which appear from May to August. It is characterised by pubescent stems and yellow corolla assembled in terminal spikes. The plant can be found in the cracks of lime rocks from sea level up to 1000 m (2). In Italy it is mostly present in Liguria, Sicily and Sardegna. The leaf, flower and fruit oils are characterized by a predominance of sesquiterpenes, such as  $\beta$ -caryophyllene, germacrene D,  $\beta$ -bisabolene (2). *Teucrium fruticans* L. is a shrub native to the Mediterranean region. In Italy it grows along the Tyrrhenian coasts up to Naples, in Sicily, Sardinia and in almost all the minor islands (3). In Sicily it is known to have antifeedant activity against *Spodoptera littoralis* (4). *T. fruticans* L. is widely used as an ornamental plant due to the attractive contrast of its striking blue flowers with its evergreen foliage, which is gray-green above and silver-white beneath (5). Chemical studies on *T. fruticans* L. also reported the presence of neo-clerodane diterpenoids, flavonoids, fatty acid esters and important essential oils such as  $\beta$ -myrcene (5-6). Most *Teucrium* species are bitter, astringent and anti-rheumatic herbs used for gastric ulcer and intestinal inflammation, to stimulate the digestion and as diuretic, antiseptic, antipyretic and antihelminthic agents (5, 7). *Teucrium* species were also used as alimentary plants and some of them are currently used in the preparation of flavoured wines, herbal teas, bitters and liqueurs, as well as leaf and flower infusions are used for flavoring beer in some countries (8). Recent investigations on chemical composition revealed that *Teucrium* species are characterized by mono and sesquiterpenes hydrocarbon compounds, flavonoids, fatty acid esters and essential oils (9-10). Many species of this genus show antimicrobial, antioxidant and antifungal activities, rendering them useful as natural preservative ingredients (11). In view of the interesting biological properties reported for *Teucrium* species, objective of this study was to investigate the *in vitro* antibacterial activity of inflorescence extract of *T. flavum* L. and *T. fruticans* L. 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 extracts was also evaluated. Antiradical activity of *T. flavum* L. and *T. fruticans* L. extracts was performed by DPPH test and by superoxide anion scavenging capacity. The effects of *T. flavum* L. and *T. fruticans* L. extracts 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 extracts have both antioxidant *in vitro* activity and antimicrobial ability against Gram-positive and Gram-negative strains but with different effectiveness probably due to the variation in composition of active compounds.

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4.2 = ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITY OF *FERULAGO CAMPESTRIS* ESSENTIAL OIL

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*Ferulago campestris* (Besser) Grecescu (Apiaceae) is one of the three species of the genus *Ferulago* widely spread throughout the Italian peninsula. Different species of *Ferulago* have been used as spices and flavorings and in Turkish folk medicine as sedatives, tonics, digestive aids and antihelmintic and in the treatment of hemorrhoids. Moreover, extracts of the root of some species of *Ferulago* are known as aphrodisiac. In a recent work the essential oil of *F. campestris* obtained by hydrodistillation of the fruits from two collection sites in central Italy has been analyzed. The oil yield, calculated on the dry weight, was in the range 5.9-7.1%, indeed very interesting for the purposes of a possible industrial use. Quantitative analysis was performed by GC-FID and GC-MS and 27 components were identified. The main fraction of the oils is represented by monoterpene hydrocarbons (78.8-80.3%), such as myrcene (33.4-39.7%),  $\alpha$ -pinene (22.7-23.0%) and  $\gamma$ -terpinene (8.1-10.9%). The second fraction is given by aromatic compounds (9.4-9.8%), mainly represented by (2,3,6)-trimethyl benzaldehyde (8.6-9%) (1). Within this general framework, in the search of secondary metabolites representing a possible alternative to the use of synthetic fungicides, owing to their broad spectrum of bioactivities (2), we evaluated the antifungal and anti-inflammatory activities of *F. campestris* essential oil. The antifungal property was evaluated against five plant pathogenic fungi that usually infect our crops and the results showed that this essential oil has a good fungistatic capacity against all fungi tested. The essential oil showed no fungicidal activity. The fungistatic activity was detected at very low concentrations (50 ppm). The same oil, in a preliminary study, showed a good anti-inflammatory activity in vitro. In fact, in the 5-lipoxygenase assay the IC<sub>50</sub> values were comparable to values of the reference utilized.

Considering the results and since it is known from the literature (3) that the essential oils often have fungistatic action without presenting a biocidal effect on host tissues, one could suggest the use of the essential oil of *F. campestris* in packaging systems and in the formulations inhibiting the growth of pathogens at not phytotoxic concentrations. In fact, very often the fungicidal activity of essential oils occurs at high concentrations, which results phytotoxic as well.

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#### 4.2 = ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITY OF *PYRUS COMMUNIS* VAR. COCOMERINA EXTRACTS

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In recent years, the interest in the so-called "ancient fruits" has greatly increased. These are fruits that have been partially or completely forgotten and their recovery and subsequent scientific study might reveal a potential source of secondary metabolites representing an important step for the biodiversity conservation. In this context, our attention has been focused on a variety of *Pyrus communis* also known as "pera cocomerina". There are two types of this fruit: the early-type with fruits ripening in August, and the late-type with fruits ripening in October. The main feature of this fruit is the pink/red colored pulp. Based on this characteristic, we considered interesting to evaluate their total polyphenols (1), flavonoids (2) and anthocyanins (3, 4) content. In addition, the anti-inflammatory and antioxidant activity of the extracts obtained from these fruits was determined.

A careful evaluation of the results obtained allows us to establish that the fruits of *Pyrus communis* var. cocomerina can be a rich source of antioxidants with significant concentrations of polyphenols, flavonoids and anthocyanins that show an excellent inhibitory activity against 5-LOX (5-lipoxygenase) (5). In contrast, the radical scavenging activity demonstrated in our experiments, in agreement with other articles already present in the literature, is not significant (6). These findings are particularly significant since allow us to enhance the recovery of this fruit for the biodiversity conservation.

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#### 4.2 = VOLATILE PROFILE AND ESSENTIAL OIL COMPOSITION OF THREE SAMPLES OF *RHUS CORIARIA* L. SEEDS COLLECTED IN SICILY

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*Rhus coriaria* L., commonly known as sumac (sommacco siciliano), is a perennial shrub or small tree which reaches 1- to 4-m height in the wild in all Mediterranean areas and belonging to the Anacardiaceae family. Fruits of this tree are in the form of red or purple clusters. These berries vary in colour from brick red to dark purple, depending on where the shrub is grown and contain one seed. Berries are harvested just before they ripen, then left in the sun to dry. The dried fruits are used in cookery in some cuisine as a lemony taste to salads or meat. Prior to the introduction of lemons, the Romans used sumac as a souring agent. The Mediterranean diet is particularly rich in spices. Sumac is one example, which is widely used in Turkey, Greece and Jordan (1).

*Rhus coriaria* is largely used in the folk medicine as remedy for stomach disease, dermatitis and fever. Sumac is documented to possess antibacterial, antifungal, antioxidant, anti-inflammatory, hepatoprotective, anti-ischemic, vasorelaxant, hypoglycemic, and non-mutagenic properties (2). From an industrial point of view, sumac contains colouring matter and tannins which are used in dyeing and tanning fine leather. Leaves are also exported for this purpose. Previous phytochemical studies of this plant reported that its leaves contained flavones, tannins, anthocyanins, and organic acids (malic, citric, pyruvic acids) (3). However, it is the fruit of the plant that is typically consumed as spice after drying and grinding. The fruits contain tannins, volatile oil, various organic acids (such as malic, citric, and pyruvic acids), anthocyanins and fixed oil. There are several studies in the literature on chemical composition of essential oil (4) and aldehydic components, along with terpenes and sesquiterpenes were found to characterize the typical aroma of sumac.

The present study deals with the volatile composition of three fruit samples of *Rhus coriaria* collected in the wild in Sicily [Monterosso Almo (RG), Castronuovo di Sicilia (PA) e Chiaramonte Gulfi (RG)] in the same period (December 2013) and air dried. The hydrodistilled essential oils were analysed by GC-MS techniques. Differences in the chemical class of constituents were evidenced in the three samples: the percentage of oxygenated monoterpenes is higher in plants collected in Monterosso (13.64%) than in the other two (Castronuovo, 4.87% and Chiaramonte, 0.66). Sesquiterpene hydrocarbons showed the same trend, ranging from 28.64% in Monterosso to 18.32% in Castronuovo and 8.9% of Chiaramonte. An opposite behaviour was found in the amount of non terpenic compounds. Among pure constituents cembrene was present in high percentage in Monterosso and Castronuovo samples (10.60% and 15.14%, respectively), while *p*-anisaldeide was the most abundant component in Chiaramonte sample (20.79%).

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#### 4.2 = PHARMACOGNOSTIC STUDY OF *STYLOSANTHES GUIANENSIS* REVEALING THE OCCURRENCE OF MAYOLENE LIPIDS WITH ANTIPROLIFERATIVE PROPERTIES

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*Stylosanthes* species are widespread native legumes of tropical Central and South America. Species of this genus are also grown in Africa, Asia and Australia as a pasture and cover crop, being well adapted to a range of soil and climatic conditions. Unlike many legumes, *Stylosanthes* is recognized for its retention of nutritive value also in the dry season (1), and as a result, this crop is in expansion in many African countries. This legume is largely grown for forage meal production also in China (2). Therapeutic uses of *S. fruticosa* and *S. erecta* can be found in the traditional medicines of different countries, especially in Africa and India, while the medicinal properties of *S. guianensis* have not been reported hitherto.

In this study, commercial samples of *S. guianensis* were analyzed by both microscopic and molecular techniques to confirm identification at the species level. Micro-morphological investigations of the leaf epidermis showed the occurrence of paracytic stomata, a large amount of crystalliferous idioblasts made of calcium oxalate, and uni- and multiseriate hairs. These latter were broad at the base and tapering above. In the leaf mesophyll the occurrence of mucilaginous idioblasts as well as of idioblasts containing phenolic substances were detected (3).

Molecular identification by DNA barcoding approach involved the use of three different markers: *matK*, *trnH-psbA* and *ITS*. The comparison with the NCBI database has enabled the correct identification of the species and supports micro-morphological data.

After taxonomical assessment, a bioassay-guided search of biologically active phytochemicals was carried out. Dried specimens were minced and extracted with 95% aq. 2-propanol. The crude extract was partitioned on an RP-18 column and eluted with H<sub>2</sub>O-MeCN mixtures.

The various fractions obtained were individually tested for their bioactivity. In vitro cultures of HeLa and A431 cancer cell lines were incubated with increasing extract concentrations for 24 h, and thereafter, their viability was evaluated with the calcein-acetoxymethylester assay by using a fluorescence plate reader. Based on IC<sub>50</sub> values, two among the late-eluted fractions showed strongest cytotoxicity.

NMR and LC-MS analyses of these fractions showed the presence of linoleic acid, along with hydroxyl derivatives of linolenic and/or linoleic acids. These latter are endogenously present in the plant as mayolenes, i.e. labile compounds formed by esterification of the hydroxyl function by a second fatty acid. Mayolenes have been rarely found in nature so far, having been described as defensive lipids of Lepidopteran larvae (4, 5). Our finding confirms their strong biological activity and opens new perspectives for possible pharmacological uses of *S. guianensis*.

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#### 4.2 = TOXIC PLANTS: THE ROLE OF A PHARMACEUTICAL BOTANIST AS A SUPPORT OF THE EAD (EMERGENCY ALERT DEPARTMENT) HOSPITAL

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Plants has always represented a source of food, medicine, cosmetics, dyes, flavourings and more for man. In the course of history and the centuries, the man has also identified a group of plants, the poisonous plants, which can also be a valid food, drug or other. In general, people have little knowledge about the potential toxicity of the plants. A great confidence in natural products usually prevails considering them able to solve any health problem and that in any case allow us to live better. Unfortunately, it is not always the case. It has recently been noted an increase in the number of cases of poisoning due to the careless use of plants, considered mistakenly as safe and harmless (1, 2).

The Poison Control Center of Milan for over 40 years responds 24h to the problems of poisoning and/or exposures of private citizens, hospital doctors, teachers, etc., providing answers and advice to 65-70% of cases occurring in Italy (3). The poisoning and/or exposures monitored due to plant material are about 1000 per year (1). A close cooperation between botanists and the medical staff of the Hospital Poison Center is essential to provide an effective information service to medical staff and to help in the prevention and management of the deleterious effects of toxic plant on human health. The first and mandatory point is a rapid identification of the poisonous plant.

The cases of poisoning due to ingestion of wild plants are those that most need the expertise of a botanist, since it is not possible to know in advance which will be the botanical specimen to examine: we can have only some parts of fresh plant, or just fragments of cooked plant or still it is necessary to examine the outcome of vomiting. It is also crucial to know the season in which the poisoning occurs, in order to correlate it with the plant ontogeny, and, equally important to know the geographical location: the Italian territory from North to South has very different climatic zones and habitats.

Usually, the poisonings that occur in the early spring are mainly due to the different morphology that the plant has in the juvenile stage rather than when it is fully developed (such as late spring and summer). In the early spring the plant has not yet completed its growth and it looks completely different from what we assume, and only, once it becomes adult with flowers, it presents the same morphology, that is usually described in the books of Botany. This contribution will present and discuss some most frequently occurring together with unusual cases..

In conclusion it should be emphasized that both public and health professionals should be trained in the identification of potentially toxic plants. However, the poisonous plants are very useful to man, especially from in the pharmaceutical field since they contain highly poisonous secondary metabolites, which can profitably be used to prepare drugs sometimes even lifesaving.

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#### 4.2 = RADICAL SCAVENGING AND ANTIOXIDANT ACTIVITIES OF EXTRACTS FROM *HYPERICUM PERFOLIATUM* L.

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To the genus *Hypericum* (Guttiferae) belong more than 400 species, diffused in warm-temperate areas throughout the world and well represented in the Mediterranean and the Near East area (1). *Hypericum perforatum* L. is a perennial plant usually growing in shady places among rocks (2, 3).

Important pharmacological properties have been attributed to extracts of *Hypericum* species: they have been reported to possess antidepressant, anxiolytic, antiviral, wound-healing and antimicrobial activities (4).

Previously, our research group carried out studies on the antimicrobial (5) and on the antidepressant activities in rats of four different species of *Hypericum* (6).

In this work, four extracts of *H. perforatum* were investigated for their free radical-scavenging and antioxidant activities and for total phenolic content. These extracts, obtained from the whole plant in an increasing polarity solvent system, were analyzed for radical scavenging and antioxidant activities using the DPPH test. Chloroform/methanolic (9:1) and methanolic extracts demonstrated the highest antioxidant activity and can be considered as potential sources of natural antioxidant compounds. Moreover, the quantification of phenolic content has been performed using the Folin-Chiocalteu reagent; results show an interesting polyphenolic profile.

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#### 4.2 = CHEMICAL COMPOSITION AND ANTIMICROBIAL ACTIVITY OF ESSENTIAL OILS OF WILD AND CULTIVATED *ORIGANUM SYRIACUM* PLANTS GROWN IN SINAI, EGYPT

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The antimicrobial and antioxidant properties of essential oils (EOs) have been known for a long time, and a number of investigations have been conducted on antimicrobial activities using various bacteria, viruses and fungi (1). *Origanum syriacum* L. is an aromatic, herbaceous and perennial plant growing wild in the Sinai Desert of Egypt (2). The genus *Origanum* is well known for its volatile oil and constituents and is characterised by a large morphological and chemical diversity. Forty-nine taxa divided into 10 sections belong to this genus, most of them having a very local distribution around the Mediterranean basin (3). In folk medicine, *Origanum* species are used as powerful disinfectants, flavoring agents, in perfumes and in scenting soaps (4). They are also useful as a source of antimicrobial compounds (5). Due to these properties, spices and herbs have been added to food since ancient times, not only as flavoring agents but also as preservatives (6).

The essential oil of oregano is well known for the presence of carvacrol and/or thymol as its dominant components, followed by  $\gamma$ -terpinene, *p*-cymene, linalool, terpinen-4-ol and sabinene hydrate (7).

The chemical composition of EOs of *Origanum syriacum* L., collected from cultivated and growing wild plants in South Sinai, Egypt, was determined by GC-MS. The effectiveness of these essential oils as antibacterial was also evaluated on several Gram positive and Gram negative bacterial strains. The antimycotic activity of these EOs was tested against eight fungal strains isolated from different sources. Forty-six compounds were identified in the EOs, dominated by carvacrol in cultivated type. Thymol,  $\gamma$ -terpinene, linalool and 4-terpineol were the most represented constituents in *O. syriacum* collected from wild populations. Both EOs showed antibacterial activity with varying magnitudes, while EO from cultivated *O. syriacum* showed the highest antibacterial activity against *S. aureus* with an inhibition zone of 32 mm. Both EOs showed good antifungal activity against all fungal strains. *O. syriacum* EO from cultivated plants showed the lowest MIC 0.25 mg/L with *A. fumigates* clinical strain isolated either with *A. flavus*.

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#### 4.2 = PRELIMINARY STUDY OF THE PLANTS USED IN THE FOLK MEDICINE IN THE MOLISE SECTOR OF THE ABRUZZO, LAZIO AND MOLISE NATIONAL PARK (ITALY)

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An ethnobotanical research was carried out in the Castel San Vincenzo, Colli a Volturmo, Filignano, Fornelli, Pizzone, Rocchetta a Volturmo and Scapoli municipalities, included in the Molise sector of the Abruzzo, Lazio and Molise National Park and in the neighboring area. The old settlements have a medieval origin and there are remnants of the presence of the Samnite people (megalithic walls). The inhabitants' number ranges between 334 (Pizzone) and 1941 (Fornelli), and the average age is 46.5 years (1).

The territory is located in the heart of the central Apennines (Mainarde Mountains) and the landscape is prevalently characterized by mountain chains, hills, karst phenomena and rivers (the altitude ranges from 460 m to 2124 m a.s.l.).

Despite the area is very important as a model for nature conservation and biodiversity safeguard, it has been the object of very little investigation concerning the officinal plants (2, 3). In particular, this study reports data on the plants used in the folk medicine.

These data were collected during 2012-2014 through semi-structured interviews given to people, which had strong links with the traditional activities of the area. The interviews were conducted on both single person and groups. Occasionally, fresh plants gathered from surrounding areas were displayed. The informants were requested to provide for each plant: the local name, the use, the preparation, the parts used, the period of gathering, the related recipes and an indication of whether the uses were still practiced.

In total 128 interviews were collected, the age range of the informants is between 21 and 95 years, with an average value of 72 years.

Voucher specimens were collected and stored in the *Herbarium Universitatis Aeserniae* (IS) of the University of Molise. The nomenclature of the plants follows the Italian checklist (4, 5).

The ethnobotanical inventory included a total of 414 records, belonging to 89 taxa and 44 families, and the informants reported data on 70 different human and veterinary medical uses. The 72.3% of the informants does remember from 1 to 4 useful species, 21.3% remembers from 5 to 9 useful species, while only the 6.4% of the informants reports more than 10 useful species.

16 taxa are cultivated and among these the most cited species are: *Allium cepa*, for insect stings, to heal or purge the wounds, to promote urination; *Allium sativum* as an anthelmintic; *Solanum tuberosum*, whose tubers are applied fresh in case of burns and *Triticum* sp. to purge the wounds after an operation, for pimples in the ears, as an analgesic, to cure chilblains. It is interesting that one record referred to *Aloe arborescens* Mill., a species originating from the deserts of South Africa, is used as an antiseptic for bruises and wounds.

Among the wild species, the most cited are: *Malva sylvestris*, used for gastrointestinal diseases, respiratory apparatus affections, urogenital system inflammations, dermatitis, toothache, and dental abscesses; *Matricaria chamomilla* used to promote sleep, as an antiseptic, for irritated eyes; *Urtica dioica* as an antiseptic, to reduce hairloss, for rheumatic and muscular pain; *Laurus nobilis* used as a digestive, against stomach ache and aerophagia. For a great part of the local people, it is still in use to prepare infusions with these species collected in the study area, alone or in a mixture with other species and dried figs or apples to improve the taste.

Peculiar species, normally not used in the folk medicine of central Italy are: *Teucrium polium*, used for gastrointestinal diseases and stomach ulcers; *Sempervivum tectorum*, employed for wasp stings, skin soothing, engorgement and sprain; *Portulaca oleracea* for renal colic and *Pteridium aquilinum* for lower high blood pressure (nevertheless, this last use can be dangerous).

The results obtained demonstrate the ethnopharmacological importance of this specific area in the Molise region.

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4.2 = ANTI-INFLAMMATORY PROPERTY OF *VITIS VINIFERA* L. TENDRIL EXTRACTS

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Grapevine (*Vitis vinifera* L.) is well known not only for its dietary uses and for wine production but also for the use of seeds and leaves in herbal medicine. Grape tendrils until recent times haven't been studied for their composition and anti-inflammatory activity, while they are also used in some countries for dietary purposes (1). Polyphenolics have been shown to have anti-inflammatory properties in different cell lines, but their anti-inflammatory mechanisms involving microRNA (small non-coding RNAs able to regulate gene expression) in the inflammation process, have not been widely examined so far (2). In this study we decided to inquire on the composition and possible anti-inflammatory effect of grape tendril extract (TVV). Total and qualitative polyphenol contents have been determined. We performed our investigation on cells implicated in the inflammation process and in vascular damage, such as monocyte (U937) and senescent endothelial (HUVEC) cells. The analyses of miR-126, miR-146a and their related targets, allowed us to evaluate the inflammatory profile of LPS-stimulated cells treated with the TVV extract. The TVV extract analyses revealed high contents of polyphenols ( $66.2 \pm 5.8$  mg/ml); flavonoids ( $15.8 \pm 1.7$  µg/ml) and anthocyanins ( $125.6 \pm 9.7$  µg/g fresh weight). Different classes of compounds with anti-inflammatory properties were detected: flavonols (rutin, quercetin-3-glucuronide); phenolic acids (caffeic, ellagic and caftaric acids); fumaric and citric acids. The main findings induced by TVV on U937 and HUVEC cells were: (i) the decreased expression of IL-6; (ii) the increased expression of miR-146a and miR-126; (iii) the modulation of miRs targets responsible for the IL-6 secretion during TLR4 signaling activation (LPS stimulation). These TVV extract effects confirm our hypothesis about the anti-inflammatory property and may play an important role in the prevention of endothelial dysfunction and aging related diseases.

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4.2 = HAIRY ROOTS INDUCTION IN DIFFERENT *SALVIA* SPECIESCARLOS ALBERTO GARIBAY INFANTE<sup>1</sup>, LAURA BASSOLINO<sup>2</sup>, BARBARA RUFFONI<sup>2</sup><sup>1</sup>Department of Agrobiologia, Universidad Michoacana de San Nicolás de Hidalgo, Paseo Gral. Lázaro Cárdenas y Berlín S/N, Col. Viveros, C.P. 60170, Uruapan, Michoacán, México; <sup>2</sup>Ornamental Plant Research Unit, Consiglio per la Ricerca e la Sperimentazione in Agricoltura - Cra-Fso, Corso Inglese 508, 50132 Sanremo (Im), Italy

*Salvia*, a genus belongs to *Labiatae* family, includes more than 900 species cultivated worldwide for use in folk medicines as well as for culinary purposes (1). Some species are very valuable because of their secondary metabolic content; in fact they can produce diterpenoids (2, 3, 4), tanshinones (5, 6), tannins (7) and flavonoids (8) and many efforts have been done to increase their relative concentration *in planta*. Hairy roots (HRs) are a powerful biological system for the production of valuable compounds from medicinal plants (9). The hairy roots phenotype, characterized by high growth rate and genetic stability, is induced after *Agrobacterium rhizogenes* infection leading to neoplastic roots development. These genetically transformed root cultures can be sub-cultured in a bioreactor for the production of high levels of secondary metabolites and thus can be exploited by pharmaceutical industry for phytochemical preparations (10). In this work, we aim to screen different *Salvia* spp. in terms of their capacity to develop hairy roots. We selected six different species, namely *S. jamensis* “la luna”, *S. corrugata*, *S. cinnabarina*, *S. elegans* “ananas”, *S. sclarea* and *S. dolimitica*. The selected species were micropropagated by growing shoots and internodes on MSO supplemented with 1.33  $\mu$ M BA. After one month of *in vitro* growth, leaves and stems were excised from parental tissues and used as starting material for HRs production. Transformation mediated by *Agrobacterium rhizogenes* wildtype strain 15834 ATCC (American Type Culture Collection provided by LGC standards) was performed according to Savona *et al* (11). Several putative HR lines were selected and grown on MSO supplemented with 100 mg/L cefotaxime (Cx) and then verified through PCR analysis. Once confirmed, few clones per each sage have been selected and transferred to a liquid hormone free medium to establish HR liquid culture for secondary metabolites extraction and analysis. In future, will be interesting to assess the differences in terms of secondary metabolites-derived hairy roots among the selected *Salvia* spp.



Fig 1. *Salvia* species selected for this study. a) *S. sclarea*, b) *S. cinnabarina*, c) *S. dolimitica*, d) *S. jamensis* “la luna”, e) *S. corrugata*, f) *S. elegans* “ananas”

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#### 4.2 = “ABBONDANZA APPLE” CLONE WITH RED PULP: POLYPHENOLS AND ANTHOCYANINS CONTENT, ANTIOXIDANT AND ANTI-INFLAMMATORY ACTIVITIES

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In recent years, fruit and vegetable products, in particular the forgotten fruits (ancient fruits) were evaluated for their importance in food and for their health properties. In fact, particular attention is focused on the antioxidant and anti-inflammatory properties of many fruits, including apples. The attractiveness of apples to consumers is determined both by appearance and by internal attributes of firmness, taste, and health benefits. Polyphenol compounds contribute to both fruit color and human health. These compounds are known as natural antioxidants. Accordingly, antioxidants, abundant in foods, have received great attention and have been studied extensively, since they can reduce the risk for cardiovascular diseases or several types of cancer. Apples are one of the main sources for flavonoids and, in red-colored cultivars, for anthocyanins (1, 2). Considering that the market is interested in creating the apples with red flesh with a pleasant taste, this work was aimed at studying a particular apple the Romagna region (Italy). The main characteristics of this derived clone are the colour of the skin and flesh that are red. On the basis of these observations, in this study we evaluated the polyphenols (3) and anthocyanins (4, 5) content. In addition the anti-inflammatory and antioxidant activity of ethanolic extracts obtained by peel and pulp was determined. Preliminary data show the presence of high concentrations of polyphenols and anthocyanins when our extracts were compared with extracts of other cultivars of apples. This feature determines the peculiarities of this ancient fruit. In addition, all extracts showed good inhibition of lipid peroxidation with the assay of 5-LOX (5-lipoxygenase) (6). The investigation results that this clone can be used not only as an accessible source of natural antioxidants but also as an ingredient of the functional food. For all these reasons, one might assume the possible intensification of cultivation of this clone, which was forgotten for a long time.

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#### 4.2 = WILD EDIBLE PLANTS OR HERBAL MEDICINE? PRELIMINARY ETHNOBOTANICAL INVESTIGATION ON ASTERACEAE IN TBK OF SARDINIA ISLAND, ITALY

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This research is a result of a preliminary ethnobotanical investigation carried out in Sardinia Island, Italy. Due to its geographic isolation and the Mediterranean climate, Sardinian flora includes a high number of taxa (2408), with several endemic species (14,4%) (1, 2). Since there is a well-established culture on the consumption of wild edible plants in this territory, it is interesting to evaluate folk uses and cultural relevance of such species in order to obtain a rational organization of the Traditional Botanical Knowledge (TBK) of the Sardinian inhabitants (3, 4). An ethnobotanical investigation on spontaneous plants used as "*alimenta urgentia*" (phytoalimurgy) was performed and compared with therapeutic purposes.

This investigation was carried out through semi-structured interviews with the local population integrated with a literature review (5, 6, 7, 8, 9), focusing specifically on the *Asteraceae* family. For each species, vernacular name, preparation of the edible parts and folk uses as medicine were specified. Results showed that the most cited Tribes were *Cichorieae* (52%) followed by *Cardueae* (35%). Moreover, among all the edible plants 87% were consumed raw, and 67% cooked. In particular, among the raw preparations 88% of the species were preferably prepared as salad, instead 32% of the cooked vegetables were used as soup's ingredient. About 63% of the species had also a folk use as medicine, of which 76% was prepared as decoction and 72% as infusion.

In conclusion, this study can be considered as a preliminary step for a future broad-spectrum research performed in different subregions of the Island, bound to the valorization of edible plants. Moreover, the subsequent goal would be to analyze the species from a phytochemical and biomolecular point of view. This approach could give more information about the chemical composition and biological activity of the extracts, in order to validate the traditional health beneficial effects. Nevertheless, the research of molecular markers would contribute to the valorization and characterization of the species under study (10).

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## 4.2 = EXTRACTION OF BIOACTIVE POLYSACCHARIDES FROM CEREALS FOR THE PREPARATION OF FUNCTIONAL PASTA

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The increased interest in functional foods has significantly stimulated research finalized to formulation of dry pasta enriched with fiber. Different sources of soluble and insoluble dietary fibers were mixed in a variable ratio with the semolina to get functionalized pasta (1).

In this work a protocol for (1→3),(1→4)- $\beta$ -D-glucan extraction from wholemeal oat flour has been developed. The dry extract (Fig. 1) containing 33-36%  $\beta$ -glucans was mixed with durum wheat semolina, cultivar Pietrafitta, in a ratio 1:10 by weight and used in pasta-making tests. Pasta made with 100% semolina (control) or with a blend semolina/barley flour (11%  $\beta$ -glucans) 6:4 by weight were also made and assayed for total carbohydrates (2) and  $\beta$ -glucans. The amount of  $\beta$ -glucans in raw and cooked functionalized pasta was lower than theoretically expected but still higher than the minimum amount (1 g/serving) imposed by EFSA for placement of the health claim on the label (3). The three types of raw pastas (fusilli shape) showed evident color differences, with that prepared adding extracted oat  $\beta$ -glucans more intensely yellow than control and semolina/barley flour pastas (Fig. 2). Release of  $\beta$ -glucans in cooking water was negligible in both functionalized pastas. Differences in structural characteristics of control and functionalized types of pastas were also evidenced by scanning electron microscopy (SEM) (Fig. 2).



Fig. 1. Dry extract of oat  $\beta$ -glucans

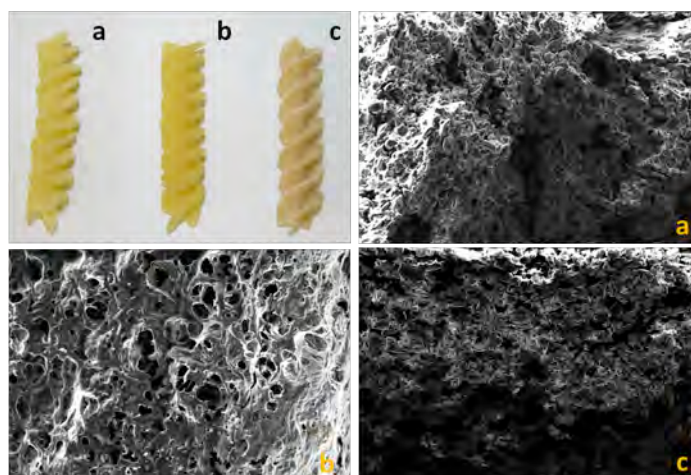


Fig. 2. Macroscopic appearance and scanning electron microscopy structure of pastas made with: a) 100 % semolina, b) 1:10  $\beta$ -glucans extract/semolina, c) 6:4 semolina/barley flour.

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#### 4.2 = NEW DIARYLHEPTANOIDS WITH ANTIOXIDANT ACTIVITY FROM THE LEAVES OF THE PGI PRODUCT “NOCCIOLA DI GIFFONI” (*CORYLUS AVELLANA* L.)

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Typical products are characterized by a strong identification with the land they come from. PDO (Protected Designation of Origin), PGI (Protected Geographical Indication) and TSG (Traditional Specialities Guaranteed) labels in Campania contribute significantly to agricultural economy. In the region, there are currently 16 registered agriculture products, among which the PGI product “Nocciola di Giffoni” (*Corylus avellana* L.) (1). Even if the nutritive features of this product are well known, to our knowledge little is known about its metabolome and its by-products.

Hazelnut (*Corylus avellana* L.), which belongs to the family Betulaceae, is one of the most popular tree nuts on a worldwide basis and ranks second in tree nut production after almond. Italy is the second producer of hazelnut with over 13% after Turkey. Campania is the first Italian region in the hazelnut production, and in Salerno the 90% of the production is represented by “Nocciola di Giffoni”. The hazelnut hard shell, containing a kernel, is the nut of commerce. The product features the following characteristics: spheroidal shape nut measuring not less than 18 mm, medium thickness, more or less deep brown shell with darker streaks, white firm flesh with a pleasant fragrance. Hazelnut skin, hazelnut hard shell, and hazelnut green leafy cover as well as hazelnut tree leaf do not have any commercial value. However, hazelnut green leafy covers and tree leaves are used as organic fertilizers for the hazelnut trees and vegetables upon composting. Hazelnut is known as a source of nutritious food with a high content of healthful lipids. Although some papers have been published regarding the antioxidant activity of phenolic constituents of hazelnut kernel, little is known about the phytochemical composition and the biological activity of its by-products (2).

The leaves of *C. avellana* L. have been used in traditional medicine for varicose veins and haemorrhoidal symptoms, and also for their slight antimicrobial effect (3). Previous investigations on the phenolic constituents of *C. avellana* leaves focused on the main flavonoid and caffeic-acid derivatives (3).

In order to achieve deeper insight into the chemical composition of the by-products of the PGI Campania product “Nocciola di Giffoni” and to highlight the occurrence of biologically active compounds, the phytochemical investigation of the leaves has been carried out. The MeOH extract has been submitted to different chromatographic steps affording new phenolic compounds. Their structures have been elucidated by extensive spectroscopic methods including 1D- (<sup>1</sup>H and <sup>13</sup>C) and 2D-NMR (DQF-COSY, HSQC, HMBC, TOCSY, ROESY) experiments as well as ESIMS analysis. Isolated compounds have been determined as diarylheptanoid-type molecules, characterized by oxygenated functions at different positions of the heptanoid chain, named giffonins A-I.

On the basis of the anti-oxidant activity reported for diarylheptanoids isolated from plants belonging to the Betulaceae family (4), the effect of the MeOH extract and giffonins A-I (**1-9**) 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> have been tested and compared with the activity of the known antioxidant curcumin. Lipid peroxidation has been quantified by measuring the concentration of TBARS. All compounds and curcumin have been tested at concentrations ranging from 0.1 to 100 μM. Most of the compounds were more active than curcumin at the same concentration.

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## 4.2 = ETHNOBOTANICAL SURVEY IN PROCIDA ISLAND (NAPLES, ITALY)

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The present research is an ethnobotanical investigation on the uses of wild and cultivate plants in Procida island (Naples, Italy) (PI) and it represents a tribute to the ethnobotanical knowledge in Southern Italy. Procida is a volcanic island 3.9 km<sup>2</sup> wide; it's far from Naples and Ischia island only 3 km; it has only one municipality, Procida, with about 10.500 inhabitants. The climate is typically mediterranean, characterized by mild winter and hot and dry summer. PI is characterized by Mediterranean vegetation restricted only in some places of the island due to the widespread human settlements and to the presence of many crops.

The investigation was carried out interviewing local older people. In order to avoid mistakes in the identification of species and considered that the same vernacular name is often referred to more species sometimes botanically quite different, interviewed people were asked to show wild and cultivated plants reported by them to have folk uses. Thus, only reports for which the informant was able to indicate and collect the plants were taken into account. For each plant we required to furnish vernacular name, folk use, used parts, gathering period, related recipes, the preparation and the possible association with other plants in its use. Each specimen has been identified: the nomenclature of the plants is according to Tutin et al. (1) and Pignatti (2); plants families were classified according to Judd et al. (3).

This study recorded 106 plant species used for ethnobotanical purposes, belonging to 100 genera and 57 families. The most represented genus are *Allium*, *Cichorium*, *Citrus*, *Prunus* and *Quercus*, with 2 entities; the most represented families are Asteraceae (9 entities), Lamiaceae (8), Rosaceae (7), Fabaceae (6), Poaceae and Solanaceae (4).

Among the investigated species, 81 have an human medicinal use, 40 food use, 15 veterinary use, 15 ritual use, 12 handcraft use, 12 cosmetic use, 10 agricultural use and 45 other uses; several species are multipurpose.

Regarding medicinal utilisation, cough and cold are the most treated ailments (9%), followed by teething (5%), gastrointestinal diseases (5%), constipation (3%), acne (3%), skin diseases (3%), rheumatic pains (3%) and flu (3%); some plants are also used as depurative (5%). Decoction is the most diffused preparation (32%), followed by topic use (22%), poultice (8%) and infusion (8%).

Some species have revealed a very particular use, e.g. *Agave americana* L. and *Olea europaea* L. against warts, *Arbutus unedo* L. and *Malva sylvestris* L. to relieve chilblains, *Carpobrotus acinaciformis* (L.) L. Bol. to solve oedema and haemorrhoids, *Cynodon dactylon* (L.) Pers. against abdominal pains, *Lippia citriodora* Kunth as laxative, *Castanea sativa* Mill. for wounds healing, *Cynara scolymus* L. to increase milk production, *Opuntia ficus-indica* (L.) Mill. against cough, *Parietaria officinalis* L. to relieve sprains, *Petroselinum sativum* Hoffm. for discoloring skin marks and *Vicia faba* L. as astringent and against colitis.

In alimentary field, direct assumption (26%) and liqueur preparation (20%) are the most frequent utilisation, followed by the flavouring use (11%) and as jam ingredient (10%).

Regarding other uses, it's interesting to underline that some species had an important role in the past, such as *Cannabis sativa* L., used to make ship ropes, *Populus alba* L., to make baskets, *Linum usitatissimum* L., as textile, *Sonchus asper* (L.) Hill, as dying, and *Euphorbia dendroides* L. for illegal fishing.

In the study area, the research has shown a good knowledge of ethnobotanical uses, in spite younger generation are losing the heritage about plant uses. In any case, the relative isolation of the island has permitted to preserve a good ethnobotanical tradition which elsewhere has been lost. This kind of research is important to strength literature information of territory and to furnish valuable tools to promote activities in tourism and educational field.

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#### 4.2 = PHYTOCHEMICAL AND BIOLOGICAL ACTIVITY INVESTIGATIONS ON *SIDERITIS ITALICA* EXTRACT.

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*Sideritis italica* (Mill.) is a medicinal plant, endemic in Italy (1), that was traditionally used as diuretic and digestive. The aerial parts collecting from blooming plants are used to prepare decoctions or infusions. The scientific literature confirm that the species *S. italica* was only marginally investigated. This work describes the results of chemical investigation on primary and secondary plant metabolites focused on pigments, amino acid, total proteins and main class of phenols distribution in *S. italica*. We also compared the composition of water, ethanol and hydroalcoholic extracts. The biological activity of extracts was also investigated as antioxidant in chemical assays as well as in in vitro pharmacological assays on myoblast cell line as modulators of oxidative stress.

The ultrastructure of aerial parts and quantitative distribution of pigments, including chlorophylls and amino acids, as well as the main class of secondary metabolites were investigated. The extracts were tested by radical scavenging assays and pharmacological assays (antiproliferative activity, ROS and DNA damage induced by hydrogen peroxide) for their effects on C2C12 cell line.

The SEM confirms the presence of pharmacognostic characteristics, such as glandular and non-glandular trichomes on aerial green parts. The chemical analysis indicates that the leaves are the most important part of the plant, and ethanol/water 70/30 is the preferable extraction solvent because the highest concentration of all metabolites founded in 70% ethanol extract of leaves. The presence of glandular trichomes justifies the pleasant smell of *S. italica* and the small numbers can be related to low yield in essential oil (2). The antiradical assays and the in vitro tests on mouse myoblast cells C2C12 confirm the biological activities of the extract. C2C12 culture medium supplemented with extract, at doses (5-200µg/ml) not interfering with cell viability, was seen to modulate the ROS production and balance the increased oxidative stress induced by hydrogen peroxide. The treatment of C2C12 cells with 200 µg/ml of extract results in a high percentage reduction of ROS, compared to untreated and hydrogen peroxide treated groups. The quantitative reduction of 8-hydroxy-2'-deoxyguanosine, which is a biomarker of free radical DNA damage, confirms the protective effect of *S. italica* extract on oxidative stress at basal condition as well as in presence of exogenous stimulus. The results obtained support the rational base for the medicinal use of plant and extracts in modulating the free radical metabolism and to balance the oxidative stress.

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#### 4.2 = THE MORPHOLOGY OF ROOT AND LEAF OF THE TROPICAL INVASIVE SPECIES *CROTALARIA SPECTABILIS* (FABACEAE)

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*Crotalaria spectabilis* Roth (Fabaceae) is a species originary of India and later naturalized in South America (prevalingly in Brazil) and later in southern USA, where it assumes an invasive behaviour. This species is traditionally known for its property as cause of pulmonary hypertension in rats (1).

Moreover, more recently, *C. spectabilis* revealed to be an effective species in reducing the nematods in the soil, to an extent that it can be compared to chemical products (2). Such remarkable properties may be of biotechnological interest and our study aimed to understand in which organs this plant accumulates the alkaloid considered responsible of its pharmacological properties, the Monocrotaline (3).

The microscopic observation showed the presence of idioblasts particularly in the leaf of *C. spectabilis* where such cells show small vacuoles containing a toluidine blue matrix with some white bodies (Fig. 1). We suggest that these cells may be responsible of the accumulation of the alkaloid Monocrotaline, responsible of the pharmacological properties of the plant.

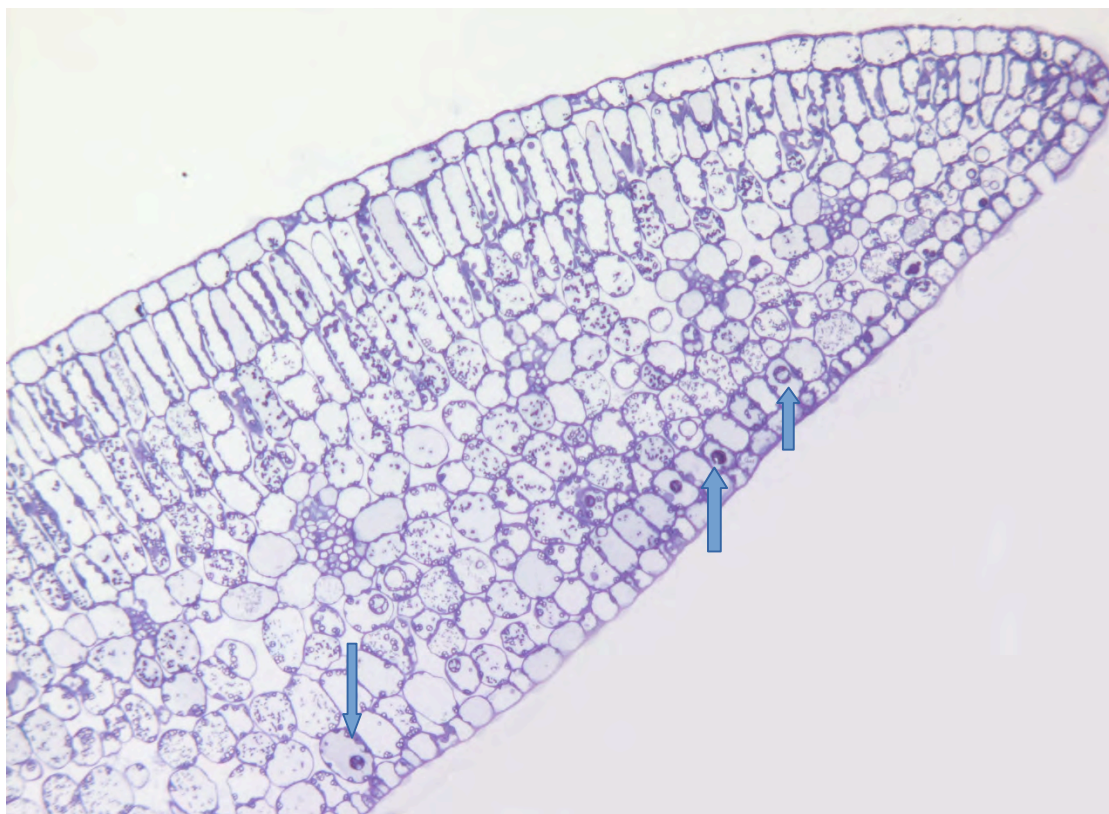


Fig. 1. *Crotalaria spectabilis* leaf. Arrows indicate idioblasts containing small vacuoles.

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#### 4.2 = METALS IN *UNDARIA PINNATIFIDA* (HARVEY) SURINGAR AND *SARGASSUM MUTICUM* (YENDO) FENSHOLTID FROM VENICE LAGOON: AN UPDATE

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**BACKGROUND:** From 1992, the occurrence of two brown algae *Undaria pinnatifida* (Harvey) Suringar and *Sargassum muticum* (Yendo) Fensholt has been documented in the Lagoon of Venice (1, 2). These are the two most abundant invasive species that colonize the hard substrates of the historical center of Venice (3). The cause of the introduction of these species in the new environments is mainly due to maritime traffic or import and breeding of shellfish from Japan (4, 5). In this work the metal contents (Fe, Zn, Cu, Cd, Ni, Pb, Cr, As, Al, Sr, Mn, Ba, Ca, Mg, Co) in samples of *U. pinnatifida* and *S. muticum* collected in different sites of Venice lagoon are reported.

**MATERIALS AND METHODS:** fresh samples, harvested in spring 2013, were washed and lyophilized. Five algae samples were sampled in six areas of the Lagoon of Venice. A suitable microwave digestion procedure was used to mineralize the algae samples (6). All elements were measured by using inductively coupled plasma coupled to a mass spectrometer.

**RESULTS AND DISCUSSION:** Ca and Mg were the predominant elements and the respective contents in the two algae were comparable. No significant differences were observed among samples from different sites, reflecting their role mainly in physiological processes. All the data were processed with principal component analysis. Two principal components were extracted explaining around 50 % of the total variance. Although differences were observed among samples of the same species from the same site, probably due to the different development stage of the algae, in the corresponding score plot, at a glance, a clear separation between the *U. pinnatifida* and *S. muticum* samples was observed. The cluster of *S. muticum* compared to that of the *U. pinnatifida* is in the area of the principal components where the content of elements is greater and it is possible to note the formation of sub clusters due to the sampling zone.

These results suggest a different role of brown algal polysaccharides in metal binding due to the different structure of the alginate in determining selectivity among various metals. In this work, the emphasis is on outlining the interest to evaluate the capacity of *U. pinnatifida* and *S. muticum* to bioaccumulate not only essential but also toxic elements such as certain heavy metals, also in account of their increasing commercial value in pharmaceutical and food products.

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#### 4.2 = MORPHOLOGICAL AND CHEMICAL CHARACTERIZATION OF *HUMULUS LUPULUS* CV. SAAZ CULTIVATED IN NORTHERN ITALY

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*Humulus lupulus* is a climbing and dioecious plant, belonging to Cannabaceae family and native of Eurasian continent. Hop is widespread in the temperate zones of all continents and its cultivation is traditionally concentrated in Europe (especially Germany, Czech Republic, Poland), though USA, China, Australia contribute to the worldwide production. The botanic components of major interest for brewery are the so-called 'hop cones', the female inflorescences characterized by peltate glands on perianth surface and bracts accompanying single flowers (1,2). In Italy, beer brewing is developing in the last years as a small-scale production of excellence, with a particular attention toward local or regional high quality hops to add value to their productions. Among these, *H. lupulus* cv. Saaz is a very traditional aroma hop that has been grown in the Czech Republic for centuries. It is classified as one of the four true Noble varieties. Alpha acids are low and its primary use is for its distinct mild spice aroma and mild flavor.

Our investigation aimed at the morphological and chemical characterizations of *H. lupulus* cv. Saaz recently cultivated in Italy. Open-field treatments with chitosan, an elicitor of the plant defence mechanisms, copper hydroxide and a combination of both products were carried out on plants grown at farm La Morosina (Abbiategrosso, Milan), to study their effects on morphological and phytochemical traits of hop (4).

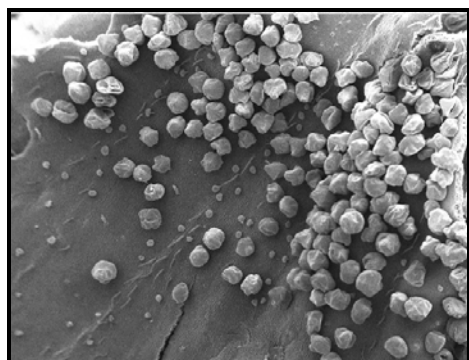


Fig. 1. Peltate glands in abaxial bract

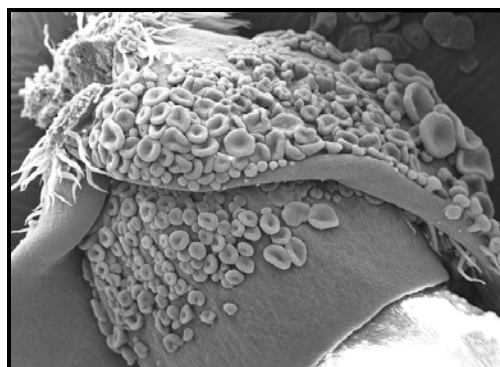


Fig. 2. Peltate glands in abaxial bracteole and perianth

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#### 4.2 = CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITIES OF THE ESSENTIAL OIL FROM *ANREDERA CORDIFOLIA* GROWN IN BRAZIL

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*Anredera cordifolia* (Ten.) Steenis (Basellaceae) is a plant native to South America, from Paraguay to Southern Brazil, Uruguay and Northern Argentina (1). The specific name, *cordifolia*, refers to the heart-shaped leaves of the species, which has a climbing slender stem about 30 cm long, and dark green leaves with small and numerous white flowers. This plant presents small, irregular, green or light brown air tubers (1,2). It is known by common names such as “Madeira vine” (South America), “Binahong” (Indonesia) and “Dhen San Chi” in China (3). It is used traditionally to treat skin disease, hypertension, inflammation and gout (3).

Martinevski and coworkers (2) reported the nutritional value of *A. cordifolia*, known as *bertalba* in Brazil, where it is considered an unusual vegetable, with high nutritional value and known as spinach gaúcho, leaf-fat and leaf-santa (4).

Because of some studies have reported different biological activities of the plant (5, 6, 7), we decide to study the chemical composition of the essential oil obtained from the leaves of Brazilian *A. cordifolia*, to evaluate its possible *in vitro* effects against germination and initial radicle elongation of *Raphanus sativus* L. (radish), *Sinapis arvensis* L. (wild mustard) and *Phalaris canariensis* L. (canary grass), the potential antimicrobial activity against ten selected microorganisms and the eventually antioxidant.

In all, 19 compounds were identified, accounting for 91.6% of the total oil; hydrocarbons were the main constituents (67.7%). The essential oil, at 1.25 µg/mL and 0.625 µg/mL, significantly promoted the germination of *S. arvensis*. Finally, it showed a weak inhibitory activity against the Gram-positive pathogens and it hadn't antioxidant activity.

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#### 4.2 = CHEMICAL COMPOSITION OF THE ESSENTIAL OIL OF *CLADANTHUS SCARIOSUS* (ASTERACEAE) WILD GROWN IN MOROCCO

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*Cladanthus* Cass. [Syn. *Ormenis* (Cass.) Cass.] is a genus of the family *Asteraceae*, endemic to the Mediterranean region and related to the tribe *Anthemideae* (1). It comprises 15 species including *C. scariosus* (Ball) Oberpr. & Vogt [Bas. *Santolina scariosa*; Syn. *Ormenis scariosa* Litard. & Maire] from Morocco where it concentrates 1/3 of the species of the genus (2). Some of these are perennial, suffruticose and strongly aromatic plants. *C. mixta* (L.) Chev. is used in Morocco as chamomile and this is commonly called Moroccan chamomile. In the same country, *C. scariosus* is fairly common in open places, on sandstone substrates (3) and is characterized by a strong aromatic character, this has motivated the authors – some of which were previously occupied by other species of the same genus (4) – to undertake such phytochemical study.

In this study, the authors present the results of chemical composition of the essential oil of *Cladanthus scariosus* wild grown in many regions of Morocco (5).

Hydrodistillation of *C. scariosus* aerial parts, collected on the thermo-mediterranean belt of the central High Atlas – Oukeimeden, from Marrakech to Quarzazate – during the flowering phase gave a pale yellow oil. Overall, sixty-four compounds were identified, representing 92.7% of the total components. The main class of the oil was represented by sesquiterpene hydrocarbons (39.8%) with germacrene D (20.7%) as the most abundant component of the class and of the oil. Monoterpene hydrocarbons, oxygenated monoterpenes and oxygenated sesquiterpenes were present in similar amount (14.8%-15.1%). In these classes the main products were  $\alpha$ -pinene (4.8%) and sabinene (6.9%) among the monoterpene hydrocarbons, (*E*)-chrysanthenyl acetate (8.3%) among the oxygenated monoterpenes and  $\tau$ -muurolol (4.2%) and (*E,E*)-farnesyl acetate (3.9%) among the oxygenated sesquiterpenes. It is also noteworthy the good presence of chamazulene (7.1%).

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4.2 = CHEMICAL COMPOSITION AND BIOLOGICAL ACTIVITIES OF *ZINGIBER OFFICINALE* ROSCOE ESSENTIAL OIL FROM AMAZONIAN AND CHINESE PLANTS

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*Zingiber officinale* Roscoe (Zingiberaceae) is an herbaceous perennial plants with underground rhizomes (crude drug) having annual leafy stems, about one meter (3 to 4 feet) tall, indigenous to southern China, from where it is spread to the Spice Islands and other Asiatic regions, West Africa and Caribbean. Flowers with yellow with dark purplish spots borne on a spike, condensed, oblong and cylindrical with numerous scar bracts, bisexual, epigynous, ovary inferior, three carpellary. The fruit is an oblong capsule, seeds glabrous and fairly large. The crude drug rhizomes, commonly known as ginger, are commonly used as spice for foods and beverages, and as traditional health remedy for many kinds of diseases, such as arthritis, atherosclerosis, hypercholesterolemia, ulcers and depression, just to mention a few (1, 2). The ancient, traditional and modern uses of ginger crude drug are mainly suggested by Indian folk medicine. In fact, India and China are currently the Countries where *Z. officinale* is widely cultivated producing important economic relaunch. Because of its ancient health use, *Z. officinale* crude drug has been in depth studied for the biological capacity of its extracts, essential oil included, pointing out the potential role as cancer preventive tool, together with its antioxidant and anti-inflammatory activities (3). It is known that the same kinds of plant extracts from the same species, but grown in different geographical regions and/or in regions characterized by high levels of biodiversity, could present remarkable discrepancies in chemical composition with consequent dramatic differences in the expression of their biological efficacy and safety. Amazonian basin is one of the most important biodiversity hot-spot of the Earth and Amazonian plant species have been studied for years by our research group to point out their chemical characteristics and biological properties, with particular attention to their health implications. In light of these premises, Amazonian Ecuador *Z. officinale* essential oil has been chemically characterized by GC-FID-MS and assayed for its antioxidant capacity and cytotoxicity towards CaCo2 cell line. Results were all compared to the essential oil obtained from commercial Chinese *Z. officinale* crude drug. Both essential oils were obtained by steam distillation using a Clevenger-type apparatus. Amazonian ginger essential oil presented 37 identified components (98.0% of the total) among which  $\alpha$ -zingiberene (14.45%), camphene (14.72%), 1,8-cineole (8.81%) and ar-curcumene (6.86%) were the most abundant. Chinese ginger essential oil, instead, showed 39 compounds mainly represented by limonene (11.77%), ar-curcumene (9.12%),  $\alpha$ -zingiberene (7.89%), camphene (7.75%) and  $\beta$ -sesquiphellandrene (6.2%). Therefore, chemical characterization highlighted interesting differences to be verified through biological strategies, and for this reason, preliminary antioxidant and cytotoxicity assays have been performed. To this end, antioxidant capacity with DPPH and ABTS radicals was tested for both essential oils through spectrophotometric and bioautographic strategies. In both assays, Chinese essential oils showed the best performances, mainly due to the less abundant geranyl acetate,  $\alpha$ -bisabolene,  $\gamma$ -eudesmol and  $\alpha$ -eudesmol as evidenced by HP-TLC bioautography (4). The Amazonian essential oil, which exhibited a different minor-compound profile than Chinese one, with neral, geranial,  $\alpha$ -farnesene and  $\beta$ -bisabolene, showed instead a lower antioxidant capacity. Cytotoxic activity of Amazonian and Chinese essential oils was checked on human colon carcinoma cells, CaCo-2, by MTT test. Amazonian ginger essential oil evidenced, after 72 h of exposure, a more interesting efficacy on CaCo-2 cell line ( $IC_{50}=0,002\%$ ) than Chinese one ( $IC_{50}=0,003\%$ ). In light of these preliminary but promising evidences, further investigations to determine anticancer active components and a wider biological activity profile of Amazonian and Chinese essential oils and are currently in progress.

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#### 4.2 = PHYTOCHEMICAL CHARACTERIZATION AND CYTOTOXIC PROPERTIES OF THE POLAR EXTRACTS FROM THE LEAVES OF *ISATIS TINCTORIA* L. COLLECTED IN SICILY

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The spontaneous flora of Sicily includes several species belonging to the Brassicaceae family, potential source of bioactive compounds. *Isatis tinctoria* L. (woad) is a herbaceous biennial species which grows wild mainly in Southern and North-western Italy, as well as on the major islands. The stems are erect, up to 120 cm in height, simple below and branched above. Basal leaves are oblong-lanceolate and long-petioled. Cauline leaves, narrower than basal and gradually reduced upwards, are simple, entire, sagittate, usually amplexicaul, with acute auricles (1). *I. tinctoria* L. has been cultivated and used in Europe since antiquity for production of the blue dye indigo and as medicinal plant, until it fell into oblivion due to the import of cheaper indigo and the disappearance of woad cultures. Nowadays *I. tinctoria* L. is employed, together with the closely related *Isatis indigotica* Fort., in traditional Chinese medicine (2).

This study was designed to characterize the phytochemical profile and to investigate the cytotoxic properties of the polar constituents of *I. tinctoria* L. leaves collected at different times of the year, January (It-J) and April (It-A). Cauline leaves, picked from *I. tinctoria* L. grown wild around Acireale (Catania, Sicily), were lyophilized and sequentially extracted with dichloromethane and methanol 70%. The HPLC-PDA-ESI-MS analysis of It-J and It-A hydroalcoholic extracts revealed a similar phenolic fingerprint, being flavonoids the most abundant constituents, although some differences in the content of individual compounds were found; vicianin-2 was the main flavonoid in both extracts. Glucosinolates (GLSs) were identified by LC/MS analysis, and the indole GLS glucobrassicin was detected as the major compound in It-J only.

The anti-proliferative effect of It-J and It-A extracts was evaluated *in vitro* on human leukemia cell line (MOLM-13). After the treatment for 24 and 48 hours, to assess viability, cells were stained with Annexin V and 7-amino-actinomycin D and fluorescence was evaluated by flow cytometry. It-J extract demonstrated good cytotoxic effect at both time points, with an IC<sub>50</sub> of 0.3 mg/mL at 24 hours and of 0.2 mg/mL at 48 hours. It-A extract didn't prove to be active and could barely reach an IC<sub>50</sub> value even after a longer treatment. The potential cytotoxic activity of It-J and It-A was tested using *Artemia salina* lethality bioassay, too (3); both extracts did not display any cytotoxicity against brine shrimp larvae (LC<sub>50</sub> > 1000 µg/mL).

In the last decades, antioxidant compounds have received increased attention from nutritionists and researchers for their potential activities in the prevention of several degenerative diseases such as cancer. The antioxidant and cancer protective properties of polyphenols have been well documented in several studies (3). In a previous work, we showed the *in vitro* antioxidant activity of *I. tinctoria* L. leaves hydroalcoholic extracts, higher for It-A than It-J, which seems to be related to their total phenolic content (99.36 ± 0.17 mg GAE/g and 79.00 ± 0.64 mg GAE/g, respectively) (4). In spite of this, our experimental data show that there isn't correlation between the anti-proliferative activity of *I. tinctoria* L. leaves extracts against MOLM-13 cells and the antioxidant phenolic compounds. It's well known that isothiocyanates, bioactive compounds derived from the hydrolysis of GLSs, possess chemopreventive properties in a variety of cell and animal models (5). Hence, it can be hypothesized that GLSs are involved, almost in part, in the cytotoxic activity of It-J extract. A more extensive analysis is ongoing to better dissect the promising cytotoxic properties of It-J extract and further investigate its anti-cancer applications.

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#### 4.2 = VOLATILE COMPOSITION AND ANTIRADICAL CAPACITY OF ESSENTIAL OIL FROM *ACHILLEA MOSCHATA* WULFEN AERIAL PARTS

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*Achillea moschata* Wulfen (Asteraceae) is an endemic herbaceous plant distributed along the Alpine region and traditionally used for its aromatic properties in several medicinal and food preparations. The investigated samples were collected during summer 2013, on the Retiche Alps, Northern Italy, at three different locations of the Sondrio province, namely Valfurva, Valmalenco and Valchiavenna, between 2000 and 2400 m a.s.l. The essential oils obtained by steam distillation from the dried aerial parts of the whole plants were investigated by GC/FID and GC/MS. Several compounds were identified belonging to different chemical classes, including monoterpenes and sesquiterpenes as the most abundant constituents, together with ketones, alcohols, phenols, acids and esters. A variation in the quantitative composition of several constituents was recorded in the oils from the three different collecting areas. The antioxidant potential of the obtained volatile oils was evaluated by *in vitro* methods using 2,2-diphenyl-1-picrylhydrazil (DPPH) and [(2,2'-azinobis (3-ethylbenzothiazoline-6-sulfonic acid)] radical-scavenging assays. All the investigated oils possess a good antioxidant activity, and a correlation between the two employed tests was also obtained.

4.3 = *TRICHODERMA* POTENTIAL INTO POLLUTED SOILS DETOXIFICATION

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Oil dispersion is nowadays one of the most severe environmental issue. Petroleum hydrocarbon pollution may arise from oil well drilling production operations, transportation and storage in the upstream industry, and refining, transportation, and marketing in the downstream industry. Due to the diversity of hydrocarbon compounds a range of remediation technologies may be applicable. Among these, microbial biodegradation is one of the most important processes involved in the eventual removal of petroleum from the environment, particularly of the non-volatile components. Bioremediation technologies that take advantages on these microorganisms' natural activities appear to be among the most promising methods for dealing with a wide range of organic contaminants, particularly petroleum hydrocarbons. In this context, a *Trichoderma atroviride* strain able to grow on media contaminated with hydrocarbons was investigated.

*Trichoderma* species are widespread and highly competitive soil-borne fungi. They display a successful antagonism against soil-borne plant pathogens. Moreover, they produce extracellular enzyme systems including cellulolytic and chitinase activity (1), and extracellular laccases (2).

In this context, this strain was supposed to be able to produce enzymes able to detoxify the soils (3).

In order to test the detoxifying capacity of the *Trichoderma* strain we have proceeded as follows. The germination index of *Lepidium sativum* on a low sulphur crude oil (LSCO) polluted medium at various concentrations was evaluated, in order to find out the LD50. *L. sativum* seeds were distributed on the paper and left for germinating. After three days a LSCO concentration of 5% w/v led to inhibit the germination of half of the seeds (LD50).

Gardening soil samples were, than, artificially polluted with a LSCO concentration of 5% w/v, by mixing the soil with hydrocarbons, solved into dichloromethane as solvent, and distributing in 40 jars of 50 ml. Twenty of the jars were inoculated with fresh culture of *Trichoderma atroviride* suspended into sterile water, and left for forty days at room temperature in sterile plastic bag. The other twenty jars were left without the fungus at the same condition. After that time, three seeds of *L. sativum* for each jars were sown and the germination index was recorded each three-four days for two weeks. A significantly different germination index was observed between the soil treated by *Trichoderma* and the control, after 6 days. In the jars with the fungus the germination index was 20% higher than the untreated soil (p value <0,05) (4).

The germination index reached the plateau after 6 days in the treated jars and after 11 days in the control jars, reaching the same values (70%). Taking the time of the germination index for reaching the plateau, as a parameter the polluted soil treated with *Trichoderma atroviride* was less toxic compared with the untreated one of about the 45%. The strain of *Trichoderma atroviride* utilized in these tests was able to halve the toxicity of LSCO after forty days of growth and activity.

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#### 4.3 = STUDY OF BIOREMEDIATION. CYANOBACTERIA AND MICROALGAE FOR WASTEWATER TREATMENT

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Urbanization, agricultural practices and industrialization have led to a gradual increase and accumulation of nutrient elements such as Nitrogen and Phosphorus (N, P) [1] in the aquatic environments. Nutrient enrichment is one of the main causes of eutrophication, which involves an increase in growth of algae and aquatic plants, loss of biodiversity and alterations of natural ecosystems.

In accordance with the EU Water Framework Directive regulations, the N and P concentrations in the effluent must be reduced from the current EU discharge requirements of 10 mgN L<sup>-1</sup> and 1 mgP L<sup>-1</sup> to concentrations appropriate for discharge to water bodies. Current technologies of wastewater treatment cannot be considered as sustainable and suitable methodologies, because of the utilization of chemical processes [2].

Microalgae and cyanobacteria are planktonic or benthic photoautotrophs, naturally present in water bodies. Besides natural aquatic environments, they also occur in artificial man made water bodies as wastewater treatment plants where they grow using the nutrients present in the water body [3]. For this reason, they have been investigated as an alternative biological treatment to reduce the concentration of N and P from wastewater.

In the Laboratory of Biology of Algae, at the University of Rome "Tor Vergata", studies are carried out in order to investigate the ability in P-removal of cyanobacteria and microalgae isolated from natural sites and cultured in different growth systems: batch, photobioreactor, flow incubator. In addition, the algal biomass produced is reused for the extraction of compounds of commercial interest (biodiesel, polysaccharides, pigments) [4, 5].

Among the strains tested, the green microalga *Scenedesmus* sp. is very promising with a percentage P-removal of 97% when grown in the photobioreactor. In addition, the use of benthic cyanobacteria, growing adherent to surfaces (biofilm-forming), appears particularly interesting due to the ease of biomass harvesting.

The ability in bioremediation of microalgae and cyanobacteria, offers the possibility to create an integrated system that couples the water purification with the recycling of algal biomass for biotechnological applications, realizing a virtuous circle ecologically and economically sustainable.

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### 4.3 = BIOREMEDIATION OF A HYDROCARBON POLLUTED SOIL: ISOLATION AND CHARACTERIZATION OF NATIVE DEGRADING BACTERIA

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Contamination by the petroleum-based products, resulting from the activities related to the petrochemical industry, are currently one of the major environmental problems. Unintentional releases in the environment of these products are of particular concern causing long-term damage to water and soil ecosystems. The accumulation of these pollutants in animals and plant tissues are inextricably linked to human health since they belong to the family of carcinogens and neurotoxic substances. The issue of contamination of soils is more and more relevant also due to their steady increase. Conventional technologies commonly used for the soil remediation comprises, for instance, chemical oxidation, thermal desorption and excavation with off-site disposal in landfill. These technologies are however expensive and can also lead to incomplete decomposition of contaminants whereas the bioremediation that uses microorganisms to detoxify or remove pollutants through their diverse metabolic capabilities offers many advantages over traditional remediation technologies also because it can be applied *in situ* without the need to remove and transport the contaminated soil and is usually less expensive and less labour-intensive. Several indigenous microorganisms isolated from contaminated soils have proved to degrade hydrocarbon contaminants making this technology promising for the treatment of oil polluted sites leading to complete mineralization of organic contaminants into carbon dioxide, water and inorganic substances or to the transformation of complex organic contaminants into other simpler carbon-based compounds (1, 2). Biodegradation by natural populations of microorganisms represents one of the primary mechanisms by which petroleum and other hydrocarbon pollutants can be removed from the environment and bioremediation is therefore a viable developing technology.

Our study was carried out with a soil from a decommissioned refinery, located in Italy, contaminated by petroleum hydrocarbons > C12. Through enrichment cultures performed with this soil and by using diesel oil as sole carbon source, 36 indigenous bacteria have been isolated.

These microorganisms, characterized by 16S rRNA gene sequencing, have been proved to belong to the class of Proteobacteria and to different genera among them *Pseudomonas*, *Achromobacter*, *Stenotrophomonas*, *Sphingobium*, *Comamonas* and *Acinetobacter* known for their ability to breakdown many toxic organic pollutants such as PCBs, PAH, pesticides, lindane. The classification of the isolates is thus consistent with the type of hydrocarbons (high molecular weight with complex structure molecules) present in the soil, showing that the indigenous microbial community is potentially capable of degrading the pollutants and can therefore be exploited for a bioremediation approach. Experimental data of the isolation and characterization of these hydrocarbon degrading bacteria will be presented.

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#### 4.4 = THE GENUS *FESTUCA* S.L. (POACEAE) IN ITALY: NOVELTIES AND ACHIEVEMENTS IN THE NEW EDITION OF “FLORA D’ITALIA”

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The genus *Festuca* L. s.l. represents one of the most intricate and neglected critical groups of the Italian and Euro-Mediterranean flora. A combination of intrinsic high phenotypic variability and nomenclatural complexity, produced by two centuries of heterogeneous taxonomic interpretations, affected a comprehensive distributional and systematic knowledge of the whole genus in Europe, with substantial differences between the single countries (3). As recently observed (3), systematic researches on this genus had been very scarce in Italy during the 110 years period between Hackel’s monography (6) and Pignatti’s treatment (8). The situation changed from the middle 1990’s, when our research team started taxonomic investigations on this genus in Italy, through an approach involving the study of type material and nomenclature, new field collections, morpho-anatomical, karyological and molecular analyses.

The forthcoming new edition of “Flora d’Italia” by Sandro Pignatti is a crucial occasion to summarize and evaluate the current systematic knowledge on *Festuca* s.l. (including *Drymochloa*, *Leucopoa*, *Patzkea* and *Schedonorus*) in Italy. In comparison with the 1982 edition, some major differences are worth considering, most importantly the total number of taxa, which increased from 68 to 89 (76 species with 40 infraspecific taxa) (Fig. 1). This recruiting process is due either to the recent description of new taxa, the majority of them Italian endemics (e.g. *F. riccerii* Foggi & Gr.Rossi, *F. veneris* Gr.Rossi, Foggi & Signorini) (7), and to the recording of taxa previously passed unnoticed on the Italian territory (e.g. *F. nigricans* (Hack.) K.Richt., *F. rupicaprina* (Hack.) A.Kern.). Even though this trend appears lower than the French one (3), it should be noted that a number of taxa has been excluded from the Italian flora (e.g. *F. gracilior* (Hack.) Markgr.-Dann., *D. drymeja* (Mert. & W.D.J.Koch) Holub subsp. *drymeja*) as a consequence of recent specific taxonomic revisions (4, 5). The distribution of some lesser known species has been improved (e.g. *F. violacea* subsp. *puccinellii* (Parl.) Foggi, Gr.Rossi & Signorini and *F. robustifolia* Markgr.-Dann. resulted to occur in a more restricted area than previously stated) and the most recent nomenclatural updates have been taken into account, such as the new treatment at genus level of the “broad-leaved” fescues (1, 5).

Additional advantageous features are represented by updated identification keys and descriptions, summing up the major papers on Italian fescues published after 1982. A modern iconography replaces the old one acquired from (2) (in conflict with the current taxonomic concept of some species) and new original line drawings of leaf blade sections are provided (Fig. 2). This new treatment, although innovative and outlining the first updated scenario on *Festuca* s.l. in Italy after more than 30 years, is not to be considered exhaustive: some relevant groups are still obscure and under study (e.g. *F. circummediterranea* Patzke) and new explorations and new revisions are needed to pass from  $\alpha$ - to  $\Omega$ -taxonomy.

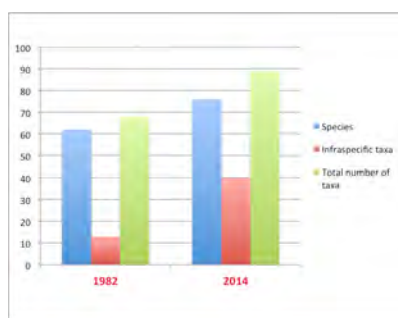


Fig. 1. Taxa of *Festuca* s.l. in Pignatti’s actual and “future” floras



Fig. 2. Picture and drawing of a leaf section (by N. Ardenghi)

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#### 4.4 = AN INTEGRATED MORPHOLOGICAL, MORPHOMETRIC AND MOLECULAR APPROACH TO BIOSYSTEMATICS OF CARNIVOROUS EUROPEAN *UTRICULARIA* SPECIES (LENTIBULARIACEAE)

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The bladderworts (*Utricularia* L., Lentibulariaceae Rich) are carnivorous plants occurring in five out of the six continents. Their most striking feature is the bladders, tiny modified leaves, working as traps for the prey capture. In Europe just seven native out of the 220 known species occur, all aquatic, inhabiting wet biotopes often threatened by human activities: *U. australis* R.Br., *U. bremii* Heer, *U. intermedia* Hayne, *U. minor* L., *U. ochroleuca* Hartman and *U. stygia* Thor, and *U. vulgaris* L. (1). *U. australis*, *U. bremii*, *U. ochroleuca*, and *U. stygia* are generally sterile; likely, they represent not one, but several morphologically slightly different vegetative apomicts and many populations do not flower, or rarely do (1). Due to this aspect and to the close resemblance of the vegetative parts, it is not easy to correctly identify not flowering individuals, so that in much of the literature the situation is confused and many herbarium specimens were actually misidentified. Summarizing the data available in literature regarding the vegetative parts (1, 2, 3, 4, 5), it is possible to distinguish *U. australis* and *U. vulgaris* by the occurrence (or not) of the teeth, from which setulae arise, on the lateral margin of the ultimate leaf segments. Concerning *U. intermedia*, *U. ochroleuca* and *U. stygia*, the former can be discriminated from the latter two species by the number of teeth on the leaf margin and the ultimate leaf segment apex shape, while *U. ochroleuca* and *U. stygia* can be distinguished by the angle formed by the short arms of the trap quadrifid glands and the shape of apical leaf segment. The vegetative parts of *U. bremii* and *U. minor* are basically identical.

One of the most intriguing arguments is about the use of the quadrifid glands inside the traps as diagnostic tool. Thor (2) assessed that all the Scandinavian species might be distinguished by the features of these glands, but some other authors are skeptic (1, 4, 6, 7, 8, 9).

In this study, investigations were mainly focused on the presence and aspect of teeth on the leaves and on the shape of quadrifid glands occurring inside the traps. The quadrifid glands analysis were performed by means of geometric morphometrics (10). Besides the morphological investigations, also molecular analysis were performed, aimed to the detection of DNA short species-specific sequences, a popular approach known as DNA Barcoding (11), and to the reconstruction of the phylogenetic relationships between these species. One nuclear marker, the ITS, and two plastidial markers, the *trnL-trnF* IGS and the *rps16* intron, were used. Some characters tested revealed to be potentially useful for discrimination of species, while some other resulted quite unreliable. For instance, the morphometrics analysis of the quadrifid glands showed that this feature cannot discriminate between the whole set of European *Utricularia* species. Indeed, it was not possible to find significant difference between the shape of *U. bremii/U. minor* glands, as well as for *U. australis/U. vulgaris*. Barcoding approach gave no appreciable results, since different haplotypes/ribotypes can be found within the same species or different species share the same haplotypes/ribotypes. However, interesting hypothesis could be derived from phylogenetic trees obtained, including hybridization events to explain the rise of the mostly sterile *U. australis*, *U. bremii*, *U. ochroleuca* and *U. stygia*.

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## 4.4 = ROBERTO DE VISIANI'S HERBARIUM DALMATICUM: RECOVERY, REORDER, CATALOGATION AND VALORIZATION OF AN HISTORICAL COLLECTION

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Among the Phanerogamic Herbaria present inside *Herbarium Patavinum* (PAD) (5), one of the most consulted is *Herbarium Dalmaticum*, made by Prof. Roberto de Visiani (1800-1878), Prefetto of the Orto Botanico di Padova between 1836 and 1878. Talented and tireless systematic, he dedicated time and efforts to his *Herbarium Dalmaticum*, for whose realization he took advantage of the help of many correspondents and collaborators of various social extractions, nations, and languages, such as Neumayer and Pantocsek, whose full *Herbaria* Visiani bought in 1873. The collection, which so contains *exsiccata* not only from Dalmatia (land of the ancient Republic of Venice), but also from the Veneto and the Balkan Peninsula, allowed him the publication of his magistral opera: *Flora Dalmatica* (1842 - 1852), published by Hofmeister in 3 parts (Leipzig, 1872) and completed with numerous Supplements (72 - 77 - 82).

De Visiani put his *Herbarium* in elegant, dark green boxes, with the sheets containing the various *exsiccata* collected in titulated folders, one per each species, and alphabetically disposed inside the Orders. The taxonomic nomenclature used, such as in *Flora Dalmatica*, is different from the current one, but the names of the Orders, usually with different endings, somewhat recall modern Families (1, 4).

Unfortunately, over the years, many of the various sheets have been removed from the boxes (of which remain only the pieces with the Orders heading), the *exsiccata* detached, and transferred on sheets of different size to uniform them with those of *Herbarium Generale* (HG PAD) and *H. Venetum* (HV PAD), and inserted there with the related tags, accurately but with scarce scientific competence (4).

In 1977/78, G. G. Lorenzoni, then Professor of Systematic Botany of the University of Padua, who understood the importance of this *Herbarium*, because of the many requests for viewing its samples, decided on a series of operations, necessary for a better accessibility and easier consultation, and entrusted these tasks to Prof. Elisabetta Dal Col (1).

So, a recovery was undertaken of the samples present inside the two cited *Herbaria*, a patient cleaning of the *exsiccata* from DDT powder, and copying of all the tags, usually handwritten in different languages and styles and sometimes illegible, most times matched with a de Visiani's confirmation tag ("*Flora Dalmatica*" and "Visiani" printed, and species and gathering location handwritten).

The *Herbarium Dalmaticum*, after this huge work, is now constituted by 40 folders (or packs) of multiple sheets, rarely more than 100, clustered by species in 2502 cases, titled and numbered and alphabetically sorted. The present species, for a total of about 10,000 samples, represent about 2,500 taxa, inside which the author cites several *Typi*.

This recovery work, which also implied a taxonomical update (2, 3, 6, 7, 8, 9), a complex filing and catalogation available for informatization, allows for an easier and accessible fruition of a historically important cultural resource, necessary for documenting changes in the natural environment.

In-depth study of the *Herbarium Dalmaticum*, also, has allowed the activation of many new starting points for research: in the future, it is expected a typization of some of its samples, the revision of critical entities, and the recovery of *exsiccata* belonging to the collection from national and international *Herbaria*.

1) Dal Col E., 1982. *Un botanico dell'800: Roberto De Visiani*. *Natura e Montagna*, 29:23-292) Euro+Med, 2006-2014. Euro+Med PlantBase - the information resource for Euro-Mediterranean plant diversity. Published on the Internet <http://ww2.bgbm.org/EuroPlusMed/>3) Guiry M.D. & Guiry G.M., 2014. *AlgaeBase*. World-wide electronic publication, National University of Ireland, Galway. <http://www.algaebase.org>4) Lorenzoni G. G., 1983. *L'Erbario della Flora Dalmata di R. De Visiani*, in *Zbornik Roberta Visianija Sibencanina*. Sibenik, Muzej Grada Sibenika, pp.181-1845) Pedrotti F., 1995. *L'Erbario fanerogamico dell'Orto Botanico di Padova*, in A. Minelli, *L'Orto Botanico di Padova (1545-1995)*, Marsilio Editori, Venezia, pp. 245-2596) Pignatti S., 1982. *Flora d'Italia*. Edagricole, Bologna, 3 Voll.7) The Plant List, 2014. Version 2. Published on the Internet, <http://www.theplantlist.org/>8) The International Plant Names Index (2014). Published on the Internet <http://www.ipni.org>9) Tutin T.G. et Al., 1964-1980. *Flora Europaea*. Cambridge University Press, Cambridge, Voll. I-V



#### 4.4 = ANALYSIS OF THE NATURALISTIC ELEMENTS IN THE *STUDIOLO* OF FRANCESCO I IN PALAZZO VECCHIO, FLORENCE, ITALY

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The city of Florence hosts an art heritage of exceptional influence. To carry out interdisciplinary researches in Florentine museums, in order to identify natural elements represented in artworks - plants, animals, soil types, landscape features – and to trace the numerous connections that arose in Florence among art, science, agriculture and patronage, is an effort of great scientific and cultural interest.

The University of Florence (Department of Biology and Museum of Natural History) and CNR-Ibimet have set up a multidisciplinary research group aimed to the identification of natural elements in cultural heritage, thanks to the experience acquired by some of the components with studies on different artworks hosted in Florence, such as Ghiberti's *Porte del Paradiso* (Baptistry), Bachiacca's frescoes in the *Scrittoio* of Cosimo I (Palazzo Vecchio), Benozzo Gozzoli's *Cavalcata dei Magi* (Palazzo Medici Riccardi), Botticelli's *Primavera* (Uffizi), the base of Fontana del Porcellino by Tacca (Museo Bardini).

On these issues, a working group including botanists, zoologists and soil scientists was recently established. An agreement is currently to be finalized with the City of Florence, for the study of naturalistic elements in artworks housed in Palazzo Vecchio, also in order to propose naturalistic/artistic itineraries. Palazzo Vecchio, which in the past has already been investigated by researchers of this University, is one of the places that best preserve testimonies of some figures of the Medici family - especially Cosimo I and Francesco I -, who were patrons of artists, experimenters and scientists, as well as collectors of marvels and curiosities about natural world. In particular, in the *Studiolo* of Francesco I 36 paintings and eight statues are kept, most of them also containing images of natural elements: vegetables and fruits, ornamental and medicinal plants, plants related to the myth, forests, birds, mammals, molluscs, rocks, etc.

With this research activity trying to link artworks with naturalistic elements, the research group aims to offer an original reading of the artistic heritage to visitors. Tools will be provided to track the many threads that between the 16th and 17th century tied Florence and the Medici to the birth of modern natural sciences and to changes in cultivation of fruits, flowers and horticultural plants, also retracing evidences linking the Medici family to Florentine institutions such as the Botanical Garden of Florence University, founded in 1545 for want of Cosimo I.

AGNOLETTI M., SIGNORINI M. A., 2011 - Il paesaggio della 'Cavalcata dei Magi'. Pacini ed., Firenze

CLAUSER M., NEPI C., 2011 - La Fontana del Tacca osservata dal botanico: identificazione delle piante raffigurate intorno al "Porcellino". In: Nesi A. (ed) *Il Porcellino di Pietro Tacca - le sue basi, la sua storia*. Polistampa, Firenze

LEVI D'ANCONA M., SIGNORINI M. A., CHITI BATELLI A., 2000 - Piante e animali intorno alla Porta del Paradiso. M. Pacini Fazzi ed., Lucca

NEPI C., SIGNORINI M. A., 2008 - Forme e colori d'ogni specie di frutti. Bartolomeo Bimbi e la tradizione pomologica e botanica alla corte medicea. In: Baldini E. (ed) *Mito, arte e scienza nella Pomologia italiana*, pp. 99-124. CNR, Roma

NEPI C., SIGNORINI M. A., 2010 - Cosimo I e il Rinascimento della botanica europea. Commenti sull'identificazione dei vegetali nella bordura degli arazzi. Lista delle specie vegetali dell'arazzo 'Lamento di Giacobbe'. In: Godart L. (ed.) *Giuseppe negli arazzi di Pontormo e Bronzino. Viaggio tra i tesori del Quirinale*. Pp. 175-187. Tecnostampa, Loreto

SIGNORINI M. A., 1993 - Sulle piante dipinte da Bachiacca nello scrittoio di Cosimo I a Palazzo Vecchio. *Mitt. Kunsthist. Inst. Florenz*, XXXVII: 396-407

SIGNORINI M.A., RICCI C., VIVONA L., 2010 - Erborizzando nei quadri dei musei. I vegetali nell'arte e nuove riflessioni sulla 'Primavera' di Botticelli. *Atti e memorie dell'Accademia toscana di scienze e lettere La Colombaria*. n. s., 61 (2009), 75: 152-175

VANNI S., NISTRI A., 2002 - I Serpenti della Medusa (pp. 61-62, 2 figs). In: Caneva C. (ed), *La Medusa del Caravaggio Restaurata. Retablo Cultura-Arte-Immagine*, Roma, 184 pp.

#### 4.4 = INTEGRATED TAXONOMY TO IDENTIFY SARDINIAN PLANTS WITH NUTRACEUTICAL PROPERTIES

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The aim of this project is to develop an integrated taxonomic approach that allows the identification of the entities present in the plant flora of Sardinia, which possess nutraceutical properties, through the use of morphological and molecular analyzes. The first step of the research has been directed to the development of an identification system based on the approach molecular DNA barcoding. This methodology is based on the analysis of one (or more) regions of DNA capable of distinguishing unambiguously a given species. This region should be amplifiable by universal primers, dimensions have relatively short (no longer than 700 bp) and present a high level of genetic variability that can discriminate phylogenetically related taxa. Although numerous candidate markers have been proposed, both plastid and nuclear origin, the data currently available did not allow to identify a universal marker (1). On the basis of these premises, the work was initially focused on the analysis of different markers in a sample group of species from Sardinia.

After the first stage of retrieval of plant samples and their morphological analysis we proceeded to the extraction of DNA from a group of 30 species belonging to different families. The DNA obtained was used to perform the amplification of three markers plastid: *matK* and *rbcL* and *trnH-psbA*.

They were also used for nuclear markers to solve any problems in case of congeneric species, hybrid or complex taxa. We proceeded to the amplification and sequencing of the ITS region and the analysis of some genes COS (conserved orthologous genes) as *sqd1* and *at103*. To choose the most suitable markers for the project was initially necessary to evaluate the success of amplification of the markers chosen and the quality of the sequences obtained. From the early investigations show that the chloroplast markers are able to produce the best yield and provide amplification sequences of good quality; among these the most variable marker gene is the spacer *trnH-psbA* as seems to be the most universal marker *rbcL*.

In the case of nuclear markers, although the ITS region has been widely used in phylogenetic studies, this marker has often paralogous forms that disturb the quality of the amplified sequences. As regards the analysis of new nuclear markers based on single-copy genes present there is a need to develop a system of universal amplification beyond that to verify their variability intra and interspecific.

1) P. M. Hollingsworth, S. W. Graham, D. P. Little (2011) Choosing and Using a Plant DNA Barcode *A. Plosone* 6(5): e19254. doi:10.1371/journal.pone.0019254

#### 4.4 = PHYLOGENETIC ANALYSIS OF THE SICILIAN *HIERACIUM* TAXA (ASTERACEAE) USING “DNA BARCODING”: PRELIMINARY DATA

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*Hieracium* L. s. str. (*Asteraceae*) is represented in Sicily by 11 taxa (1), 9 endemic to the island (*H. busambarensis* Caldarella & al., *Hieracium hypochoeroides* subsp. *montis-scuderii* Di Grist. & al., *H. lucidum* Guss. subsp. *lucidum*, *H. lucidum* subsp. *cophanense* (Lojac.) Greuter, *H. murorum* subsp. *atrovirens* (Guss.) Raimondo & Di Grist., *H. pallidum* subsp. *aetnense* Gottschl. & al., *H. racemosum* subsp. *pignattianum* (Raimondo & Di Grist.) Greuter, *H. schmidtii* subsp. *madoniense* (Raimondo & Di Grist.) Greuter, *H. symphytifolium* Froel.), the remaining 2 with a wider range (*H. pallidum* Biv. and *H. racemosum* subsp. *crinitum* (Sm.) Rouy). These taxa are well differentiated by both morphological and ecological features and belong to sections *Bifida* (Arv.-Touv.) Clapham, *Grovesiana* Gottschl., *Italica* (Fr.) Arv.-Touv., *Oreadea* (Fr.) Arv.-Touv., *Pulmonaria* Monnier. Within the genus, the endemic chasmophyte to Mt Gallo (Palermo), *H. lucidum*, represents a significant taxon interesting not only from a phytogeographic point. In fact, being one of the few sexual species of the whole genus, it could be probably considered common ancestor to many European entities of *Hieracium* (2, 3, 4).

The aim of this study was to define the phylogenetic relationships among the above mentioned Sicilian taxa using the approach of “DNA barcoding”.

The analysis has so far involved only 7 taxa (*H. lucidum* subsp. *cophanense*, *H. murorum* subsp. *atrovirens*, *H. pallidum*, *H. racemosum* subsp. *crinitum*, *H. racemosum* subsp. *pignattianum*, *H. schmidtii* subsp. *madoniense*, *H. symphytifolium*) out of 11 total. Genetic identification was performed following the international protocols of the CBOL (*Consortium for the Barcode of Life*) (5). In the present study, we evaluated the performance of 3 plastid DNA regions: *rbcl*, *matK* and *trnH-psbA*; the last sequence is an effective and reliable region to discriminate between morphologically closely related species (6).

On the basis of the obtained specific *multi-locus* genetic divergence, our preliminary results showed that the current taxonomic treatment – based on morphological characteristics – not always corresponds to molecular data instead suggesting different arrangements. As a matter of fact, the higher genetic affinity of *H. racemosum* subsp. *pignattianum* with *H. lucidum* subsp. *cophanense*, rather than with *H. racemosum* subsp. *crinitum*, seems inconsistent as well as that one between *H. pallidum* and *H. schmidtii* subsp. *madoniense*; the last two taxa, indeed, are clearly autonomous and independent due to obvious morphological, ecological and karyological differences (7, 8).

1) E. Di Gristina, G. Gottschlich, R. Galesi, F.M. Raimondo, A. Cristaudo (2013) *Fl. Medit.*, 23, 49-55

2) S. Pignatti (1979) *Webbia*, 34(1), 243-255

3) S. Pignatti (1982) *Flora d'Italia*, 3, Edagricole, Bologna

4) S. Pignatti (1994) *Ecologia del Paesaggio*, UTET, Torino

5) CBOL Plant Working Group (2009) *Proc. Natl. Acad. Sci. USA*, 106, 12794-12797

6) W.J. Kress, K.J. Wurdack, E.A. Zimmer, L.A. Weight, D.H. Janzen (2005) *Proc. Natl. Acad. Sci. USA*, 102, 8369-8374

7) E. Di Gristina, A. Geraci, F.M. Raimondo (2005) *Inform. Bot. Ital.*, 37(1), 26-27

8) F.M. Raimondo, E. Di Gristina (2007) *Pl. Biosyst.*, 141(1), 86-92

#### 4.4 = SYSTEMATIC INVESTIGATIONS CONCERNING *SAXIFRAGA GRANULATA* S.L. IN THE TUSCAN ARCHIPELAGO (NORTHERN TYRRHENIAN SEA): EVIDENCES FOR DESCRIBING NEW TAXA IN CAPRAIA AND MONTECRISTO ISLANDS

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The genus *Saxifraga* L. (*Saxifragaceae*) has a holarctic distribution and counts more than 440 species, including about 120 living in Europe (1). Seven species with four additional subspecies are currently reported within the Series *Saxifraga* (2). Multi-year floristic and systematic research both in the wild in the Tuscan Archipelago (Central Italy) and in herbaria allowed to investigate some interesting populations of *Saxifraga* living in Capraia, Elba and Montecristo islands and known since the end of the nineteenth century. The territory covered by the three populations is located on the border between the distribution areas of *S. granulata* L. and *S. corsica* (Ser.) Gren. & Godr. Over the years the taxonomic treatment concerning these populations has undergone various interpretations, sometimes considering them as belonging to *S. granulata* group and sometimes as belonging to *S. corsica* group. The Capraia population was formally taken into account by Sommier (3), who described a new infra-specific taxon with the name *S. granulata* var. *brevicaulis* Sommier.

The three populations were analyzed both from the morphological and caryological points of view. Morphological investigation was carried out both on living cultivated plants and on dried specimens (exsiccata) stored in several herbaria (FI, FIAF, GDOR, GE, PIAGR, PI). Morphologically the three populations can be assigned to two distinct groups: the Elba population presents the typical features of *Saxifraga granulata* s.s., whereas the populations of Capraia and Montecristo certainly belong to the group of *S. granulata* s.l. (including *S. corsica*); however they show numerous peculiar characteristics that make it difficult to identify them within *S. granulata* s.s. or *S. corsica* s.s. (plant height less than 20 cm, corymbiform inflorescence, style-stigma complex shorter than the calyx, stigma flattened and elongated rather than rounded).

Cytological studies in the genus *Saxifraga* present many practical difficulties (4), further complicated by an extremely marked intraspecific aneuploidy, leading to different chromosome numbers within populations taxonomically considered as individual species (5). The ongoing caryological investigation showed low chromosome counts for the Elba and Montecristo populations and higher counts for the Capraia population. These results and other morphometric evidences as well highlight the differences between the populations of Capraia and Montecristo.

The affinity of the Elba population with *S. granulata* s.s., widely distributed in the Italian peninsula, can be well explained by the geographic contacts between the Elba Island and the mainland till the last glacial period. Similarly, the isolation of Capraia and Montecristo, dating back to the Upper Messinian, may have allowed the differentiation and stabilization of individuals distinguishable from the original pool.

In conclusion, based on the results of the present study, and on the geographical and ecological isolation, we propose to assign the populations of Capraia and Montecristo to two new taxa, that will be described in shortly, separated either from *S. granulata* s.s. or *S. corsica* s.s.

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2) D.A. Webb, R.J. Gornall (1989) Saxifrages of Europe. Christopher Helm, London

3) S. Sommier (1898) Aggiunte alla flora di Capraia. Nuovo Giorn Bot Ital n.s. 5, 106–139

4) C. Favarger (1965) Notes de caryologie alpine, IV. Bull. Soc. Neucheloise Sci. Nat. 88, 15-23

5) S. Kumar, S. Kumari, R.C. Gupta, V.K. Sharma (2013) Additions to the cytology of *Saxifraga* (*Saxifragaceae*) from the Western Himalayas, India. Botanica Serbica 37(2), 147-154

4.4 = PHYLOGENETIC ANALYSIS OF  $\beta$ -GLUCURONIDASES GENES IN ANGIOSPERMS

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$\beta$ -glucuronidases (GUS) are glycosyl-hydrolases (GHs) which catalyse the hydrolysis of the glycosidic bond between glucuronic acid and other carbohydrates or molecules different from sugars, termed aglycones.

GUSs have been identified in all the living organisms and, according to their aminoacid sequence, have been classified in three families: GH1 GH2 e GH79.

Only members of the GH79 family have been demonstrated to be present in plants, where they are widely distributed. The first sequenced *GUS* was in *Scutellaria baicalensis* (1), whilst in *Arabidopsis thaliana* three *GUSs* (*AtGUS1*, *AtGUS2* and *AtGUS3*) have been identified (2). This work is aimed to investigate the evolution of *GUS* in plants.

*GUSs* cDNA sequences were obtained, experimentally in *N. tabacum* and by GenBank analysis in a large number of Angiosperms and also from Gymnosperms, seedless vascular plants and Bryophyta.

These data were used for phylogenetic analysis and the possible inferences drawn by the analysis of the resulting tree are discussed.

1) Morimoto S., Tateishi N., Matsuda T., Tanaka H., Taura F., Furuya N., Matsuyama N., Shoyama Y. (1998) J. Biol. Chem., 273, 12606-12611

2) Woo H.H., Faull K.F., Hirsch A.M., Hawes M.C. (2003) Plant Physiol, 133, 538-548

#### 4.4 = ANTIOXIDANT ACTIVITY AND ESSENTIAL OIL COMPOSITION OF THREE SUBSPECIES OF *SIDERITIS LIBANOTICA*

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The genus *Sideritis* (Lamiaceae) consists of more than 150 species of aromatic herbs or subshrubs or shrubs, occurring mainly in the Eastern and Western Mediterranean regions, and its taxonomic classification is rather complex. Many species of the genus have been used for a long time in the traditional folk medicine of several countries, especially in the Middle East, as a herbal tea to treat different illnesses. The chemical investigations on *Sideritis* genus concern essential oils, flavonoids, and especially diterpenoids that occur in almost all the species and show a remarkable variability of carbon skeleta. In the continuation of our investigations on the essential oils of *Sideritis* species (1, 2), we now report the composition and the antioxidant activity of the essential oils obtained from aerial parts of *S. libanotica* ssp. *libanotica* (*Lb*), *S. libanotica* ssp. *linearis* (*Ln*) and *S. libanotica* ssp. *microclamys* (*Lm*) growing wild in Lebanon.

The essential oils from dried samples were isolated by hydrodistillation in a Clevenger apparatus and analyzed by gas chromatography-mass spectrometry (GC/MS). More than 100 components belonging to monoterpenes, sesquiterpenes, diterpenes, aliphatic and aromatic compounds in variable amounts were detected and identified. Antioxidant activity of the oils was evaluated using both chemical DPPH and FRAP assays. The Folin & Ciocalteu assay and Zhishen method were applied to evaluate the polyphenolic and the flavonoids content respectively.

According to the DPPH test the essential oils of all *Sideritis* studied don't possess any antiradical activity, while as regards FRAP test the antioxidant activity seems to be higher for all the samples above mentioned, demonstrating that these samples are not active in catching free radicals but they have an average ferric reducing/antioxidant power. The essential oils of all the subspecies of *Sideritis* studied were found to be significantly more active when assayed by FRAP test than DPPH. The sample that seems to be the most active in ferric ion reduction is *S. libanotica* ssp. *linearis* while the minor action has been showed by *S. libanotica* ssp. *libanotica*.

1) A. Basile, F. Senatore, R. Gargano, S. Sorbo, M. Del Pezzo, A. Lavitola, A. Ritieni, M. Bruno, D. Spatuzzi, D. Rigano, M.L. Vuotto (2006), J Ethnopharmacol. 107, 240-248

2) C. Formisano, D. Rigano, F. Senatore, G.C. Tenore, M. Bruno, F. Piozzi (2010) Nat Prod Res., 24, 640-646

#### 4.4 = THE SAF HERBARIUM AT THE DEPARTMENT OF AGRICULTURAL AND FOREST SCIENCE (UNIVERSITY OF PALERMO)

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In the wide scenario of herbaria housed in the Italian Universities there are very few examples of structures mainly devoted to forest sciences and fungi in forest ecosystems (1). The “Regio Istituto Superiore Forestale di Vallombrosa” (Tuscany), established in 1869, was the first example of a structure that housed an herbarium with *exsiccata* of forest trees (2). In Italy, the southernmost laurea degree course in “Environmental and Forest Sciences” concern the Department of Agricultural and Forest Sciences in the University of Palermo (Sicily). Two herbaria (CAT and PAL) are currently present in Sicily with the following specialties: a) Vascular plants, bryophytes, and algae of Mediterranean area, especially Italy, Sicily, and Cyrenaica (Libya) [CAT]; b) Sicily; Mediterranean basin; Canary Islands; South America; Somalia; South Africa; Australia [PAL]. As a result of University reform introduced by the Italian Government, faculties were transformed into mega-Departments. The Department of Agriculture and Forestry was thus established in January 2013 as a substitute for the Agricultural Faculty. The Department includes botanists, agronomist and foresters whose research activities in the field provides for the collection and identification of numerous herbarium specimens of forest trees and fungi. Besides in the subject “Forest Botany and Mycology” all students are required to submit for the final exam an herbarium of dried plants and fungi collected during the lessons in the field including at least 25 species. The new herbarium currently holds ca. 5131 specimens (plants, shrubs, fungi) but this number is expected to increase rapidly. The fungal section is a valuable resource that holds also voucher collections of macro- and microfungi including: 1) plant pathogenic fungi and dried organs of diseased plants showing symptoms and signs, 2) fungi that are deadly poisonous to humans when consumed, and 3) fungi that are useful for commercial applications (e.g. industrial processing, bioremediation, medicinal, antagonists of plant parasites, cultivable edible mushrooms). The newly-formed forest trees section is particularly rich in specimens from the Mediterranean maquis. A relevant section is represented by *Tamarix* L. species as a result of numerous collections of tamarisk carried out in several Italian regions (3, 4, 5). Specimens of plants are pressed, dried, and mounted on sheets while cryptogams are stored in folded paper envelopes. The herbarium is temporarily housed in the former library of the Institute of Plant Pathology of the former Faculty of Agriculture but the future goal is to have a larger space that includes also other types of scientific collections.

1) F. Taffetani (2012) Nardini Editore, Firenze, pp. 814

2) U. D’Autilia, S. Greco (2012) Il Forestale, 71, 24-26

3) G. Venturella, B. Baum, G. Mandracchia (2007) Fl. Medit., 17, 25-46

4) G. Venturella, M.L. Gargano, G. Mandracchia (2012) Pl. Biosyst., 146(2), 480-485

5) M.L. Gargano, G. Mandracchia, G. Venturella (2009) Inform. Bot. Ital., 41(1), 125-128

4.5 = SOME BIOLOGICAL ACTIVITIES OF *TAGETES LUCIDA* PLANT CULTIVATED IN EGYPT

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*Tagetes lucida* is an aromatic herb used as a spice, for medicine, and as insecticide (1). Bioactive extracts of different plant parts exhibit nematocidal, fungicidal and insecticidal activities (2). Mexican marigold can play an important role in food preservation, food preparation and as an excellent food spice. It is an important, nutritious plant and an effective herbal medicine as antifungal (3).

Mexican tarragon (*Tagetes lucida*, Family Asteraceae) seeds were introduced to be cultivated as one of the medicinal plants in Egypt. This work aimed to study the antioxidant, antimicrobial, insecticidal and Nematocidal activities of *T. lucida* plant. Antioxidant activity of the different concentrations of *T. lucida* extract was near that of ascorbic acid and increasing the used concentration increased the antioxidant activity. IC<sub>50</sub> of *T. lucida* extract was 109 % of ascorbic acid which means that their IC<sub>50</sub> were very close to each other. The essential oil of *T. lucida* was active against all tested microbial strains. *Candida albicans* and *Staphylococcus aureus* were very sensitive to the essential oil than the other strains. Their inhibition zone diameters were more or less similar to that obtained with Streptomycin (10 mcg). The ethanolic extract of *T. lucida* showed high reduction against the aphid (*Aphis brassicae*) during the first six days after application. After nine days, the population of aphids started to increase. The ethanolic extract of *T. lucida*, inhibited ( $P \leq 0.05$ ) motility, visible flexing of all plant-parasitic nematode genera tested. Immobility of *Meloidogyne incognita*-J2 and filiform stages of *Criconebella* spp., *Helicotylenchus* spp, and *pratylenchus* spp. was higher ( $P \leq 0.05$ ) after 24 and 72 h in mg and mg/2 dilutions of *T. lucida* roots than their corresponding herbal parts. *M. incognita*-egg-hatching followed the same trend. From the nematological point of view, this study revealed that *T. lucida* is a promising starting and new material for the production of bio-nematicides in Egypt.

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#### 4.5 = PRODUCTION OF FREE AND GLYCOSYLATED ISOFLAVONES *IN VITRO* SOYBEAN (*GLYCINE MAX* L.) HYPOCOTYL CELL SUSPENSIONS AND COMPARISON WITH INDUSTRIAL SEED EXTRACTS

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*Glycine max* is one of the major sources of phytochemicals and in particular of isoflavones (1), phytoestrogens having ascertained healthy effects on human (2). In the present study, *in vitro* callus production from soybean hypocotyl seedling explants and cell suspensions were optimized. Time-courses having 20, 40 and 60 g/L of initial cell inoculum were performed in order to determine the concentration most suitable for isoflavone production. The amount of total polyphenols, total flavonoids and the antioxidant capacity of both cell and culture media fractions were measured by means of spectrophotometric methods. The levels of aglycone and glycosylated isoflavones (didzein, genistein, glycitein, didzin, genistin, glycitin), as well as of ferulic acid, vanillic acid and vanillin, were determined by HPLC-DAD. On average soybean cell suspensions showed the 93.5% of aglycones over the total (cells plus media) detected isoflavones. Concentrated cell cultures as well as industrial soybean seed extracts were enzymatically hydrolyzed in order to release the aglycones and their metabolic profiles were analysed by HPLC-DAD. In contrast to cell suspensions, undigested seed extracts evidenced 83.2% of glycosides over the total isoflavones amount. After enzymatic treatment the antioxidant capacity increased by 30 and of 33%, respectively, in concentrated cell and seed extracts, demonstrating the presence of a larger amount of bioactive metabolites after digestion. At the present extraction conditions, soybean concentrated cell suspensions yielded 5.8-fold more total isoflavones (mostly in the free form) than seed extracts, leading to hypothesise their possible use as ingredients for cosmetic and nutraceutical applications.

This work have been recently published: Sansanelli et al., *Plant Cell Tiss Organ Cult*, DOI 10.1007/s11240-014-0534-0.

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#### 4.5 = ANALYSIS OF ESSENTIAL OIL PRODUCTION IN *SALVIA. DOLOMITICA* PLANTS CULTURED *IN VITRO*

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*Salvia* L. is the largest genus of Lamiaceae (or Labiatae) family with more than 900 species of which ca. 26 are native to southern Africa like *S. dolomitica* Codd an aromatic perennial shrub found in the northeast province of Transvaal (1). This sage is also drought resistant (2) and extremely fragrant. *S. dolomitica* essential oil (EO) has been used in traditional medicine to treat different disorder such as malaria, inflammation, microbial infections as well as sickness (3). Previously reported data confirmed good antiplasmodial and antiinflammatory activities (4), antimicrobial, antimycobacterial (2) and anticancer cell line specific (5) properties.

This study aims to establish protocols for *in vitro* manipulation of selected *S. dolomitica* plants for fast propagation, biomass production in controlled conditions and as genetic improvement support. Second objective is the characterization, in comparison with *in plein air* mother plants, of the *in vitro* essential oil and volatile components production and profile and the foliar morphology with particular care to secretory structures.

We obtained micropropagated plants, callus and cell line culture (Fig. 1) and after 5 weekly subcultures the FDA test demonstrated that cells were totally viable (Fig. 2) and that could be suitable for the establishment of transformation procedures as well as for scale up of biomass useful for metabolite production and extraction. Moreover, we evaluated the direct neo-organogenesis ability of *S. dolomitica* leaves. At the same time, we performed qualitative and quantitative phytochemical analysis of the essential oil of various cultivated plants. *S. dolomitica in vitro*-derived plantlets showed differences in the quali-quantitative composition of the essential oil compared to the open field grown plant. The investigation of the effect of high light treatments on volatile compounds compositions demonstrated that this stress can slightly affect the essential oils composition. The results suggest that tissue culture can be exploited by researchers to drive the accumulation of selected components of the essential oils.

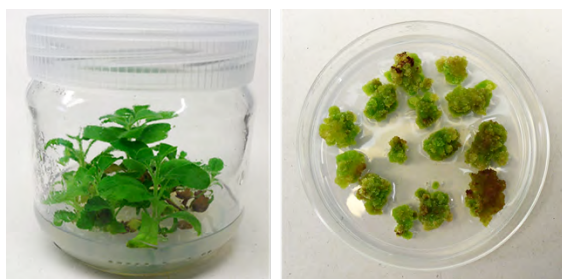


Fig. 1a-1b. *In vitro* production of *S. dolomitica* biomass

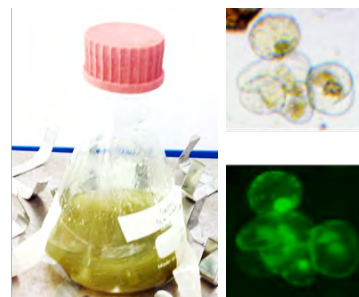


Fig. 2. Cell suspension culture and FDA assay.

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## 4.5 = NEW APPROACHES FOR IMPROVING OLD VARIETIES OF SCENTED CUT ROSES

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The cultivation of the rose as cut flower in Western Liguria began in 1872, at the turn of the century the first shelters and greenhouses were used to cultivate new varieties and in the thirties rose growing on the Riviera Ligure received great outburst from the research of two great Italian rose-breeders, Domenico Aicardi and Quinto Mansuino, of international importance. Nowadays, rose is still one of the most economically important ornamental crop worldwide and breeding programs are mainly focused to improve disease resistance, recurrent blooming, flower morphology and scent production. In the context of the project “Innesto” (1), a study has been conducted to optimize the production methods of old varieties of scented cut roses, already grown in Western Liguria in the first half of 1900, applying innovative techniques. Different rootstocks were provided by local nurseries and their identification and discrimination has been carried out by using morphological characters in combination with molecular markers. Morphological characters were chosen in accordance to UPOV (International Union for the Protection of New Varieties of Plants) Guidelines for *Rosa* L. The Start Codon Targeted (SCoT) polymorphism has recently become the marker of choice in the case of genetic diversity studies: primers are designed according to the short conserved region surrounding the ATG translation start codon (2). The SCoT analysis has been employed for the genetic characterization of seven rootstocks locally named: *Rosa* ‘Indica Locale’, *Rosa* ‘Indica Major’, *Rosa* ‘Indica Francia’, *Rosa* ‘Israele’, *Rosa hybrida* ‘Brea’, *Rosa hybrida* ‘Natal Briar’ and *Rosa canina* L. PCR conditions were determined using genomic DNAs extracted from young leaves. Three historical scented varieties, *Rosa* ‘Gloria di Roma’ (Domenico Aicardi, 1937, Italy, Hybrid Tea), *Rosa* ‘Ulrich Brunner Fils’ (Antoine Levet, 1882, France, Hybrid perpetual) and *Rosa* ‘Souvenir de H. A. Verschuren’ (Verschuren and Sons, 1922, The Netherlands, Hybrid Tea) were bud-grafted on the cuttings of the seven rootstocks. The efficiency of grafting has been evaluated by measuring the percentage of developed scion-rootstock unions. Moreover, in order to maintain and preserve plant genetic resources, the old varieties were conserved in the Roses Collection in the Hanbury Botanical Gardens and in the micropropagation laboratories of the Unità di ricerca per la Floricoltura e le Specie Ornamentali (Fig. 1).



Fig. 1.a *Rosa* ‘Gloria di Roma’ and 1.b *Rosa* ‘Ulrich Brunner’ in the book *Histoire des roses* of André Leroy (1954) and in the micropropagation laboratories (2014).

1) The project “Innesto” (Innovazione, Introduzione e Valorizzazione Commerciale di Rose Storiche nel Ponente ligure) has been funded by Regione Liguria (PSR 2007-2013, Fondo europeo agricolo per lo sviluppo rurale, L’Europa investe nelle zone rurali, Misura 124 Cooperazione per lo sviluppo di nuovi prodotti, processi e tecnologie nei settori agricolo, alimentare e in quello forestale) and includes the participation of seven Ligurian rose-growers, who grow old rose varieties in open air and in cold greenhouses

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#### 4.5 = EVALUATION OF THE DNA BARCODING APPROACH IN *HYPERICUM* SPP. DISCRIMINATION

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*Hypericum* genus, with more than 450 species, is widespread in temperate zones all over the world.

In Italy 30 taxa are known, 26 species and 4 subspecies; 10 of them are native of Sicily, in addition to *H. calycinum* which was found as naturalized.

*Hypericum* biochemical compounds (flavonoids, coumarins, glycosides, sesquiterpenes, tannins, volatile oils) are well recognized for many pharmacological activities: anti-inflammatory, improving blood flow, against traumas, in wounds and burns recovering. The most important activity is ascribed to the hypericin, a compound especially derived from *Hypericum perforatum* L., with successfully application in anti-depressive phytotherapy.

The medical field relevance and the related commercial interest led to the input for improving the taxonomic identification method to dispose of certain plant material. Methods for fast and accurate identification of the plant species are required to support morphological characterization.

In this study the potential of the “DNA Barcoding” molecular method was investigated in discriminating the Italian *Hypericum* taxa in order to develop an easy authentication assay helpful in solving taxonomic doubts or in commercial trade traceability of whole plants, portions or derived products.

The samples range was mainly recovered from native habitats in Italy, during the flowering period. Some samples were also sourced from certified herbarium collection.

The DNA extraction was carried in three biological replicates, according to CTAB protocol for plant material (1). The DNA bank and also the *ex-situ* collection are stored at CRA-SFM of Bagheria.

The three plastid regions, *rbcL*, *matK* and *trnH-psbA*, were assessed, according to the CBOL Plant Working Group indications (2). Phylogenetic analysis of each molecular marker were conducted by comparing sequences including those available from international databases (BOLD/NCBI) based on Kimura 2-parameter (Kimura, 1980). The preliminary results indicate the effectiveness of the method in discriminating the taxa of *Hypericum*, suggesting the possibility to build a fast and accurate molecular identification method by barcode.

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2) CBOL Plant Working Group (2009). *Proceedings of the National Academy of Sciences USA* 106: 12 794–12 797

#### 4.5 = A MOLECULAR APPROACH TO IMPROVE *EX SITU* CONSERVATION STRATEGIES OF FIVE ENDANGERED WILD PLANT SPECIES IN THE AOSTA VALLEY (NORTHWEST ITALY)

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As recognized by the Convention on Biological Diversity (<http://www.cbd.int>), as well as by other important international documents, *i.e.* the Global Strategy for Plant Conservation (<http://www.cbd.int/gspc/>) and the European Strategy of Plant Conservation (1), *ex situ* long-term conservation of rare and endangered wild plant species can ensure their survival. Indeed, samples collected in seed bank can be used in reintroduction or reinforcement plans, as well as in habitat restoration. In order to optimize protection strategies, seed collections should be conducted to acquire samples as representative as possible of the genetic diversity of the species concerned (2). Accordingly, the number and type of populations, as well as the minimum number of plants to be sampled within each population, are currently deduced from the biology and eco-geographical range of the selected species. However, a population genetic analysis prior to seed banking would be desirable (3). Levels and distribution of genetic variation revealed by molecular analysis are well correlated with the levels and distribution of variation for loci affecting traits of future adaptive importance (4). Although simple sequence repeat (SSR) loci represent one of the most informative and versatile DNA-based markers used in plant genetic research, their use for studies on population genetic of wild plant species has been so far limited by the difficult and costly process required for their development. Nonetheless, the emerging next-generation sequencing (NGS) technologies allows for a rapid and cost-effective discovery of SSR loci (5).

The present study, which is part of a wider project supported by the European Regional Development Fund (ERDF) and the European Social Fund (ESF), is aimed at (i) evaluating, through SSR markers, the population genetic diversity of five rare and endangered wild plant species in the Aosta Valley and (ii) optimizing the sampling strategies for their *ex-situ* conservation by using the genetic data derived through this study.

The five selected plant species, which are declared in need of strict protection at a regional level, included: *Astragalus alopecurus* Pall. and *Cypripedium calceolus* L., which are reported in the annexes II and IV of the Council Directive 92/43/EEC; *Aethionema thomasianum* J. Gay, *Epipactis palustris* (L.) Crantz and *Potentilla pensylvanica* L., which are deemed as vulnerable or endangered at a national level (6). After the determination of the size and the number of populations detected for each species in the Aosta Valley, a randomly sampling of 10-20 individuals was conducted in each collecting site.

The detection of SSR loci will be performed through GS-FLX sequencing technologies and at least 30 SSR markers will be tested for each species. Highly polymorphic loci will be used to evaluate the genetic diversity within and among populations. The genetic data will be used to plan seed collection strategies for the *ex-situ* conservation of most of the genetic resources available for each species in the Aosta Valley. The molecular markers which will be developed in this study would represent a rapid and useful tool to investigate and compare genetic diversity of these wild plant species from other countries and seed banks.

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#### 4.5 = FUNCTIONAL CHARACTERIZATION OF *OesDHN* IN TRANSGENIC PLANTS OF *ARABIDOPSIS THALIANA*

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Abiotic stresses such as water deficit and soil salinization adversely affect plant growth and crop productivity (1,2).

DHNs are a member of a large group of highly hydrophilic protein, known as protein LEA (Late Embryogenesis Abundant), initially, identified as group II of the LEA proteins (5;6,7) which belong to common protective compounds. They have been observed in several independent studies on drought stress, cold acclimation and salinity stress (3; 4).

A member of the gene dehydrin family, called *OesDHN* has been previously identified from a cDNA library obtained from leaves of plants of *Olea europaea* subsp. *europaea* var *sylvestris* and interestingly its expression levels were found to be up-regulated in wild olive plants exposed to water stress conditions and cold (5).

In order to clarify the functional role of *OesDHN* in relation to water stress, we generated transgenic *Arabidopsis thaliana* lines, overexpressing *OesDHN*. The expression pattern of *35S::OesDHN* and its potential role in osmotic tolerance were analyzed in T3 homozygous lines with 1 T-DNA locus of *Arabidopsis* plants. Two lines with the highest expression levels were selected for an osmotic tolerance experimental set up and, accumulation of *OesDHN* transcripts, was detected in whole seedlings by RT-PCR analysis. The osmotic stress was experimentally induced by adding mannitol 25 mM to the culture medium. The obtained results showed an increased tolerance of transgenic plants to this specific stress condition. According to these data, the *in silico* analysis revealed the presence of two putative regulatory elements stress-inducible type ABRE and MYB, located in the promoter region of *OesDHN*.

GFP transgenic lines *35S::GFP:OesDHN* and *35S::OesDHN:GFP* were also generated to investigate the subcellular localization of gene encoded product. In line with the *in silico* analysis, showing the presence of a nuclear target peptide in the deduced *OesDHN*, fluorescent signal was mainly localized at nuclear level.

Taken together, these results allowed us to confirm the role of selected gene in plant tolerance to water stress conditions. In addition, information on the putative target of gene action was also obtained. The prospect of long-term research is addressed to broaden the knowledge useful to define possible strategies to increase font of tolerance / resistance in important crop species or not.

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#### 4.5 = THE BIORICE EUROPEAN PROJECT: BIOTECHNOLOGY FOR THE RECOVERY OF VALUABLE PEPTIDES FROM INDUSTRIAL RICE BY-PRODUCTS AND PRODUCTION OF ADDED VALUE INGREDIENTS FOR NUTRACEUTICALS, FUNCTIONAL FOODS AND COSMETICS

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The nutraceutical, functional food and cosmetic EU and worldwide markets are rapidly evolving sectors always in need to develop new classes of products. One of the most important market trends is the use of new bio-based ingredients obtained by environment-friendly extraction processes and testing methods. Of particular importance is the re-cycling and valorization of agro-food industry by-products as feedstocks for the isolation of bioactive molecules.

The BIORICE project aims to fill the gap of knowledge of the involved Small and Medium Enterprises (SMEs) on rice protein by-product pre-treatment, peptide isolation and relative bioactivity and safety testing. In particular, BIORICE research activities will produce added value bioactive ingredients (semi-purified digestates and small molecular weight peptides) starting from protein by-products contained in the processing water of the rice starch production stream. The protein by-products will be pre-treated via biotechnological approaches (enzymatic proteolysis, microbial treatments) and different small molecular weight (5-15 kDa) peptides will be isolated by means of innovative eco-sustainable and not degrading techniques. A new method for in vitro evaluation of the skin sensitization potency will be developed so that the products will be tested without using laboratory animals. The BIORICE project brings together 6 partners distributed in 3 EU Member States and 1 Associated Country. In BIORICE 3 RTD Performers will make their multidisciplinary and complementary expertise in the areas of plant biotechnology, downstream processing and human tissue engineering available to 3 SMEs operating in the nutraceutical, food and cosmetic markets.

The aim of the present project is to obtain and to characterize peptides from rice protein by-products and evaluate their bioactive properties. The project results are also expected to have a significant impact on the competitiveness of SME Participants that will be able to expand their business by adding to their product range new bioactive ingredients and protocols enabling new product formulations applicable in food, cosmetic and nutraceuticals sectors. The BIORICE project started on 1st November 2013 and will last 24 months. The project is co-funded by the Seventh Framework Programme (FP7-Capacities Research for the Benefit of SMEs, 2007-2013) of the European Union and is coordinated by Dr. Annalisa Tassoni, Department of Biological, Geological and Environmental Sciences, University of Bologna, Italy.





#### 4.5 = IN VITRO ROOT CULTURES OF DIFFERENT *HYPERICUM* SPECIES: A PROMISING SYSTEM FOR THE PRODUCTION OF ANTIFUNGAL XANTHONE-RICH EXTRACTS

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*Hypericum perforatum* L. (Hp) is a medicinal plant considered an important natural source of secondary metabolites with a wide range of pharmacological activities (1). It contains naphthodianthrones, acylphloroglucinols, flavonoids, biflavones, phenylpropanes, xanthenes and an essential oil rich in sesquiterpenes (2).

In the last years, our research group has been focused on the study of xanthone biosynthesis in *in vitro* root cultures of Hp. We demonstrated that Hp root cultures grown in liquid medium containing 1 mg/l indole-3-butyric acid produced xanthenes at concentrations (5 mg/g DW) higher than the plant roots (traces) (3). The treatment of root cultures with the elicitor chitosan enhanced xanthone biosynthesis (16 mg/g DW). The effect of the elicitor increased with its concentration, and the best results were obtained with 200 mg/l chitosan (4). The extracts obtained from elicited roots showed an interesting antifungal activity against some human pathogenic fungi, such as *Candida* spp., *Cryptococcus neoformans*, and dermatophytes (1).

Recently we have started new root cultures from seeds collected from different individuals of Hp, in order to select the most productive genotypes. At the same time we have initiated root cultures from other species belonging to the *Hypericum* genus whose xanthone profile was never been investigated, such as *H. pulcrum*, *H. annulatum*, *H. koutchense*, *H. tetrapterum*, *H. humifusum*, *H. maculatum*, *H. rumeliacum* e *H. tomentosum*.

HPLC chemical analyses showed that xanthone content in the root cultures of the above mentioned *Hypericum* species ranged from 0.057 mg/g DW (*H. koutchense*) to 2.8 mg/g DW (*H. annulatum*). These concentrations were very low with respect to that found in the new Hp root cultures (20 mg/g DW in the most productive cultures). The obtained results suggest that Hp root cultures are a promising system for biotechnological xanthone production.

Currently, investigations are in progress to evaluate the antifungal activity of the extracts obtained from root cultures of different species of *Hypericum* against human pathogenic fungi.

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#### 4.6 = EPIGEOUS AND HYPOGEOUS MACROFUNGI IN THE HISTORICAL “HANBURY” BOTANICAL GARDENS

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The historical botanical gardens “Hanbury” are located in Liguria (North-Western Italy) on the promontory of “Capo Mortola”, in the province of Imperia, few kilometers far from the boundary between Italy and France. These gardens, founded in 1867, represent an interesting site where a high number and diversity of flowers, plant species and collections can be found. They also represent a regional protected area covering over 19 hectares. About half of this surface is cultivated with plants from different areas with subtropical or warm-temperate climates, while the other half is characterized by semi-natural Mediterranean vegetation, with the presence of *Pinus halepensis* Mill. woods and other habitats. Despite the gardens are well-known because of the richness and diversity of plant species and vegetation types, no information is available on macrofungi. The knowledge on macrofungal species can surely represent an important tool for a conservative perspective, due to the crucial ecological roles played by fungi, as well as the symbiotic (mutualistic or pathogen) interactions that they can establish with plants. The scarce information on macrofungal component associated to botanical gardens encouraged us to carry out mycological surveys aimed to describe and characterize the macrofungal species occurring in the site.

Mycological investigations, focused on both epigeous and hypogeous species, were performed in autumn (September–November) and in spring (April–June). During the field surveys, notes on morphological characters and ecology of the fresh specimens (along with photographic documentation of sporomata), were taken. Taxonomical identifications were performed *in situ* and in laboratory. All the species records were inserted in the database A.L.C.E. (Advanced Liguria Check-list of Ectomycorrhizal and other fungi) and the relevant specimens were deposited at the GDOR (Herbarium of the Museo Civico di Storia Naturale Giacomo Doria, Mycology Section, Genoa, Italy).

One year of study allowed us to identify 38 species: 8 *Ascomycota*, 29 *Basidiomycota* and 1 *Glomeromycota*. The observed species belong to 9 different orders, 23 families and 32 genera. Among these, *Genea* Vittad., *Lepista* (Fr.) W.G. Sm., *Pluteus* Fr., *Stereum* Hill ex Pers. and *Tuber* P. Micheli ex F.H. Wigg. were the richest in number. From an ecological point of view, all the recorded species were split into functional groups (3), as follows: 15 ectomycorrhizal, 13 soil (humus or litter) decay, 2 parasitic, and, 8 wood decay fungi. With the reference to national (1; 2) and regional macrofungal checklists (4; 5), a value of geographic distribution was given for each species. Accordingly, the highest percentage of the species found corresponds to widespread and common species, such as: *Amanita ovoidea* (Bull.) Link, *Boletus subtomentosus* L., *Byssomerulius corium* (Pers.) Parmasto, *Clathrus ruber* P. Micheli ex Pers., *Lepista nuda* (Bull.) Cooke, *Phanerochaete velutina* (DC.) P. Karst., and, *Psathyrella candolleana* (Fr.) Maire. It is worth noting the presence of the infrequent species *Clavaria fragilis* Holmsk., *Hemimycena cucullata* (Pers.) Singer, *Hygrocybe acutoconica* (Clem.) Singer, *Pisolithus tinctorius* (Mont.) E. Fisch., and, *Pluteus salicinus* (Pers.) P. Kumm.. Moreover, some numerous records of hypogeous species should be emphasized, such as *Gautieria morchelliformis* Vittad., *Genea fragrans* (Wallr.) Sacc., *G. verrucosa* Vittad., *Glomus microcarpum* Tul. & C. Tul., *Reddellomyces donkii* (Malençon) Trappe, Castellano & Malajczuk, *Tuber brumale* Vittad., and, *Tuber excavatum* Vittad.. Finally, two parasitic species, *Fuscoporia torulosa* (Pers.) T. Wagner & M. Fisch. and *Ganoderma australe* (Fr.) Pat., were identified.

Future mycological investigations are planned in order to improve the knowledge on the macrofungi of Hanbury Botanical Gardens.

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## 4.6 = THE PALMETUM, A NEW SECTOR IN THE PALERMO BOTANICAL GARDEN

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The Palermo Botanical Garden has evolved over a period spanning more than 200 years. Thus, the evolution of the Plant Sciences, both regarding Systematics as well as changing visions of the utility of plants, can be seen in the layout and organization of the Garden's collections.

Today, the Botanical Garden is made up of two principal bodies; the oldest area, built at the end of the seventeen-hundreds next to the historic "*Gymnasium*", where the collections are laid out according to Linnaeus's system of plant classification, and the most recently built area, added on between the mid eighteen-hundreds and the beginning of the nineteen-hundreds near the Botany Department, and where Engler's classification system can be found. Various organizational methods are used throughout the garden, including a systematic layout, a taxonomic layout, an ecological layout, a phytogeographical layout, as well as layouts based on plant uses (food, medicine, coloring agents, and textiles) (1).

One of the most prized areas that follows a taxonomic layout is the *Cycadetum*, dedicated to the Cycadopsida group (2). Just next to the Cycad collection, we can find one of the most recent areas developed in the Botanical garden (which is still in progress) the Palm collection. The *Palmetum*, whose construction began in 1990 in an area that then belonged to the experimental sector, has greatly increased the number of species represented in respect to the few generainherited from the precedent century.

Today, the Palermo Botanical Garden's *Palmetum* hosts an important outdoor collection, holding genera that are spread throughout the continents in nature. Thanks to the area's favorable climate, specimens from more than one hundred species have been planted in this large area in the middle of the Garden.

The Garden's collection currently contains 140 *taxa* for a total of 630 live specimens. Of these, 110 are planted in the ground and 30 are in pots.

As for their origins, 54% are American, 23% are Asian, 16% come from Australia, and the rest come from Africa and the Mediterranean.

Some noteworthy specimens in the collection are *Roystonea regia* from Cuba, *Bismarckia nobilis*, *Dyopsis decaryi*, and *Ravenea rivularis* all from Madagascar, *Serenoa repens* (well known for its medicinal properties) from Florida, *Wallichia densiflora* from the Himalayas, and *Cryosophila argentea* from Central America. A large part of the *Phoenix* genus is also represented, including *P. theophrasti* from the eastern Mediterranean, as well as *P. humilis* and *P. sylvestris*, which are both Indian; there are also some *Arenga* species, including *A. engleri* from Taiwan and *A. caudata* from Thailand, as well as some *Caryota* species (*C. maxima* from Java, *C. ochlandra* from the Himalayas), *Trithrinax* species (*T. brasiliensis* and *T. campestris* from Brazil and Argentina respectively), *Archonthophoenix* species (*A. cunninghamiana* and *A. alexandrae* from Australia) and also various species of the *Chamaedorea* genus.

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4.6 = THE PALERMO BOTANICAL GARDEN'S *FICUS* GENUS (MORACEAE) COLLECTIONGIUSEPPE BAZAN<sup>1</sup>, MANLIO SPECIALE<sup>2</sup>, PIETRO MAZZOLA<sup>1</sup><sup>1</sup>Department of Agricultural and Forest Sciences, University of Palermo, Italy; <sup>2</sup>Orto Botanico, University of Palermo, Italy

Figs could very well be considered the symbolic plants of the Palermo Botanical Garden as well as of the City itself. With their exuberant growth, over time these trees have become identifying elements of Sicilian Historic Gardens, giving them a strong tropical connotation. An extraordinary example of this can be seen in the *Ficus macrophylla* subsp. *columnaris* of the Norman Palace that clutches a much older historic specimen of *Pinus pinea* in its buttress and aerial roots: the exotic element declares its supremacy over the Mediterranean one and prevails. In fact, it would be that uncontrollable Romantic spirit that would dominate the late Baroque illuminist formalism during the Eighteen hundreds; here we see *Sturm und drang* in full action.

The Palermo Botanical Garden had a central role in the introduction, acclimation, and diffusion of the various species of the *Ficus* genus present in the ornamental flora of Mediterranean Italy. The Royal Botanical Garden Bulletins (*Bollettini del Regio Orto botanico di Palermo*) give us a detailed retelling of the introduction of the different species that are now present in the collection (1, 2, 4).

The activity centered on the introduction and cultivation of various species of *Ficus* in the Garden at the end of the XIX century was also motivated by the quest for a possible source of rubber derived from the latex in these trees, even if chemical studies of the latex of the *Ficus* species cultivated in Sicily reveal the presence of a small amount of elastic rubber (3).

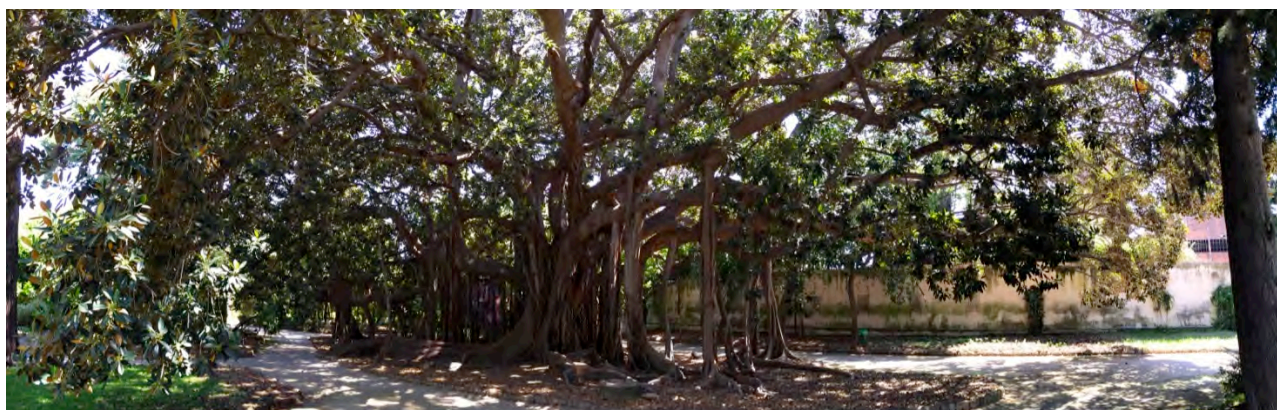
Currently, *Ficus* collection of Palermo Botanical Garden includes 127 plants belonging to 40 *taxa*, of which 23 species, 3 subspecies, 1 variety and 13 plants that are still taxonomically unidentified. 91 of these are planted in the ground, while the remaining 36 are in pots.

*Ficus watkinsiana*, *F. macrophylla* subsp. *columnaris* and *F. microcarpa* are the most abundant species in the collection with respectively 14, 10, and 9 specimens.

The most relevant *taxa* planted in the ground, aside from the immense *Ficus macrophylla* subsp. *columnaris* are: *F. benghalensis* and *F. religiosa*, two trees that are sacred in the Buddhist and Hindu religions; *F. sycomorus*, tree cited in the Old Testament; *F. rubiginosa*, which forms a real jungle, an admirable *F. altissima*; but also *F. watkinsiana*, *F. aspera* var. *parcelli*, *F. magnifolia* and *F. bibracteata*.

Many other *taxa* are present: *F. erecta*, *F. virens*, *F. microcarpa*, *F. indica*, *F. longifolia*, *F. pumila*, *F. umbonata*, *F. aurea*, *F. baileyana*, *F. citrifolia*, *F. gigantea*, *F. habrophylla*, *F. populifolia*, *F. bracteata*, *F. cordata* subsp. *salicifolia*, *F. deltoidea*, *F. frigida*, *F. heterophylla*, *F. hispida*, *F. lutea*, *F. lyrata*, *F. morifolia*, *F. natalensis* subsp. *leprieurii*, *F. palmata*, *F. sur*, *F. theophrastoides*, *F. cyathistipula* and *F. carica*.

Taken as a whole, and considering its monumentality, the *Ficus* collection in the Botanical Garden of Palermo University can certainly be counted amongst the richest and most significant ones present in open air in European botanical institutions.



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## 4.6 = DIAGNOSTIC ANALYSIS FOR THE RESTORATION OF THE CLOISTER OF THE FIFTEENTH-CENTURY FORMER MONASTERY OF SANT'ULDARICO IN PARMA

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The monastery, owned by the Benedictine order, was built between the seventh and eighth century on the ruins of the ancient Roman theater. The nuns were in charge of the management of the mills, not far from the monastery, and to accommodate pilgrims and travelers coming to Parma. At the end of the tenth century Uldarico, Bishop of Augsburg, traveling to Ravenna to meet the Emperor Otto I, was housed in the Monastery's hospital. Two years after his death, on July 4, 973, the nuns elected Uldarico owner and patron of the church and monastery, even before the Pope canonized him. In the fourteenth and fifteenth centuries the church was rebuilt in Romanesque style and finally restored in eighteenth-century style in the eighteenth century. Following the Napoleonic edicts, in 1809, the nuns lost all rights on the monastery that was expropriated among state assets. In the early twentieth century, the parish priest Don Bernardi, after the First World War, removed the walls erected by the army to close the arches of the lodges, restoring the original appearance of the Cloister. In 1923 the State Property let the parish use the Cloister, but kept the property and in 1971 the parish priest Don Alberto Baroni bought from the State the remainder of the old monastery. The choir, built in 1505 by Abbess Cabrina Carissimi, and the wonderful cloister, completed in 1449 (1), are the only remaining parts of the original building. The cloister has terracotta arches supported by columns of sandstone, except for some in Verona red marble, probably the remains of the ancient Roman theater, with bases and capitals also in sandstone. The colonnade is based on the characteristic low brick wall, the cobbled courtyard paving is still in good condition, with a well preserved and six-feet deep well at ground level. The floor of the lodge is made of bricks, very worn, evidence of the long history of this monastery. The complex today is located in the city centre, in an area where significant sources of anthropogenic pollution were found such as high population density and high-traffic roads, with the consequent emissions in the atmosphere. Biological and non-biological degradation phenomena were detected (2) using the Normal 1/88, 3/80, 24/86 and UNI 10923:2001 Recommendations (3) as a reference for the macroscopic alterations at the surface, for diagnostic analysis and sampling techniques of biodeteriogens. The observation revealed a significant discoloration and blackening of terracotta arches, sandstone and marble columns; the sandstone columns also have a massive exfoliation both in bases and capitals. The external walls and low walls - which were originally covered with plaster - currently missing in various points - show frequent cracks and fractures. Stains and patinas both of biological and abiological nature are present with greater frequency on the east and south sides of the cloister (Fig. 1), where a larger biological colonization (4) has also been noticed. Various species have been identified among algae, lichens, bryophytes and higher plants. The higher presence of biodeteriogens is on the south side of the cloister, constantly shaded, and in the southeast corner, where a percolation is present due to a broken rainpipe. The patina of algae is widespread and the observation with an optical microscope revealed the presence of unicellular (*Chlorella vulgaris*) and filamentous (*Ulothrix* sp.) green algae. The porosity of the material of the low wall and the constant humidity favour the development of liver (*Lunularia cruciata*) and mosses (*Tortula muralis*, *Bryum* sp.). The lichen colonization is restricted and concentrated on the south side (*Lecidella elaeochroma*). Higher plants colonize the cobblestones (*Senecio vulgaris*, *Capsella bursa-pastoris*, *Poa annua*, *Calamintha nepeta*, *Oxalis corniculata*, *Cymbalaria muralis*), the cracks in the low wall and the brick pavement of the lodges (*Parietaria judaica*, *Oxalis fontana*, *Saxifraga tridactylites*, *Hedera helix*).

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## 4.6 = ARCHAEOBOTANY FOR RECONSTRUCTION OF GARDENS

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Our Laboratory carries out the reconstruction of green spaces of the past through archaeobotanical analysis integrated with evidence from other disciplines. For the Roman Period, research on *domus* of Modena (1) and Forlì (2) reported ornamental species, some frequently recurring (such as box, acanthus and myrtle); for the suburban area was rebuilt green furniture around a fish farming (3). The Middle Ages and Renaissance presents the most interesting cases: in Ferrara, the *Giardino delle Duchesse* of Este family (4, 5), the green space of the Benedictine Monastery of S. Antonio in Polesine (6) and the kitchen gardens of via Vaspergolo/corso Porta Reno (7, 8); in Modena, the green space of the Bishop's Palace (9). For the Modern Age it was investigated the *Giardino dei Semplici* of the *Ospedale Maggiore* of Milan (10) and the gardens of *Palazzo Giardino* in Sabbioneta and *Palazzo Te* in Mantua. Some investigations have provided information on the history of important crops and ornamental plants (eg. 11, 12, 13).

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## 4.6 = COLLECTIONS ENHANCEMENT AT THE BOTANIC GARDEN OF MODENA: SOME CONSIDERATIONS ABOUT THE HISTORIC HERBARIUM

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As part of the national project for the Dissemination of Scientific Culture (law 6/2000) “Botanical Garden and territory: botanic collections and vegetal biodiversity in the Emilia territories”, the digitization of the phanerogamic herbarium of the Modena Botanic Garden has been undertaken. This herbarium, started by Gaetano Rossi (founder of the Garden) solely for teaching exigences, then founded by Giovanni De Brignoli di Brunnhoff, director of the Botanic Garden from 1817 to 1856 (1), consists of about 50.000 sheets, mostly with XIX century samples; all the genera are numbered according to Pfeiffer’s method (2).

From the first analyses (only 10% of the herbarium has been scanned) we can reconstruct an intense exchange activity with other botanists and botanic gardens, both Italian and foreign. The major collections, as known (3), regard the province of Modena and are attributed to illustrious scientists of the XIX and early XX century, such as Giuseppe Gibelli, Pietro Romualdo Pirota, Antonio Mori, Antonio Vaccari. There are also samples provenient from other territories (Piedmont, Lombardy, Trentino, Veneto, Tuscany, France, Austria, Germany, Hungary, Libya, Australia...) and collected by different researchers, such as Abbot Carestia, Gaetano Savi, Giuseppe Acerbi, Stéphen Sommier, Enrico Gelmi, Otto von Penzig, Emilio Chiovenda, Augusto Béguinot. We mention the numerous samples of the *Flora Italica Exsiccata*, *Flora Bellunensis* by Sebastiano Venzo, pharmacist from Lozzo di Cadore, *Flora Berolinensis*, *Flora Transsilvanica*, *Flora Ossolana*, *Herbarium Formationum Coloradensium*, *Erbario della R. Scuola Superiore di Agronomia in Milano*, and less known collections such as the *Herbarium Orsini* by Antonio Orsini from Ascoli Piceno, who explored the upper valley of the river Tronto and the Monti Sibillini in the middle XIX century. We also remember specimens of the *Regio Erbario Atestino in Modena*, managed by Ettore Celi, director of the Botanic Garden of Modena from 1856 to 1873. The *exsiccata* of the early 1800, quite abundant, often in a very good state of preservation (much better than samples collected a century later!), in many cases were collected by Giovanni De Brignoli, mostly in Friuli-Venezia Giulia, but also in the Modena province. Finally we have to cite various samples obtained from plants cultivated in the Botanic Garden, as it was normal during the XIX century.

The labels, oftentimes entirely hand-written, not always report *Legit* and *Determinavit*, but a simple signature, especially when the collector is famous (e.g. Adriano Fiori, Giuseppe Gibelli, Romualdo Pirota, Antonio Vaccari). In the specific case of Giovanni De Brignoli, instead, labels often show the annotation *ipse legi* (= I myself collected). Reading these nineteenth-century labels, one can see a widespread lack of specific indications concerning the collection place, often substituted by generic notes about the species presence in the territory: for example, *Carex praecox* Schreb. was found in the «grasslands around Castelfranco» (*Leg. Pirota*, 1882), «extremely frequent along the ditches margins, neighborhoods of Modena» (s.d., 1852); *Stratiotes aloides* L. was simply indicated for the «Lakes of Mantua» (*Leg. Adr. Fiori*, 1883; *Leg. Vaccari*, 1888) and «in the marshes of the Valli Ostigliesi» (*Leg. Gibelli*, 1875); *Nuphar lutea* (L.) Sm. grew in the «marshes of the lowlands of Modena» (*Leg. Pirota*, 1879). In most cases they are aquatic species, whose habitat has been seriously compromised by drainage, agriculture, urban sprawl and pollution (4), which in the past were found nearly everywhere and now are confined to a few areas, or even disappeared (3): one can think that such a difference in the geographical indications is not due to the past Authors’ imprecision or approximation, but to the great abundance of these species in that territories, whose presence was so common that it was not noteworthy for the botanists of the XIX century.

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2) L. Pfeiffer (1870) *Synonymia Botanica locupletissima generum, sectionum vel subgenerum ad finem anni 1858 promulgatorum. In forma conspectus systematici totius regni vegetabili schemati endlicheriano adaptati*. Cassellis, sumptibus Theodori Fischer

3) A. Alessandrini, L. Delfini, P. Ferrari, F. Fiandri, M. Gualmini, U. Lodesani, C. Santini (2010) *Flora del Modenese. Censimento, Analisi, Tutela*. Provincia di Modena, Istituto per i Beni Artistici, Culturali e Naturali della Regione Emilia-Romagna

4) F. Buldrini, D. Dallai, P. Torri (2013) *Ann. Bot. (Roma)* 2013(3), 245-254

#### 4.6 = A NEW COLLECTION OF *HAWORTHIA* AT THE BOTANICAL GARDEN MUSEUM OF ROME: MORPHOLOGY AND *EX-SITU* CONSERVATION

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In the autumn of 2012 a project was presented for setting up a plant collection of the genus *Haworthia* in the French Greenhouse of the Botanical Garden Museum of Rome.

The first plants ascribed to the genus *Haworthia* were described in 1753 by Linnaeus (1) in the *Species plantarum* and were included in the genus *Aloe*.

Then, in 1804 (2), Haworth made the first systematic classification of *Haworthia* confirming them in the genus *Aloe*. Duval, in 1809 (3), described the genus *Haworthia* and the genus *Gasteria*, separating them from *Aloe*. In 1976, Bayer (4) made its first revision of the genus *Haworthia*, changing the previous classifications and proposing three subgenus (*Haworthia*, *Hexangulares* and *Robustipedunculares*), as suggested by Uitewaal (5) based on the morphological characteristics of the flowers. In 1998, Breuer (6) began to study *Haworthia*, confirming the division into the three subgenera proposed by Bayer and introducing new species. Currently the most important scholars of *Haworthia* are Ingo Breuer (7, 8), to whom we refer the collection of plants displayed in the French Greenhouse, Bruce Bayer and Masahiko Hayashi.

**MORPHOLOGY** – The *Haworthia* have a leaf structure peculiar to many South African succulents, for which they are called ‘fenestrate plants’. *Haworthia truncata*, with its particular distichous and truncated leaves, is one of the most representative example of ‘fenestrate plants’.

The fenestrate leaves have a flat and transparent apex and are usually located at ground level so that the remaining part of the plant is protected from sunlight and excessive heat, allowing to limit water loss. These plants remain underground thanks to the presence of contractile roots.

This evolutionary adaptation is very useful to compensate the reduction of light. The leaves function as an optical system allowing the light to pass through the fenestrate, to reach the inner part photosynthetically active. In this way, with a minimum surface area exposed to the external environment, a larger part of photosynthetically active tissue is ensured.

The contractile roots have the characteristic of "pulling" the plant deep into the ground to protect it from sun and heat in the dry season. As the stem grows, the roots attract the plant into the soil so that it remains underground or to a certain level with respect to the soil surface. In addition to this, continuous root decomposition and regeneration create interstices facilitating the development of the plant into the soil.

**FIELD NUMBER AND *EX-SITU* CONSERVATION** – The Field Number is a code formed by the combination of letters and numbers assigned to plants or seeds collected in nature and associated with the habitat information (geographical coordinates, soil characteristics, climate, etc.). Thus the field number can trace the specific geographical point the plant originated from.

This information is essential for those populations suffering a reduction of the natural distribution area or under extinction threat. Thanks to the Botanical Gardens activity these plants, with support of specific projects, could be reintroduced in nature or, in any case, preserved in specific structures for *ex-situ* conservation.

1) Linneo, C. 1753. *Species plantarum*. Laurentii Salvii. Stockholm

2) Haworth A.H. 1804. A new Arrangement of the Genus *Aloe*, with a chronological Sketch of the progressive Knowledge of that Genus, and of other succulent Genera. The Linnean Society of London, 7(1):1-28. London

3) Duval H.A. 1809. *Plantae succulentae, in horto Alenconio*. Paris Apud Gabon et Socios

4) Bayer B. 1999. *Haworthia Revisited*. A revision of the Genus. Umdaus Press, Hatfield, SA

5) Uitewaal, A.J. 1947. A first attempt to subdivide the Genus *Haworthia* based on floral characters. *Desert Plant Life* 19:133-138

6) Breuer I. 1998-2002. *The World of Haworthias* Vol. 1 and 2. I. Breuer and Arbeitskreis für Mammillarienfreunde e. V., Niederzier e Homburg/Saar

7) Breuer I. 1999. *Haworthia* photographs used to typify taxa described by Dr. Karl von Poellnitz. I. Breuer and Arbeitskreis für Mammillarienfreunde e. V. Niederzier. Homburg/Saar

8) Breuer I. 2011. *The Genus Haworthia Book2* (Part 1+2). *Alsterworthia International*



## 4.6 = FOREST CANOPY GRADIENT AND MONUMENT CONSERVATION IN THE ANGKOR ARCHAEOLOGICAL SITE

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The biological colonisations on Khmer temples in Angkor (Cambodia), after the abandon occurred many centuries ago, gave rise to the development of different biological communities reaching in some cases a final luxuriant forest. After the restoration activities carried out in some areas, a different canopy gradient can be observed and the biological communities colonising the stones now also vary in relation to the local environmental conditions. Then, field and laboratory microscopic observations of the biodeterioration patterns were carried with the aim to explore such phenomena along a forest canopy gradient and to quantify their frequency and ecological characteristics.

Ta Prohm temple was selected for the presence of a dense forest canopy; Ta Nei and Bayon temples, for their intermediate conditions of semi-shadowing and relatively humid environment, and Ta Keo temple, in sunny and dry conditions, due to the forest cutting (1, 2). For each temple the different biodeterioration pattern present in the different expositions was estimated through quali-quantitative surveys according to the phytosociological method and analyzed with a statistical approach.

The field observations of the biodeterioration patterns correspond to six communities of microflora until higher plants. The first community growing with a minimum level of the water and overall in shady conditions is the reddish community of *Trentepohlia*. Increasing water and in conditions of high solar radiations, the blackish patinas of cyanobacteria (*Scytonema-Gloeocapsa*) develop. In condition of low light and more water availability, three different communities of lichens have been recognized, related to different water and light requirements: respectively dominated by *Lepraria*, *Pyxine coralligera* and *Cryptothecia subnidulans*. Finally, the colonization of mosses, and ferns and higher plants establishes with increased shadow condition and higher level of water (3). Such communities also show a different biodeteriogenic activity, which was estimated in a previous work (4).

Hierarchical cluster analysis applied to the eight communities found in the investigated temples produced a clear pattern based on two distinct groups: communities typical of forested sites characterized by low tolerance to dryness and a general preference for humid habitats and communities of non-forested sites formed by cyanobacteria and *C. subnidulans* more resistant to dry conditions and xeric habitats.

The analysis thus indicates that temples are well separated on the basis of their typical biological colonization, which follows a sequence related to different forest canopy. This variable seems very significant and it constitutes the driving factor of the biological colonization of the stone, influencing humidity, lighting and temperature.

The knowledge of these relationships seems very useful for evaluating the possible use of indirect control methods against the various biological colonizers, and thus to identify the best microclimatic conditions for stone conservation.

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2) M.M. Kanade, P.T. Lakshmi, Ta Prohm temple: a conservation strategy, published by director general archaeological survey of India, Janpath New Delhi, dec. 2006

3) G. Caneva, S. Ceschin, O. Salvadori, H. Kashiwadani, K.H. Moon, Y. Futagami, Biodeterioration of stone in relation to microclimate in the Ta Nei temple – Angkor (Cambodia), in: 12th International Congress on the Deterioration and Conservation of Stone, New York 22-26 October 2012, Session XIII, pp. 12-27

4) F. Bartoli, A. Casanova Municchia, Y. Futagami, H. Kashiwadani, K.H. Moon, G. Caneva, Biological colonization patterns on the ruins of Angkor temples (Cambodia) in the biodeterioration vs bioprotection debate, International Biodeterioration & Biodegradation, in press



#### 4.6 = A HISTORICAL GARDEN IN FLORENCE: FASCINATION AND DISCOVERY OF *GIARDINO TORRIGLIANI*

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The *Giardino Torrigiani* is the largest privately owned garden in Europe situated within city boundaries. Its origin dates back to 1414, as stated by the records of the Historical State Archive of the city of Florence. In the early nineteenth century Pietro Guadagni, later Marquis Pietro Torrigiani, inherited the family name and the property and started acquiring the surrounding land. He decided to transform the estate into a 'romantic park' in the English style, adhering to the fashion of the time. Torrigiani appointed the landscape architect and public officer of the Granduca di Toscana Luigi de Cambray Digny, who had recently realised two further gardens in Tuscany: the garden of *Orti Oricellari* in Florence and that of *Scornio* in Pistoia. In the Torrigiani garden Cambray Digny combined natural elements with artificially made, landscaping and managing to include many symbols of an esoteric nature. Several traits make *Giardino Torrigiani* unique: its six hectares of park, meadows and flowers are hidden in the heart of Florence and surrounded by the city walls. Further, it is the unique English garden entirely included within the urban area and it is a perfect example of Masonry symbolism, for the presence and distribution of both botanical and architectural elements. The visitor is lead on a sentimental journey through the dark 'sacred' wood surrounding the crypt, symbol of the transience of earthly life, to the open spaces around the temple of Arcadia, symbol of pastoral life. In this garden, named *Giardino de' Boffi*, the botanist Pier Antonio Micheli, founded in 1716 the Florentine Botanical Society. Today the Torrigiani family is still committed to preserve and maintain the beauty and the fascination of the garden. In our work we performed the botanical inventory of trees, shrubs and hedges and described their position by GPS (Global Positioning System); a comparison with previous catalogues was made, thus tracing record of losses and new acquisition of plants. Further, we analysed the phytosanitary condition preparing a plan of wealth restoration. Finally, we compiled guidelines for the conservation and management of the garden.

## 4.6 = THE BOTANICAL COLLECTION OF HISTORIC GARDENS IN EASTERN SICILY

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This contribution sets out to illustrate the results of research aimed at understanding the botanical heritage of historical gardens in eastern Sicily. Historical villas conserve precious collections of plants that lend the garden an added value, linked not only to its aesthetic significance but also to its cultural function. In Sicily, the aristocratic and middle classes promoted the spread of a system of villas with gardens having a dual role: *locus amoenus*, in which to entertain illustrious guests and a productive area, sometimes inserted in the geometric network of the agrarian landscape. The research aimed recognising historic gardens and parks in eastern Sicily, especially in the provinces of Catania and Messina. In these two investigated provinces there are both public gardens and numerous “smaller” examples, mostly private, with historical origins and morphological, typological and botanical features that are differentiated according to their geographical-cultural setting. The study was carried out on 101 gardens of villas or private buildings, from the end of the 18<sup>th</sup> to the early 20<sup>th</sup> centuries. Some of the investigated villas are subjected to Environmental and Architectural Heritage restrictions *ex lege* 1089/39. The research has been undertaken through the identification of the villas, vascular plant census and analysis of floristic data. The identification of the villas was done by examining aerial photographs and historic documentation. The vascular plant census was carried out by field surveys and the identification of the plant entities. Then, the phase of elaborating and analyzing the data collected and, lastly, that of interpreting the results. Field observations were also conducted in several periods of the year and voucher herbarium specimens were collected to help identifying plant species. The floristic list was compiled reporting botanical family, the biological form and the geographic origin of introduced plants. The analysis of the flora has highlighted the plant wealth expressed by 131 botanical families, 364 genus and 596 taxa; of these, a good percentage belongs to common entities of the historic Sicilian garden and a small part instead belongs to rare or ancient entities. Among the plants of special botanical value for their rarity, particular mention should be made of *Allocasuarina verticillata* (Lam.) L. A. S. Johnson (1), noted only in the garden of Villa Ingham di Racialia in Marsala (Trapani), the park of Donnafugata in Ragusa and the public garden ‘Vittorio Emanuele’ of Caltagirone (2), *Bosea amberstiana* (Moq.) Hook. f., *Myrsine africana* L. and *Prunus caroliniana* (Mill.) Aiton, found in the gardens of Caltagirone (1); *Nolina longifolia* Hemsl. and *Searsia lancea* (L. f.) F. A. Barkley, observed in Caltagirone and in the historic garden of Villa Paternò Castello (San Giovanni La Punta, CT). Other rarities are *Acacia podalyrifolia* A. Cunn. ex G. Don, *Alstroemeria psittacina* Lehm., *Calia secundiflora* (Ortega) Yakovlev, *Corymbia citriodora* (Hook.) K.D. Hill & L.A.S. Johnson, *Corynocarpus laevigatus* J.R. Forst. & G. Forst., *Cotoneaster pannosus* Franch. *Kleinia anteuophorbium* (L.) Haw. and *Ochrosia elliptica* Labill., observed in the gardens of Milazzo (ME) (3). Botanical peculiarities of the Catania and Milazzo gardens are also *Doryalis caffra* (Hook. f. & Harv.) Warb., *Kleinia nerifolia* Haw. and *Vachellia farnesiana* (L.) Wight & Arn. Notable in the Catania gardens are *Camellia japonica* L. cv. ‘Fimbriata’ (4), *Haemanthus coccineus* L., *Oreopanax dactylifolium* T. Moore and *Schefflera elegantissima* (hort. Veitch ex Mast.) Lowry & Frodin. Spectacular for size and/or bearing and noteworthy for their age are *Calocedrus decurrens* (Torr.), *Chamaerops humilis* L., distinguished by unusual creeping stipes, *Cinnamomum camphora* (L.) J. Presl, *Cycas revoluta* Thunb, *Cupressus sempervirens* L., *Dracaena draco* (L.) L., *Eucalyptus globulus* Labill., *Lagunaria patersonia* (Andrews) G. Don, *Morus alba* L., *Pittosporum undulatum* Vent., *Syzygium australe* (J. C. Wendl. ex Link) B. Hyland, *Tamarix gallica* L. and *Taxus baccata* L. Among this botanical patrimony, the presence of a rich collection of *Camellia japonica* L. has already been highlighted. Even if there are numerous gardens hosting it today, the “Garden of the Mulberry” in Milazzo (ME), with its 132 specimens, represents an exceptional feature for the Island (5).

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2) G. Bazan, A. Geraci, F.M. Raimondo (2005). Quad. Bot. Ambientale Appl. 16, 93-126

3) A. Cristaudo & S. Catara, 2014. Proceedings 6<sup>th</sup> International Congress "Science and Technology for the Safeguard of Cultural Heritage of the Mediterranean Basin". Athens, Greece. Vol. I (III): 388-394

4) G. Cattolica, A. Lippi, P.E. Tomei, 1992. Pacini Editore, Pisa

5) S. Catara, A. Cristaudo, E. Allevato, G. Di Pasquale, 2012. Proceedings 5<sup>th</sup> International Congress "Science and Technology for the Safeguard of Cultural Heritage of the Mediterranean Basin". Istanbul, Turkey. Vol. IV (IV): 50-55

#### 4.6 = PLANTS IN THE POETICAL WORKS OF GIOVANNI PASCOLI: CRITICAL ANALYSIS AND A “PASCOLIAN WALK” IN THE BOTANICAL GARDEN OF FLORENCE

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“La flora pascoliana: presenze botaniche nella poesia di Giovanni Pascoli” (“The Pascolian flora: botanical presences in Giovanni Pascoli’s poetry”) is the title of a thesis by Marcella Pozzi (University of Fribourg, Switzerland).

Taking this text as a starting point, an inter-disciplinary group of research has been formed, with the aim of further investigating the subject from a botanical point of view, to improve cultural connections between naturalistic and literary disciplines and to create a “Pascolian walk” in the Botanical Garden of Florence. This group includes botanists, agronomists, forestry researchers and italianists, from different Italian universities.

The first step of the research was a critical analysis of the plant list reported by Pozzi in order to verify the botanical identification of each plant. From this study some difficult cases emerged, also due to the fact that Pascoli used common plant names that are often too generic to be precisely identifiable and can vary according to the time period and the locality. To overcome such problems, a multidisciplinary approach was needed. In some examples references to flowering periods, growth habits and morphology found in the original texts were helpful for a correct identification. In more dubious cases, other sources were used: lists of common plant names used in the Italian regions more connected to Pascoli’s life and works (Tuscany, Romagna); names still in use in the same regions, obtained from the literature, from interviews or from personal knowledge of the researchers; Pascoli’s own writings (first drafts, notes, glossaries) and textbooks he used to cultivate his botanical interests.

The second step consisted of a critical analysis of all the plants cited in Pascoli’s poetic works. For this purpose, a database including different kinds of information for each identified species was compiled. Information for each plant includes: botanical family, growth habit (herb, shrub, tree...), native/introduced and spontaneous/cultivated status, ethnobotanical (medicinal, food...) uses if any, data about the presence of the plant in Pascoli’s works (frequency, name variations), etc.

The third step will be the creation of a “Pascolian walk” in the Botanical Garden of Florence: a brochure with a map of the garden showing a selection of plants cited by the poet and accompanied by extracts from poems related to a significant presence of the species (in terms of quality and quantity).

We can affirm that the meeting of different competences was essential to deal with some critical cases with a comprehensive approach and that the research group will take advantage of this collaboration in additional areas of interest, especially in view of the publications of the results of this study in a book.

#### 4.6 = CASTLES OF LAGNASCO: GARDEN OF THE WEST SIDE, PROJECT FOR THE PROPOSAL OF A “GIARDINO DELLE ESSENZE”

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Inside the area of the western side of the Castle, the project aims to propose a “Garden of Essences” through the cultivation of aromatic and medicinal herbs, and fruit and vegetable crops.

The project is part of a wider initiative of territorial development, which aims to create a cultural heritage network sharing a strong and significant link with the territory, a network of landscapes, gardens and fields characterized by the cultivation of herbs, medicinal plants and fruit and vegetable crops from ancient tradition. The identified assets will form a potential system for creating a “network of routes of flavours and fragrances in the Savoy land” to preserve the territorial cultural identity of the places thanks to a perfect integration in land valorization system. The initiative will concern the following projects: implementation of a Roman Kitchen Garden in the archeological site of *Augusta Bagiennorum* (Bene Vagienna, CN) for educational purpose; reconstruction of the garden of the West side of Tapparelli d’Azeglio of Lagnasco Castle (Cuneo province); restoration of “Giardino delle Foglie” designed by Arch. Mirella Macera and Arch. Paolo Pejrone and of “Il Giardino dei Principini”, in front of the neogothic greenhouse in La Margaria in the parkland of Racconigi Castle (Cuneo province).

In Lagnasco the project aims to complete the recent restoration of the outdoor area of the Castles by proposing a “Giardino delle Essenze” in the southern side. The enclosed space, where the garden will be set up, reminds the atmosphere of *Hortus Conclusus*, and will propose a cultivation typical of the Middle Age *Hortus Simplicium* in order to maintain, study and recognize medicinal and aromatic plants; the medieval terminology “simplices” means the healing principles directly obtained from nature. Important design suggestions were drawn from a unique landscape affresco, located inside the castle and dated XVC “Il castello di Lagnasco e i giardini cinquecenteschi” by Pietro and Giovanni Angelo Dolce. The project is supported by the Sovrintendenza per i Beni Architettonici e Paesaggistici per le Province di Torino, Asti, Cuneo, Biella e Vercelli. The layout of the garden has been designed in rectangular raised beds edged with chestnut wooden fences. Furthermore there are pergolas, for the cultivation of vines and espalier orchard for apple and pear trees. Among its objectives the project intends to redevelop the area and to promote new educational initiatives for students, scholars and visitors. Considering that Lagnasco area is particularly devoted to fruit trees cultivation, old varieties of fruit trees will be planted in the garden. In the *Hortus Simplicium* – rearranged for educational purposes - the plants will be distributed according to their most common use. This classification is not exhaustive, since many plants have different uses, and therefore will be located in several areas. The visit of the Garden of Simples seeks to inspire the desire to learn about the medicinal plants in their historical use and in the modern therapy.

#### 4.6 = “BOTANIC GARDEN AND TERRITORY”: AN EDUCATION PROJECT ON BOTANICAL COLLECTIONS AND VEGETAL BIODIVERSITY IN THE TERRITORIES OF EMILIA

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In the context of a national project for spreading scientific culture (L.6/2000) a cooperation between two University Botanic Gardens and a Botanic Garden managed by a no-profit organization (NPO) was started in 2012, with the goals of developing education and public awareness on biodiversity conservation, testing methods for teaching science and history of science, creating exhibitions at the Gardens/Botanical Gardens or in the involved territory, running environmental education programs and working towards the implementation of Target 14 of the Global Strategy for Plant Conservation (1).

University Botanic Gardens of Modena and Parma are ancient Duchy Gardens, founded in XIII century (2). Like most of similar Institutions in the world, they are now involved in several projects for the promotion of scientific culture in schools at all levels, enhancing the use of scientific laboratories and multimedia tools to spread awareness of the importance of the natural, ecological, biological and agricultural sciences in everyday life and sustainable social development.

They are rich in ancient collections, consisting in herbaria and other plant samples, generally the most considerable historical/scientific heritage (4). Notably, the herbarium collections are, among others, irreplaceable instruments to deepen the knowledge on the past floristic presences in an investigated area (3).

Botanical Garden “La Pica” is a no-profit organization born in 2007, with the aim to promote the protection and enhancement of nature and the environment of the lowlands of Modena. The association cooperates with the local Municipalities (San Felice sul Panaro and Mirandola - as well known severely damaged by 2012 earthquake) in environmental education activities. In this project, the role of this Garden is to support with concrete actions the activities of the academic Gardens, starting cultivations and organizing educational activities in order to increase the knowledge of the most characteristic aspects of Emilia territory, as a part of the complex Italian landscape and cultural mosaic. The project action plan consists of different interventions specifically targeted to historical collections (herbaria, wood, seeds and fruits), living collections (parterre, arboretum, ducal greenhouses, plant nursery, conservation collection, didactic itineraries and structures), initiatives on the territory (i.e. from *ex situ* to *in situ* conservation, interactive guide to the plant identification, the Drainage Consortium Herbarium), networking activities (toward a Network of Botanical Gardens of Emilia Romagna, to collaborate with landscape management Authorities, parks and reserves, schools, scientific and cultural associations, mass media).

The carried out experiments demonstrate the wide impact of these initiatives on local public. At the same time, the participation of a botanical garden created by volunteers in the territory of the Valli di Mirandola (La Pica NPO), deeply linked to the culture, tradition and rural aspects of the territory, can contribute to improve social awareness of a responsible use of resources. Moreover, in the frame of the reconstruction after the 2012 earthquake, it can help to regain interest in “beautiful things, such as plants, flowers and nature” as properly affirmed by local Authorities.

(1) <http://www.cbd.int/gspc/targets.shtml>

(2) P.Meda (1996) *Guida agli Orti e Giardini Botanici*. Editoriale Giorgio Mondadori, Milano

(3) A. Alessandrini, L. Delfini, P. Ferrari, F. Fiandri, M. Gualmini, U. Lodesani, C. Santini (2010) *Flora del Modenese. Censimento, Analisi, Tutela*. Provincia di Modena, Istituto per i Beni Artistici, Culturali e Naturali della Regione Emilia-Romagna

(4) D. Dallai (2008) *Orto Botanico Universitario Estense di Modena*. In: Russo A., Corradini E. (a cura) “Musei Universitari Modenesi”. Ed. Moderna, Bologna (ISBN 978-88-8863-214-8): 93 -122

(5) C. Santini, D. Dallai, M. Gualmini, E. Sgarbi, con il contributo di F. Fiandri, L. Delfini, U. Lodesani (2009) *La flora del territorio modenese, alla luce delle trasformazioni urbane e agrarie del Novecento*. In Bulgarelli V. & Mazzieri C. (a cura) “La città e l'ambiente. Le trasformazioni ambientali e urbane a Modena nel Novecento. Annale dell'Atlante storico ambientale urbano di Modena, Comune di Modena, APM ed., (Carpi – MO): 227 – 243. ISBN 978-88-89109-33-5

## 4.6 = PLANTS OF THE BIBLE

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We synthetize here the results "of a work of herborization conducted among the pages of the Bible, searching the plants in the land of Israel both in biblical and modern times and their meaning"(from the words of Ing. Mario Caiola).

The research was conducted from 1999 to 2013, through the study of literature on the topic, and surveys in Israel, and we were able to fulfill the book "The plants of the Bible" (ed. Gangemi, 2013). Text, 110 cards, colored images of the described plants, 170 references about literature and locations, as well as 12 summary tables (counts, the symbolism of biblical plants and the names of the main plants of the Bible in Hebrew, Greek, Latin and Arabic) can be found among its 208 pages.

The reference Bible was the version of CEI-UELCI (1). The plants mentioned in the Bible by common name add up to 83. 77 are found in the Old Testament (OT), 54 of which are exclusive of OT; 29 in the New Testament (NT), 6 of which are exclusive of the NT; 23 are both in the Old Testament that in the NT. Moreover 27 collective names of plants must be added to this number.

Among the books of OT the highest number of botanicals quotes can be found in the Pentateuch and in those of the Prophets (Isaiah 34 citations) and the most recurring plants are wheat (175 quotes), frankincense (159), cedar of Lebanon (70) and linen (68). The 110 identified species belong to 54 families and 97 genera, with one or few species indicated: this shows the diversity of environments. There are also 113 plants quoted only by their scientific binomial, that had required particular attention and alternative researches, in order to bring them to the mentioned groups.

Among the components of the biblical flora we noted: native plants (eg *Commiphora gileadensis* L. C. Chr, *Pistacia palaestina* Boiss), plants with Mediterranean distribution, also present in Italy, particularly in the South (eg, *Quercus ithaburensis* Decne, *Sarcopoterium spinosum* Spach), African Plants (eg *Commiphora myrrha* (Nees) Engl., *Ficus sycomorus* ), Asian plants from ancient Palestine through the Via Maris or the Silk Road (eg *Amomum subulatum* Roxb., *Nardostachys jatamansi* (D. Don) D.C.).

The introduction to the volume reported the number of plants counted in the Bible by 7 among the principal authors of these studies in the period 1952-1999 (2,3).

The iconographic study (for which we thank in particular the Israeli botanist Avinoam Danin) has allowed us to find some images of biblical plants hardly represented in other volumes, eg. nard, the agallocha, the amomo. In the book the individual species cards report descriptions, uses, biblical quotations of the plants as well as information derived from texts by authors of ancient greek-roman period (Columella, Pliny, Theophrastus, etc.). The volume is intended to help both visitors to the Botanical Gardens where there are areas dedicated to biblical plants, and enthusiasts, both fans of the biblical botany, and teachers that put effort in reading the Bible at schools.

1) C.E.I.-UELCI, 2008. La Sacra Bibbia. Libreria Editrice Vaticana, Città del Vaticano

2) Włodarczyk, Z. 2007. *Review of plants species cited in the Bible*. Folia Horticulturae 129/1:67-85

3) Zohary M. 1982. *Plants of the Bible*. Cambridge, UK. Topic III: Biotechnology and Natural Resources. Symposium 2

#### 4.6 = FLORINTESA, A PROGRAM AGREEMENT FOR THE ITALIAN BOTANICAL GARDENS AND THE NATIONAL FLORISTIC HERITAGE

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OUR CLAIM: *"The Botanical Gardens are, par excellence, responsible for carrying out the important mission of the conservation of our flora, through specific actions on live plants and their seeds, along with education and outreach aimed at spreading a new environmental culture, more careful and respectful of the essential needs of life, more sensible to the aesthetic and scientific value of the national flora"*

To overcome the isolation and strengthen their role in our society, the University Botanical Gardens have become promoters of national and European consortiums: examples are the working group "Botanical and Historic Gardens" of the Italian Botanical Society (1) and, on the global level, the Botanic Garden Conservation International (2).

Aims and tasks of the Botanical Gardens in the second millennium have been the topic of a thorough debate, with specific references to the provisions of the United Nations Programme for a sustainable development. In particular, the Action Plan for Botanic Gardens in the European Union (3) identifies the following major assets: scientific research, conservation of plant diversity, public advisory services on it, environmental education related to it. To pursue these objectives, initiatives are constantly needed to enhance the visibility of the institutions involved and help them to perform their functions. In this context was born FLORINTESA.

FLORINTESA is a program agreement, funded by the Italian Ministry of Education (MIUR) with identification code ACPR12\_00201, involving as partners ENEA, Plinianum Forum and the Italian Botanical Society, which contributes to bridge the still existing gap between the scientific research, the technical action of conservation and preservation of plant diversity and the public awareness on such themes.

The main objectives of the FLORINTESA can be summarized as follows:

- Establishing an institutional network service for information and dissemination on the flora of Italy, with reference to the role of Botanical Gardens as centers of research and knowledge on the national flora, as well as on the assessment and conservation of its rarest species,;
- Disseminating and publicizing the activities of the University Botanical Gardens for the flora of Italy;
- Increasing the visibility of the Italian Botanical Society and of its working group on "Botanical and Historic Gardens";
- Disseminating the achievements and helpful assistance offered by the Botanical Gardens in the implementation of the National Strategy for Biodiversity (4) and the Natura 2000 Network (5), through initiatives such as the Italian Germplasm Banks Network (6), the International Foundation pro Herbario Mediterraneo (7), the pan-Mediterranean Genmeda network (8), the Horti Mediterranei Educational Network (9).

The flora of Italy will be the leitmotif of the actions envisaged in FLORINTESA, highlighting the unique role of the University Botanical Gardens and their respective institutions as "engines of knowledge" on the national flora heritage, as "engines of awareness" on the important issue of conservation of flora and habitats, as "engines of passion" for the grateful acknowledgement of the role of plants as primary producers not only of resources in the natural ecosystems, but also of inspiring beauty in the human cultures.

The kick-off meeting, open to all members of the Italian Botanical Society, will take place in Rome, at the Auditorium of the Accademia Nazionale dei Lincei, on October 24, 2014.

1) <http://www.ortobotanicoitalia.it>

2) <http://www.bgci.org>

3) <http://www.botanicgardens.eu/index.html>

4) <http://www.minambiente.it/pagina/strategia-nazionale-la-biodiversita>

5) <http://www.minambiente.it/pagina/rete-natura-2000>

6) <http://www.reteribes.it>

7) <http://unipa.it/herbmed/foundation.html>

8) <http://www.genmeda.org/it/home.php>

9) <http://www.hortimediterranei.it/index.php>

## 4.6 = LOOKING AT MEDITERRANEAN GARDENS AS EVIDENCE OF HISTORIC AND CULTURAL LANDSCAPE

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The Mediterranean landscape has been strongly affected and shaped by human imprint from ancient times. Sicily, in particular, can be defined as the "core essence" of this landscape, because its strategic central position in the Mediterranean made it a crossroads of people and historical events. Results from such connection are clearly readable both in urban and rural settings, where the time sequence of human activities produced a great diversity of features and land assets. Gardens as planned places including both natural and man-made materials are good indicators of the man/land relations. As a matter of fact, gardens have the capability to give physical form to human experiences, memories, and ideas which reflect the awareness of current and past life, as well as the perception of surrounding environment and landscape. Mediterranean gardens in Sicily effectively reproduce the singular mixture of historical, social and cultural processes occurred over the time and clearly show layered signs of changing lifestyles and environmental contexts. In this respect, eastern Sicily offers an interesting case study since very different geographic layouts, bioclimatic conditions, socio-economic and cultural background discriminate these territories from the western part of the island. Particularly, the prevailing lack of large rural estates (*latifondi*) and the early partitioning of lands to smallholders, as well as the emergence and spread of a rural high bourgeoisie gave rise to a significant representation of garden models, often small sized, with original design, materials, and plant collections (1). Far from providing a full check of the existing garden diversity, a representative frame can be summarized by the following types.

— *Monastic gardens*, with a cloister inside and a fruit/vegetable garden outside the walls; an adjoining natural park, called *silva*, was often integrated part of church lands. Many of them have become public gardens after the ecclesiastical confiscations in 1866. Examples still occur in Piazza Armerina (now Giardino Garibaldi), in Catania (San Nicolò La Rena), Siracusa (Latomia of Capuchins) and Taormina (San Domenico), although deeply altered and rearranged in their plant scheme and composition (2, 3).

— *Country houses* of different relevance and size, typical of the rural Mediterranean landscape; firmly rooted in the agriculture productivity, they were farm estates mostly devoted to crop production and/or livestock breeding, while the ornamental garden, next to the main dwelling, was reserved to the private recreational use of the owner's family. Examples varied from big productive complexes, old centres of rural communities, like Villa Fegotto (Chiaromonte Gulfi, RG), Nelson's Duchy (Bronte, CT), Villa Zirilli (Milazzo, ME), to minor mansions with smaller plots of land and farming incomes, well represented both in the hyblaean and etnean countryside, e.g. Villa Gisana (Modica, RG), Villa Casalotto and Villa Previtiera (CT).

— *Holiday and residential villas* in the suburban or urban areas, common expression of a comfortable and wealthy lifestyle related to aristocracy and then to high middle class; gardens represented beautiful escape from the summer heat, but mainly they incorporated combinations of good social standing, reputation, influence, and honour of their owners. These are the most preserved and representative historic gardens, such as Villa Elvira del Principe Bonaccorsi (Milazzo, ME), Villa Falconara and Casa Cuseni (Taormina, ME), Villa Bellini and Villa Consoli Marano (CT), Villa Patti (Caltagirone, CT), Villa Reimann (SR), Donnafugata Castle and Villa Palmeri di Villalba (RG) (4).

— *Green promenades and tree pathwalks*, born as first types of public green spaces; they were designed to provide recreational opportunities for leisure, walking and gathering of citizens, both in main and small cities, and in time were enlarged to become typical public gardens, e.g. Villa Pacini (CT), Villa Belvedere (Acireale, CT), Giardino Ibleo (RG), Villa of Palazzolo Acreide (SR).

As usual, these main garden types can be further characterized following a temporal range from the 18<sup>th</sup> to the 20<sup>th</sup> century, and a spatial extent North-South of eastern Sicily (*Valdemone* and *Val di Noto*).

Regarding the time scale, gardens in E Sicily show style changes varying from the typical Italian design, to the romantic and eclectic forms, all sharing the common Mediterranean use of fruit trees, aromatic plants and palms as main botanical collections (5). Geographic gradient, instead, results in the use of different materials (stone, clayey, terracottas, wood), decorations and handcrafts, strictly related to the landscape and local customs, as well as in the plant composition depending on specific microclimate contexts, cultural trends and exchanges of garden owners or gardeners with botanical gardens, science community and plant collectors.

1) AA.VV. (2012) Mediterranean Gardens from Sicily to Malta, Morrone Ed., Siracusa

2) Guglielmo A., Pavone P., Salmeri C. (2006) Quad. Bot. Ambientale Appl., 17/2, 89-98

3) Salmeri C., Guglielmo A. (2012) Acts A.I.Ar. Workshop, Palermo 2009, 53-67

4) Cristaudo A., Catara S. (2014) Acts 6<sup>th</sup> Int. Congress of "Science and Technology for the Safeguard of Cultural Heritage of the Mediterranean Basin", 1(3), 388-394. Athens.

5) Guglielmo A., Pavone P., Salmeri C. (2006) Acts A.I.Ar. Workshop, Siracusa 2005, 229-244, ARACNE Ed., Roma



## 4.6 = MORPHOLOGICAL ANALYSIS OF ANCIENT GRAPE SEEDS FROM A SINK IN THE MIDDLE-AGE TOWN OF PALERMO

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The archaeological excavations in Piazza della Vittoria, in the Roman-Middle Age town of Palermo (Sicily) put in light a sink 3.20 m deep and 1 square m. large, partially filled by thin organic sediments. Grape seeds (grapestones), fish scales and few vertebrate bones have been found in specific strata sealed under a stratum chronologically attributed to Islamic Middle-Age period (a *post-quem* limit). The finding of well preserved grape seeds is peculiar and their study opens the opportunity to improve the actual knowledge about evolution, cultivation, use and trade of *Vitis* L. in the Mediterranean area.

This preliminary work focuses on morphologic and morphometric analysis of the ancient grape seeds with two aims: *i*) systematically describe the remains collection and, *ii*) define seeds typology and a consequent morphotaxonomic attribution.

Over 200 seeds have been carefully dry cleaned (soft brush), photographed and analyzed for total breadth (B), total length (L) and length of stalk (LS) parameters, the most efficient for typological attribution (1, 2); Stummer index has been also calculated (1). Apical notch length (AN) has been for the first time evaluated. Measurements on digital images have been performed using ImageJ 1.31 platform; morphological parameters have been assembled in a dedicated database. Descriptive analysis and linear correlations have been performed using SYSTAT 10. Analysis of variance (ANOVA) and Tukey's HSD (5% level of significance,  $\alpha = 0.05$ ) have been applied.

All the parameters approximate a normal distribution. Major variation has been observed in LS (c.v. = 35.6%) and AN (c.v. = 35.6%), while B and L showed a c.v. of 9.5% and 12.6% respectively. All the analyzed parameters behave as independent variables with the exception of a significant correlation between Stummer index and L ( $R^2 = 0.45$ ;  $y = 8.17 - 0.047x$  with  $y = L$  and  $x = \text{Stummer index}$ ). This correlation reveals that Stummer index depends more from the L and not from the B parameter. On the base of LS measures three subgroups have been arbitrarily created in relationship with the LS: LS1 < 0.50 mm (45 seeds), LS2 from 0.51 to 0.89 mm (109 seeds), LS3 > 0.90 mm (35 seeds). Analyzing together the LS groups toward AN, we have found a proportional and significant correlation ( $p = 0.05$ ) between the extremes LS1 and LS3.

In the entire collection, Stummer index varies from 55.76 to 100.86; in the LS groups, the range is 68.38-97.87 in LS1, 61.02-100.86 in LS2 and 55.76-81.70 in LS3. A small group (17) of seeds has been excluded for the impossibility to measure the stalk.

The analyzed ancient grape seeds show a wide range of variability for all the considered parameters, revealing a polymorphic collection. In general, the seeds have a rounded heart-like shape, with a noticeable pointy stalk and a very invaginated apical notch. This typical shape is more marked in LS3 group. On the base of LS measures, LS1 is ascribable to wild grapevines, while LS2 and LS3 seem to be ascribable neither to wild nor to cultivated autochthonous *Vitis*. Furthermore, these seeds differ from those already described in other archaeological horizons in Italy (3) and in France (2). The Stummer index varies highly, exceeding the known range of wild *Vitis vinifera* (4), although values close to 100 have been already found in wild grapevines in Spain (5) and values above 80 have been also described in Extra-European *Vitis* species (6). A deep evaluation of the sample, including isotopic analysis and aDNA studies, is in progress.

1) T. T. Korenčič, J. Jakše, Z. Korošec-Koruza (2008) *Veget. Hist. Archaeobot.*, 17(Suppl. 1), S93-S102

2) L. Bouby, I. Figueiral, A. Bouchette, N. Rovina, S. Ivorra, T. Lacombe, T. Pastor, S. Picq, P. Marinval, J. F. Terral (2013) *PLoS ONE*, 8(5), e63195

3) C. Milanese, F. Antonucci, P. Menesatti, C. Costa, C. Faleri, M. Cresti (2011) *Interdisciplinaria Archaeologica – Natural Sciences in Archaeology*, II(2), 95-100

4) A. Stummer (1911) *Mitt. Anthropol. Gesellschaft Wien*, 41, 283-296

5) F. M. De Toda, J. C. Sancha (1999) *Am. J. Enol. Vitic.*, 50(4), 443-446

6) D. Rivera, B. Miralles, C. Obón, E. Carreño, J. A. Palazón (2007) *Vitis*, 46(4), 158-167

#### 4.6 = ENVIRONMENTALLY AND ECONOMICALLY SUSTAINABLE MANAGEMENT OF THE ENGLISH HISTORICAL GARDENS IN THE LIGURIAN RIVIERA AND CÔTE D'AZUR

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The Hanbury Botanical Gardens in La Mortola, Ventimiglia, Italy, is a listed botanical and historical garden, regional protected area, scientific structure and public property. The Botanical gardens that cover 18 hectares (ca. 44.5 acres) and the protected area are managed by the University of Genova. The gardens feature botanical collections of xerophytic plants, architectural garden elements and historical buildings, thus making the simultaneous management of all the different aspects being a really challenging issue. Management is further complicated by the Mediterranean maritime climate, with hot summer, mild winter and summer drought, which makes difficult both preserving collections and meet visitors' expectations.

The GBH were founded in 1867 by Sir Thomas Hanbury as acclimatization gardens, and now they are listed as historical landscape and garden. They have been a rare mix of beauty, science, history, archaeology, landscape architecture and research since their establishment (1).

Local environment and climate are not friendly to a gardener, so the founder himself and his gardeners were forced to deal with an early sustainable management because of the summer heat, the low annual rainfall and the poor soils. Today the sustainable management of the Gardens is linked to several issues, such as water saving, a moderate usage of pesticides, organic matter recycling, conservation of historical and natural values and therefore the need of controlling the number of visitors. Probably because of the well known aesthetical value of the garden, some visitors do not appreciate the natural and unconventional appearance of plants in the garden, which is the result of sustainable management and the related compromises.

Further problems of management are linked to the incompatibility of Italian laws on cultural heritage with the management requirements of a site, that is a botanical garden and in the meantime an English garden and a historical one. As a place of science, research and education would require more space for action, although it has to be very limited because of the historical background. As a historical place, restoration and conservation would require the replacement of historical species which have proved unsuitable with the climate, so their replacement would be both contrary to the mission of the botanical garden, and to the wishes of the founders themselves, who wanted a trial garden of acclimatization. As an English landscaped garden, the respect and preservation of the garden structure would require careful attention to new planning plantation and even impossibility of planting new species because of the wrong shape or size for the site. All these aspects are clearly difficult to match, and the same happens to other English gardens along the Riviera, such as Villa Piacenza Boccanegra (2) (3) and Serre de la Madone at Menton (4). They are both acclimatization gardens, and they are focalized on the same aspects. New species are introduced and tested, experiencing what would suit to other Riviera Gardens. Experiments imply the study of the different areas of the gardens, with the aim to understand their characteristics and develop them in balance with sustainable management, planning new plantings and restoring historical garden features.

The three Gardens pursue the sustainable management of their cultural heritage, but raising people awareness of the value of resources and of the true meaning of garden sustainability is necessary to a full effectiveness, through communication and education. The implementation and development of communication skills aimed to public education and learning, particularly Italian one, is urgent, so that the "sustainable garden" and its compromises could be correctly interpreted and accepted.

1) Zappa E., 2011. Spunti dalle fonti per lo studio delle dinamiche di sviluppo dei Giardini, in F. De Cupis, E. Ragusa (edit.), *La Mortola e Thomas Hanbury*, Atti della Giornata di studio del 23 novembre 2007. Allemandi editore, Torino, pp.125 – 140

2) Piacenza G., 2004. *Villa Boccanegra. Giardino Piacenza*. pp. 8

3) Salghetti Piacenza, U. (2009). *Ellen Willmott a Boccanegra* in D. Gandolfi (edit.), *Apronia Marcella e le altre*. Fratelli Frilli Editori pp. 93-100

4) Jones L., Angers N., 2002. *Serre de la Madone Menton*. Actes Sud. pp. 48

## 4.6 = ALPINE BOTANICAL GARDEN OF CAMPO IMPERATORE (GRAN SASSO, ITALY): 60 YEARS OF ACTIVITY

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The Alpine Botanical Garden of Campo Imperatore was established in 1950 by the National Research Council on the initiative and pressure of Prof. Vincenzo Rivera, prominent political and founder of the University of L'Aquila in 1952. After an initial intense activity by making a concrete contribution to many fields of the botanical research, the Botanical Garden had a long period (1963-1970) of neglect. After the acquisition by the University of L'Aquila in 1971, the Garden started to take on an increasingly strong connotation of education and teaching, directing its efforts to raise public awareness and to inform about the activities carried out inside, in a scientifically correct and accessible way. The Alpine Garden of Campo Imperatore "V. Rivera", is located in the Gran Sasso massif, within the "Gran Sasso e Monti della Laga" National Park, at 2117 m asl. It plays an essential role in the conservation of high altitude plant species, with a particular attention to the endangered ones (1). The Gran Sasso is the highest mountain of the Central Apennines; from a geological point of view, it is composed by a broad carbonate platform originally developed on the margin of a lost ocean called Tethys. The cold climatic phases occurred during the Pleistocene greatly influenced the morphology of the central Apennines, where it is possible to recognize erosional and depositional features of glacial origin (2). The flora of the "Gran Sasso e Monti della Laga" National Park is important not only quantitatively but also qualitatively. In this region, in fact, there are more than 2400 entities, including many endemic and relict species of the quaternary glaciations, located in the high mountain areas (3). In addition to the considerable floristic richness, there is a wide articulation of vegetation types, related to various types of morphology, to several climate, lithological and soil types and to human activities. These plants and communities have acquired several adaptations to the difficult environmental conditions of high peaks during the long biological evolution (4). Among the most interesting species of high altitude vegetation, there are those of screes, cliffs, nival valleys, various pasture types, whose richest collection is located on the Altopiano of Campo Imperatore. The conservation of plant biodiversity, for both the species of agronomic interest and the native flora, is a topic currently drawing an increasing attention. Climate change is considered one of the major risk factors for biodiversity, as it affects the distribution of species and determines the displacement of distribution areas, both in latitude and in altitude. Therefore, the alpine flora appears to be endangered in the low and hot mountains, such as pre-Alps and Apennines, where there is no possibility of going up in altitude and where species in the highest peaks are merely isolated populations. Being able to halt the loss of biodiversity is a complex task, because the different levels of biological organization (genes, individuals, populations, species and habitats) must be preserved, as well as the interactions existing between them and the outside environment, considering, at the same time, the anthropic pressure. The Alpine Botanical Garden of Campo Imperatore is involved both in the collection of scientific data for the identification of high altitude species and in the cultivation and propagation of entities at risk (5). The areas of scientific research sponsored by the Alpine Garden are those relating to systematics, biology, physiology, phytogeography, ecology, high altitude flora. The Garden is managed by Department MeSVA in collaboration with the National Forest Service L'Aquila – Biodiversity Office.

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## 4.6 = A “FREUD GARDEN” AT THE UNIVERSITY TOWN OF PALERMO (SICILY)

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Several gardens in Italy have been named after Sigmund Freud (1856-1939), the founder of psychoanalysis. There are found at Rome, Pisa, Bologna, Arezzo and Ravenna; the latest has just been open in the Parco d'Orléans, the Palermo University town. It has been located in a partly green area in front of the building where the Department of Psychology is found. It has been carried out on the initiative of the Italian Society of Psychoanalysis supported by the Psychology and BIONEC Departments of Palermo University, with the aid of the Botanical Garden, the Palermo Municipality, and its service company AMAP. It has been strongly desired by the president of Palermo Psychoanalysis Centre, Professor Malde Vigneri, to remember the Freud stay at Palermo and his great interest in plants and flowers he was used classifying and describing.

The peculiarity of this garden consists of a flowerbed where the plants in the Freud dreams are cultivated: from musk to lilac (*Syringa vulgaris*), cardoon (*Cynara cardunculus*), lily of the valley (*Convallaria majalis*), lily (*Lilium candidum*), asphodel (*Asphodelus microcarpus*), violet (*Viola odorata*), carnation (*Dianthus caryophyllus*), cherry tree (*Prunus avium*), Bengal rose (*Rosa chinensis* s.l.), camellia (*Camellia japonica*). These plants were in part collected in the field and transplanted *ad hoc*, then labelled using their both popular and scientific names. Some preexisting trees, such as bitter orange (*Cytrus aurantium*), white mulberry (*Morus alba*) walnut (*Juglans regia*), remember some famous personalities of psychoanalysis: among these, the pupil of Freud princess Alessandra Wolff (1894-1982) – born in Lettonia, married to Giuseppe Tomasi di Lampedusa, she was living in Palermo – together with the whole Italian school including Francesco Corrao (1922-1994), Francesco Siracusano (1919-2007), Edoardo Weiss (1897-1989), Nicola Perrotti (1897-1970), C. Luigi Musatti (1897-1989), Eugenio Gaddini (1916-1985), Luciana Nissim Momigliano (1943-1998) and, in addition, Anna Freud (1895-1982) and Melanie Klein (1882-1960) from Austria, Sandor Ferenczi (1873-1933) from Hungary, Wilfred Bion (1897-1979) and Donald Woods Winnicott (1896-1971) from England, Jaques Lacan (1901-19818) and André Green (1927-2012) from France, Carl Gustav Jung (1875-1961) from Switzerland. A wild olive tree (*Olea europaea* var. *sylvestris*), was transplanted in the opening morning, in memory of Sigmund Freud.

The analyst of dreams reached Palermo on 9 September 1910; like many learned Middle-European people he was attracted by the South. He was astonished by the wonder city that was magnificent during the Belle Epoque – also thanks to economic rising of the Florio family –. In that year, several important cultural institutions arose in the capital of Sicily: among these, the modern art Gallery and the Philosophy library founded by Giovanni Gentile, while the Statue of Liberty was erected. Freud wrote his wife praising the beauties of landscape and the luxuriant Mediterranean vegetation: “I do not say you how many beauties I saw and whatever fragrances I enjoyed. Owing to magnificent flowers, parks and public gardens autumn is forgotten”.

The Freud Garden in the university town, owing to its diversity and well defined floristic elements, is identified as a little garden related to the Palermo University and, consequently, its Botanical Garden.

#### 4.6 = THE ROLE OF HISTORIC GARDENS IN THE CONSERVATION OF LICHEN BIODIVERSITY. THE CASE STUDY OF THE BOTANICAL GARDEN OF ROME

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In urban and peri-urban areas, historic gardens represent islands of plant diversity. This is even more true considering the lichen flora: the epiphytic species find refuge from the drought and rising temperatures characteristic of the city and have access to unusual substrates consisting in several kinds of phorophytes, also centenary trees, which are usually not common outside the gardens. The epilithic and endolithic lichens, while taking advantage of the same favorable environmental conditions, are often hosted in optimal microhabitats, e.g. fountains surrounded by vegetation.

The Botanical Garden of Rome, founded in 1883 on the existing seventeenth-century garden of Villa Corsini, is a typical case. Located in the centre of Rome, it covers an area of 11 hectares and occupies the north-eastern slopes of the Janiculum and part of the underlying fluvial terraces of the Tiber. The garden has little changed in the last 250 years, many of the architectural elements date from the eighteenth century: the Fountain of the Tritons was built around 1750 by Ferdinando Fuga, the Monumental Staircase, also by Fuga, dates from 1732.

Lichens of the Botanical Garden have been studied for the epilithic colonization of the fountain (1) and the staircase (2), the first studies on the epiphytic flora dating back to 1999 (3). A total of 72 taxa were detected. The most interesting woody flora for the study of lichens consists of the largest and oldest trees planted at the beginning of the century, after the transformation of the garden of Villa Corsini, and some pre-existing specimens, such as oriental planes of the monumental staircase. There is also a small group of evergreen oak and other deciduous trees, which are fragments of the ancient forest on the slopes of the Janiculum, survived to the present day under subnatural conditions.

Many epiphytic lichens, e.g. *Hyperphyscia adglutinata*, *Physcia adscendens*, *Amandinea punctata*, *Candelaria concolor*, are common in urban areas or where human activity is intense, while other species are of both ecological and biogeographical interest (4): *Lecanactis amylacea* and *Dimerella tavaresiana* usually occur in ancient, undisturbed forests; *Chaenotheca hispidula* is a pin lichen which grows in the crevices of old trees; *Anisomeridium bifforme*, *Bacidia circumspecta*, *Opegrapha niveoatra*, *Waynea stoechadiana* are rare in Italy. Among epilithic species, in addition to the most common lichens, a few are rare: e.g. *Opegrapha mougeotii* which abundantly grows on the containment walls of the staircase, especially on north-facing wall.

A clean-up of lichens on the monumental staircase (Fig. 1) was recently carried out (5). The restoration, motivated by the chromatic impact they caused, has effectively depleted the lichen biodiversity. Recently, several initiatives have been launched – e.g. the "Adopt a Monument" of the Italian Lichen Society (6) - aimed to increase awareness of the institutions and superintendents of Cultural Heritage, to promote the conservation of the biological element, if there is no manifest biodeterioration.



Fig. 1a-1b. Before and after restoration

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#### 4.6 = THE PASSION FLOWER COLLECTION AT THE HANBURY BOTANICAL GARDENS: HISTORICAL INVESTIGATION AND A PLAN FOR FUTURE INTRODUCTIONS

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Passion flowers are among the most ornamental of the historical collections at Hanbury Botanical Gardens at la Mortola (Ventimiglia, IM, I)

Due to the beauty attributes of the exotic flowers and leaves, and their flowering all the year round together with the abundance of blooms and exuberant foliage, Passion flowers have been used in European greenhouses and gardens since their introduction to the Old World around 1625 (1).

Passion flowers were introduced at la Mortola by Thomas and Daniel Hanbury in the autumn of 1867 (2) and are mentioned among the plants procured from Hyères that would not grow at all in the open air in England (T. Hanbury, in 3).

They experimented also grafting of rare and tender species and hybrids on the robust *Passiflora coerulea* (D. Hanbury, MS.).

In two different letters (21<sup>st</sup> and 22<sup>nd</sup> October 1868) T. Hanbury wrote about Passion flowers growing on the arch and walls of the garden and the year after (12<sup>th</sup> February 1869) on the plantation of one *Passiflora* 'Impératrice Eugénie' on the South face of the old terrace wall (T. Hanbury, in 2).

Passion fruit *Passiflora edulis* is among the fruiting plants first introduced in Italy and the Riviera at La Mortola to study their commercial potential (4). Ludwig Winter, curator and head gardener at la Mortola forwarded to T. Hanbury some passion fruits (*Passiflora edulis*) writing "of which there was a great quantity" (L. Winter, in *litteris*).

Historical investigation on catalogues of the plants cultivated in the garden [(5), 18 records; (6), 15 records; (2), 19 taxa; (7) 20 taxa; (8) 8 records], articles (9) and ancient documents of the Hanbury Archives (*Accounts for plants and seeds and for garden works/ Quietanze di piante e semi e di lavori per il giardino, 1877-1906, GBH, AH; Orders to gardeners 1869 to 1884/ Istruzioni ai giardinieri dal 1869 al 1884 IISL, AH; letters, IISL, AH*), compared with some more recent records, enabled us to reconstruct, to a certain degree, the history and the dynamics of the original collection.

The results of the research can help us to identify species and cultivars that could be successfully reintroduced in a plan for the restoration of the collection.

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#### 4.7 = GAS EXCHANGE AND CHLOROPHYLL *a* FLUORESCENCE AS SCREENING TOOLS TO EVALUATE THE RESPONSE OF DIFFERENT WILLOW CLONES TO ZINC STRESS

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The importance to protect soils is key issue in their management in order to improve the services they provide. The fundamental ecosystem services provided by soils, with extensive economic, ecological, and sociological influences on the wellbeing of the human society, are jeopardized by many contaminants, and among these, the heavy metals are the most common in the environment (1). In this context phytoremediation approach, which uses the plants to decontaminate the soils by heavy metal, may be used (2). The potential European and US markets for phytoremediation was estimated about 36–54 billion US\$, with a share of 1.2–1.4 billion US\$ for the removal of heavy metals from soil (3).

The objective of this study was to evaluate the ability of *Salix* sp., used in the removal of contaminants in riparian zones, to withstand different toxic levels of Zn in soil. Two clones of *Salix alba* (“1962” and “1968”) and two hybrid clones of *Salix matsudana* (“Drago” and “Levante”), used for biomass production in Northern Italy and selected for Short Rotation Coppice (SRC), and one clone of *S. alba*, coming from the natural growing population of the contaminated site in Sacco River Valley (Central Italy) were analyzed. In particular, the effect of Zn on gas exchange, chlorophyll *a* fluorescence, relative photosynthetic pigment content and biometric parameters has been examined. Rooted cuttings were treated for 15 days in pots, under controlled environmental conditions (Fig.1), with 0 (control), 300 mg/kg, 750 mg/kg and 1500 mg/kg of Zn added to the soil as zinc chloride (ZnCl<sub>2</sub>). The willow clones exhibited different responses to increasing Zn concentrations in the soil: no effects on ecophysiological parameters were observed at the low Zn concentration (300 mg/kg), while a decrease of gas exchanges was measured at the medium and high Zn concentrations (750 and 1500 mg/kg). In particular, after 3 days of the exposure, a reduction of assimilation rate (A) due to stomatal limitation was observed in Levante and 1962 clones at 750 mg/kg and in all clones at 1500 mg/kg. 1962 resulted to be the most sensitive to metal stress at both concentrations: at the end of the treatment with 1500 mg/kg, it showed an inhibition of stomatal conductance (g, Fig.2) and a decrease of photosynthetic performance (PI<sub>total</sub>), in agreement with other studies conducted under abiotic stress (4), as well as a reduction of the relative chlorophyll content and leaf area. In a different way, the maintenance of assimilation rate and the increase of photosynthetic Performance Indices (PI<sub>abs</sub> and PI<sub>total</sub>) in the Sacco clone, at both medium and high Zn concentrations may be regarded as a photosynthetic compensatory mechanism, favoring acclimation to the environmental stresses. Therefore, the *S. alba* cutting obtained from the contaminated area (Sacco clone) resulted less sensitive to Zn and it showed the higher capability to withstand elevated Zn levels than the other studied clones, which are selected to produce high biomass in natural sites. For this reason, the Sacco clone could be investigated as a potential restorer of areas contaminated by Zn, in view of the possible use in the phytoremediation in the field.



Fig.1. Willow clones

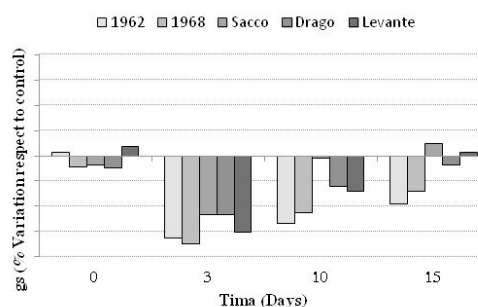


Fig.2. Stomatal conductance (gs) in plants treated with 1500 mg/kg of zinc

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## 4.7 = RECOVERY AND MANAGEMENT OF SECONDARY GRASSLANDS OF THE HABITAT 6210\*

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The recovery and conservation of secondary grasslands of the habitat 6210\* “Semi-natural dry grasslands and scrubland facies on calcareous substrates (*Festuco-Brometalia*) (\* important orchid sites)” in Central Southern Apennines (Italy) presents many difficulties.

In general, there are two main aspects: the grasslands have been completely abandoned or have been under-utilized. In both cases, the first colonisation is by *Brachypodium rupestre* or *B. genuense* (the first species below 900 m a. s. l. and the other at higher altitudes), and then the invasion of shrubs. In the second case, grazing and mowing are maintained but the surfaces used are very small, and then the same dynamic occurs in the patches of the pastureland surfaces not used.

The elimination, partial or total, of the species that have invaded the grasslands, are herbaceous or shrubby, however, it is not simple. Some studies have been carried out on these subjects, according to the following methodology.

- i) Assessment of the qualitative characteristics of the grasslands in terms of phytocoenotic biodiversity and status of the post-abandonment dynamics;
- ii) Analysis of the dynamics of the processes of invasion of herbaceous and shrub species;
- iii) Monitoring of bird species before and after restoration measures;
- iv) Improvement of the grasslands and simultaneously recovery of areas invaded by shrubs that are appropriate to the restoration of the grazing;
- v) Preservation of the natural balance by maintaining groups of shrubs and conservation and eventual recovery of the wetlands present;
- vi) Absolute respect of the hydrogeological status through the preservation of the soil from erosion, promoting water drainage of surface waters.

The recovery of grasslands was more difficult than expected as we have encountered considerable difficulties. We believe that it is necessary to remove the herbaceous species, especially the grasses of *Brachypodium* genus which do not whet animals appetite due to the consistency of their leaves that are rich in silica and lignin (1, 2) and long rough hairs. Moreover, animals risk to die if they are obliged to feed with these plants (3).

The reduction of shrubs is also a complex phenomenon to be implemented, indeed the cutting is not sufficient because some species reject from the root.

In the market, seeds of native herbaceous species are not available, so the reseeded with these seeds is dangerous because they can hybridize with native species and endemic species, typical and characteristic of Apennine communities are completely absent in these commercial seeds. Indeed, it is very difficult to achieve a trade of native herbaceous species that could be used for this aim as for many other purposes.

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## 4.7 = LANDSCAPE DIVERSITY OF THE NORTHERN CAMPANIA SANDY COAST

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Sandy coast ecosystems own high naturalistic and landscape values due to the various aspects of their biodiversity (1). Nevertheless they are among the most globally threatened and vulnerable due to direct and indirect human pressures (2, 3). The anthropogenic pressure results in the fragmentation or the loss of the coastal ecosystems as well as the services that they can provide (4). Most of them can include habitats *sensu* Directive 92/43/CEE and listed in the annex I because considered of Community interest and in need of conservation measures.

The sandy coast of northern Campania extends for more than 45 km from the Garigliano plain to the Phlegrean Fields and it marks the western boundary of the Campanian Plain. The landscape has been shaped since the ancient times by settlements, marshes drainage, deforestation and afforestation. In the last decades human pressure has increased dramatically by intensive urbanization, touristic exploitation and intensive farming. However the resulting landscape still preserves traits of high floristic and vegetation values (5,6, 7, 8, 9; 10). Therefore along the coast eight Sites of Community Interest (SCI) according to the above mentioned European Directive, have been established.

The present study aims to:

- i) produce a detailed land cover map as a tool for the investigation on the landscape pattern and processes;
- ii) analyse the biodiversity in species, communities and habitats for the traits of the coast affected by different levels of human pressures;
- iii) define management priorities and emergencies for an effective conservation strategy of the coastal habitats.

The first results showed that the landscape is affected by a simplification of the natural habitats spatial patterns and suffers a decrease in species richness, as recorded for other areas (11). Moreover a generalized fragmentation of the landscape pattern and a high frequency of the Artificial-Natural contacts is reported.

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## 4.7 = LOW CO(A)ST HABITAT NATURA 2K RESTORATION AT KM 0

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The Low Coast dune system is the result of erosion of land surfaces due to the action of the water. The quantity of sand present along the coast is in relations mainly with geomorphology and geology; reflects the climate and sea activities; is often influenced by anthropic factors in the water catchment areas. These main factors are integrated by the action of the wind and plant communities that contribute to the establishment of complex system where is often difficult to plan an actions being certain for final results.

The Southern Ionian coast of Calabria hosts the most important nesting area of the loggerhead sea turtle (*Caretta caretta*) in Italy, and the LIFE CARETTA project aims to implement multiple and integrated actions for the conservation of the species and its habitats.

The project area extends along the coast from Melito Porto Salvo up to Capo Bruzzano and includes entirely or partially 6 SCI. The area is characterized by the vegetation series typical of the Mediterranean sea dunes, with communities referable to *Cakiletea maritima*, *Ammophyletea*, *Helichryso-Crucianelletea maritima*, *Tuberarietea guttata* and to the following Natura 2000 habitat types: 1210 Annual vegetation of drift lines; 2110 Embryonic shifting dunes; 2120 Shifting dunes along the shoreline with *Ammophila arenaria* (white dunes); 2210 *Crucianellion maritima* fixed beach dunes; 2230 *Malcolmietalia* dune grasslands; 2260 *Cisto-Lavanduletalia* dune sclerophyllous scrubs.

In spite of the strategic importance of the area for *Caretta caretta* and other Natura 2000 species, the dunal complex has been strongly modified by human activities. The increasing of sea erosion is evident and, together with direct human pressure, it provokes the chaotic dynamics of dunal vegetation in the series.

For the scope of the project, taking in consideration the amplitude of the different environmental factors in GIS environment, four areas have been selected for restoring actions. The first step was to infer the real chance of success for direct actions of conservation in different ecological conditions, the prioritization and the cost-benefit evaluation. Secondly an accurate sociological analysis is carried out for the stakeholders involvement in the process of conservation and restoration actions.

In order to deal with biotic elements of the systems, two main approaches have been adopted: the facilitation of the natural evolution and, when necessary, the complete restoration of the dunal vegetation. For both techniques local populations of edificatory species are individuated and a plan for germplasm collection from the so individuated source areas was prepared.

The target species for germplasm collection are dune-building and Mediterranean scrub species. Collections will be carried out within the project areas and in adjacent areas, according to species presence and population dimensions. In this way, the genetic purity of local populations will be protected and genetic erosion avoided. Prior to multiplication, seed germination will be tested in laboratory. On the basis of germination tests results, it will be chosen the best protocol for seed multiplication. Then, plants will be multiplied in a protected environment (i.e. glasshouses) or directly in the field to be restored. Similarly, the actual multiplication of plant through vegetative propagation will be preceded by tests aimed to identify the best protocols. Multiplication will be carried out in the same areas where plants will be used for restoration or in adjacent areas, possibly without building new facilities, in order to reduce costs and CO<sub>2</sub> emissions. Multiplied germplasm will be used for the restoration of target habitats. At first, small-scale experiments will be tried; revegetation will include both plots where seeds will be sown, as well as transplanting of plants. Then, depending on sites features, plant reproductive traits and the results of the experimental plots, germplasm will be re-introduced in wider areas, not covering the whole area of intervention, and it will be exploited the ability of plants to widen their area of presence through seed dispersion. This process will be repeated and restored areas will be constantly monitored in order to both identify the areas that require further interventions and correct the protocols on the basis of the information collected.

During all phases of the project implementation the local communities will be try to be involved. Especially the young generations will be targeted with communication programs. Simultaneously, all phases of the actions will be critically analyzed from administrative point of view in order to integrate the obtained results into the acts at regional and local level.

#### 4.7 = ECOLOGICAL AND SOCIOLOGICAL VALUES: THE BASIS FOR A INTEGRATED MANAGEMENT OF URBAN GREEN SPACES. THE CASE STUDY OF FLORENCE CITY

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In Europe 75% of population lives in towns and it is expected that in 2020 people residing in urban environment will be 80% (1). Preservation and increase of diversity of habitats and species in urban areas is a crucial theme of the strategy to halting the loss of biodiversity after 2010 (2): 97 Nature sites exist in 32 big European towns (3), including some capitals (London, Paris, Prague, Rome). The national law (n.14 January 2013) "Standards for the development of the Urban Greenery" indicates some essential for management of urban green that overcomes an exclusively aesthetic vision and supports ecosystem functions performed by green spaces (maintenance of biodiversity, prevention of hydrogeological, water conservation, air quality improvement). However, many of the recreational activities conducted in the green areas of the city are in conflict with the actions of conservation of habitats and species and processes remain uncertain and the means by which to increase the quality of urban ecosystems in contexts where it is often a high level of public interest and social participation. The study investigates the interactions between social and ecological functions assumed by the green areas in the urban environment, in order to develop guidelines for management aimed at reconciling these two aspects. Based on the paradigm of the urban ecosystem as a complex of habitats, it is possible to think of the green spaces of the city as part of a system that fits into a larger space and takes a real role at the landscape level: the macro-areas, connected with the suburban matrix, may constitute "source"; others play the role of "sink"; others will form the connection elements. They are part of this system public and private areas, indistinguishable from the ecological point of view even if they are just spaces managed by government the most suitable places in which to experiment models ecologically and economically sustainable.

The first part of the research has focused on the identification of some parameters can succinctly express the level of ecological function and biological richness of the green areas of the city. For this purpose, sampling was carried out of 64 green areas (gardens or public parks) of the City of Florence, spread into the urban fabric. The data collected can be divided into 1) quality: number of habitats and number of plant species distributed in the tree layer (A), intermediate (I) and herbaceous 8 (E); 2) quantity: coverage (%) of 3 layers (A, I, E), relative cover (%) of native species, naturalized and alien in each of the 3 layers (A, I, E); Soil sealing (% of land built; % paved ground with asphalt; % paved ground with stones; % bare soil, % vegetated soil). The collected data were used to develop a synthetic index (IAV) that can be used to identify specific guidelines for the environmentally sound management of urban green areas. The parameters involved IAV merged into 4 main aspects: the floristic quality (QF), habitat quality (QH), soil quality (SQ) surface of the green (A). In addition to characters of composition, IAV also considers the quality of the surroundings in which it is immersed, the green area (QC), through the degree of naturalness / artificiality and contrast with the patches contained within a circular buffer ( $r = 500$  m) built around the centroid of the area under consideration. The second part of the study focused on a macro-town area that includes urban and hilly and where there are different types of green spaces. These were also applied according to a composite index that evaluated the social features: Quality and offer facilities for recreation, value recognition, service and decor, Safety Factors pressure, Accessibility. Through a careful collection of experiences and research developed in other national and European contexts has been defined a classification of urban green areas in Florence and was a simulated planning process for the redevelopment of urban green areas in key ecological and social perception, identifying some protection objectives and outlining the priority for each of these lines of best management practices that they considered the advantages, problems and social implications and informative.

1) EEA, European Environment Agency (2010). Report No 10/2010, Urban sprawl in Europe. The ignored challenge. European Commission/Joint Research Centre. file:///C:/Users/fitogeo/Downloads/eea\_report\_10\_2006.pdf. Downloaded on: 6 August 2014

2) K. Sundseth, G. Raeymaekers (2006). Biodiversity and Natura 2000 in urban areas. Nature in cities across Europe: a review of key issues and experiences. Brussels, Ecosystems LTD, 2006

3) N. Müller, P. Werner, J. G. Kelcey (2010) Urban Biodiversity and Design, Blackwell Publishing Ltd.

4.7 = ETHNOBOTANICAL USES OF *MOPANE* WOODLANDS IN SOUTHERN ANGOLA

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*Mopane* ecosystem play an important role in the livelihood of people living in southern Angola, contributing to satisfy basic human needs such as food, medicine, fuelwood and building materials. However, over-exploitation of plant resources and unsustainable harvest practices can potentially degrade forests. The aim of this study was to document the use of *Mopane* plant products in local communities, with a discussion of the ecological importance and conservation status of the species used. Fieldwork took place in 7 communities in the municipality of Bibala, province of Namibe (13°21' S, 14°46' E), southern Angola. The climate is seasonal semi-arid, with an average annual temperature of 21.6°C and an average rainfall of 300 to 600 mm. Vegetation is mostly composed of woodlands (*mata de mutuatê*), with *Colophospermum mopane* (Kirk ex Benth.) Kirk ex J. Léonard (*mutuatê*) as the dominant species alone or in association with *Adansonia digitata* L. (*imbondeiro*). We conducted semi-structured interviews with 69 informants (51±14 years, 35 men (51%) and 34 women (49%)) about their knowledge, use and harvesting practices of useful *Mopane* plants. Quantitative ethnobotanical indices such as *Cultural Value Index* (CV), *Relative Importance Index* (RI), *Use Value Index* (UV) and *Informants Consensus Factor* (ICF) were applied in order to identify the most used and relevant species within the studied area. A survey on local *Mopane* vegetation was also carried out in order to assess abundance and distribution of woody plants cited in the interviews. At this purpose a total of 36 plots (surface area: 500 m<sup>2</sup>) were established in the study area. A Conservation Priority index (Dzerefos and Witkowski, 2001) was also applied to rank conservation values of each locally used woody species

One hundred and twenty three plants cited by the informants were botanically identified; 1651 citations, for 8 sectors of use and 37 categories of use, were totally recorded. The species belong to 55 families, among which the most represented is Fabaceae (20 species), while the largest number of citations was recorded for Malvaceae with 259 citations. The most cited species were: *mukua* (*Adansonia digitata* L.) (60 informants, RI=0.53, CV=0.09), *omumbe* (*Berchemia discolor* (Klotzsch) Hemsl.) (50 inf., RI=0.66, CV=0.25), *mumpeke* (*Ximena americana* L.) (48 inf., RI=0.75, CV=0.34) and *kuanana* (*Aristolochia albida* Dunch) (41 inf., RI=0.4, CV=0.04). Mainly parts of the plant used are underground organs (roots, bulbs, tubers, rhizomes) (16 %, UV=0.96), fruit (16%, UV=0.94) and leaves (16%, UV=0.91).

Among the woody species observed in vegetation plots, 11 were cited as useful during the interviews (33% in the medicinal category, 33% in the agropastoral category, 14% in the food category, 3% in the handicraft category and 5% in both veterinary and ritual categories). Results of conservation ranking showed that most of species can be moderately collected within a careful conservation management.

In conclusion, this study shows that the communities investigated rely heavily on local forest products for their daily subsistence requirements in medicines, food, firewood/charcoal and building materials. However, over-exploitation and destructive collection seems to threaten the survival of some of the woody species used. A sustainable approach including the involvement of local communities in the management of woody species is recommended.

We are indebted to all the communities' villagers who shared with us their knowledge on plant uses. This research was funded by NGO COSPE (Cooperazione per lo Sviluppo dei Paesi Emergenti) through the PIDEFA (Integrated Project for the Protection and Development of Angolan Coastal Forests) Program financed by European Community.

1) Dzerefos CM, Witkowski ETF: Density and potential utilization of medicinal grassland plants from abe bailey nature reserve, South Africa. *Biodivers Conserv* 2001, 10: 1875–1896

## 4.7 = HABITAT PECULIARITY AND MANAGEMENT PROBLEMS OF SILA MT. GRASSLANDS

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The Sila Massif (SIItaly) is a territory of remarkable phytogeographic interest because of its geographical position (centre of the Mediterranean region), geology (a granitic “island”) and peculiar bioclimatic characteristics. According to Rivas-Martinez & Loidi Arregui (1999), the bioclimate of the upper parts of Sila is characterized by supra-temperate submediterranean thermotype and marginally also by the supramediterranean one, with subhumid or humid ombrotypes. The mountain vegetation of Sila, between 1000-1927 m asl, is characterized by beechwoods, often alternating with Calabrian pine (*Pinus nigra* subsp. *calabrica*) forests, thorny cushion-like shrub communities, meadows and wetlands. Geomorphology, structure of soil, water gradient, snow persistence and grazing influence the distribution and structure of the pastures and determine a high diversity of herbaceous communities in few meters. Some of them can be referred to N2K habitat types and motivated the institution of 25 SCIs, included in the National Park of Sila Mt. Peculiarity of Sila grasslands is due to the mixture of Mediterranean elements with Temperate ones. The latter is particularly evident in mesophilous and wet meadows, that represent a refuge for many relict circumboreal species at their Southern distribution limit (*Viola palustris*, *Molinia caerulea*, etc.). In turn, xerophilous grasslands are dominated by endemics belonging to oro-Mediterranean groups such as *Astragalus parnassi* subsp. *calabricus*, *Armeria brutia*, *Anthemis cretica* subsp. *calabrica*, *Hypericum calabricum*, etc. The interpretation of this plant community mosaic is not simple and needs deep phytosociological studies for the individuation of the syntaxa, their ecological role and their relations with NATURA 2000 habitat types.

The knowledge for the oro-mediterranean xerophilous grassland (*Festuco-Brometea*) of the area of study is revised and integrated through here proposed *Hyperico calabricae-Festucion paniculatae*. This vegetation together with *Festuca circummediterranea* ones are analyzed in regarding to the dry cushion like vegetation and their relation to the Central Apennines and Sicily mountains. The main contributions to the *Molinio-Arrhenatheretea* regards *Secalo strictae-Arrhenatherion elatioris* belonging to *Arrhenatheretalia elatioris* where the high grasses are dominant. The techniques for hay production altered to the pasture in addition to the soil water availability give origin of four well differentiated associations three of them described for the first time. The geomorphology of the territory favorite the presence of extended periodically dried shallow water bodies populated by hygrophilous e mesohigrophilous communities. The *Deschampsia caespitosa* and *Carex hirsuta* formations are described in relation of the water gradient and pasture.

An adequate habitat mapping is necessary for a correct quantification of main threats and the implementation of conservation measures and monitoring programs. The choice of an appropriate scale and map units, is not always simple, and in many cases it is not possible to identify the area occupied by a singular element of the mosaic. On the whole, with different intensity, all habitat types are influenced by grazing (mostly cattle). On the other end, abandonment of pastoral systems favors vegetation evolution towards forest types and provokes a general reduction of pastures.

We can definitely say that grazing and its appropriate regulation and monitoring are the key for a long-term conservation of the peculiar complex of Sila grasslands and its rich biodiversity pool.

Meadows are also seriously influenced by any changes in hydrographic functioning. The progressive reduction of relictual patches of mires (7140) and their connected habitats (6410, 6430) can be attributed to the simultaneous effect of direct human pressures (grazing and water exploitation) and global climate change.

In order to obtain successful conservation results and to reach Dir.92/43 objectives, for the oro-Mediterranean pastures, it is important to consider an adaptative and dynamic synphytosociological approaches taking in consideration all elements of the grassland mosaic.

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