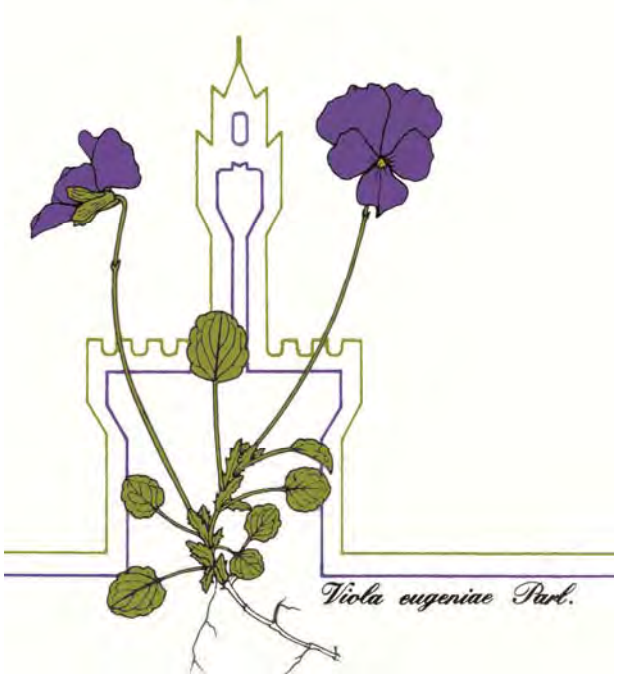
112° Congresso della Società Botanica Italiana

IV INTERNATIONAL PLANT SCIENCE CONFERENCE (IPSC)

Parma, 20 - 23 September 2017



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UNIVERSITÀ DI PARMA

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112° Congresso della Società Botanica Italiana onlus

IV INTERNATIONAL PLANT SCIENCE CONFERENCE (IPSC)

Parma, Campus Universitario, 20 - 23 September 2017

Programme

Wednesday 20 September 2017

8:30-9:30 Registration and poster installation

9:30-11:00 Opening Ceremony

General Session

(chairpersons: L. Sanità di Toppi, M. Tomaselli and C. Siniscalco)

11:00-11:45 • **Stephan Clemens**, University of Bayreuth, Germany (40 + 5 min)
"ZINC-ing about plant metal homeostasis and tolerance"

11:45-12:30 • **Rob Brooker**, James Hutton Institute, Aberdeen, Scotland (40 + 5 min)
"Facilitation in plant communities: understanding processes and consequences from mountain tops to crop fields"

12:30-14:00 Lunch

12:30-13:00 Meeting of the Working Groups and election of new board members

Symposium 1

FROM SPECIES ECOLOGY TO FUNCTIONAL TRAITS IN A "GLOBAL CHANGE" CONTEXT

(chairpersons: L. Bragazza and M. Marignani)

Key words: plant ecology, plant diversity, functional traits, climate change, land use change

14:00-14:35 • **Eric Garnier**, Centre d'Ecologie Fonctionelle & Evolutive, CNRS, Montpellier, France (30 + 5 min)
"Functional traits in plant ecology: advances, limits, challenges"

14:35-15:35 **Communications**

- **Stefano Chelli,** Michela Marignani, Elena Barni, the Italian Plant Traits Consortium (12 + 3 min)
"Plant Traits 2.0 in Italy: back to the future"
- **Juri Nascimbene**, Renato Benesperi, Peter Othmar Bilovitz, Alessandro Chiarucci, Matteo Dainese, Paolo Giordani, Lorenzo Marini, Helmut Mayrhofer, Pier Luigi Nimis (12 + 3 min)
"Patterns of functional traits elucidate lichen community assembly under a global change scenario"
- **Giacomo Puglielli**, Francisco J. Cuevas Román, Rosangela Catoni, José M. Moreno Rojas, Laura Varone, Loretta Gratani (12 + 3 min)
"Do changes in leaf morphology control the photosynthetic process in Mediterranean *Cistus* spp. under suboptimal air temperature and subsequent recovery?"
- **Enrica Zalloni**, Giovanna Battipaglia, Paolo Cherubini, Matthias Saurer, Veronica De Micco (12 + 3 min)
"Tree-ring Intra-Annual-Density-Fluctuations as functional indicators of responses to different environmental conditions in *Quercus ilex* L. (Vesuvius, southern Italy)"

15:35-15:55 Coffee break

15:55-16:30 • **Bjorn J.M. Robroek**, Biological Sciences, University of Southampton (UK) (30 + 5 min)
"Decoupling taxonomic and functional turnover in European peatlands"

16:30-17:30 **Communications**

- **Andrea Mondoni**, Maria Tudela-Isanta (12 + 3 min)
"Do germination traits contribute to species distribution in alpine habitats?"
- **Michele Carbognani**, Marcello Tomaselli, Alessandro Petraglia (12 + 3 min)
"Reproduction of two alpine tundra species under climate warming"
- **Ludovica Oddi**, Edoardo Cremonese, Gianluca Filippa, Marta Galvagno, Mirco Migliavacca, Umberto Morra Di Cella, Consolata Siniscalco (12 + 3 min)
"Long-term monitoring of a subalpine grassland: inter-annual variations in phenology, biomass production and plant community structure among nine growing seasons"
- **Matteo Petit Bon**, Katarina Gunnarsdotter Inga, Freja Fagerholm, Tove Aagnes Utsi, Ingibjörg Svala Jónsdóttir, Kari Anne Bråthen (12 + 3 min)
"Herbivore assemblages and seasonality determine nitrogen and phosphorous content in tundra plants"

17:30-18:30 Poster session

18:30-19:00 Meeting of the Working Groups and election of new board members

Thursday 21 September 2017

Symposium 2

PLANT AND FUNGAL RESPONSES TO ABIOTIC STRESS

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

Key words: stress response, environmental pollution, cell signaling, plant-environment interactions

09:00-9:35 • **Ilse Kranner**, University of Innsbruck, Austria (30 + 5 min)
"Elevated temperature and drought stress trigger molecular switches in developing seeds that affect seed quality"

9:35-10:35 **Communications**

- **Lara Perrotta**, Antonio Maniga, Malvina Urbani, Diego Albani (12 + 3 min)
"Characterization of the MAPK and MKK gene families of *Lactuca sativa* and analysis of their expression under abiotic stresses"
- **Maria De Benedictis**, Luigi Sanità di Toppi, Olena K. Vatamaniuk (12 + 3 min)
"Phytochelatin synthase 1 regulates iron homeostasis in *Arabidopsis thaliana*"
- **Marianna Pacenza**, Leonardo Bruno, Antonella Muto, Adriana Chiappetta, Maria Beatrice Bitonti (12 + 3 min)
"Impact of DNA methylation status on plant response to Cd explored through a transcriptomic analysis"
- **Grazia Cecchi**, Pietro Marescotti, Andrea Ceci, Simone Di Piazza, Anna Maria Persiani, Mirca Zotti (12 + 3 min)
"Interactions between microfungi and sulfide mineralizations"

10:35-10:55 Coffee break

10:55-11:30 • **Francisco J. Corpas**, Consejo Superior de Investigaciones Cientificas, Granada, Spain (30 + 5 min)
"Peroxisomal nitric oxide (NO) metabolism under adverse environmental conditions"

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

- **Luuk W. Muthert**, Luigi G. Izzo, Giovanna Aronne (12 + 3 min)
  "Gravity vs. other external stimuli in seedling root tropisms"
- **Alessandro Silvestri**, Valentina Fiorilli, Laura Miozzi, Massimo Turina, Gian Paolo Accotto, Luisa Lanfranco (12 + 3 min)
  "Do arbuscular mycorrhizal fungi possess an RNA silencing machinery?"
- **Angela Cicatelli**, Francesco Guarino, Karina B. Ruiz, Stefania Biondi, Stefano Castiglione (12 + 3 min)
  "Effects of salinity and chromium on growth and element uptake and accumulation in the halophytic plant *Chenopodium quinoa* Willd."
- **Lorenza Rugnini**, Giulia Costa, Roberta Congestri, Laura Bruno (12 + 3 min)
  "Cu and Ni removal from aqueous media by green microalgae in single and bimetal solutions"

12:30-13:30 Lunch

13:30-14:30 Poster Session

Symposium 3

QUALITY, EFFECTIVENESS AND SAFETY OF BOTANICALS

(chairpersons: R. Bruni and P. Rubiolo)

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

14:30-15:05 • **Robert Anton**, Faculty of Pharmacy, University of Strasbourg, France (30 + 5 min)
"The role of traditional knowledge in the safety assessment of botanical food supplements"

15:05-16:05 **Communications**

- **Mariangela Marrelli**, Giancarlo Statti, Filomena Conforti (12 + 3 min)
  "*Muscari comosum* (L.) Mill.: metabolite profiling, anti-obesity and antioxidant potential of bulbs and aerial parts"
- **Manuela Mandrone**, Francesca Esposito, Angela Corona, Enzo Tramontano, Ferruccio Poli (12 + 3 min)
  "Application of metabolomics to improve *in vitro* bioactivities assays for plant crude extracts"
- **Maria Fernanda Taviano**, Khaled Rashed, Salvatore Ragusa, Angela Filocamo, Paola Dugo, Francesco Cacciola, Natalizia Miceli (12 + 3 min)
  "Antioxidant properties and phenolic profile of a hydroalcoholic extract obtained from the leaves of *Ficus vasta* Forssk. (Moraceae)"
- **Cinzia Sanna**, Daniela Rigano, Giuseppina Chianese, Andrea Maxia, Carmina Sirignano, Angela Corona, Carmen Formisano, Mauro Ballero, Enzo Tramontano, Francesca Esposito (12 + 3 min)
  "Secondary metabolites from *Limonium morisianum* Arrigoni, endemic species of Sardinia (Italy), as potential dual HIV-1 reverse transcriptase-associated RNase H and Integrase activities inhibitors"

16:05-16:40 • **Mauro Serafini**, Dipartimento di Biologia ambientale, Sapienza Università di Roma (30 + 5 min)
"The substantiation of claims for botanical food supplements in relation to traditional use"

16:40-17:40 **Communications**

- **Camilla Badiali**, Alessio Valletta, Giulia De Angelis, Gabriella Pasqua (12 + 3 min)
  "Effect of chitosan oligosaccharides and other biotic elicitors on root cultures and *in vitro*-grown plantlets systems of *Hypericum perforatum* L."
- **Marco Biagi**, Valeria Nencioni, Paolo Governa, Elisabetta Miraldi, Giulia Cettolin, Laura Cornara, Daniela Giachetti (12 + 3 min)
  "Leaf extract from Mount Amiata chestnut as a novel anti-photoaging and skin care agent"
- **Lidia Leporini**, Claudio Ferrante, Lucia Recinella, Giustino Orlando, Annalisa Chiavaroli, Sara Martinotti, Simone Carradori, Marcello Locatelli, Giulia Vecchiotti, Luigi Menghini (12 + 3 min)
  "Evaluation of protective effect of *Harpagophytum procumbens* DC. ex Meisn. root water extraction by microwave, biological activity on *ex vivo* rat colon inflammatory model and microscopic investigation"
- **Barbara Sgorbini**, Valeria Cagno, Cinzia Sanna, Cecilia Cagliero, Mauro Ballero, Andrea Civra, Manuela Donalisio, Carlo Bicchi, David Lembo, Patrizia Rubiolo (12 + 3 min)
  "*Salvia desoleana* Atzei & V.Picci: an essential oil with an interesting activity against Herpes Simplex Virus-2"

17:45-19:00 General Meeting of the Italian Botanical Society (members only)

21:00 Congress social dinner

Friday 22 September 2017

Symposium 4

MARINE AND TERRESTRIAL BIODIVERSITY STUDIES IN THE TIME OF BIOLOGICAL INVASIONS
(chairpersons: C. Totti and L. Peruzzi)

Key words: floristics, systematics, alien species, phycology, evolutionary ecology

9:00-9:35 • **Mark van Kleunen**, University of Konstanz, Germany (30 + 5 min)
"Global patterns and drivers of plant naturalization"

9:35-10:35 **Communications**

- **Gianniantonio Domina**, Gabriele Galasso, Fabrizio Bartolucci, Riccardo Guarino (12 + 3 min)
  "Ecological outline of the alien flora of Italy through the use of the Ellenberg's indices"
- **Angelino Carta**, Francesco Roma-Marzio, Marco D'Antraccoli, Brunello Pierini, Giovanni Astuti, Gianni Bedini, Lorenzo Peruzzi (12 + 3 min)
  "Assessment of invasive alien plants distribution through phylogenetic measures of biodiversity and environmental predictors: estimating current IAS diversity centres in Tuscany"
- **Federico Selvi**, Elisa Carrari, Andrea Coppi (12 + 3 min)
  "Impact of pine invasion on the taxonomic and phylogenetic diversity of a relict Mediterranean forest ecosystem"
- **Chiara Montagnani**, Rodolfo Gentili, Sandra Citterio (12 + 3 min)
  "Kudzu: subtle threat to native flora of a clear invasive alien plant"

10:35-10:55 Coffee break

10:55-11:30 • **Christine Maggs**, University of Bournemouth, UK (30 + 5 min)
"How should biologists respond to the global increase in invasive alien seaweeds?"

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

- Giuseppina Alongi, Clelia Gialli, **Francesca Toscano** (12 + 3 min)
  "On the occurrence of *Halophila stipulacea* in the Augusta Gulf (eastern Sicily, Mediterranean Sea)"
- **Anna Santin**, Emanuela Moschin, Maurizio Lorenti, Maria Cristina Buia, Isabella Moro (12 + 3 min)
  "Invaders in a changing world: *Caulerpa cylindracea* Sonder and ocean acidification"
- **Antonio Manghisi** (12 + 3 min)
  "Detection of alien macroalgal species in the Mediterranen sea using the DNA barcoding tool"
- **Rossella Pistocchi**, Laura Pezzolesi, Stefano Accoroni, Giorgio Honsell, Cecilia Totti (12 + 3 min)
  "Allochtonous microalgae as carriers of new toxins"

12:30-13:00 Congress Closure

13:00-14:15 Lunch

Post-Congress at the Botanical Garden of Parma University, via Farini 90

"Gli Orti Botanici e il loro ruolo nella terza missione dell'Università"

Special session organized in collaboration with "Gruppo di Lavoro per gli Orti Botanici e i Giardini storici della SBI" and "Settore Musei dell'Università di Parma"

15:00

- **Cristina Lo Giudice**, Pietro Pavone
  "L'Orto botanico, luogo di cultura *hortus apertus* alla città e ai suoi bisogni socio-culturali"
- **Gianni Bedini**
  "Orti Botanici verso la terza missione, per vocazione e non per trasformismo"
- **Lorenzo Peruzzi**
  "Il crescente impegno dell'Orto e Museo Botanico di Pisa nelle attività della terza missione, a seguito dell'inserimento nel Sistema Museale di Ateneo"
- **Mauro Mariotti**
  "I Giardini Botanici Hanbury e la terza missione"
- **Loretta Gratani**
  "Le azioni strategiche per la terza missione al Museo Orto botanico di Roma"
- **Giovanna Giomaro**, Anahi Elena Ada Bucchini, Laura Giamperi
  "L'Orto Botanico di Urbino: tra storia, immagini e terza missione"
- **Paolo Caputo**
  "La percezione dell'Orto botanico e le richieste della comunità: la terza missione all'Orto botanico di Napoli"
- **Michele De Sanctis**, Michela Pacifici, Ferdinand Bego, Petrit Hoda, Lulëzim Shuka, Stefano Martellos, Marjol Meço, Ermelinda Mamhutaj, Carlo Rondinini, Fabio Attorre
  "Developing Natura2000 network in Albania: methods and approaches"

Saturday 23 September 2017

8:00-15:00 Post-Conference Excursion at the Pietra di Bismantova (Reggio Emilia), with lunch offered by the National Park of the Tuscan-Emilian Apennines.

The timetable may be subject to changes

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III. GARNIER ERIC "Functional traits in plant ecology: advances, limits, challenges"
IV. ROBROEK BJORN J.M. "Decoupling taxonomic and functional turnover in European peatlands"

Symposium 2. Plant and Fungal responses to abiotic stress

V. KRANNER ILSE "Elevated temperature and drought stress trigger molecular switches in developing seeds that affect seed quality"
VI. CORPAS FRANCISCO J. "Peroxisomal nitric oxide (NO) metabolism under adverse environmental conditions"

Symposium 3. Quality, effectiveness and safety of botanicals

VII. ANTON ROBERT "The role of traditional knowledge in the safety assessment of botanical food supplements"
VIII. SERAFINI MAURO "The substantiation of claims for botanical food supplements in relation to traditional use"

Symposium 4. Marine and terrestrial biodiversity studies in the time of biological invasions

IX. VAN KLEUNEN MARK "Global patterns and drivers of plant naturalization"
X. MAGGS CHRISTINE "How should biologists respond to the global increase in invasive alien seaweeds?"

COMMUNICATIONS

Symposium 1. From species ecology to functional traits in a "global change" context

i. CHELLI STEFANO, MARIGNANI MICHELA, BARNI ELENA, THE ITALIAN PLANT TRAITS CONSORTIUM "Plant Traits 2.0 in Italy: back to the future"
ii. NASCIMBENE JURI, BENESPERI RENATO, BILOVITZ PETER OTHMAR, CHIARUCCI ALESSANDRO, DAINESE MATTEO, GIORDANI PAOLO, MARINI LORENZO, MAYRHOFER HELMUT, NIMIS PIER LUIGI "Patterns of functional traits elucidate lichen community assembly under a global change scenario"
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iv. ZALLONI ENRICA, BATTIPAGLIA GIOVANNA, CHERUBINI PAOLO, SAURER MATTHIAS, DE MICCO VERONICA "Tree-ring Intra-Annual-Density-Fluctuations as functional indicators of responses to different environmental conditions in *Quercus ilex* L. (Vesuvius, southern Italy)"
v. MONDONI ANDREA, TUDELA-ISANTA MARIA "Do germination traits contribute to species distribution in alpine habitats?"
vi. CARBOGNANI MICHELE, TOMASELLI MARCELLO, PETRAGLIA ALESSANDRO "Reproduction of two alpine tundra species under climate warming"
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Symposium 2. Plant and Fungal responses to abiotic stress

ix. PERROTTA LARA, MANIGA ANTONIO, URBANI MALVINA, ALBANI DIEGO "Characterization of the MAPK and MKK gene families of *Lactuca sativa* and analysis of their expression under abiotic stresses"
x. DE BENEDICTIS MARIA, SANITÀ DI TOPPI LUIGI, VATAMANIUK OLENA K. "Phytochelatin synthase 1 regulates iron homeostasis in *Arabidopsis thaliana*"
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xv. CICATELLI ANGELA, GUARINO FRANCESCO, RUIZ KARINA B., BIONDI STEFANIA, CASTIGLIONE STEFANO "Effects of salinity and chromium on growth and element uptake and accumulation in the halophytic plant *Chenopodium quinoa* Willd."
xvi. RUGNINI LORENZA, COSTA GIULIA, CONGESTRI ROBERTA, BRUNO LAURA "Cu and Ni removal from aqueous media by green microalgae in single and bimetal solutions"

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xviii. MANDRONE MANUELA, ESPOSITO FRANCESCA, CORONA ANGELA, TRAMONTANO ENZO, POLI FERRUCCIO "Application of metabolomics to improve *in vitro* bioactivities assays for plant crude extracts"
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1. From species ecology to functional traits in a "global change" context

1.1 Plant ecology

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6 GIANGUZZI LORENZO, PAPINI FABIO "Small-scale and high-resolution in geobotanical mapping: the case study of Sicily"

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1.4 Climate change

14 CASAZZA GABRIELE, MINUTO LUIGI, DAGNINO DAVIDE "Between past and future: legacy and fate of an important centre of endemism for the Mediterranean region"

15 D'ARCO MATTEO, VELLI ANDREA, FERRONI LUCIA, SPERANZA MARIA "The green roof's thermal performance in the cold season: first results from different plant covers"

16 DI MUSCIANO MICHELE, STANISCI ANGELA, FRATE LUDOVICO, CARRANZA MARIA LAURA, DI CECCO VALTER, DI MARTINO LUCIANO, FRATTAROLI ANNA RITA "Nurse plant effect along mountain vertical gradients. An insight in central Apennine"

17 MINUTO LUIGI, CASAZZA GABRIELE, DAGNINO DAVIDE, MACRÌ CARMELO NICODEMO "Future adaptations of plant species to environmental changes"

18 ONGARO SILVIA, CAREDDA ALBERTO, MARTELLOS STEFANO, CORTIS PIERLUIGI "Modelling present and future climatic niche of most common Sardinian orchids"

19 VITALE ERMENEGILDA, CIRILLO CHIARA, ARENA CARMEN, BASILE BORIS, SCOGNAMIGLIO PASQUALE, ZALLONI ENRICA, CAPUTO ROSANNA, ERBAGGIO ARTURO, DE MICCO VERONICA "Effect of grafting on xylem efficiency/safety modulation and photosynthetic performance in *Vitis vinifera* 'Piedirosso': concerns in a climate change scenario"

1.5 Land use change

20 BARNI ELENA, DAVÌ ELISA, W. FORTE T'AI G., BRUNO GIAMPAOLO, LA MORGIA VALENTINA, NOVARA CRISTINA, SINISCALCO CONSOLATA "Monitoring the effects of grazing-management on performance and survival of orchids in xerothermic grasslands"

21 FLORENZANO ASSUNTA, MONTECCHI MARIA CHIARA, RATTIGHIERI ELEONORA, TORRI PAOLA, MERCURI ANNA MARIA "The landscape of Sicily: pollen insight into the agro-pastoral system of Philosophiana and Villa del Casale (UNESCO Roman site)"

22 LAZZARO LORENZO, LASTRUCCI LORENZO, BENESPERI RENATO, GONNELLI VINCENZO, VICIANI DANIELE, COPPI ANDREA "Changes in species assemblages in the transition grasslands - shrublands: does phylogeny match the different conditions in term of ecological factors?"

23 MERCURI ANNA MARIA, FLORENZANO ASSUNTA, TORRI PAOLA, BOSI GIOVANNA, MONTECCHI MARIA CHIARA, RATTIGHIERI ELEONORA, RINALDI ROSSELLA, CLÒ ELEONORA, FORNACIARI RITA, MAZZANTI MARTA "The agro-sylvo-pastoral system of 3600-3200 years ago (Terramare, Po Plain; SUCCESSO-TERRA project)"

24 TORRI PAOLA, FORNACIARI RITA, RICCIARDO DEVID, MERCURI ANNA MARIA "Morphology of *Vitis* pollen from cultivars and wild grapevine and the *Vitis* pollen from the Terramara Santa Rosa di Poviglio (project SUCCESSO-TERRA)"

2. Plant and Fungal responses to abiotic stress

2.1 Stress response

25 GUZZON FILIPPO "Drought avoidance adaptive traits in seed germination and seedling growth of *Citrullus amarus* landraces"

26 ROCCOTIELLO ENRICA, ROSATTO STEFANO, RIGGI ALEX, DOZZA DENISE, ZOTTI MIRCA, MARIOTTI MAURO "Effect of nickel stress on seed germination in hyperaccumulator plants and related species"

27 RUSSO FABIANA, SICILIANO ANTONIETTA, CECI ANDREA, MAGGI ORIANA, GUIDA MARCO, PERSIANI ANNA MARIA "Oxidative stress responses induced by Hexachlorocyclohexane (HCH) in saprotrophic soil microfungi"

28 TOMMASI FRANCA, DIPIERRO NUNZIO, LEUCI FEDERICA, POZZESSERE LAURA, PARADISO ANNALISA, D'AQUINO LUIGI, ORAL RAHIME, PAGANO GIOVANNI, TRIFUOGGI MARCO "Rare earth elements as new pollutants: responses to light and heavy different rare earth elements in different biological models"

2.2 Environmental pollution

29 BONANNO GIUSEPPE, DI MARTINO VINCENZO "Seagrasses *Posidonia oceanica* and *Cymodocea nodosa*: trace element accumulation and biomonitoring of marine pollution"

30 CECI ANDREA, RUSSO FABIANA, MALUSÀ ELIGIO, MAGGI ORIANA, TARTANUS MAŁGORZATA, ŁABANOWSKA BARBARA HELENA, PERSIANI ANNA MARIA "Stress response and tolerance to DDT: soil fungal species isolated from polluted agricultural areas of Poland and their potential in fungal bioremediation"

31 CICCARELLO SEBASTIANO, SPADARO VIVIENNE, MAZZOLA PIETRO "Allergens in the park and garden flora of Sicily"
32 GRECO GIUSEPPE, CAPELLO MARCO, CECCHI GRAZIA, CUTRONEO LAURA, DI PIAZZA SIMONE, VAGGE GRETA, ZOTTI MIRCA "*Aspergillus sydowii* in the Port of Genoa (Liguria, NW Italy)"
33 GUARINO FRANCESCO, CICATELLI ANGELA, BRUNDU GIUSEPPE, CASTIGLIONE STEFANO "Assisted phytoremediation of a polluted soil using an elite clone of *Arundo donax* L.: hypotheses on reclaim capability and detoxifying molecular mechanisms"
34 ROSA ESTER, COMITE ANTONIO, MARIOTTI MAURO, ZOTTI MIRCA "Wastewater mycoremediation"
35 RUFFINI CASTIGLIONE MONICA, SPANÒ CARMELINA, MUCCIFORA SIMONETTA, BELLANI LORENZA, BOTTEGA STEFANIA, TASSI ELIANA, SIRACUSA GIOVANNA, DI GREGORIO SIMONA, CASTILLO-MICHEL HIRAM, GIORGETTI LUCIA "TiO_2 nanoparticles and sludge from wastewater treatment plants: a new concern for crops?"
36 SORRENTINO MARIA CRISTINA, VICIDOMINI ANTONIO, CAPOZZI FIORE, GIORDANO SIMONETTA, SPAGNUOLO VALERIA "Evaluation of morpho-physiological alterations in three cultivars of *C. cardunculus* grown on a polluted soil in view of its use for phytoremediation"

2.3 Cell signaling

37 BRUNO LEONARDO, FORGIONE IVANO, SLATTER FELICITY, PACENZA MARIANNA, VAN LIJSBETTENS MIEKE, BITONTI MARIA BEATRICE "Interplaying between epigenetic mechanism controls leaf development in *Arabidopsis thaliana*"

2.4 Plant-environment interactions

38 ADAMO MARTINO, CALEVO JACOPO, BAZZICALUPO MIRIAM, GIOVANNINI ANNALISA, CORNARA LAURA, GIRLANDA MARIANGELA "A look inside *Laelia purpurata* seeds: from seed micromorphology to plant-fungus interactions"
39 CERRI MARTINA, SAPKOTA RUMAKANTA, COPPI ANDREA, FOGGI BRUNO, GIGANTE DANIELA, LASTRUCCI LORENZO, VENANZONI ROBERTO, NICOLAISEN MOGENS, FERRANTI FRANCESCO, REALE LARA "Oomycete diversity in relation to *Phragmites australis* die-back"
40 DE MICCO VERONICA, IZZO LUIGI GENNARO, AMITRANO CHIARA, STINCA ADRIANO, ARONNE GIOVANNA, BARILE ROSSELLA, CONTI PAOLA, ARENA CARMEN "Anthropogenic impact in the Vesuvius National Park: effect of dust accumulation on leaves of C*entranthus ruber* (L.) DC. subsp. *ruber*"
41 ROSATTO STEFANO, ROCCOTIELLO ENRICA, CECCHI GRAZIA, ZOTTI MIRCA, MARIOTTI MAURO "Rhizosphere response to nickel in hyperaccumulator and non-hyperaccumulator species"

3. Quality, effectiveness and safety of botanicals

3.1 Medicinal plants

42 ACQUAVIVA ROSARIA, DI GIACOMO CLAUDIA, TOMASELLO BARBARA, MINAFRA LUIGI, PISCIOTTA PIETRO, TRINGALI ROBERTA, MARCHESE VALENTINA, DI MAURO MARIA DOMENICA, RAGUSA SALVATORE, CAMMARATA FRANCESCO PAOLO "Potential application of *Betula etnensis* Raf (Betulaceae) alcoholic extract as sinergist in radiotherapy"
43 ANGELINI PAOLA, ROSIGNOLI PATRIZIA, BISTOCCHI GIANCARLO, ARCANGELI ANDREA, TIRILLINI BRUNO, FABIANI ROBERTO, VENANZONI ROBERTO "Antioxidant, antifungal and chemo-preventive properties of *Fomitopsis pinicola* (Sw.) P.Karst. extract"
44 BENNADJA SALIMA, ATI SAMIRA "Quantitative analysis and antioxidant activity of three Algerian endemic *Genista* species"
45 CHIOCCHIO ILARIA, MANDOLFO ANTONIO L., LIANZA MARIACATERINA, GOVERNA PAOLO,MARCO "Wound healing activity of five *Sedum* species collected in Emilia Romagna (Italy)"
46 DEGOLA FRANCESCA, REFIFÀ MEHER, MARZOUK BELSEM, GALATI SERENA, MOLTALBANO SERENA, DRAISCI ROBERTO, RESTIVO FRANCESCO M., BUSCHINI ANNAMARIA "Biological activity of *Citrullus colocynthis* (L.) Schrad. tegument and plantulae extracts"
47 GERMANÒ MARIA PAOLA, RAPISARDA ANTONIO, D'ANGELO VALERIA, D'AMBOLA MASSIMILIANO, DE TOMMASI NUNZIATINA, BADER AMMAR "Phytochemical study and anti-angiogenic activity of *Daphne mucronata* Royle subsp. *linearifolia* (Hart) Halda (Thymelaeaceae)"
48 GOVERNA PAOLO, BIAGI MARCO "Anti-*Helicobacter pylori* activity and characterization of C*opaifera langsdorffii* Desf. extracts"
49 MALFA GIUSEPPE, ACQUAVIVA ROSARIA, DI GIACOMO CLAUDIA, RENIS MARCELLA, ADDAMO ALESSANDRO PAOLO, RAGUSA SALVATORE, TUNDIS ROSA, DI PAOLA MARTINA, LONGO MARIA, GENOVESE CARLO "*In vitro* evidence for biological acitivities of Sicilian *Orobanche crenata* Forssk. leaf extract"
50 MENGHINI LUIGI, FERRANTE CLAUDIO, ORLANDO GIUSTINO, LOCATELLI MARCELLO, CARRADORI SIMONE, CHIAVAROLI ANNALISA, RECINELLA LUCIA, VECCHIOTTI GIULIA, LEPORINI LIDIA "Optimization of productive cycle of *Crocus sativus* from Abruzzo, investigating innovative use and application of different flower parts"
51 PASTORINO GIULIA, RODRIGUES FRANCISCA, PINTO DIANA, BELLOTO DE FRANCISCO LIZZIANE MARIA, OLIVEIRA MARIA BEATRIZ, LAURA CORNARA "Possible use of *Melilotus officinalis*, *Lespedeza capitata* and *Glycyrrhiza glabra* in human's health"
52 RAGUSA SALVATORE, BONESI MARCO, LOIZZO MONICA R., PASSALACQUA NICODEMO G., ACQUAVIVA ROSARIA, MENICHINI FRANCESCO, TUNDIS ROSA "*In vitro* antioxidant and hypoglycaemic activities of *Hyssopus officinalis* L. subsp. *aristatus* (Godr.) Briq. (Lamiaceae) from Southern Italy"
53 SALINITRO MIRKO, VICENTINI RENZO, BONOMI COSTANTINO, TASSONI ANNALISA "Traditional knowledge on wild and cultivated plants in the Kilombero Valley (Morogoro Region, Tanzania)"
54 SMERIGLIO ANTONELLA, RAGUSA SALVATORE, MONFORTE MARIA TERESA, D'ANGELO VALERIA, CIRCOSTA CLARA "*Euphorbia dendroides* L. (Euphorbiaceae) latex: polyphenols characterization and evaluation of antioxidant and radical scavenging properties"
55 SPADARO VIVIENNE, FORMISANO CARMEN, RAIMONDO FRANCESCO MARIA, SENATORE FELICE(†), RIGANO DANIELA "Chemical composition of the essential oils of some taxa of the genus *Clinopodium* (Lamiaceae) from Sicily"

56 SPAMPINATO GIOVANNI, PAGLIANITI ISABELLA, MUSARELLA CARMELO MARIA "New ethnobotanical records on medicinal plants for humans and animals in the territory of Poro and Preserre Calabresi (Vibo Valentia – S-Italy) "

57 TLILI- AIT-KAKI YASMINA, CHOUIA AMEL, AOUADI RHOZLENE, BENHADID RYM, BENNADJA SALIMA "Research for an antibacterial activity of the essential oil of *Cupressus sempervirens* L. from Algeria, on some bacterial strains"

58 TSIOUTSIOU EFTHYMIA ELENI, GIORDANI PAOLO, HANLIDOU EFFIE, BIAGI MARCO, CORNARA LAURA "Traditional use of medicinal plants in the regions of Edessa and Naussa (Central Macedonia, Greece)"

3.2 Plant secondary metabolites

59 ACQUADRO STEFANO, ESPOSITO FRANCESCA, SANNA CINZIA, CAREDDA ALESSIA, MARENGO ARIANNA, CAGLIERO CECILIA, TRAMONTANO ENZO, BICCHI CARLO, SGORBINI BARBARA, RUBIOLO PATRIZIA "*Punica granatum* leaves as a potential source of bioactive molecules"

60 ASCRIZZI ROBERTA, FLAMINI GUIDO, FRATERNALE DANIELE "The antiaging properties of *Helichrysum italicum* (Roth) G.Don essential oil: collagenase and elastase inhibition activities"

61 BISIO ANGELA, PEDRELLI FRANCESCA, ARAGO DOUGUÉ KENTSOP ROMÉO, FRATERNALE DANIELE, RUFFONI BARBARA, DE TOMMASI NUNZIATINA, SCHITO ANNA MARIA "Antibacterial activity of roots of *Salvia corrugata* Vahl."

62 D'URSO GILDA, SHAKERI ABOLFAZL, MASULLO MILENA, MONTORO PAOLA, PIZZA COSIMO, IRANSHAHI MEHRDAD, PIACENTE SONIA "In depth chemical investigation of *Glycyrrhiza triphylla* Fisch & C.A.Mey roots guided by a preliminary HPLC-ESIMS^n profiling"

63 FICO GELSOMINA, GIULIANI CLAUDIA, LUPI DANIELA, ASCRIZZI ROBERTA, TASSERA GIACOMO, SANTAGOSTINI LAURA, MALECI BINI LAURA, GIOVANETTI MANUELA, FLAMINI GUIDO "Linking volatile profile, glandular trichomes and pollinators in *Salvia verticillata* L. (Lamiaceae)"

64 IMPELLUSO CARMEN, CATARA STEFANIA, GALESI ROSARIO, DONSÌ FRANCESCO, STRANO TONIA, RUBERTO GIUSEPPE, CRISTAUDO ANTONIA "Chemical polymorphism and phytotoxic activity of the essential oils of *Thymus* taxa: a case study of some wild species from Sicily and Calabria"

65 MARENGO ARIANNA, SANNA CINZIA, MAXIA ANDREA, BALLERO MAURO, BERTEA CINZIA, GAVIRA CAROLE, BOURGAUD FRÉDÉRIC, BICCHI CARLO, CAGLIERO CECILIA, RUBIOLO PATRIZIA "*Ptilostemon casabonae* (L.) Greuter: some updates on a little known endemic species from Sardinia"

66 MILELLA LUIGI, CASTRONUOVO DONATO, LIBONATI ROSSANA, RUSSO DANIELA, CANDIDO VINCENZO, ANDRADE PAULA, DE TOMMASI NUNZIATINA "Effects of irradiation on yield, phytochemical profiles and on the antioxidant activity of *Ocimum basilicum* L. extracts"

67 STINCA ADRIANO, DI SALVATORE MINA, CARAFA ANNA MARIA "*Ballota pseudodictamnus* (L.) Benth.: micromorphological diversity of trichomes"

3.3 Phytochemistry

68 CAMERO MUÑOZ CESAR, DE LEO MARINELLA, TEMRAZ ABEER, DE TOMMASI NUNZIATINA, BRACA ALESSANDRA "New limonoids from *Aphanamixis polystachya* (Wall) R.Parker (Meliaceae) leaves"

69 FERRI BENEDETTA, CIONI PIERLUIGI, PISTELLI LUISA "Aroma profile and essential oil characterization of five healthy and infected Polish hop cultivars"

70 GIORDANI PAOLO, DRAVA GIULIANA, MINGANTI VINCENZO, BRIGNOLE DANIELE, FERRETTI ILARIA, MALASPINA PAOLA, CORNARA LAURA "Trace element bioconcentration and translocation in edible herbs: a case study for *Plantago lanceolata*"

71 HADEF YOUCEF, GOUASMI Z'HOR, LAKHEL SAMIA, TAHAR BENMOUSSA MOHAMED, CHEFROUR AZEDDINE "Synergistic effects between the essential oil of *Thymus numidicus* Poir. and Amphotericin B"

72 MASULLO MILENA, DE SOUZA SANTOS CLISIANE CARLA, CERULLI ANTONIETTA, MARI ANGELA, DOS SANTOS ESTEVAM CHARLES, PIZZA COSIMO, PIACENTE SONIA "Phytochemical investigation of *Schinopsis brasiliensis*, a Brazilian medicinal plant"

73 NAJAR BASMA, PISTELLI LUISA, CERVELLI CLAUDIO, FICO GELSOMINA, GIULIANI CLAUDIA "Aroma profile and morphological analysis of *Salvia broussonetii* Benth."

3.6 Food plants

74 AMITRANO CHIARA, ARENA CARMEN, VITAGLIONE PAOLA, ARONNE GIOVANNA, PUGLIESE MARIAGABRIELLA, DE MICCO VERONICA "Combined effect of ionizing radiation and light on morpho-anatomical and nutritional traits of soybean seedlings intended as integrators for astronauts' diet"

75 ANTOGNONI FABIANA, MANDRIOLI ROBERTO, RENZI ELISA, BELLELLI SARA, LUGLI STEFANO, TARTARINI STEFANO "Characterization of bioactive compounds in new sweet cherry (*Prunus avium* L.) cultivars made in UNIBO through a breeding programme"

76 BATTIATA ANDREA, CLAUSER MARINA, PETRINI GIOVANNI "Bioactive garden in the Botanical Garden of Florence"

77 CASCINI ALBERTA, PAPPALARDO SIMONA, GRILLO FEDERICA, MASTRACCI LUCA, CORNARA LAURA "Seeds for everyone's taste"

78 CERULLI ANTONIETTA, NAPOLITANO ASSUNTA, PIZZA COSIMO, PIACENTE SONIA "Multi-class polar lipid profiling of *Corylus avellana* (cultivar "Tonda di Giffoni") hazelnut kernel by LC-ESI/LTQOrbitrap/MS/MSn"

79 CORNARA LAURA, SMERIGLIO ANTONELLA, DI GRISTINA EMILIO, FRIGERIO JESSICA, LABRA MASSIMO, TROMBETTA DOMENICO "The problem of misidentification between a wild edible plant and a poisonous one: the case of borage"

80 DI CECCO VALTER, DI MUSCIANO MICHELE, IANIERI ANDREA, DI SANTO MARCO, DI MARTINO LUCIANO, CASAVECCHIA SIMONA, SILVERI DONATO, FRATTAROLI ANNA RITA "First result of morphometric analysis on different populations of Solina grain (*Triticum aestivum* 'Solina')"

81 DI PIAZZA SIMONE, VERSEUX CYPRIEN, BILLI DANIELA, TOSI SOLVEIG, ZOTTI MIRCA, ONOFRI SILVANO "Cultivation of mushroom on food waste: a possible solution to improve the Life Support Systems (LSS) during long-term mission and spaceflight"

82 GIORGI ANNAMARIA, PENTIMALLI DANIELA, GIUPPONI LUCA, PANSERI SARA "Saffron (*Crocus sativus* L.) quality of the Alpine areas"

83 IZZO LUIGI GENNARO, ARENA CARMEN, DE MICCO VERONICA, ARONNE GIOVANNA "Light quality enhances anthocyanins modulation in red and green cultivars of *Atriplex hortensis* L."

84 MUSARELLA CARMELO MARIA, CUZZUCOLI FEDERICA, ROGOLINO FRANCESCA, SPAMPINATO GIOVANNI "New ethnobotanical records on food plants in the territory of Southern Aspromonte (Reggio Calabria – Southern Italy)"

85 MUTO ANTONELLA, ARANITI FABRIZIO, MARRELLI MARIANGELA, CONFORTI FILOMENA, CEVERISTA CHIAPPETTA ADRIANA ADA, BITONTI MARIA BEATRICE, BRUNO LEONARDO "Genetic and metabolic profile of wild cardoon populations (*Cynara cardunculus* L. subsp. *cardunculus*) in Calabria region"

86 RENNA MASSIMILIANO, PARADISO VITO MICHELE, CASTELLINO MARIA, LEONI BENIAMINO, CAPONIO FRANCESCO, SANTAMARIA PIETRO "Crenate broomrape (*Orobanche crenata* Forssk.): from parasitic plant to traditional agri-food product of Puglia (Southern Italy) with interesting nutritional traits"

4. Marine and terrestrial biodiversity studies in the time of biological invasions

4.1 Floristics

87 BEDINI GIANNI, PIERINI BRUNELLO, ROMA-MARZIO FRANCESCO, GESTRI GIOVANNI, CAPARELLI KATIA F., BONARI GIANMARIA, D'ANTRACCOLI MARCO, PACIFICO GIULIANO, PERUZZI LORENZO, CARTA ANGELINO "An assessment of geographic sampling in Wikiplantbase #Toscana"

88 BULDRINI FABRIZIO, GENTILINI MATTEO, SANTINI CLAUDIO, BOSI GIOVANNA "A first contribution to the knowledge of the urban flora of Modena"

89 CAMBRIA SALVATORE "First report of *Cyclamen africanum* Boiss. & Reut. in Italy"

90 DELLAVEDOVA ROBERTO, ARDENGHI NICOLA M.G., ROSSI GRAZIANO "Historical and recent investigation of Val Grande National Park's Vascular Flora (Piemonte, NW Italy)"

91 GIUPPONI LUCA, GIORGI ANNAMARIA "Floristic heritage of the Mt. Cavallo path (Orobie Bergamasche Regional Park)"

92 PERUZZI LORENZO, PIERINI BRUNELLO, BEDINI GIANNI "Antique floristics: the status of knowledge of Tuscan flora (Central Italy) at the beginning of twentieth century"

93 RAIMONDO FRANCESCO MARIA, MAZZOLA PIETRO, DOMINA GIANNIANTONIO, SPADARO VIVIENNE "Taxonomic, ecological and chorological remarks on *Clinopodium minae* (Lamiaceae), critical and rare plant of the Sicilian flora"

4.2 Systematics

94 BEZERRA CHIAVEGATTO RAQUEL, ARANTES CHAVES ANA LUISA, GANDOLFI BENITES FLÁVIO RODRIGUES, PERUZZI LORENZO, TECHIO VÂNIA HELENA "Distribution of repetitive DNA sites in *Cynodon* (Poaceae)"

95 D'ANTRACCOLI MARCO, PERUZZI LORENZO "Disentangling infraspecific taxonomy of *Cirsium vulgare* (Savi) Ten. (Asteraceae). A first contribution from Tuscany"

96 DAGNINO DAVIDE, MINUTO LUIGI, CASAZZA GABRIELE "Niche divergence between putative taxa: ecological niche models in taxonomic researchs"

97 ROMA-MARZIO FRANCESCO, DOLCI DAVID, PERUZZI LORENZO "Contribution to the taxonomy of *Polygala flavescens* group (Polygalaceae): karyological and morpho-colorimetric data"

98 SAITTA MARIA, LE GALL LINE, MORABITO MARINA, MANGHISI ANTONIO "A molecular-assisted investigation of the Halymeniales (Rhodophyta) collected in two recent expeditions in Madagascar and Guadeloupe reveal hidden taxonomic diversity"

99 SPAGNUOLO DAMIANO, MORABITO MARINA, GENOVESE GIUSI, MANGHISI ANTONIO "Cryptic diversity of Bangiales (Rhodophyta) along the Italian coasts revealed by DNA barcoding"

4.3 Alien species

100 BIANCO ILEN, VIAGGIU EMANUELA, SARNO DIANA, CONGESTRI ROBERTA "Phytoplankton alien species in marine and freshwater ecosystems from Latium Region (Middle Tyrrhenian Sea, Italy)"

101 CECERE ESTER, PORTACCI GIUSEPPE, BELMONTE MANUELA, RUBINO FERNANDO, PETROCELLI ANTONELLA "How much close is the connection between non-indigenous species and human activities? An example from a Mediterranean coastal zone"

102 COGONI ANNALENA, BRUNDU GIUSEPPE, CADDEO ALESSANDRA, LOI MARIA CECILIA, MARIGNANI MICHELA, IIRITI GIANLUCA, VAQUER GIULIANO "LIFE ASAP - increasing awareness on alien species in Italy"

103 MANNINO ANNA MARIA, BALISTRERI PAOLO, DEIDUN ALAN "Role of Sicily and circum-Sicilian Islands as recipient and donor area for alien marine macrophytes in the Central Mediterranean Sea"

104 MARIOTTI MAURO, BONECHI FRANCO, CARACCIOLO DANIELA, CRESTA PAOLO, RAINERI VALTER "First assessment of the Box tree moth (*Cydalima perspectalis*) invasion in NAT2000 Habitat 5110 in Liguria"

105 SFRISO ADRIANO, BUOSI ALESSANDRO, WOLF MARION, SFRISO ANDREA "Inventory of the distribution and abundance of alien macroalgae in the Venice Lagoon: *Gracilaria vermiculophylla*, an emblematic case"

106 WAGENSOMMER ROBERT PHILIPP, ALBANO ANTONELLA, MEDAGLI PIETRO "The increase of plant invasion in the Mediterranean area: the allochthonous vascular flora of Puglia (SE-Italy) as a case study"

4.4 Phycology

107 ARMELI MINICANTE SIMONA, MANGHISI ANTONIO, GENOVESE GIUSEPPA, LE GALL LINE, LOPEZ-BAUTISTA JUAN, MORABITO MARINA "SeaVe - Seaweeds of Venice Lagoon: a Barcode of Life Data System project revealing hidden macroalgal diversity"

108 BONANNO FERRARO GIUSY, BLASI MONICA FRANCESCA, MANGHISI ANTONIO, GARGIULO GAETANO MAURIZIO "Epizoic algae on Loggerhead turtle (*Caretta caretta* Linnaeus) from Aeolian Archipelago (Southern Tyrrhenian Sea, Italy)"

109 CAMBREA GIUSEPPE, CROSCA ALICE, GEMELLI FEDERICA, GARGIULO GAETANO MAURIZIO "Karyomorphology and DNA quantification in the marine angiosperm *Halophila stipulacea* (Forssk.) Asch. (Alismatales, Hydrocharitaceae) from Red Sea"

110 CARONNI SARAH, CALABRETTI CHIARA, CECCHERELLI GIULIA, DELARIA MARIA ANNA, GRECHI MATTEO, MACRI GIOVANNI, NAVONE AUGUSTO, OCCHIPINTI AMBROGI ANNA, BASSO DANIELA "Short term interactive effects of mucilage and sedimentation on encrusting coralline algae"

111 CROSCA ALICE, MANGHISI ANTONIO, GARGIULO GAETANO MAURIZIO "The genus *Antithamnion* (Ceramiales, Rhodophyta) in the Mediterranean Sea: nomenclatural and taxonomical problematics"

112 PAPINI ALESSIO, SANTOSUOSSO UGO "Geographic Profiling: how a criminological method can help to find the origin of a biological invasion by a green alga (*Caulerpa prolifera* Sonder) in combination with other analytical techniques"

113 PEZZOLESI LAURA, PIGOZZI SILVIA, CANGINI MONICA, BURATTI SARA, SERVADEI IRENE, GUERRINI FRANCA, PISTOCCHI ROSSELLA "Ballast waters as a prominent vector for the transfer of harmful aquatic organisms: the study of phytoplankton and dinoflagellate cysts in the Italian port of Bari (BALMAS project)"

4.5 Evolutionary ecology

114 ASTUTI GIOVANNI, PRATESI SANDRO, CARTA ANGELINO, PERUZZI LORENZO "Comparative reproductive biology of Tulipa pumila Moench and T. Sylvestris L. (Liliaceae)"

115 CORTIS PIERLUIGI, COGONI ANNALENA, LUSSU MICHELE, DE AGOSTINI ANTONIO, CAREDDA ALBERTO, PUTZOLU MARCO SEBASTIANO, MASSESSI KATIA, SCOPECE GIOVANNI "Pollen transfer efficiency is a characteristic of species rather than populations in the Mediterranean food-deceptive orchid Orchis anthropophora"

116 LUSSU MICHELE, COGONI ANNALENA, MARIGNANI MICHELA, CORTIS PIERLUIGI "A comparative study on floral traits in two Mediterranean orchids with a different pollination strategies"

117 ROMA LUCA, CAFASSO DONATA, SCHLÜETER PHILIPP, SCOPECE GIOVANNI, COZZOLINO SALVATORE "Genomic scan reveals cladogenetic and biogeographic patterns in an orchid biodiversity hotspot"

5. Free Topic

118 ABELI THOMAS "A review of the success in wetland plant translocations"

119 AMICUCCI ANTONELLA, GIAMPERI LAURA, ROSSI CLAUDIO, RICCI DONATA, BUCCHINI ANAHI ELENA ADA "The genus *Cortinarius* in Italy: molecular studies for taxonomic and nutraceutical reassesment"

120 ARDENGHI NICOLA M.G., BRACCO FRANCESCO, ROSSI GRAZIANO "When art meets botany: Fulgenzio Vitman's herbarium in Pavia (PAV)"

121 BERTACCHI ANDREA, FAGARAS MARIUS "Comparison of dune habitats of North Mediterranean coasts - Tuscany (IT) with those of Black sea coasts -Dobrogea (Ro, Bg): similarities and differences"

122 BRUNO GIOVANNI LUIGI, CARIDDI CORRADO, TOMMASI FRANCA "Physiological alterations in olive trees affected by Co.Di.R.O.: preliminary studies"

123 CALEVO JACOPO, COPETTA ANDREA, MARCHIONI ILARIA, BAZZICALUPO MIRIAM, GIRLANDA MARIANGELA, GIOVANNINI ANNALISA "Improvement of *in vitro* development for epiphytic orchids: comparison between culture *media*"

124 CANELLA MARCO, MONDONI ANDREA, ORSENIGO SIMONE, VAGGE ILDA, GUZZON FILIPPO "Seed heteromorphy influences seed longevity in *Aegilops*"

125 CASILLI MARZIA, PANNUNZIO GIOVANNA, PACE LORETTA "Pollen morphology of two fasciated plants of the family of *Asteraceae*"

126 CIANI FRANCESCO, GIULIANI CLAUDIA, FOGGI BRUNO, GIACHI GIANNA, ARANGUREN BIANCAMARIA, MARIOTTI LIPPI MARTA "The Paleolithic site of Poggetti Vecchi (Grosseto): a paleopalynology research"

127 DAGLIO GABRIELE, MASSA NADIA, CESARO PATRIZIA, LAZZARI MASSIMO, BERTA GRAZIELLA "Can an optical sensor detect the symptoms of Flavescence Dorée and Esca disease in *Vitis vinifera*?"

128 DEL MONDO ANGELO, DE NATALE ANTONINO, PINTO GABRIELE, POLLIO ANTONINO "Microcosm experiment and polyphasic approach for the assessment of *Fusarium solani* mycelial growth on a stone substratum"

129 FOSSATI FABRIZIA, BERTOLI LORENZO "Biological analysis, for conservation purposes, of Matildic Parish of Santa Maria Assunta (XI century) located in Sasso di Neviano Arduini, Parma Appennines"

130 GIAMPERI LAURA, GIOMARO GIOVANNA, RICCI DONATA, BUCCHINI ANAHI ELENA ADA "Role of Botanic Garden of University of Urbino in the application of inquiry-based education"

131 MARINO MATTIA LETIZIA, CAMPISI PATRIZIA, DIA MARIA GIOVANNA "Bryophyte diversity in some Sicilian protected areas"

132 MARINO PASQUALE, BAZAN GIUSEPPE, LUCZO STEPHEN JAMES "Protecting biodiversity on private property: an experience in Corleone (Sicily)"

133 MENICUCCI FELICIA, BELLI MATTIA, RISTORI SANDRA, GONNELLI CRISTINA, PAPINI ALESSIO "Developing lipid-based nanodevices from *Pyrocystis lunula* and *Arthrospira platensis* for biomedical applications"

134 MULAS BONARIA, FOGU MARIA CATERINA, PUDDU DAVIDE, TATTI ALESSIA, COGONI ANNALENA "A Census of the Mycological *Herbarium* CAG: Macromycetes from the Mediterranean area"

135 NOVARA CRISTINA, ASCARI LORENZO, PALESTINI GIOVANNA, DUSIO VIRGINIA, SINISCALCO CONSOLATA "Long term monitoring of airborne pollen concentration and phenology of hazel"

136 ORI FRANCESCA, LEONARDI MARCO, LEONARDI PAMELA, PACIONI GIOVANNI, ZAMBONELLI ALESSANDRA, IOTTI MIRCO "Ectomycorrhizal fungal community of a *Tuber borchii* orchard established in a non-typical environment"

137 PALLA FRANCO, ROTOLO VALENTINA, BARRESI GIOVANNA, DI CARLO ENZA, GIORDANO AMBRA, LOMBARDO GIOVANNA, BRUNO MAURIZIO "Plant products to control biodeterioration of cultural assets"

138 PEZZOTTA MARCO, VALLETTA ALESSIO, PASQUA GABRIELLA "The bizarre carnivorous plant *Genlisea*: *in vitro* propagation and microscopical investigation of glandular structures"

139 POPONESSI SILVIA, VENANZONI ROBERTO, MANELI FABIO, ALEFFI MICHELE, GIGANTE DANIELA "Bryophytic component from Mediterranean temporary ponds in Umbria (Central Italy): state of knowledge on biodiversity and vegetation"

140 RESTUCCIA ALESSIA, FONTANAZZA STEFANIA, SCAVO AURELIO, ABBATE CRISTINA, MAUROMICALE GIOVANNI "Importance of cover crops for weed control in a sustainable mediterranean agroecosystem"

141 ROSSI GRAZIANO, GARGANO DOMENICO, MONTAGNANI CHIARA, FENU GIUSEPPE, PERUZZI LORENZO, ALESSANDRINI ALESSANDRO, ABELI THOMAS, ARDENGHI NICOLA M.G., ASTUTI GIOVANNI, BACCHETTA GIANLUIGI, BARTOLUCCI FABRIZIO, BERNARDO LILIANA, BRULLO SALVATORE, CASTELLO MIRIS, COGONI DONATELLA, CONTI FABIO, DOMINA GIANNIANTONIO, FOGGI BRUNO, FRATTINI SILVIO, GENNAI MATILDE, GENTILI RODOLFO, IBERITE MAURO, LASEN CESARE, MAGRINI SARA, NICOLELLA GIANLUCA, SANTANGELO ANNALISA, SELVAGGI ALBERTO, VILLANI MARIA CRISTINA, WAGENSOMMER ROBERT PHILIPP, VAGGE ILDA, PROSSER FILIPPO, WILHALM THOMAS, BOVIO MAURIZIO, BLASI CARLO, PERRINO ENRICO V., DUPRÈ EUGENIO, TARTAGLINI NICOLETTA, ORSENIGO SIMONE "The Red List of Italian endemic vascular plants"

142 SALVIOLI DI FOSSALUNGA ALESSANDRA, DESIRÒ ALESSANDRO, VENICE FRANCESCO, GHIGNONE STEFANO, BONFANTE PAOLA "An Arbuscular Mycorrhizal Fungus possesses the potential for the biosynthesis of secondary metabolism compounds"

143 SARIGU MARCO, UCCHESU MARIANO, SANNA IGNAZIO, DEL VAIS CARLA, D'HALLEWIN GUY, GRILLO OSCAR, BACCHETTA GIANLUIGI "Earliest evidence of *Prunus domestica* L. in Italy during the Phoenician and Punic periods (6^{th}-2^{nd} century BC)"

144 SCIALABBA ANNA, DI TRAPANI DOROTEA, DI NOTO GIUSEPPE, CIULLA EMMANUELE R., SALMERI CRISTINA "Plant Conservation Centres in Sicily: the 'Valle Maria' Regional Centre (Godrano, Palermo) and the case study of the rare *Centaurea erycina* (Asteraceae)"

145 VENANZONI ROBERTO, WAGENSOMMER ROBERT PHILIPP, BISTOCCHI GIANCARLO, ARCANGELI ANDREA, RUBINI ANDREA, PERINI CLAUDIA, ANGELINI PAOLA "Red List of Ascomycota (Pezizomycotina) in Umbria"

KEYNOTE LECTURES

ZINC-ING ABOUT PLANT METAL HOMEOSTASIS AND TOLERANCE

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Transition metals such as zinc (Zn) and iron (Fe) are essential for virtually every biological process. For example, current estimates assume that around 10% of all eukaryotic proteins are Zn-dependent. Our understanding of acquisition and distribution pathways for these micronutrients, however, is still limited. Plants have to acquire essential microelements from soil solutions that can vary in concentrations of the respective ions by orders of magnitude. Not only deficiency is a threat but also toxicity. An excess of Zn or other metal ions can inhibit growth due to their tendency to strongly interact with various cellular component. A homeostatic system comprising metal transporters, metal ligands and regulatory proteins maintains the concentrations of essential elements within rather narrow physiological ranges inside plant tissues.

Relevant as environmental factors for plants are not only macro- and microelements, but also potentially highly toxic elements without biological function, for example cadmium (Cd) and arsenic (As). Nonessential toxic elements are present in the environment either because of natural causes or because of man-made pollution.

Human well-being depends in many ways on the ionome of plants, i.e. the concentrations of essential and nonessential elements especially in edible tissues. Plant-derived food is a major source of micronutrients and an estimated three billion people around the world are threatened by Zn or Fe deficiency. Furthermore, most of the human Cd intake and a large fraction of the As intake is due to the consumption of plants. Thus, we need to better understand the pathways determining metal accumulation in plants.

We are pursuing different approaches to dissect metal homeostasis. One of them focuses on the ability of certain plant species to hyperaccumulate Zn and Cd up to levels more than 1000fold higher than in non-hyperaccumulating plants. Our model is *Arabidopsis halleri*, a close relative of *A. thaliana,* and growing in old mining areas in Central Europe (1, 2). A second approach is aiming at identifying components of metal tolerance in *A. thaliana* (3). Many genes contributing to metal tolerance are part of the homeostatic network controlling metal accumulation and distribution.

A key process is the synthesis of chelating molecules that control the mobility of essential as well as non-essential elements in plants. Prominent ligands in plants are the non-proteinogenic amino acid nicotianamine and phytochelatins, glutathione-derived peptides.

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FACILITATION IN PLANT COMMUNITIES: UNDERSTANDING PROCESSES AND CONSEQUENCES FROM MOUNTAIN TOPS TO CROP FIELDS

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Over the past twenty years, the study of plant facilitation – those beneficial interactions that occur between two plants, often neighbouring – is a topic that has moved from the periphery to the mainstream of plant ecology. During this time the focus of my own research has also shifted from arctic and alpine environments to more recent studies of crop systems. This geographic shift has been accompanied by a shift from trying to understand fundamental ecological principles - how plants fit together and function in communities - to studying key applied issues, including food security and sustainable crop production.

This presentation will follow this path from the mountain tops down to the crop fields. Starting with a brief history of the concept of facilitation, and some basic definitions of the concept, it will then consider facilitation in arctic and alpine environments. It will look in particular at a number of major studies in these systems which focussed on exploring the generality of facilitative interactions[1] and their role, relative to other major biogeographic processes, in regulating global patterns of alpine biodiversity[2]. It will then consider in more detail the concept of the Stress Gradient Hypothesis, a conceptual model linking the severity of the environment to the dominant types of plant-plant interactions that are occurring in plant communities, including the relationship of this concept to other over-arching plant community ecology frameworks such as Grime's CSR model[3].

The presentation will then consider how such conceptual frameworks can be applied to food production. An obvious starting point is intercropping, where the beneficial effects of neighbouring plants are well understood and explored, but might also be enhanced by integration of new ecological knowledge[4]. We'll then look at how understanding interactions between plants within crop systems can provide us with new fundamental ecological insights, as well as being relevant to addressing some of our major societal and environmental challenges[5].

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FUNCTIONAL TRAITS IN PLANT ECOLOGY: ADVANCES, LIMITS, CHALLENGES

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Biological diversity, the variety of living organisms on Earth, is traditionally viewed as the diversity of taxa, and species in particular. However, other facets of diversity need to be considered for a comprehensive understanding of evolutionary and ecological processes. Here, I will show the advantages of adopting a functional, trait-based approach to diversity.
By concentrating on traits, which are generalizable properties of organisms, it is possible to move between scales of biological organization and between geographical locations despite the idiosyncratic details of each site, taxonomic assemblage, or geographical location. There is a growing consensus that such an approach based on the use traits has a strong potential to address several pending questions in ecology. A non-exhaustive list includes: (1) the functioning of organisms and how it relates to the environment, (2) the understanding of unsolved questions in community ecology such as the identification of rules governing the assembly of communities, and (3) the understanding of how the functioning of organisms scales up to that of ecosystems and controls some of the services they deliver to humans, including those delivered by agriculture. These different aspects will be illustrated using examples taken from various types of plants and ecosystems. Limits to the trait-based approach in plant ecology and challenges ahead will be discussed.

DECOUPLING TAXONOMIC AND FUNCTIONAL TURNOVER IN EUROPEAN PEATLANDS

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Current projections on climate change may alter peatland carbon cycles, through changes in environmental conditions or by alterations in the plant community composition. Our understanding on the effects of climate change on C cycles is growing, yet the mechanism underlying apparent plant controls are not fully known.
In the first part of this presentation we present the results from a plant functional type removal study aiming to understand the role of vascular plant functional groups on peatland carbon cycling. The second part of the presentation will focus on the effects of environmental change on plant community composition and its consequences for the functional composition of the peatland plant community. We show that plant community composition strongly drives the composition of the microbial community, with subsequent consequences for carbon dioxide and methane turnover. Using plant community composition data from 56 peatlands across Europe, we show that plant community composition is largely affected by environmental conditions. Surprisingly, plant species aggregate in two major clusters based on their response to environmental conditions. Both clusters show distinct but contrasting responses to temperature, precipitation and atmospheric deposition, indicating a strong cluster–specific environmental filtering. The observed species turnover across these environmental gradients did, however not result in a change in the plant community functional identity, nor in its functional redundancy. Our results suggest a cluster–specific species substitution to underlie the conservation of functional redundancy at the community level along the environmental gradients. In other words, species loss by environmental filtering appears to be compensated by a deterministic gain of functionally alike species. Taken together, our results show taxonomic and functional turnover to be decoupled, which may indicate a high robustness of peatland ecosystem functioning to future environmental changes.

ELEVATED TEMPERATURE AND DROUGHT STRESS TRIGGER MOLECULAR SWITCHES IN DEVELOPING SEEDS THAT AFFECT SEED QUALITY

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Seeds are pivotal to agricultural productivity and ecosystem conservation, yet there remain substantial gaps in our understanding of the critical role that the environment plays during seed development and storage, and its effect on seed quality. The looming challenges of climate change and food security require new knowledge of how stress impacts on seed quality, as well as a re-appraisal of optimal storage conditions. These issues were addressed in an EU-funded project entitled “Impacts of Environmental Conditions on Seed Quality” (EcoSeed). Here we report on the effects of the maternal environment on seed quality in four plant species, *Arabidopsis thaliana*, *Brassica oleracea*, *Hordeum vulgare* and *Helianthus annuus*. Seed quality is defined as the sum total of all seed traits that are acquired from seed development on the mother plant to seed germination, including seed vigour and viability, maturity and desiccation tolerance, longevity and dormancy, and the commercial estimates seed yield and 1000-seed weight. Plants were subjected to suboptimal temperatures or drought. Changes in water availability and temperature were close to those predicted in climate change scenarios but were not detrimental to the plants or to seed production. Our results indicate that the maternal environment affected plant and seed physiology in a species- and genotype-specific manner. However, both stresses applied individually impacted strongly on seed yield. Further effects of drought and temperature stress were not entirely consistent across the four species tested, whereby *H. annuus* and *H. vulgare* were less affected by the applied stresses than *A. thaliana* and *B. oleracea*. Temperature stress had the strongest effect on the species tested: elevated temperature during seed production led to reduced thermodormancy in *A. thaliana* and *H. vulgare*, and affected mean seed size and quality in *A. thaliana* and *B. oleracea*. We used a set of biochemical and molecular approaches, including transcriptomics, proteomics, metabolomics and post-"omics" approaches, such as nuclear and chromatin compaction, DNA repair, and oxidative and post-translational modifications to macromolecules, with a view to defining regulatory switchboards that underpin the seed phenotype, and selected results will be presented in this lecture.

Fig. 1
EcoSeed was a €3m FP7 project with a run time of 4 years (2012-2016). Coordinated by the University of Innsbruck, Austria, the project involved collaborations of 11 Partners with well-established track records in seed biology and converging sciences from the United Kingdom, France, Spain, Germany and Austria. The main aim was to obtain an in-depth view of the effects of elevated and drought, stress factors that are predicted to increase due to climate change, with a focus on identifying important molecular regulatory pathways and key cellular switches that mediate seed environmental sensing and signalling, and their importance for downstream seed quality and seedling establishment.

PEROXISOMAL NITRIC OXIDE (NO) METABOLISM UNDER ADVERSE ENVIRONMENTAL CONDITIONS

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Peroxisomes are single membrane-bound subcellular compartments present in almost all types of eukaryotic cells. The basic enzymatic constituents are catalase and H_2O_2-producing flavin oxidase which illustrates its prominent oxidative metabolism (1). Peroxisomes are also characterized by metabolic plasticity, as their enzymatic content can vary according to the organism, cell/tissue type, development stage and environmental conditions. In plant cells, these organelles house a large number of antioxidative enzymes, such as catalase, superoxide dismutase (SOD), components of the ascorbate-glutathione cycle and several NADP-dehydrogenases, involved in different functions (2). Nevertheless, accumulating data have shown that plant peroxisomes have the capacity to generate nitric oxide (NO) through an L-arginine-dependent nitric oxide synthase (NOS) which strictly depends on NADPH and requires calmodulin (CaM) and Ca^{2+}. Furthermore, peroxisomal NO, together with other related RNS such as peroxynitrite, has been shown to participate in the response to abiotic stresses such as salinity, arsenic, cadmium or drought indicating that peroxisomes have an active nitro-oxidative metabolism (3, 4). As part of this metabolism, the numbers of peroxisomal proteins which undergo NO-mediated post-translational modifications such as nitration and *S*-nitrosylation have been also demonstrated under physiological and stress conditions (5). All these data demonstrate the existence of a very dynamic nitro-oxidative metabolism into plant peroxisomes indicating the relevance of these organelles.
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THE ROLE OF TRADITIONAL KNOWLEDGE IN THE SAFETY ASSESSMENT OF BOTANICAL FOOD SUPPLEMENTS

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The safety assessment of botanicals has been subject of a number of publications and recommendations in recent years. These publications have proposed flow charts for the safety assessment of botanicals and identified data that would be required to perform such risk assessments. An important element, often ignored or undervalued in the various systems proposed for the safety assessment, is information and experience from the traditional use of the plant or plant preparation.
This presentation explores the various systems proposed and aims to identify the critical and essential requirements that should observe to verify and assure the safety of botanical food supplements.

THE SUBSTANTIATION OF CLAIMS FOR BOTANICAL FOOD SUPPLEMENTS IN RELATION TO TRADITIONAL USE

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Over centuries, plants have been used for various properties, both in foods and medicinal products.
Their effects on humans have been gathered through experience, and the knowledge has been passed from generation to generation.
Legislation that has been developed to cover these products differs from country to country, which makes harmonisation complex and difficult.
Communication on the properties of plants is a key element. For medicinal use this communication focuses on indications and conditions of their use to
treat or prevent diseases. For non-medicinal use, communication focuses on nutritional and/or health benefits.
For botanical food supplements it is therefore essential that such communication is possible under the form of a claim.
Properties of plants relating to both the treatment of disease and the maintenance of health have been based largely on traditional use. In order to judge the validity of traditional use, a number of elements need
to be considered. These elements have been described elsewhere.
This presentation explores how traditional use can be combined with the applicable legislation on botanical food supplements in the European Union (EU) to ensure meaningful information to consumers.

GLOBAL PATTERNS AND DRIVERS OF PLANT NATURALIZATION

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The search for drivers of plant naturalization can be done at global, regional and local scales. Through the Global Naturalized Alien Flora (GloNAF) database, we now have the first overview of the worldwide extent and patterns of plant naturalizations (1, 2). So far, this has revealed that plant naturalizations are not happening at random with respect to geography and taxonomy (1, 2), and that bilateral trade plays a pivotal role in the distribution of naturalized alien plants around the world (3). We could also show that the ability to self-fertilize is one of the traits associated with the global naturalization success of plants (4). In current analyses, we identify the global hotspots of plant naturalization and test the effects of naturalization on homogenization of the global flora.

A major pathway for introduction of alien plants into new regions is horticulture, which might result in the biased introduction of ornamental species with certain characteristics. We used path analysis of trait, introduction and naturalization data to unravel the direct and indirect effects of traits on naturalization success of ornamental plant species in Germany (5). Furthermore, using a data set of Asian woody species that have been introduced to Europe, we could show that climatic suitability is one of the major drivers of naturalization success (6). Given that many of the non-naturalized ornamental plants in Europe originate from warmer regions, they may have a head start under climate change. In several studies, we are currently addressing the naturalization potential of ornamental plants under climate change.

While analyses of global and regional databases provide important clues about the potential drivers of plant naturalizations, and could inform management, the actual naturalization process (population establishment) happens at the local community scale. Therefore, I will end my presentation with several examples of local introduction experiments that are currently underway in my group. These studies address the role of functional traits, phylogenetic relatedness and responses to climate change, and should provide insights into the mechanisms driving the local, and consequently regional and global naturalization success of plants.

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HOW SHOULD BIOLOGISTS RESPOND TO THE GLOBAL INCREASE IN INVASIVE ALIEN SEAWEEDS?

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Invasive seaweeds are increasingly rapidly in Europe and globally, both in terms of numbers of species and biomass, spread by aquaculture and other anthropogenic activities. International law mandates control and/or eradication of invasive species (CBD Aichi Biodiversity Target 9 and EU Regulation 1143/2014 on Invasive Alien Species) and this has been translated into various national policies. However, the consequences of removal of invasive species are poorly understood, with potentially unexpected changes to other ecosystem components. Previous successful eradications of invasive species have mostly been terrestrial, on islands and at small spatial scales, focussed on extreme examples of ecological damage, whereas invasive seaweeds are well-established over large ranges. For marine algae, mechanical control efforts have mostly been totally unsuccessful, and biological control has not even been attempted. Despite their abundance and rapid spread, there have been very few demonstrations of serious negative impacts of invasive seaweeds on native species and communities. Increasingly, invasive species are seen as potential resources in the invaded area, such as the use of *Gracilaria vermiculophylla* for in Integrated Multitrophic Aquaculture and harvesting it to produce agar.

COMMUNICATIONS

PLANT TRAITS 2.0 IN ITALY: BACK TO THE FUTURE

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Plant Functional Traits (PFTs) are defined as any morphological, anatomical, biochemical, physiological or phenological heritable feature measurable at the individual level, from the cell to the whole-organism level that impact fitness (1, 2). PFTs mediate the response of plants to environment (3), affect the trophic levels and influence ecosystem processes and services (4). Accordingly they are used in many studies ranging from comparative plant ecology to community and functional ecology, from evolutionary biology to biogeochemistry. After face-to-face networking during the workshop "Plant traits 2.0" (SBI, February 9-10, 2017, Bologna), we decided to review the main findings of the Italian studies related to PFTs of vascular plants, lichens and bryophytes. We were able to collect 106 papers published in national and international peer-reviewed journals until May 2017. Review articles are usually an important source of information where scientific findings, methods, gaps and perspectives are gathered and summarized. Italy represents a highly interesting model region, since it covers a remarkable latitudinal and climatic gradient spanning three biogeographic regions (Alpine, Continental, Mediterranean), which shelter a broad variety of habitats from close-to-natural, to managed semi-natural and agro-ecosystems, and host a high plant species richness.
Almost the entire scientific production was performed during the past decade and studies were mainly carried out in the Continental biogeographic region (40%), followed by the Alpine (34%) and the Mediterranean region (26%). Secondary grasslands (35%) and forests (23%) resulted the most studied ecosystems, while few studies have dealt with Mediterranean shrublands and silvopastoral systems (3.5%), coastal habitats (8%) and montane primary grasslands or shrublands (9%). A similar frequency in the use of whole plant (25%), leaf (20%) and seed traits (18%) was observed. These broad groups encompass traits inherent to major ecological strategy theories, such as the three key traits related to the Leaf-Height-Seed plant ecology strategy scheme (5): specific leaf area, plant height and seed mass. Additionally, leaf traits (i.e. leaf area, specific leaf area, leaf dry matter content (6)) are used to classify plants according to the CSR strategy scheme (7). Phenological and clonal traits were also well represented (respectively 16% and 12%), while few papers dealing with root traits were evident (2%). Plant traits were primarily measured from samples collected in the field. The variation of these plant functional traits has been studied mainly along environmental gradients (79%) and climate manipulation experiments (11%). Among environmental gradients, climate and elevation were the most investigated, followed by land-use/management and soil nutrient/moisture. This first survey of Italian papers dealing with plant functional traits demonstrates a surprisingly high scientific production. Accordingly, we propose the enhancement of collaboration among research groups, focusing on a few recommendations for future research in Italy:
a) increase the research activity in the Mediterranean biogeographic region, with particular regard to Mediterranean shrublands, silvopastoral systems and coastal areas;
b) extensively investigate belowground traits (i.e. root and clonal traits), since these are informative with regard to fundamental mechanisms, including plant-plant, plant-soil and plant-climate interactions;
c) further investigate the effect of climate change on plant functional traits, at species and also community levels. In this regard, experimental approaches in the field or in controlled environments could be useful to disentangle the effect of land-use change in managed semi-natural ecosystems and agro-ecosystems.

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PATTERNS OF FUNCTIONAL TRAITS ELUCIDATE LICHEN COMMUNITY ASSEMBLY UNDER A GLOBAL CHANGE SCENARIO

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A promising avenue to better comprehend community dynamics involves the integration of functional traits with traditional approaches based on species richness and composition. Species functional traits are expected to directly link to environmental factors providing mechanistic insights on the ecological processes ruling community assembly along natural and anthropogenic gradients. In a number of studies across Italy (e.g. 1-5), we applied a functional trait approach to better elucidate and interpret assembly mechanisms of lichen communities. Lichens are among the most climate- and nitrogen pollution-sensitive organisms, while their pioneering capacity put them among the most important organisms triggering plant succession in early deglaciated areas. Therefore, the exploration of their diversity patterns along natural and anthropogenic gradients may provide early warning signs regarding biodiversity and functional changes in forest and high elevation ecosystems as a consequence of global change. Results of our research indicate that community dynamics are influenced by a process of selection of some important and easily identifiable functional traits, such as thallus growth form, photobiont type, and dispersal strategy, that mediate the relationships between lichens and environmental conditions at different spatial scales, from local to regional and national level.

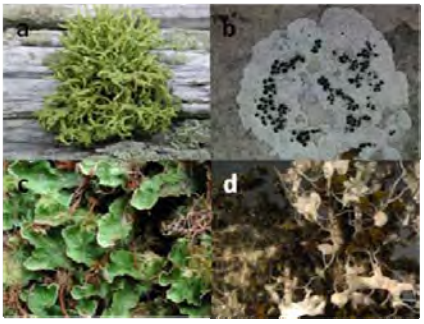

Fig. 1
Examples of different thallus growth forms: (a) fruticose, (b) crustose, (c) foliose with large lobes, (d) foliose with narrow lobes.

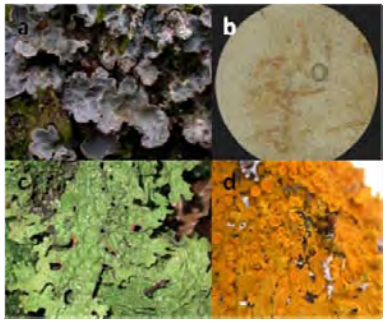

Fig. 2
Examples of lichens with different photobionts: (a) with cyanobacteria, (b) Trentepohlia, (c) mixed with coccoid green algae and cyanobacteria, (d) with coccoid green algae.

Fig. 3
Examples of different reproduction strategies: (a) vegetative by soredia, (b) sexual by ascospores (fruiting bodies), (c) vegetative by isidia, (d) vegetative by thallus fragmentation.

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DO CHANGES IN LEAF MORPHOLOGY CONTROL THE PHOTOSYNTHETIC PROCESS IN MEDITERRANEAN *CISTUS* SPP. UNDER SUBOPTIMAL AIR TEMPERATURE AND SUBSEQUENT RECOVERY?

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During the winter season, Mediterranean plant species can experience stressful conditions due to a combination of high irradiance and suboptimal growth air temperatures (i.e. between 0 and 10 °C) (1). The genus *Cistus* comprises 21 summer drought semi-deciduous shrub species with a predominantly Mediterranean distribution (2). *Cistus* spp. show an increase in leaf mass area (LMA) under suboptimal air temperature (3,4). Nevertheless, a greater LMA implies higher construction costs per unit leaf area (5), mainly deriving from its negative relationship with instantaneous carbon assimilation (1). This is payed-back in terms of leaf-longevity, which is generally higher than in leaves with a lower LMA (5). However, *Cistus* spp. are characterized by a mean leaf longevity of about six months (3,4). We questioned if the expected changes in leaf morphology through the winter season are related to changes in the instantaneous carbon assimilation given the short leaf-life span of *Cistus* spp. To answer this question, the stable medium-term (months) adjustments of leaves photosynthetic rate, the stable carbon isotope composition and leaf morphological parameters from November 2015 to April 2016 were investigated in three *Cistus* species: *C. salvifolius* (CS), *C. creticus* subsp. *eriocephalus* (CE) and *C. monspeliensis* (CM). The sampling dates defined four climatically different experimental periods (EPs) covering three sub-optimal periods (EPI, EPII and EPII) and one favorable period (EPIV). LMA increased by 10% (on average for the considered species) from EPI to EPII. In EPIV, LMA decreased in CS (1%) and in CE (17%) while increased in CM (5%), compared to the values measured in EPII. Changes in LMA across species were due to changes in both leaf thickness (LT) (R^2= 0.75, P = $2.2e^{-16}$) and leaf dry matter content (LDMC) (R^2= 0.40, P = $6.7e^{-8}$). Across species, LMA and its components scaled with photosynthetic capacity revealing a decreased net photosynthesis per unit leaf dry mass (A_{mass}) with increasing LMA and a lack of significance for the relationship between LMA and net photosynthesis per unit leaf area (A_{area}). However, the relationships did not hold through each EP to the same extent. Within each EP, the relationships LMA-LDMC and LMA-LT were weaker in EPII (R^2 = 0.13 and 0.41; P = 0.194 and 0.01, respectively) compared to the rest of the EPs (mean R^2= 0.70 and 0.80 for the relationships LMA-LDMC and LMA-LT, respectively, P<0.05). LDMC-LT, which was not significant for pooled data (R^2 = 0.0276; P = 0.852), changed dramatically within each EP (R^2 ranged between 0.07 and 0.66 and slopes between -0.41 and 0.54). The relationships LDMC-LT (R^2 = 0.66; P = 0.001) and A_{mass}-LMA (R^2 = 0.80; P = 0.003) were significant only in EPIV. The relationships δ^{13}C-LMA and δ^{13}C-LT were only significant in EPI (R^2= 0.26 and 0.28; P = 0.036 and 0.044, respectively) and in EPII (R^2= 0.36 and 0.29; P = 0.023 and 0.048, respectively). The relationship δ^{13}C-LDMC was not significant within each EP.

Changes in LMA, LT and LDMC in the considered species reflect a certain degree of leaf hardening with no effects on the return of carbon fixed under sub-optimal conditions. However, the positive scaling of δ^{13}C with LT, and LMA, in EPII revealed that changes of LT in determining LMA variations in EPII may drive internal adjustments in order to reduce the resistance to CO_2 diffusion within the leaves.

The results highlight that *Cistus* spp. do not mirror changes in leaf morphology with a longer payback time for construction costs under sub-optimal conditions, and this is in accordance with their ecology. Nevertheless, when temperature becomes favorable, then the relationship LMA (and its components)-A_{mass} was significant and driven by species specific differences. Our results therefore suggest that the analyzed functional relationships can follow seasonal changes in *Cistus* spp, possibly affecting species leaf turnover strategies during the favorable period. The highlighted functional switch during the transition from unfavorable to favorable environmental conditions deserves particular concern in a global climate change context which is forecasted to modify the length of the favorable period in Mediterranean ecosystems.

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TREE-RING INTRA-ANNUAL-DENSITY-FLUCTUATIONS AS FUNCTIONAL INDICATORS OF RESPONSES TO DIFFERENT ENVIRONMENTAL CONDITIONS IN *QUERCUS ILEX* L. (VESUVIUS, SOUTHERN ITALY)

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Understanding plant reactions to past climatic variability is important, especially to forecast how different species will cope with forthcoming changes (1). "Intra-Annual-Density-Fluctuations" (IADFs), recently listed among wood functional traits (2), appear as unexpected changes in wood density within tree rings and result from complex interactions between tree physiology and climate during the growing season (3). IADFs could be a source of proxy data for reconstructing tree response to intra-annual variations in environmental conditions, once the relationship with the triggering factors is clarified. IADF formation is frequent within tree rings of species living in the Mediterranean area (4), which is considered to be one of the climate change hot spots (5). The main scope of this study is to analyse the properties of IADFs occurring in tree rings of *Quercus ilex* L. growing on two opposite slopes of the Somma-Vesuvio volcanic complex (Southern Italy) (Fig. 1) in order to acquire knowledge on its plasticity to cope with seasonal alternation of climate conditions (Fig. 2). Specific aims of this study are: 1) to explore IADF frequency, IADF width, and intra-annual variation of anatomical traits and carbon isotopes in *Q. ilex* tree rings, and 2) to find out the relationships between such traits and temperature and precipitation data. Dominant trees were sampled (two cores per tree) in the south-slope and in the north-slope site in order to date them and to identify IADF presence/absence, width and anatomical traits. Tree-ring width, IADF frequency, IADF width and vessel size, wood density and vessel frequency were measured. Time-series climate analyses were performed with precipitation and temperature data. Carbon isotopes were quantified in the different portions of tree rings with and without IADFs in order to provide information about intra-annual tree physiological responses. North-slope trees were found to have wider tree rings. Considering the common period of the two sites (1966-2015), IADFs were found to be more frequent, wider, with smaller and less frequent vessels and a higher wood density in the north-slope compared to the south-slope site. A decrease in IADF frequency over time was shown in both sites. Spring precipitation was the main driver of ring width for both sites, with the south-slope site more sensitive than the north-slope one. IADF frequency and width showed to be negatively influenced by mean temperature rather than total precipitation in both sites. IADF anatomical parameters of south-slope tree rings were influenced by precipitation, while the ones of north-slope tree rings showed to be affected by temperature. The combined approach of dendrochronological and quantitative wood anatomy techniques, together with the analysis of carbon isotopes, allowed to identify main drivers for IADF formation and to propose their ecological meaning under different environmental conditions.

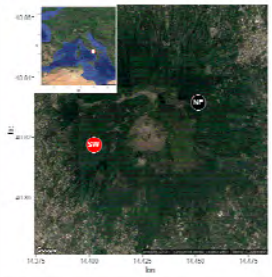

Fig. 1
Study sites

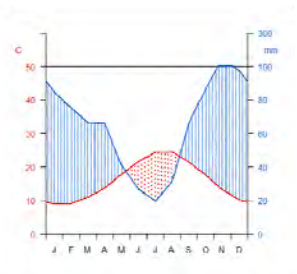

Fig. 2
Mediterranean climate

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DO GERMINATION TRAITS CONTRIBUTE TO SPECIES DISTRIBUTION IN ALPINE HABITATS?

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Functional plant traits are extendedly used in ecology to explain species distribution and community behaviour along time and space (1), including community response to climate warming. During the past years many plant traits have been registered (e.g. TRY database or LEDA Traitbase). In this context, regeneration traits and, in particular, germination traits have been neglected (i.e. appear in less than 20% of community ecology publication) (2) at the expense of other relevant but overused traits. However, species cannot be part of the community if environmental conditions are only suitable for plant growth and survival, but not for seed germination (3). In alpine habitats, regeneration from seeds is crucial for the maintenance of plant communities (4) and it is under strong environmental control, suggesting that germination traits are important in shaping alpine plant communities.
In this study, we defined the vegetative and germination trait of 14 closely related species growing in two alpine habitats- #6230- *Nardus-rich species grasslands'*; and #6170 - *Alpine and subalpine calcareous grasslands* based on Natura 2000 habitat classification (5). Germination niche derived from laboratory-controlled test, including cardinal temperatures for germination [i.e. base (T_b), optimal (T_o) and maximum (T_c) temperature], base water-potential (Ψ_b) and the pH range of germination. Meanwhile, vegetative traits was defined using species' leaf area (LA), specific leaf area (SLA) and leaf dry matter content (LDMC), by querying TRY-database. These latter functional traits were chosen as they explain most of the plant-global variability (6,7).
Habitat dissimilarities in both vegetative and germination traits were assessed using Analysis of variance (ANOVA) when data was normally distributed and Generalized Lineal Mixed Model (i.e. GLMM) when the response variable was a proportion [i.e. final germination percentage (FGP)]. Finally, two matrices, including separately vegetative and germination traits, were reduced using Principal Component Analysis (PCA). Species were clustered by habitat provenance on the new Axis defined by the PCA.
Results showed some germination traits dissimilarities according to habitat provenience, with species from habitat #6170 showing higher T_b, lower Ψ_b and lower ability to germinate under acidic pH than species from habitat #6230. Conversely, T_{op}, T_c and vegetative traits were similar in both habitats. PCA showed that germination traits separate species by habitat provenance, meanwhile vegetative traits were overlapped, showing high interspecific variation within each habitat.
The high vegetative traits convergence hints small differentiations in adult plants between habitats. Therefore, target vegetative traits did not help to explain species distribution between habitat. However, importantly, the separate habitat clusters when using germination traits and the significant differences detected when assessing habitat dissimilarities (i.e. T_b, Ψ_b and pH), suggest that species occurrence in the two habitats may be limited at the earliest stages of plant development, particularly by germination constrains. Finally, the lack of differences in germination traits (i.e. similar T_{op} and T_c) detected between habitats may indicate common plant adaptation to alpine environment that should prevent germination in late summer (8), such as narrow temperature range at the time of seed dispersal and high T_{op}.

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REPRODUCTION OF TWO ALPINE TUNDRA SPECIES UNDER CLIMATE WARMING

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Alpine plants have evolved to contend with seasonal climate and to rely on environmental cues indicating the appropriate timing for phenological phases (1). Consequently, current climate change can strongly alter plant reproduction, in particular within high-elevation ecosystems, as both observation and simulation indicate that warming rate increases with elevation (2, 3). In alpine habitats, predicted warmer temperatures coupled with a decrease in snowfall and snow-cover duration have the potential to influence plant phenology and, consequently, to affect plant sexual reproduction and regeneration from seed, which play a key role for species persistence, adaptation and migration (4, 5). Research on reproductive phenology of wild plants has been mostly based on the analysis of early-season events, whereas the responses of late-season phenology to climate change are less understood (6). For instance, under climate warming it has been predicted a counterintuitive increase of detrimental frost events during the first part of the growing season (1), due to an advanced melting of the snow-pack, which protects plants against low atmospheric temperatures. However, a warming-induced acceleration of the phenological development of reproductive structures may expose seeds to novel environmental conditions, promoting late-season germination with important implications for seedling survival and plant population and vegetation dynamics.

In this communication, based on data of two target alpine species, namely *Leucanthemopsis alpina* and *Veronica alpina*, we focused on the consequences of warmer temperatures on late season events. By integrating field, laboratory and common garden experiments, we explored how seed dispersal timing, seed germination and seedling survival are influenced by climate warming.

Experimental warming accelerated the developmental rate of reproductive structures and, consequently, advanced seed dispersal. However, phenological responses to temperature were species-specific, since the developmental rates of the plants investigated had different thermal threshold and heat-use efficiency. In addition, intra-specific variations were found for the temperature sensitivity of different phenophases and for the phenological development of sub-populations occurring along the snowmelt gradient, with higher heat-use efficiency in individuals from later snowmelt sites.

Seeds produced by maternal plants exposed to experimental warming were longer lived in both the species studied. In addition, warmer temperatures after seed dispersal increased their germination rates, but seeds of *V. alpina* were more influenced than the ones of *L. alpina*. Moreover, under warmer growing conditions the target plants showed species-specific reductions of the dormancy states, more pronounced for *L. alpina* seeds.

Seedling growth was enhanced under higher temperatures, but seedling survival to simulated snow-cover periods was not dependent on the time available for growth. On the other hand, late season frost events induced high seedling mortality, indicating that dormancy mechanisms in alpine plants may be more useful for avoiding detrimental thermal conditions in autumn than for achieving a critical size for winter survival.

In conclusion, our results suggest that in a future characterized by warmer and longer growing seasons, plant reproduction in alpine habitats may experience a release of selective pressures imposed by the length of the snow-free period and a simultaneous increase of constraints induced by both early- and late-season low temperature extremes. It is likely that these processes will have a major impact in plant population dynamics of long-lasting snow-cover communities, such as alpine snowbeds. In these habitats, changing climatic conditions may shift the primary agent of selection, promoting the fitness of cold resistant plants to the detriment of previously adapted fast growing species.

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LONG-TERM MONITORING OF A SUBALPINE GRASSLAND: INTER-ANNUAL VARIATIONS IN PHENOLOGY, BIOMASS PRODUCTION AND PLANT COMMUNITY STRUCTURE AMONG NINE GROWING SEASONS

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Grasslands are among the largest ecosystems in the world, covering more than one third of the terrestrial surface (1). They are an important carbon sink, storing up to 30% of the global reserves of C (2), and can be considered as important as forests in recycling greenhouse gases (3). Climate change has the potential to produce a shift in the role of grasslands, from C sinks to C sources, especially in alpine ecosystems which are ecologically sensitive and predicted to be severely affected by climate change (4).
This study aims to monitor the inter-annual variations among nine growing seasons in a subalpine grassland in terms of phenology, biomass production and relative abundance of different plant functional groups.
The study area is located on the Western Italian Alps in Aosta Valley, and is occupied by a subalpine grassland abandoned in 2008 and dominated by *Nardus stricta*. On average, from the end of October to late May, the site is covered by a thick snow cover (90-120 cm) which limits the growing period to about 5 months. The monitoring project started in 2009 and was carried out during the following growing seasons, approximately from May to October. Biomass samples were collected every ~10 days and then sorted in grasses, forbs and shrubs prior to further analyses. Sorted samples were scanned in order to calculate the Leaf Area Index (LAI) of each functional group, and then dried and weighted. The phenology was monitored through direct observations in field as well as the analysis of images taken by camera. Results showed a high variability among years due to snow cover duration, precipitation and air temperature. Moreover, a trend in plant community structure through the dominance of grasses was observed as a consequence of grazing exclusion.
Considering the high variability among different seasons, the effects of climate change on the C stock of alpine grasslands appears hardly predictable. Indeed, there is evidence that climatic drivers may cause direct changes of the growing season pattern even in a short period. In this framework, long-term monitoring in mountain areas is essential in order to investigate the general trend occurring in biomass production, phenology and community structure, and make reliable predictions.

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HERBIVORE ASSEMBLAGES AND SEASONALITY DETERMINE NITROGEN AND PHOSPHOROUS CONTENT IN TUNDRA PLANTS

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In an environment with pronounced seasonality and low rates of mineralization and decomposition such as the Arctic and sub-Arctic, plant-herbivore interactions play a remarkable role for the nutrient cycling. Herbivores can affect nitrogen (N) and phosphorous (P) cycling by affecting species composition, by altering nutrients in plant tissues, by removing plant nutrients and returning them to the system through faeces and urine, and by altering plant and soil micro-climate (1). The management of large herbivores, *i.e.* land use, often occurs where there are also other grazing animals. In Fennoscandia, reindeer herding has been practised for centuries within the Sámi culture. High reindeer grazing pressure has been shown to strongly influence vegetation patterns and ecological processes (2). However, small rodents, such as voles, can act in concert with reindeer in influencing vegetation structure and, in turn, ecosystem dynamics (3). Rodents can cause disturbance to the vegetation throughout the year since they remain active during the entire winter period under the snow cover. In summer 2015, we conducted a 1-year reindeer exclusion experiment in alpine tundra meadows at Ifjordfjellet (70°25' N, 27°20' E), Finnmark, Northern Norway. In addition, small rodent winter disturbance was employed as a quasi-experimental factor to disentangle the role of this herbivore assemblage in determining N and P content in tundra plant leaves. Seven sampling occasions along the summer allowed us to test for seasonality effects on nutrient content in plants.
Small rodent winter disturbance had the strongest effect on nutrient content in plant leaves. Forbs and grasses growing in disturbed patches had, in average, 11 and 26 % higher leaf N concentration and 11 and 35% higher P concentration than the ones in undisturbed plots, respectively. Furthermore, the effect of rodent winter activity on N and P concentration in leaves was consistent throughout the entire summer season. Where the vegetation was undisturbed by small rodents, summer reindeer grazing significantly enhanced N concentration in grass leaves, with plants growing in control plots having 8% higher leaf N concentration than plants excluded from reindeer herbivory. Reindeer did not directly affect N content in forbs and P content in either forbs or grasses. However, a strong interaction between the two types of herbivory dictated the dynamics of N and P in grass leaves. Where rodents had disturbed the vegetation during the previous winter, summer reindeer grazing decreased N and P concentration in plant leaves by 18 and 19%, respectively. Nutrient content in plants significantly decreased throughout the summer, with senescent leaves at the end of the growing season having 20 and 18% lower leaf N concentration and 15 and 8% lower leaf P concentration in forbs and grasses, respectively.
Our results suggest that herbivores are a key driver of ecosystem nutrient dynamics in the tundra and that different herbivore assemblages can dictate whether N and P concentration in plants increase or decrease. This latter can have huge consequences in terms of ecosystem process rates such as nutrient leaching and decomposition, which in turn lay down the foundation for the ecosystem carbon balance and nutrient cycling.

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CHARACTERIZATION OF THE MAPK AND MKK GENE FAMILIES OF *LACTUCA SATIVA* AND ANALYSIS OF THEIR EXPRESSION UNDER ABIOTIC STRESSES

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The mitogen-activated protein kinases (MAPKs) is a highly conserved signalling pathway playing important roles in plant development (1) and response to abiotic (2, 3, 4) and biotic stresses (5). Molecular characterization of the MAPK gene families have been reported for several plants, including arabidopsis (6), rice (7) and tomato (8), but no information is yet available concerning these genes and their expression in lettuce, the most cultivated leaf vegetable worldwide, for which the complete genome sequence has been reported. In this study we identified 19 MAPK and 9 MPKK genes of *Lactuca sativa*, classified on the basis of their strong homology to hortologs from *Arabidopsis thaliana*. The analysis of their expression in different organs performed by Real-Time PCR revealed distinctive patterns, with some MAPKs showing particularly strong expression in developing flowers. Expression analyses performed in plants subjected to abiotic stresses revealed that most of the MAPK and MKK genes of *L. sativa* are differentially responsive to stresses such as wounding, heat, cold, salinity and UV irradiation. Our data highlight the relevant role of some of the MAPK and MKK genes in development and abiotic stresses response in *L. sativa* plants.

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PHYTOCHELATIN SYNTHASE 1 REGULATES IRON HOMEOSTASIS IN *ARABIDOPSIS THALIANA*

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Phytochelatin synthases (PCS, EC 2.3.2.15) are cytosolic enzymes, constitutively expressed in the genome of different organisms including *Schizosaccharomyces pombe* (1), *Caenorhabditis elegans* (2,3), algae and plants (4). PCS have been explicitly studied with respect to their role and mechanism of action towards heavy metal detoxification (e.g. essential heavy metals such as zinc (Zn) as well as toxic such as cadmium (Cd) (5). Based on studies of *Arabidopsis thaliana* phytochelatin synthase 1 (AtPCS1), it is recognized that PCS catalyse the formation of thiol rich oligomers, phytochelatins (PCs), from glutathione (GSH) and related thiols in the presence of metal ions (6).
Moreover: i) AtPCS1 is involved in the catabolism of GSH-conjugates (7); ii) AtPCS1 is required for pathogen-triggered callose deposition (8); iii) its mRNA is upregulated by a mimic of eubacterial flagellin (8); iv) AtPCS1 expression might be not upregulated by heavy metal toxicity (9), but its transcript abundance in roots increases by deficiency of the micronutrient iron (Fe) (10); v) physiological (homeostatic) Fe concentrations activate the *in vitro* activity of PCS even in basal land plants (11).
The main goal of this study was to determine the *AtPCS1*-driven response towards Fe deficiency and to establish whether and how AtPCS1 is involved in Fe homeostasis. To do so, we compared the growth, Fe concentration, and the expression of Fe transporter genes in roots and shoots of the *A. thaliana cad1-3* mutant lacking functional AtPCS1 and transgenic plants overexpressing AtPCS1 (AtPCS1-OE) *vs.* wild-type, all grown either under Fe-sufficient or deficient conditions. We found that the *cad1-3* mutant accumulated less Fe in roots and was significantly more sensitive to Fe deficiency than wild-type plants. By contrast, AtPCS1-OE lines were more tolerant to Fe deficiency than *cad1-3* and wild-type plants. These results were consistent with the expression of Fe transporter genes in different plant lines. A mechanistic model of the role of AtPCS1 in Fe homeostasis will be proposed and discussed.

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IMPACT OF DNA METHYLATION STATUS ON PLANT RESPONSE TO CD EXPLORED THROUGH A TRANSCRIPTOMIC ANALYSIS

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Because of their sessile life style, plants evolved the capacity to integrate external stimuli into both developmental programs and adaptive modifications, through efficient short-term strategies which specifically rely on a rapid modulation of existing genetic information (1, 2). DNA methylation is among the main epigenetic mechanisms involved in this re-establishment of genome transcriptional potential through selective modulation of chromatin organization (2, 3).
In this context, the present work was addressed to explore how methylation-based epigenetic mechanism acts in plant response and adaptation to cadmium (Cd) stress. To this aim *drm1 drm2 cmt3* triple mutant of *Arabidopsis thaliana,* defective for three methyltransferases involved in *de novo* and maintenance methylation, was used. Cd stress was selected since the widespread presence of this non-essential element in both terrestrial and marine environment represents one of the major ecological concern. Namely, Cd is highly toxic and soluble and its accumulation in crops poses a potentially significant threat to human health (4).
Concerning Cd toxicity in plant, it has been demonstrated that, even at low concentrations, it affects several physiological and metabolic processes, including respiration, photosynthesis, nutrient uptake, antioxidant defences, hormone homeostasis thus inhibiting plant growth and development (5, 6, 7, 8). There are evidences that all these effects rely on several mechanisms, including genotoxic events, generation of free radicals, lipid peroxidation, damage to the cell membrane, enzyme inhibition, uptake competition, and more recently modifications at both transcriptional, post-translational and epigenetic levels (25, 8, 9, 10, 11).
In order to gain further insights into these aspects, we decided to compare the transcriptomic response of wild type plants (WT) and *drm1 drm2 cmt3* triple mutants of *Arabidopsis thaliana* exposed to Cd treatment (25 µM and 50 µM), by applying by RNA Seq (Illumina) technology. The aim was to explore whether a global DNA hypomethylation could differentially modulate the responses of the plant to Cd-induced stress through a different modulation of gene expression and, consequently, a different metabolic reconfiguration.
The obtained results evidenced that the number of differentially expressed genes between the mutant and the WT following Cd treatment correspond to 406 up-regulated and 229 down-regulated genes under Cd 25 µM treatment and 439 up-regulated and 861 down-regulated genes under Cd 50 µM. Among the pathways found to be differentially modulated we focused our attention on hormone-related ones. We observed that, following Cd treatment, genes related to the metabolism of several hormones, including cytokinins, gibberellins and jasmonic acid, were differentially expressed in the mutant vs WT. This result is consistent with data showing that stress sensing and the downstream plant response involves signalling molecules and plant growth regulators (2, 5, 6, 7, 8). Interestingly among the up-regulated genes in the triple mutant vs the WT, a considerable number is involved in t the regulation of transcription, DNA binding, catalytic and hydrolase activity. Globally , these results are discussed in relation the hypothesis that the hypomethylation status of *drm1 drm2 cmt3* triple mutant could directly or indirectly provide the opportunity for the plant to activate more readily the defence responses, thus limiting Cd-induced damage, consistently with the differential growth response of mutant and WT to Cd exposure.

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INTERACTIONS BETWEEN MICROFUNGI AND SULFIDE MINERALIZATIONS

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Interactions between fungi and minerals are reported from several environments and geological materials (1, 2). Recent researches have highlighted the important contribution played by fungi in sulfide rocks and mineral bioweathering, in particular in active and abandoned sulfide mines affected by Acid Mine Drainage (AMD) processes (3, 4).
This work aimed at studying the role of native microfungal strains in the minerogenetic processes (including mineral bioweathering and biomineralization), and the interactions among sulfide mineralizations and fungi.
The first experiment was carried out on pyrite-rich hardpans, sampled in a small waste rock dump from the abandoned Libiola mine (NW Italy); it was devoted to study a strain of *Trichoderma harzianum* species complex as concerns its possible capability of biomineralization.
The results evidenced that the fungal strain was able to grow and cover most of the hardpan grains on all the autoclaved samples during a two-months incubation; in this period, it produced submillimetric thick biofilm containing significant concentrations of sulfur (most likely sulfate-S) and the fungal mycelium developed a close linkage to the mineral grains and rock fragments. At the end of the experiment, acicular and elongated prisms of gypsum crystals occurred within the fungal biofilm; they formed radiating aggregates centered on subspherical amorphous masses, most likely represented by mixtures of mycelium and cryptocrystalline or amorphous minerals, which presumably acted as nucleation centers for gypsum crystals growth. Gypsum formation may be either the consequence of an active fungal biomineralization or the result of a biologically-induced indirect mineralization, where secretion of acids and secondary metabolic products by fungal mycelium may trigger chemical reactions, induce chemical gradients, and create preferential nucleation sites.
A second experiment was carried out on almost pure and unaltered pyrite mineralization. It was aimed at evaluating the potential role played by microfungi in the weathering of sulfides. Three fungal species (*Penicillium brevicompactum* Dierckx, *P. glandicola* (Oudem.) Seifert & Samson, and a strain of *Trichoderma harzianum* species complex) were used for the bioalteration tests. After one week, the pyrite grains were almost completely covered by mycelia and after six weeks the surface of pyrite crystals appeared pitted and pocked, showing signatures of fungal attack. Corrosion patterns were not related to specific crystallographic planes or weakness but they were randomly distributed in correspondence of the fungal hyphae attachment and dimensionally corresponded to fungal structures such as conidia (~3µm).
Both the experimental results highlight the paramount role of microfungi in biomineralization and bioweathering of sulfide-bearing materials. Understanding these environments, interactions and processes can increase the knowledge of these extreme habitats with environmental issues and can also yield useful applications for mycoremediation and mycomining.

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GRAVITY VS. OTHER EXTERNAL STIMULI IN SEEDLING ROOT TROPISMS

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Plants are subject to a large number of environmental factors influencing root growth and orientation. Recently, an increasing number of Space Biology studies are focused on factors and processes involved in root orientation aiming at the improvement of plant cultivation in altered gravity conditions such as those of the International Space Station (ISS), the Moon or Mars. A better understanding of root growth strategy and tropism interactions can also provide valuable information for optimisation of fertilisation and irrigation in plant cultivation on Earth. Gravity is the strongest factor which guides roots into the soil ever since plants colonised the land. However, several 'minor' tropisms have a high relative impact on the combination of influences on which root growth orientation is based. To date, many different growth responses to external stimuli have been identified for plant roots. The more extensively studied and longer known ones include gravitropism, hydrotropism, halotropism, thigmotropism, phototropism and chemotropism (1). For some of these, besides the associated responses, the molecular functioning is also documented to a certain extent, but largest part of these pathways still remains unknown. Other evidences suggest more environmental factors are influencing root growth, such as electric fields, magnetism and even sound (2). Understandably, the latter are studied in as much isolation from other tropic influences as possible. This also apply to most of research on well-studied tropisms, while it is clear that the final growth strategy under ecological circumstances relies on the integration of proportional influences of all tropic signals. So far, the particulars of these interactions have not been widely studied.
In our experiments, attempting to clarify the priorities that seedling roots give to multiple environmental cues when confronted with them simultaneously, seeds were placed in a variety of combined conditions. Strong signals related to e.g. light, water, and chemicals were tested in normal and altered gravity combining different conditions to distil the relative importance of each in orienting seedling root growth. Gravity is known to be a particularly strong tropic influencer, making it more difficult to observe the effects of 'minor' tropisms. In order to investigate the interactions between these 'minor' and 'major' tropisms, we used a uniaxial clinostat as a means of negating the constant tropic influence of gravity. While true absence of gravitropic influence for multiple days is only achievable through ISS experiments, the clinostat serves as a reliable approximation of this effect through constantly changing the gravity vector. Because most experiments on tropisms are conducted with *Arabidopsis thaliana*, and evidence for species-specific responses have been found (e.g. 3), we used seeds of different species from several Families. In this way, a broad overview of the interactions between different seedling tropisms in different species was achieved.
Our results are an important starting point for the design of plant growth chambers on the ISS, where interactions of tropisms are upset considerably by microgravity. Back on Earth, the knowledge of the priority that plants give to nutrients, water and gravity when exploring the soil and building their root architecture would provide farmers with more efficient and less resource consuming fertilization and irrigation strategies. This dual-use aspect makes the study of root tropism interactions not only an interesting, but also a valuable area of investigation.

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DO ARBUSCULAR MYCORRHIZAL FUNGI POSSESS AN RNA SILENCING MACHINERY?

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Small RNAs (sRNAs) are short non coding RNA molecules (20-30 nt) that regulate gene expression at transcriptional or post-transcriptional level in many eukaryotic organisms[1]. Beside the well established roles in the regulations of developmental processes and responses to stress[1], recent investigations have highlighted that sRNAs are also involved in interspecies communication[2, 3]. Nevertheless, almost nothing is known about sRNAs occurrence in arbuscular mycorrhizal fungi (AMF), a key component of the plant microbiota that can provide several benefits to host plants including an improved mineral nutrition and tolerance to biotic and abiotic stress.
Our interest is to characterize the RNA silencing machinery in AMF, focusing on the functional role in the silencing processes of endogenous and host plant genes and in the establishment and maintenance of the AM symbiosis. By exploiting the published genome assemblies of the AMF *Rhizophagus irregularis*[4, 5], we started the characterization of conserved RNAi genetic components. *R. irregularis* is equipped with a large number of genes encoding the typical RNAi proteins: 1 Dicer-like, 39 Argonaute and 17 RNA-dependent RNA polymerase genes. *In silico* analyses showed that some of these genes are differentially expressed in mycorrhizal roots *versus* germinating spores. At the same time, using a NGS (Next Generation Sequencing) approach, we performed a survey of the fungal sRNAs repertoire in the intra- and extraradical fungal structures in the *R. irregularis-Medicago truncatula* symbiotic association. Preliminary data suggest the occurrence of a population of functional sRNAs and a very complex fungal gene silencing machinery in AMF.

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EFFECTS OF SALINITY AND CHROMIUM ON GROWTH AND ELEMENT UPTAKE AND ACCUMULATION IN THE HALOPHYTIC PLANT *CHENOPODIUM QUINOA* WILLD.

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In natural environment, plants often face multiple stresses. Chromium (Cr) pollution and salinity can arise concomitantly in soil as a consequence of the use of non-renewable resources, industrial and agricultural activities, or salt discharge, irrigation with saline water, seawater intrusion, etc.. Singularly, Cr and salt can affect several metabolic processes in plants including photosynthesis, water balance, nutrient uptake and accumulation, or partitioning, leading to growth reduction. However, the interaction between Cr and salt may or may not be additive. In order to explore this aspect, we evaluated the cross-effects of Cr and NaCl on *Chenopodium quinoa* Willd., a facultative halophytic species whose tolerance to high doses of Cr(III) has been reported (1). A greenhouse experiment was performed to investigate the effects of 500 mg kg^{-1}soil dry weight Cr(III)) with/without 150 mM NaCl on plant biomass production and element (Cr, Na, P and Fe) uptake and distribution in roots, stems and leaves (at the end of trial, ten days after soil Cr and/or NaCl addition). Results showed that Cr(III), in the presence or absence of NaCl, had no significant effect on dry biomass (except leaf biomass which was enhanced); moreover, plants did not display any symptoms of toxicity or stress. Cr and Na were differentially accumulated in the plant organs. In particular, Cr concentration was significantly higher in roots than in shoots; interestingly, root Cr concentration was slightly but significantly enhanced when Cr and NaCl were supplied together. Even though it was mainly translocated to the leaves, Na was accumulated in all plant organs; in the combined treatment, Na accumulation in leaves was reduced as compared to the treatment with NaCl alone. The uptake of nutrients, P and Fe, was significantly influenced by Cr and/or NaCl addition, resulting in a reduction of P content in the leaves; on the contrary, Fe concentration, which was highest in the roots, increased in the presence of salt. In conclusion, quinoa is able to tolerate relatively high Cr(III) and NaCl soil concentrations, even when they are both present, suggesting that this highly stress-tolerant species can be potentially employed for phytoremediation of Cr-polluted saline soils.

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CU AND NI REMOVAL FROM AQUEOUS MEDIA BY GREEN MICROALGAE IN SINGLE AND BIMETAL SOLUTIONS

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Metal polluted waters represent a major environmental problem that poses hazards to the ecosystem and human health[1, 2]. Human activities that lead to an enrichment of metals in aquatic systems include discharges of untreated effluents from mining, chemical and metallurgical industries, while natural processes include geological weathering[3]. Cyanobacteria and green microalgae naturally growing in metal contaminated waters have proven to be the best candidates for a highly effective removal of metals from aqueous solutions [4,5].
In this study, the effect of Cu and Ni on the growth of two green strains of microalgae, namely *Chlorella vulgaris* and *Desmodesmus* sp., was evaluated along with the removal capacity from single metal solutions (by using different metal concentrations from 1.9 to 11.9 mg L^{-1}). Microalgal growth showed to decrease at increasing metal concentrations, but promising metal removal efficiencies were recorded: up to 43% and 39% for Cu by *Desmodesmus* sp. and *C. vulgaris*, respectively, with the highest sorption capacity of 33.4 mg gDW^{-1} in *Desmodesmus* sp. As for Ni, at the concentration of 5.7 mg L^{-1}, the removal efficiency reached 32% for *C. vulgaris* and 39% for *Desmodesmus* sp. Examination of the effects of multi-metal solutions is more representative of actual environmental problems than single metal studies [6] and for this reason, *Desmodesmus* sp. growth and metal removal were evaluated employing bimetallic solutions. The removal efficiency for Cu was higher than that of Ni for all the mix solutions tested with a maximum of 95%, while Ni-removal reached 90% only for the lowest concentration tested. Results suggest the occurrence of a synergistic effect for the removal of the two metals when co-present at the lower concentrations tested, and a synergistic effect only for Cu removal in the presence of significant Ni concentrations. Biosorption of both metals reached maximum removal levels within the fourth day of incubation (with metal uptakes of 67 mgCu gDW^{-1} and 37 mgNi gDW^{-1}). Observations at the confocal laser scanning microscope allowed to evaluate the intracellular bioaccumulation of metals in *Desmodesmus* sp. after DAPI staining of cells exposed or not to Cu during growth. Imaging suggested that Cu is sequestered in polyphosphate bodies within the cells, as observable also in phosphorus-deprived cultures.
The results of this study highlighted the potential of green microalgae to be employed for bioremediation of metal polluted waters.

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MUSCARI COMOSUM (L.) MILL.: METABOLITE PROFILING, ANTI-OBESITY AND ANTIOXIDANT POTENTIAL OF BULBS AND AERIAL PARTS

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Leopoldia comosa (L.) Parl. (Liliaceae) is a perennial bulbous plant largely found in the Mediterranean region (1). The edible bulb is commonly consumed, particularly in Southern Italy and Greece. In Apulia and Basilicata, where it is known as lampagioni or lampascioni, *L. comosa* is cultivated and its bulb is considered a culinary delicacy (2).
The aim of this study was to evaluate the anti-obesity activity of *L. comosa* bulbs, leaves and inflorescences. The potential health benefits in the treatment of obesity of plant extracts were verified through the assessment of the inhibition of digestive enzyme pancreatic lipase. The antioxidant potential and the chemical composition of each sample were investigated as well.
The rise of obesity is a serious health problem as this condition is associated with an increased risk of several diseases, including type II diabetes, cancer and cardiovascular events. In the last few years, different drugs have proved unsuccessful because of their serious adverse effects. Orlistat, a semisynthetic hydrogenated derivative of the natural lipase inhibitor produced by *Streptomyces toxytricini*, is the only anti-obesity medication currently approved in Europe. Orlistat is able to prevent dietary fat absorption by 30%, inhibiting both pancreatic and gastric lipase. However, because of the adverse effects associated with this available lipid-lowering agent, such as diarrhea, fecal incontinence, flatulence, bloating and dyspepsia, there is a growing interest in herbal remedies, aiming to find well-tolerated effective drugs. Different natural compounds have already been demonstrated to modulate obesity through various mechanisms of action (3).
In the present study, fresh samples of *L. comosa* bulbs, leaves and inflorescences (Fig. 1) were cut into small pieces and extracted through maceration with a hydroalcoholic solution. Total phenolic content of each sample was evaluated by means of Folin-Ciocalteau reagent. Total flavonoid content was also determined using a colorimetric method based on the formation of a complex flavonoid-aluminum. Each raw extracts was then fractionated using solvents with increasing polarity: *n*-hexane, dichloromethane and ethyl acetate. The metabolite profiling of the different plant part was detected using different chromatographic techniques, GC-MS and HPTLC.
The antioxidant properties of *L. comosa* hydroalcoholic extracts and fractions were investigated by means of DPPH and β-carotene bleaching test.
The *in vitro* lipase inhibitory activity of samples was evaluated by monitoring the hydrolysis of *p*-NPC (Fig. 2). Obtained results demonstrated the potential health benefits in the treatment of obesity of both bulbs and aerial parts of *L. comosa*, and support the hypothesis that this plant could represent a rich source of lipase inhibitors.

Fig. 1
L. comosa bulbs, leaves and inflorescence

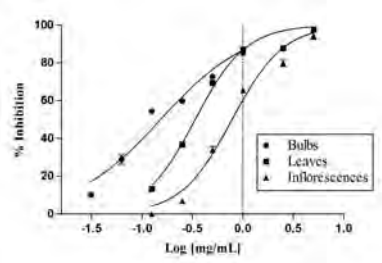

Fig. 2
Lipase inhibitory activity of ethyl acetate fractions

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APPLICATION OF METABOLOMICS TO IMPROVE *IN VITRO* BIOACTIVITIES ASSAYS FOR PLANT CRUDE EXTRACTS

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Plants have an enormous potential as a source of new bioactive molecular entities. Nevertheless, plant-based drug discovery poses some challenges, mostly due to the complexity of plants metabolome, which makes the identification of the active principles particularly complicated (1). In order to speed up the identification of active principles from plant crude extracts, a promising novel approach is provided by chemometrics-metabolomics (2). Moreover, this strategy can be employed as a dereplication procedure or to detect eventual false positive results in the bioassays. Based on this concept, in the present work, 50 medicinal plants were tested as *in vitro* inhibitors of three different enzymes, namely: collagenase, α-glucosidase and HIV-1 reverse transcriptase associated Ribonuclease H (RNase H) activity. Hence, ^{1}H-NMR-based metabolomic profiling and biological activity data were combined performing a supervised multivariate analysis (OPLS model, Fig.1), which reveled positive correlation between the presence in the extracts of tannin-related compounds (signals at δ 7.08 in the NMR spectra) and increased bioactivity. This result was confirmed preparing, by filtration on polyamide columns, tannin-free (TF) extracts of the plants previously identified as active. Hence, the TF extracts were tested in the same bioassays and the activity was compared with the one of the respective crude extract. After this procedure, all the plants previously selected as promising inhibitors of α-glucosidase and HIV-1 RT-RNase H function showed a marked loss in activity after the tannin removal procedure. Conversely, in the case of collagenase inhibition, three plants (*Alchemilla vulgaris*, *Emblica officinalis*, *Tinospora cordifolia*) retained a significant inhibitory activity after the tannins removal procedure (Fig.2). In conclusion, when crude plant extracts are tested as inhibitor of the discussed enzymes, a tannin removal procedure is advisable in order to focus on new active principles other than tannins, which are generally considered non-specific inhibitors in *in vitro* tests and can be responsible for false positive results.

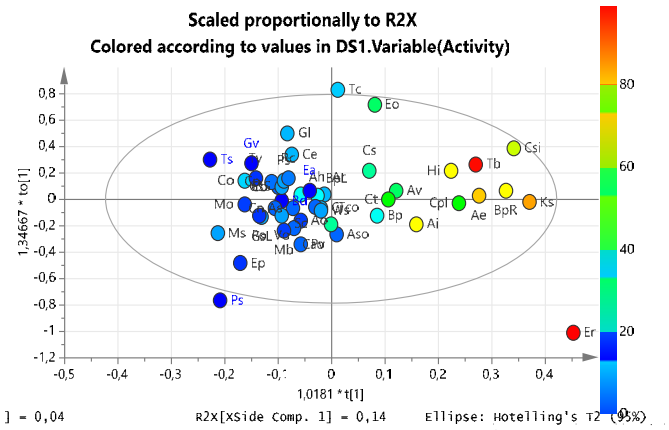

Fig. 1
Score Plot of the OPLS model for collagenase inhibition.

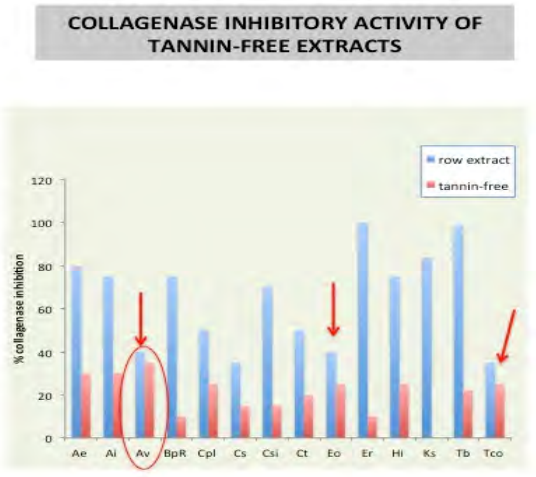

Fig. 2
Lost in anti-collagenase activity of the TF extracts.

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ANTIOXIDANT PROPERTIES AND PHENOLIC PROFILE OF A HYDROALCOHOLIC EXTRACT OBTAINED FROM THE LEAVES OF *FICUS VASTA* FORSSK. (MORACEAE)

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Ficus vasta Forssk. (Moraceae) is a very large tree growing over 25 m tall, with spreading rounded crown. Leaves are alternate, quite stiff, rough to the touch, almost circular (8-25 x 4-23 cm), margin entire, tip rounded but often with a blunt point, base rounded, heart shaped; usually glabrescent above, glabrescent, puberulous, hirsute or velutinous below (1). This species is widespread throughout the dry north and eastern Africa, Sudan, Ethiopia, Saudi Arabia and Tanzania. The leaves of *F. vasta* are traditionally used for the treatment of rheumatism, pains and intestinal worms (2).
F. vasta has been poorly studied to date; very few researches have been performed to investigate the phytochemistry and the biological activities of the leaves of this species (2-5).
The aim of this work was to evaluate the antioxidant properties and to characterize the phenolic profile of a hydroalcoholic extract (80% methanol) obtained from the leaves of *F. vasta* collected in Egypt.
In this study, three *in vitro* methods based on fundamentally different approaches and mechanisms were used to screen the antioxidant potential of *F. vasta* extract: DPPH, reducing power, and ferrous ions chelating activity assays (6). Butylated hydroxytoluene (BHT) and ethylenediaminetetraacetic acid (EDTA) were used as reference standards. In the DPPH test the extract exhibited strong radical scavenging activity, higher than BHT (IC_{50} = 0.0672±0.0038 mg/mL and 0.0821±0.0009 mg/mL, respectively). The extract showed reducing power (3.65±0.48 ASE/mL) and chelating activity (IC_{50} = 0.801±0.007 mg/mL), too; nonetheless, the effects were lower than the standards.
In order to establish the antioxidant efficacy of *F. vasta* extract in a more complex biological setting, the ability to protect bacterial growth from the oxidative stress induced by hydrogen peroxide (H_2O_2) was evaluated on *Escherichia coli* (7). The extract displayed noticeable protective effects on *E. coli* under oxidative stress; compared to H_2O_2 treated control, a total protection against H_2O_2-induced damage was observed. The activity of the extract was higher than quercetin, used as reference standard.
The quali-quantitative characterization of the phenolic compounds contained in the extract was attained by HPLC-PDA-ESI-MS. The analysis led to the identification of 11 compounds belonging to the groups of phenolic acids and flavonoids; particularly, quercetin-3-galactoside turned out to be the most abundant (81.75 mg/g±0.88% RSD), followed by gallic acid (76.36 mg/g ± 2.70% RSD) and isoquercitrin (22.5 mg/g ± 2.02% RSD).
The obtained results suggest that *F. vasta* leaves represent a potential source of natural antioxidants. Further investigations need to be carried out to isolate and identify the individual phenolic compounds responsible for the antioxidant activity.

Acknowledgements
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SECONDARY METABOLITES FROM *LIMONIUM MORISIANUM* ARRIGONI, ENDEMIC SPECIES OF SARDINIA (ITALY), AS POTENTIAL DUAL HIV-1 REVERSE TRANSCRIPTASE-ASSOCIATED RNASE H AND INTEGRASE ACTIVITIES INHIBITORS

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In early stages of the development of highly active anti-retroviral therapy (HAART), the treatments of HIV were limited to the combination therapies of reverse transcriptase (RT) and protease inhibitors. With the application of integrase (IN) inhibitors, the treatment outcome has also been improved (1). Nevertheless, although the therapy has had considerable success in controlling HIV diffusion, there are associated adverse effects and emergence of multi-drug resistance. Overall, therefore, there is still a pressing need for the development of novel antiretroviral agents, particularly with new mechanism of action. Among the novel approaches aimed to identify new HIV-1 inhibitors, the development of multi-targeted inhibitors (2, 3) is mostly attractive for the possibility of reducing the number of administered drugs and reducing their chronic toxicity (4). In addition, given the structural similarities between IN and RNase H active sites, a number of attempts have been made to identify compounds that may act on both enzymes (1). This dual approach is certainly a valid rationale for drug development.
Hence, in our research, some compounds obtained from aerial parts of *Limonium morisianum* Arrigoni, belonging to the Sardinian endemic flora, have been assayed to evaluate their HIV-1 RT and IN dual inhibition ability.
The genus *Limonium,* Plumbaginaceae family, is represented in Sardinia by thirthy-eight species, most are endemic (5). In particular, *Limonium morisianum* is a dwarf frutex exclusive of calcareous mountains of the central eastern of Sardinia (6). The dried aerial parts of *L. morisianum* were extracted using MeOH. The obtained extract was solubilized in H_2O and then subjected to liquid-liquid extraction using EtOAc as extracting solvent. The aqueous phase was further extracted using BuOH. All fractions have been purified by silica gel column chromatography and different phenolic compounds were isolated. Their structures were established on the basis of physical and spectroscopic analysis.
All compounds have been tested for their ability to inhibit the HIV-1 RNase H activity in biochemical assay and the HIV-1 IN strand-transfer catalytic activity in presence of LEDGF cellular cofactor in Homogeneous Time Resolved Fluorescence assay (7).
Three of the isolated compounds inhibited IN activity with IC_{50} values in the 1,68-1,815 µg/mL range, and RNase H activity with IC_{50} values in the 6.89 -36.46 µg/mL range, while one catechin potently inhibited the HIV-1 IN activity with IC_{50} value of 0.88 µg/mL and the RNase H activity with IC_{50} value of 0,094 µg/mL.
Overall, we report four compounds obtained from *Limonium morisianum* that display strong inhibition against two key viral enzymes, and that could be considered new and attractive leads for the development of multi-targeted antiviral agents.

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EFFECT OF CHITOSAN OLIGOSACCHARIDES AND OTHER BIOTIC ELICITORS ON ROOT CULTURES AND *IN VITRO*-GROWN PLANTLETS SYSTEMS OF *HYPERICUM PERFORATUM* L.

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Hypericum perforatum is one of the most studied medicinal plants due to its demonstrated antidepressant, anticancer and antimicrobial properties (1) and it has been used for centuries in popular medicine. Its most well-known metabolites, hypericin and hyperforin, are extracted from the aerial part of the plant (2, 3).
Although many therapeutic properties of aerial parts have been extensively studied, little information is available on the chemical composition and biological activity of root extracts. Recently, we focused on bioactive metabolites accumulated in the root, with particular interest for xanthones. These compounds exhibit numerous pharmacological activities, including antifungal (4 and references therein), antiradical and anti-inflammatory (5).
Xanthones are non-flavonoid phenolic compounds produced by some plants, fungi, lichens and bacteria. As all the other phenols, they are synthesized through the phenylpropanoid biosynthetic pathway.
In *H. perforatum* root, xanthones are accumulated at very low levels, which are not adequate for large-scale production (4). *In vitro* roots exhibit a higher content in xanthones and the production could be enhanced by treating them with both biotic and abiotic elicitors. In previous works, we demonstrated that chitosan is the most effective elicitor in increasing xanthone production in *H. perforatum* root cultures. This is a polysaccharide obtained by partial deacetylation of chitin, the main structural element of arthropod exoskeleton and of cell walls in fungi. One of the problems related to the use of chitosan is its insolubility in water at neutral pH and in other organic solvents; therefore it is generally dissolved in water acidulated with acetic acid. Although this does not limit its use for application purposes, it makes difficult to discriminate the effect of chitosan from that of its solvent, and consequently its use to determine elicitation mechanisms (4).
Recently, chitosan oligosaccharides (COS), obtained through chemical or enzymatic hydrolysis of chitosan, have gained interest for their high water-solubility at neutral pH, low viscosity, biodegradability, biocompatibility and non-toxicity (6). These features make COS promising elicitors both for application purposes and basic search.
This research aims to evaluate the effects of chitosan oligosaccharides (COS) both on *in vitro* root cultures of *H. perforatum* and *in vitro*-grown plants. Chemical analyses have been conducted on both systems to compare their elicitor-responsivity to COS in term of xanthone production. The experiments have been carried out on *in vitro*-grown plantlets with elicitors such as jasmonates and salicylic acid, which in previous tests resulted ineffective on *in vitro* cultures of regenerated roots, to verify if shoot organs are involved in the perception of elicitors. The impact of elicitor treatments was evaluated not only in relation of root-specific metabolites (e.g. xanthones), but also on shoot-specific metabolites (e.g. hypericin and hyperforin).
Further investigations regarded the expression, in COS-elicited root cultures, of genes involved in the biosynthetic polyphenols pathway, namely phenylalanine ammonia lyase (PAL), benzophenone synthase (BPS) and chalcone synthase (CHS). Previous results shown an antagonistic relationship between genes involved in the biosynthesis of chalcones (mainly flavonoids) and benzophenones (mainly xanthones), suggesting a common gene-regulation system, in agreement with (7).

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LEAF EXTRACT FROM MOUNT AMIATA CHESTNUT AS A NOVEL ANTI-PHOTOAGING AND SKIN CARE AGENT

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Modern cosmetic industry is approaching medicinal plants with ethnobotanical tradition and pre-validated use (1). We studied the Italian chestnut (*Castanea sativa* Mill., Fagaceae) from Monte Amiata's forests, Tuscany (2), focusing on its byproducts, such as leaves. In different Italian regions, fresh leaves of chestnut have a long history of use by local people for dermatologic and cosmetic applications: e.g. as external poultice against sores in Tuscany (3), to make washes for skin diseases and inflammations in Sardinia and Calabria (4, 5), as infusion against dandruff in Liguria (6) and in the Abruzzo, Lazio and Molise National Park (7). In addition, the inhibitory activity against *Staphylococcus aureus* of refined European chestnut leaf extracts has been reported, with possible applications for treatment of skin and soft tissue infections (8).
Driven by recent studies reported in literature, suggesting a scavenger and antioxidant activity of chestnut leaf extracts (9), our experimental protocol was focused on a 75% V/V ethanol extract obtained from Mount Amiata chestnut leaves, proposing its possible topical application as functional product in skin aging. Antioxidant and antiradical agents are, in fact, a useful strategy for the prevention of skin photoaging and oxidative stress-mediated skin diseases (10).
C. sativa leaf extract contains flavonoids and hydroxycinnamic derivatives, with hyperoside being the most abundant constituent of the extract. Biological tests conducted on human keratinocytes showed that the extract protects cells from chemical (hydrogen peroxide) and physical (UVA irradiation) oxidative damage. The extract activity seems to be primarily related to free-radical scavenging, since cell levels of malondialdehyde, carbonylated proteins and reactive oxygen species decreased when cells were treated with 0.1% V/V extract, while superoxide dismutase activity and Nrf-2 mRNA expression were not affected by the extract at the same concentration. The extract, incorporated in an oil/water emulsion exhibited sun protection factor booster activity. Given these results, the Mount Amiata chestnut leaf extract could be an efficient opportunity in the treatment of extrinsic aging, in which one of the main targets is the neutralization of free radicals.

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EVALUATION OF PROTECTIVE EFFECT OF *HARPAGOPHYTUM PROCUMBENS* DC. EX MEISN. ROOT WATER EXTRACTION BY MICROWAVE, BIOLOGICAL ACTIVITY ON *EX VIVO* RAT COLON INFLAMMATORY MODEL AND MICROSCOPIC INVESTIGATION

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Harpagophytum procumbens DC. ex Meisn. is a medicinal plant native to southern Africa, most commonly called devil's claw for the appearance of their hooked fruit. The plant is a weedy perennial bearing small, claw-like protrusions on the fruit and a strong central taproot growing up to 2 m deep. The secondary roots are used in decoctions and teas. This plant has been long used in folk medicine and phytotherapy as an anti-inflammatory herbal drug and as a food supplement.
The first step of the work it was the microscopic investigation to confirm the species "*procumbens*" (genus *Harpagophytum*) for the presence of pharmacognostic characters such the thick, fan-shaped or rounded slices or of roughly crushed discs, the tortuous longitudinal wrinkles that traversed the darker surface and the dark cambial zone and xylem bundles distinctly aligned in radial rows in the paler cut surface.
Considering both the capability of *Harpagophytum procumbens* to contrast inflammatory stimuli in multiple tissues and the stability of the major consituent (harpagoside) in artificial intestinal fluid, the aim of the present study was to explore the possible protective role of the plant microwave-assisted aqueous extract (1-1000 µg/mL) on mouse myoblast C2C12 and human colonrectal adenocarcinoma HCT116 cell lines, and on isolated rat colon treated with lipopolysaccharide (LPS), a validated *ex vivo* model of acute ulcerative colitis.
In this study we measure the activities of different biomarkers of colon inflammation and lipid peroxidation such as ROS, serotonin (5-HT), prostaglandin (PGE2) and 8-iso-prostaglandin (8-iso-$PGF_{2\alpha}$). In addition, we investigated the immune response modulatory effects of the plant, by measuring the gene expression of tumor necrosis factor (TNF)-α, a cytokine playing a key role in colon epithelium damage. We observed that the microwave aqueous Harpagophytum extract was effective in reducing LPS-induced production of serotonin (5-HT), tumor necrosis factor (TNF)-α and prostaglandin (PG)E2, in colon tissue.
Concluding, we demonstrated the efficacy of a microwave-assisted Harpagophytum aqueous extract in modulating the inflammatory, oxidative stress, and neuromodulatory response in pharmacological experimental models. These results suggest a possible rational in the use of Harpagophytum in management and prevention of chronic and degenerative diseases.

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SALVIA DESOLEANA ATZEI & V.PICCI: AN ESSENTIAL OIL WITH AN INTERESTING ACTIVITY AGAINST HERPES SIMPLEX VIRUS-2

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Salvia desoleana Atzei & V. Picci (Lamiaceae) is an herbaceous perennial shrub, endemic in Sardinia island. In local traditional medicine, the leaves of *S. desoleana* were used in decoction as antipyretic, and in poultice and macerated as anti-inflammatory and to heal wounds (1). *S. desoleana* is nowadays largely cultivated because of its essential oil (EO) characterized by a pleasant smell. Antimicrobial and antifungal activities of the EO have already been screened, showing a weak microbiostatic action against *Staphylococcus aureus*, *Escherichia coli*, *Staphylococcus epidermidis* and *Candida albicans* (2).
This study aimed i) at evaluating the antiviral activity against Herpes Simplex Virus-2 (HSV-2) of *S. desoleana* EO, its fractions and main components (linalyl acetate, alpha terpinyl acetate, and germacrene D), ii) at investigating its mode of action, and iii) at identifying the active ingredient(s). EO composition was characterized by GC-FID/MS analysis; the active fraction(s) and/or components were identified via bioassay-guided fractionation using *in vitro* assays on cell viability and HSV-2 and RSV inhibition.
The whole EO and a fraction enriched in germacrene D, proved to be active against acyclovir-sensitive and acyclovir-resistant viral strains suppressing HSV-2 replication after virus attachment/entry. In particular, *S. desoleana* EO inhibits both acyclovir sensitive and acyclovir resistant HSV-2 strains with EC_{50} values of 23.72 μg/ml and 28.57 μg/ml respectively. In addition, a significant suppression of HSV-2 replication was observed with an EC_{50} of 33.01 μg/ml when the EO was added post-infection. The fraction containing 54% of germacrene D showed a similar spectrum of activity as that of the whole EO with a stronger suppression in post-infection stage. These results indicated S. desoleana EO of high interest for a more in depth investigation as anti-HSV-2 products and against acyclovir-resistant HSV-2 strains.

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ECOLOGICAL OUTLINE OF THE ALIEN FLORA OF ITALY THROUGH THE USE OF THE ELLENBERG'S INDICES

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Plant invasions, resulting from the intentional or accidental transfer of taxa outside their native range through man-made activities, have become one of the main drivers of global environmental changes and the second major cause of the loss of global biodiversity after habitat loss. The studies done up to now in Italy about the non-native flora focus mostly on the number of taxa, their alien status, and a rough estimation of the biogeographic regions at regional scale (1, 2). This approach offers some clue on the habitat quality of a given region; but the study of the ecological traits of the non native taxa occurring in a given area could provide more information not only about the naturalness of this area, but also about its ecological conditions and its potential proneness to new alien invasions.
Aim of this contribution is to provide an overview of the ecological preferences of the alien flora of Italy, through the use of Ellenberg's indicator values (EIVs hereafter).
The floristic list used as basis for this study is the update (3, 4) of the "Inventory of the non-native flora of Italy" (5). Currently, this list includes 1520 taxa, along with their families, main synonyms, and indication of their alien status (invasive, naturalized, casual) for each region. These data were recently integrated by the EIVs estimated for the invasive and naturalized taxa.
EIVs have been widely adopted as proxies to estimate the influence of the main environmental drivers in determining flora and vegetation changes in a given region (6).
Ellenberg outlined the synecological preferences of all taxa belonging to the vascular flora of Central Europe by means of numerical indices referring to seven main environmental drivers: the first three EIVs refer to climatic variables: light availability (L), temperature (T), continentality (K); the last four EIVs refer to edaphic variables: moisture (F), reaction (R), nutrient availability (N), and salinity (S). These indices were adapted to the flora of Italy by extending their ranges (7), and were assigned to all the taxa included in the first edition of the "Flora D'Italia" (8). A recent update to the EIVs for the flora of Italy has been published for ferns, ferns allies, gymnosperms, and monocots (9), but the rest of the taxa currently known from the flora of Italy, but not included in the first edition of the "Flora D'Italia", is still lacking assigned EIVs.
We considered in this contribution those 694 alien taxa recorded at least in 4 administrative regions of Italy. 251 new EIVs were proposed, basing on the environmental conditions of the Italian localities in which they are known to occur.
Average EIVs for the non native flora of Italy are: L = 7.81, T = 7.64, K = 4.96, F = 4.54, R = 5.45, N = 4.49. A total of 19 taxa show a relatively low tolerance to salinity (average S = 1.63).
A direct comparison with the average indices of the whole Flora of Italy (7) suggests that the alien flora has similar light requirements (L = 7.69 vs. 7.81), higher temperature requirements (T = 7.64 vs. 6.05), higher value of continentality (K = 5.91 vs. 4.96), similar water requirements (U = 4.54 vs. 4.42), occurs on soils with pH closer to the neutrality (R = 5.45 vs. 4.44), and prefers higher nutrient availability (N = 4.49 vs. 3.25).

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ASSESSMENT OF INVASIVE ALIEN PLANTS DISTRIBUTION THROUGH PHYLOGENETIC MEASURES OF BIODIVERSITY AND ENVIRONMENTAL PREDICTORS: ESTIMATING CURRENT IAS DIVERSITY CENTRES IN TUSCANY

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Invasion by alien species is a growing concern for ecosystem stability and native biodiversity (1). Here, we examined the distribution in Tuscany of 48 invasive alien plants at regional level and of 7 invasive alien plants at European scale (2), through phylogenetic measures of biodiversity, assessing their association with environmental predictors and comparing outputs with those of the native flora. For this aims, we used 2,178 georeferenced records of invasive plants and 129,623 records of native plants currently stored in Wikiplantbase #Toscana (3), calculated standard richness measures and phylogenetic indices (4, 5), and fitted simple generalized linear models and spatial error simultaneous autoregressive models (6).
The native flora resulted negatively associated with the annual mean temperature and positively with the topographic ruggedness, an environmental index usually linked with the diversity of niches. An opposite pattern was found for the invasive alien plants, suggesting their preferences for warm sites with reduced environmental variability. The phylogenetic signals for climatic predictors were significantly different from zero, meaning that closely related taxa occupy more similar climatic niches than expected by chance. However, phylogenetic signals are rather moderate, suggesting a degree of stochasticity in site's invasion. Finally, by applying randomisation tests, we identified those areas hosting significantly high invasive diversity and we defined them as 'IAS diversity centres', subsequently classified as 'IAS hotspots' when they overlap with protected areas.
Interactions between native and alien species have been claimed as major factors affecting community invasibility and alien invasiveness (7, 8). Our results provide the baseline for future research aimed at testing this hypothesis, which requires a more balanced sampling of floristic records than is currently available (on account of the high number of critically under-sampled areas in the regional territory; 9).

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IMPACT OF PINE INVASION ON THE TAXONOMIC AND PHYLOGENETIC DIVERSITY OF A RELICT MEDITERRANEAN FOREST ECOSYSTEM

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Invasion by non-native tree species is a major driver of reduction and fragmentation of forest ecosystems, often deeply altering biodiversity components (1). However, the effects of this process on the phylogenetic structure and diversity of Mediterranean woodlands are still unknown. Phylogenetic diversity is a deep component of community diversity and has been suggested to be relevant for ecosystem functioning, service provisioning and processes such as extinction and biological invasions (2).
In a recent investigation (3), we used cork oak (*Quercus suber*) stands in northern "Maremma" (Tuscany) invaded by self-sowing populations of maritime pine locally introduced ca. 70 years ago (4) as a model system to assess the impact on the taxonomic and phylogenetic diversity of the native seed plant community. We took advantage of the mosaic-like vegetation landscape formed by the two forest types to adopt a paired sampling design based on 33 pairs of 10 × 15 m plots in comparable environmental conditions.
Taxonomic diversity was negatively affected by the pine at three levels (gamma, alpha, beta). Indicator species were significantly less numerous than in cork oak stands, and did not include two growth-forms such as herbs and vines. Phylogenetic diversity metrics (PD, NRI and NTI) were inferred from an evolutionary tree of seed plants based on a ITS-5.8S nuclear DNA dataset including original sequences from local plant material. Phylogenetic diversity (PD) was positively related to species and genus richness, showing a marked decrease in the pine stands. Seven major clades (orders) of dicot angiosperms were only represented in the cork oak community. Both the NRI and NTI indices showed a significant reduction of phylogenetic evenness in the pine forest. Here, the proportional increase of related taxa with acid-tolerance specialization suggests that soil acidification is a major driver for a ''habitat filtering" effect causing the exclusion of several understory species and genera of cork oak forests.
Progressive thinning of the pine stands is advocated to avoid further acidification and promote the re-conversion to oak woodlands by natural regeneration. This will ultimately favor the recovery of the associated plant diversity and the restoration of a vanishing forest ecosystem of the ancient Mediterranean landscape.

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KUDZU: SUBTLE THREAT TO NATIVE FLORA OF A CLEAR INVASIVE ALIEN PLANT

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Pueraria montana var. *lobata* (Fabaceae), commonly named "kudzu", is one of the 100 worst invasive species in the world (1). It is a perennial deciduous semi-woody climber and crawling vine native to Asia and probably to some western Pacific Islands (2). Kudzu has been exported all over the world with dietary, pharmaceutical, foraging, ornamental and habitat-restoring purposes (2). Today its invasive range includes all continents even if the severity of its impact varies among countries (1); beyond climate, the past rate of cultivation likely represents the key factor of spreading in new areas (3). According to the Regulation (EU) No 1143/2014, Member Countries of the European Union have the obligation to prevent, control and, when possible, eradicate this plant; in Europe, kudzu is considered a serious pest in Switzerland and Italy and it is naturalized in Ukraine (1).
Where kudzu grows uncontrolled, its impact is highly destructive for ecosystems as well as for human activities (e.g. damages to structures, buildings, forestry industry, crops). Due to its fast growth and ability of persisting in poor soils, kudzu forms massive and wide monospecific stands, transforming colonized ecosystems both at biotic and abiotic level (4). Reported impacts to native flora are usually related to its ability to: smother plants by growing on them; outcompete for resources and light, thanks to a very fast growth rate and effective allocation of resources; change edaphic parameters through a radical symbiosis with *Rhizobium* bacteria (4). Nonetheless, the wide array of observed impacts, allelopathic effect of kudzu has been poorly investigated. Allochemical compounds in kudzu have been studied mainly with bioprospecting intents (natural biocide; 5) and results are not thorough of understanding the interactions in nature. The presence of allochemicals in plant tissues as well as the field observation of inhibition zone or the decrease in number of species can be only preliminary indicators of an allelopathic interaction. Thus, in order to fully understand the real impact of *P. montana* var. *lobata*, we analysed the effect of litter (leaf and stem mulch) and viable runners of kudzu on native species (target species) common in open habitat, as those colonized by kudzu, by two different types of greenhouse bioassays. Target plants have been sown in universal potting soil, at 20° C, with 12 hours cycle of alternating light/darkness. Inhibitory effects have been investigated by measuring number of internodes, leaf area, final height and dry weight of seedling root and shoot during their development in “litter test”, whereas it was estimated by evaluating seed germination and shoot and root final height and dry biomass in "runner test".
Preliminary results showed that both kudzu litter and runner affected the growth and the final biomass of the target species. However, the magnitude of the effect was species specific. Further experiments are underway to better demonstrate and assess the negative impact of *P. montana* var. *lobata* on local flora related to the release of allochemicals.

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ON THE OCCURRENCE OF *HALOPHILA STIPULACEA* IN THE AUGUSTA GULF (EASTERN SICILY, MEDITERRANEAN SEA)

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The tropical seagrass species *Halophila stipulacea* (Forssk.) Asch. is native of the Red Sea and it is widely distributed along the western coast of the Indian Ocean, Persian Gulf, west India, tropical eastern Africa, Madagascar, Mauritius (1). After the opening of Suez Canal, *H. stipulacea* has in a short time colonized the Mediterranean Sea, where its first record comes from Greece in 1894 (1). At the beginning of its introduction, the species was distributed in the Eastern Mediterranean basin, recently the plant spread to the Eolian Islands. In Italy *H. stipulacea* appears for the first time in the harbour of Riposto (Sicily) (1) and then it is reported in the harbour of Catania (2), Vulcano Island (Eolian Island) (1), Peninsula of Maddalena (Siracusa) (3), Tindari (Messina) (3), Reggio Calabria (1), and Salerno (1).
During a floristic study carried out in Augusta Gulf (Eastern Sicily) in 2016, a seagrass meadow of *H. stipulacea* has been found. This area shows high levels of pollution due to the petrol chemical complex of Augusta-Priolo, an extensive industrialized coastal area developed in the municipalities of Augusta, Priolo Gargallo and Melilli. The presence of heavy metals in seawater and in sediments is widely documented, so the occurrence of *H. stipulacea* is remarkable. Some plants were collected in June and November 2016 and they were fixed in alcoholic solution for both morphological and reproductive phenology studies. Some leaves, preserved in seawater, have been used to investigate about the heavy metals uptake (V, Mn, Cd, Hg, Pb, Cu, Zn, Cr, Ni, As). Also sediments and water of the adjacent area have been sampled for the same purpose, as well as the uptake of heavy metals in some specimens of *Caulerpa prolifera* (Forssk.) J.V.Lamour., and a comparison between the two species has been made.
The small meadow was located in the upper infralittoral zone at 2 m depth ($\pm$ 0.5 m) on a sandy bottom in the sheltered and/or moderately wave-exposed area, protected bythe Peninsula of Magnisi.
The meadow of *H. stipulacea* appears homogenous, healthy and well-structured. The collected plants are in a good agreement with the vegetative features of *H. stipulacea* already described and illustrated (4); they show green petiolate leaves in pairs with cross veins, leading serrulate margins and each pairs of leaves accompanied by two semi-transparent scales covering the short petiole. It should be noted that numerous both male and female flowers (rarely recorded in the Mediterranean Sea for *H. stipulacea*) were found.
The Mediterranean meadows of seagrass *H. stipulacea* show high levels of genetic polymorphism and this suggests a genetic recombination through sexual reproduction (5, 6); considering that presently the female flowers have been observed only one time in Mediterranean Sea (7), the finding of abundant flowering supports the hypothesis that sexual reproduction is effective in the spreading of the species and in colonization of new habitats (6).
The analysis of the uptake of heavy metals in *H. stipulacea* has generally given evidence of their presence in low concentrations, except Mn (6.54 mg/kg) and Zn (2.81mg/kg) that are the higher values found. Uptake data for *C. prolifera* generally show the same trend of *H. stipulacea* even if Mn values are lower (1.09 mg/Kg). It is particularly relevant the high value of Mn (70.7 mg/Kg) observed for sediments.
Finally, the constant presence of *Chondria pygmaea* Garbary & Vandermeulen, epiphytic on *H. stipulacea,* detected in present study, reinforces the hypothesis that above species moved together from Red Sea, according to previous studies (2, 8).

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INVADERS IN A CHANGING WORLD: *CAULERPA CYLINDRACEA* SONDER AND OCEAN ACIDIFICATION

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The Mediterranean Sea can be considered the most impacted worldwide area by biological invasions. More than 1,000 alien taxa have been recorded; among them about 10% are well-established macroalgae, settled in the wild with free-living, self-maintaining and self-perpetuating populations (1). The green alga *Caulerpa cylindracea* Sonder is one of the best-known invaders: it is currently spread along Italian coasts, both on hard and soft substrata from 0 to 70 m depth. Its occurrence can affect the stability of recipient systems, altering the integrity of natural environments (2). This can have particular resonance in the light of climate change, in particular under ocean acidification. How this green alga can respond to the change in the water chemistry?
Volcanic CO_2 vents lower the pH of the water column and can be used as natural laboratories where to study the effects of ocean acidification on benthic systems, in particular those where alien species are already settled (3). Around the Castello Aragonese (Ischia Island, Gulf of Naples) shallow underwater vents naturally acidify the water, very close to pH values expected for 2100. In this area. it has been possible to find patches of C. *cylindracea* both at ambient (8.01) and "acid" (7.50) conditions since the beginning of 21rst century.
Through crossed transplant experiments *in situ* and by using an integrated approach we investigated short- and long- term effects of ocean acidification on *C. cylindracea.* Morphological and ultrastructural analyses were performed by using light and transmission microscopy; physiological analyses were performed by detecting chlorophyll and carotenoid contents and the *in situ* photosynthetic performance (Diving-PAM fluorometer).
Short-term transplants from 8.01 to 7.50 and *viceversa* gave opposite results. Stress effects resulted in transplants from current to lower pH values: a decrease in the number of active chloroplasts, the appearance of dilatations between thylakoidal membranes, an increase in the amount of plastoglobules. A decrease in the photosynthetic efficiency occurred, while carotenoids and non-photochemical yields were strongly increased. An opposite response was observed in transplants from lower to normal pH. However, no significant difference was evident when native plants at the two different pH conditions were compared, testifying a long-term acclimation at lower pH values.
Those preliminary results seem to confirm the high acclimation capacity of this species even in a new pH scenario, as already resulted according to variations in temperature and day length (4, 5).

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DETECTION OF ALIEN MACROALGAL SPECIES IN THE MEDITERRANEAN SEA USING THE DNA BARCODING TOOL

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The introduction of non-indigenous species (NIS) across the major European seas is a dynamic non-stop process. A wide range of vectors, such as aquaculture, shipping (fouling, ballast water), lessepsian migration, aquarium trade, fishing nets, are currently introducing a plethora of alien marine species into indigenous assemblages. At present, the knowledge of both diversity and distribution of most genera of macroalgae in the Mediterranean Sea is almost entirely based on morphological studies and published papers dealing with molecular data are limited. Due to their cryptic diversity, many algal NIS may go unnoticed and previous unrecorded taxa in a certain site could be possibly overlooked due to the variability and unreliability of accepted morphological diagnostic characters.
Our data cover mainly the area of the Strait of Messina (including Cape Peloro lagoon), Venice lagoon, Thau lagoon, Tunisia, and occasionally other sites in the Mediterranean Sea. Over the ten years, the DNA barcoding tool revealed the presence of various NIS: the Rhodophyta *Agardhiella subulata* (1), *Hypnea cervicornis* and *H. cornuta* (2, 3), *Pachymeniopsis gargiuli* (4), and *Spermothamnium cimosum* (3, 5), and the Chlorophyta *Ulva australis* (= *U. pertusa*), *U. laetevirens*, *U. ohnoi*, and the doubtful unit *U. "fasciata"* (6, 7, 8).
Accurate biodiversity assessment of marine macrophytes is essential for monitoring of biological introductions, it is critical for environmental management and to adequately evaluate temporal changes. DNA barcoding proved to be useful for such floristic knowledge (3, 8). However, the actual picture of the presence of macroalgal NIS in the Mediterranean as a whole is far to be clarified and our work aims to be a step towards its clarification. Yet, the current taxonomic, biogeographical and ecological data gaps can be filled only by cooperative work and standardized methodologies. DNA barcoding demonstrated as a fast and effective tool for: a) the accurate identification of alien species and the evaluation of their vectors (1); and b) the genetic labelling of challenging taxa, especially in those areas subjected to anthropogenic disturbances and quick changes of biodiversity. Data collected in such perspective are also valuable to increase the BOLD system catalogue (9), amplifying the biodiversity knowledge linked to geographical information, which becomes freely available for the scientific community. DNA barcodes are permanent labels assigned to specimens regardless any subsequent taxonomic or nomenclature variation.

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ALLOCHTONOUS MICROALGAE AS CARRIERS OF NEW TOXINS

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Phytoplankton species are easily transported as a consequence of natural forces (currents, typhoons) or as a consequence of human activities (ballast waters, plastic floating debris, aquaculture activities). A problem linked with the spread of alien microalgal species in coastal areas is that some are producers of toxins that are unknown or not regulated, although sometimes new toxins can be produced by indigenous species which become toxic for yet unknown reasons. The consequences of the appearance of new toxins can be dramatic, as the only method to prevent risks to human health is to avoid ingestion or contact by means of regular monitoring activities; these are carried out by all Countries and are based on microscope identification of the species and on chemical analysis of the toxins, thus allowing to assess the presence of harmful blooms in bathing areas and the presence and levels of toxins in farmed mollusks. Monitoring rules can therefore be fixed only if many biological and chemical aspects of the species are known. Morphological, ecological and growth patterns studies of toxic species, both in field and in culture, are of great help for the identification of the causative organism, for the characterization of the whole toxic profile of phytoplankton species, which is useful for mussel monitoring and, finally, the cultured biomass can provide reference material either for chemical analysis and for further studies on toxin mechanism of action and toxicology.
One of the most striking examples of these problems is represented by the appearance in the Mediterranean coasts of intense blooms of *Ostreopsis* species during summer-autumn (1, 2). Given their negative implications on both marine ecosystem functioning and human health and coastal management, several studies have been carried out leading to increased knowledge about the toxin profile, the environmental factors affecting growth and toxin production, the ecology, the cell ultrastructure and taxonomy.
To date three *Ostreopsis* species have been reported in the Mediterranean: *O.* cf. *ovata* (the most abundant and widely distributed), *O*. cf. *siamensis* (3), and the new species *O. fattorussoi* (4). *Ostreopsis* cf. *ovata* was shown to produce a large array of palytoxin analogues, i.e. isobaric palytoxin and ovatoxins (5) associated with many cases of human illness (e.g. fever, cough, dyspnea, sore throat, rhinorrhea, skin irritation, etc.) attributed to inhalation or cutaneous contact with cells or toxic aerosol (6). Cultured cells provided material for toxin structure elucidation and for the first toxicological evaluations (7, 8). A number of laboratory studies highlighted that both growth and toxin production are affected by temperature, salinity and nutrients (2). In the field, high temperature and optimal nutritional conditions were shown to trigger the *Ostreopsis* blooms typically in sheltered, shallow coastal areas characterized by rocky bottom, forming a brownish, mucilaginous mat on several types of benthic substrata (macrophytes, rocks, invertebrates, sands) (1). Ultrastructural observations of this mat showed that *Ostreopsis* mucilage is formed by acidic polysaccharides and by a very high number of trichocysts sticking together to form a complex network of filaments showing a new role of trichocysts, never described before (9).

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POSTERS

1.1 = TOWARDS A BETTER UNDERSTANDING OF THE ECOLOGY AND BIOLOGY OF THE SUBENDEMIC *IONOPSIDIUM SAVIANUM* (CARUEL) ARCANG. IN CENTRAL ITALY

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Jonopsidium savianum (Caruel) Ball ex Arcang. is an annual subendemic species, whose fragmented distribution area is limited to N-Spain (Burgos and La Rioja) and central Italy (Umbria, Latium and Tuscany) (1). It is included in the Annexes II and IV of the "Habitats" Directive (92/43/EEC and subsequent amendments). The species has been assessed as "Least Concern" in the recent Italian Red List (2), while at the EU level it is considered "Near Threatened" (3). Considering the biogeographic relevance of this very localized species, in 2010 and 2016 we carried out a study of its central-Apenninic populations, from Umbria (M. Torre Maggiore, Colle delle Centelle) and Latium (M. Tancia). Demographic, morphologic, ecological and reproductive traits of *J. savianum* have been measured. Among the considered parameters are the genet's density, size, habitus (single or multiple stems), number and size of flowers and fruits. We investigated the reproductive biology of the species, focusing on size, weight and morphology of seeds, germinability tests at different thermal regimes with several thermal stratification protocols, rate and speed of germination. The density parameters were measured within 40x40 cm^2 squares, located at intervals of 1 m along transects crossing the local stands. The macromorphological parameters were measured on eight randomly chosen individuals from each of eight transects randomly located in each of the three study site. Data and methods match with the methodological protocols officially acknowledged for evaluating the conservation status of this species (4). Correlation with ecological characteristics has been investigated, with focus on soil texture, light availability and climatic parameters. Nuclear (ITS and ITS2) and plastidial (*matK*, *rbcL*, *trnH-psbA*) DNA multi-marker barcodes were amplified, adding relevant information to the general knowledge of a *taxon* of community interest, for which only karyological data were available (5). The statistical analysis of data was performed using the software “R” (https://www.r-project.org/, 2016), in order to assess the variability and to point out any significant different distribution among the considered traits, in relation to the considered environmental characteristics.
The results obtained from this study contribute to a better understanding of the ecology and biology of *J. savianum* in central Italy and will support the periodical monitoring that Regional authorities are obliged to perform every six years, the next being expected for the end of the period 2013-2018. *Jonopsidium savianum* is a species whose conservation is strongly depending on the maintenance of traditional extensive montane farming. The present study provides useful insights for a proper planning for the preservation of viable populations of this species. It should be stressed that currently only two of the studied local populations are included in the network of Natura 2000 areas.

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1.1 = INTER-ANNUAL CONSEQUENCES OF WARMING ANOMALIES IN PHENOLOGICAL AND REPRODUCTIVE PERFORMANCES OF *GENTIANA LUTEA* L.

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Plant populations at the margins of their geographical and ecological ranges are thought to be particularly sensitive to climatic anomalies and global warming in general (1, 2). As the distance to the edge of the range decreases, individuals often experience increasingly stressful climatological conditions resulting in fewer, smaller patches of suitable habitat, or in a decreased reproduction success (3). *Gentiana lutea* L. subsp. *lutea*, a rhizomatous long-lived plant of C-S Europe, included in Annex V of European Habitats Directive, was used for this study. We analysed the phenological and reproductive performance anomalies of two phenological cycles (2013/2014 and 2014/2015) and we correlated them with soil temperatures and precipitation. In particular, we focused our attention on the hypothetical shifting plant phenology and reproductive performance in response to global warming on the Peripheral Isolated Plant Population (PIPP) of Sardinia (Western Mediterranean Basin). A special attention was paid for the drastic phenological changes experienced in the last cycle (2014/2015), which has been also reported as one of the warmest years since 1961 (4). Our main aims were to investigate in which way warming climatic conditions were related to the experienced variation of vegetative and reproductive stages and to examine how they conditioned the *G. lutea* reproductive success.
Wilcoxon signed-rank test was used to evaluate differences on climatic factors and reproductive performances between cycles and Generalized Linear Models to determine the effect of climate on six analysed aspects of reproductive performance. The Independent Effect of each predictor was also determined. Significant differences on climatic variables between the two analysed cycles were highlighted especially for maximum and mean temperatures. Such variations were reflected by a no flowering stage in two of the four analysed localities in 2015 and by significant differences on further four reproductive aspects between cycles. These results revealed that, in such unstable climatic condition that we are currently experiencing, phenological changes can be detected year by year. Phenology can therefore be a good sentinel of climatic anomalies. Considering the importance of this issue and the ease and cost effectiveness of phenological monitoring, we argue that researches in this sense can be a supporting tool for the enhancement of crucial targets for the future such as the biodiversity conservation and the climate changes mitigation. Considering the global warming trend, we put use of the anomalies to obtain information useful to bring up conclusions related to the effect of climate change on the Mediterranean mountain plants living at the edge of their distribution.

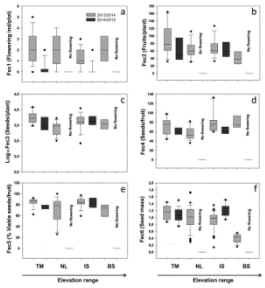

Fig. 1
Box plot of reproductive performance variables (Fec 1−6) derivated by open pollination in the two phenological cycles, 2013/2014 and 2014/2015. TM = Trainu Murcunieddu; NL = Nodu 'e Litipori; IS = Is Terre Molentes and BS = Bruncu Spina.

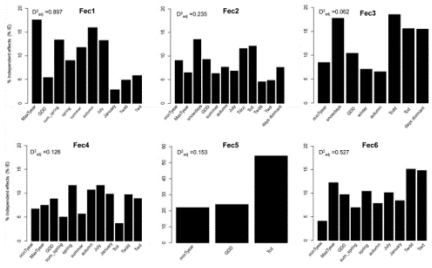

Fig. 2
Independent Effect (IE) to the total variance explained on the six reproductive performance variables (Fec 1−6) and amount of deviance explained adjusted for the number of observations and parameters (D^2adj) accounted for each significant key factor (P values < 0.05).

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1.1 = EFFECTS OF HONEYBEES ON THE REPRODUCTIVE SUCCESS OF THE PSAMMOPHYTE *HELICHRYSUM STOECHAS* (L.) MOENCH IN A COASTAL PROTECTED AREA IN TUSCANY

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Honeybees (*Apis mellifera* L.) provide a pivotal contribution to agricultural pollination ecosystem service (1). In Europe, honeybees can enhance pollination and reproductive success of native plant species in Spain (2), while they are detrimental to the abundance of wild bees in England (3).
In the Migliarino, San Rossore, Massaciuccoli Regional Park, apiaries are being installed close to the dunal system. These apiaries produce the so-called "Beach Honey", characterized by a peculiar aroma due to the presence of essential oils of the psammophyte *Helichrysum stoechas* (L.) Moench. The aim of this study is to test whether honeybees can offer an adequate or supplemental pollination service to *H. stoechas* present in the dunal system. For this purpose, we assessed the influence of honeybees on seed sets at different distances from apiaries. We sampled plant individuals in two areas of the sandy beach system hosting an apiary: a northern area called "Torre del Lago" (TDL) and a southern area, San Rossore Estate dunes (SRO). In both areas we set up a transect next to the coastline, at the nearest point to the apiary and further transects at 500 m intervals. In TDL we sampled the plants in 3 transects while in SRO in 6 transects and a control site distant from the apiary 5000 m. In each transect we collected, with a random stratified sampling method, 40 capitula bearing flowers at the anthesis stage and, just before the seed dispersal, 40 capitula containing fruits. Sampling were performed taking into account the size of plants: capitula were separately collected from small (<40 cm in diameter) and large plants (>40 cm in diameter). Flowers and fruits present in each capitulum where counted under a stereomicroscope. Fruits were divided into three different categories: unfertilized ovaries, aborted achenes, mature achenes. Seed set was calculated as follow: (number of aborted achenes + mature achenes)/ total number of ovaries. A parallel survey was carried out to identify a potential *H. stoechas* consumer insect present on corymbs. To compare distributions among transects we performed an ANOVA, while to test the effects of apiary distance, plant size and distance from the sea on seed set we fitted logistic regressions (GLM). Linear models were used to evaluate the impact of insect consumers on the seed set.
The average seed set is quite low (0.333) because it includes both mature and aborted achenes. However, seed set differs between fieldwork areas: in TDL it is 0.554, and in SRO is 0.201. The difference between the two sites is also confirmed by the homogeneity among transects, which is higher in TDL compared to SRO, likely due to coastal erosion and dune instability. GLM results demonstrate that honeybees' activity significantly increases the production of achenes while proximity to the sea negatively affects achenes production. Nevertheless, large plants may couple with this because the analyses demonstrated that they produce significantly higher quantity of fruits than small plants.
In conclusion, our findings suggest that the presence of honeybees as pollination agents can enhance the reproductive success of *H. stoechas.* Our study also suggest that honeybees activity is negatively influenced by stronger wind close to the sea, hence, these results also indicate negative effects of coastal erosion on plant reproductive success mostly caused by the reduced activity of this pollinator.

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1.1 = FORAGING ANTS AFFECT THE ACTIVITY OF EXTRA-FLORAL NECTARY AND NECTAR COMPOSITION OF *PRUNUS AVIUM* L.

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Extrafloral nectaries have been reported in about 4000 plant species that grow in a variety of temperate to tropical habitats (1, 2). They typically defend plants indirectly by attracting invertebrate predators, generally ants, the presence and activity of which reduce attacks by herbivores (3). The secretions of these nectaries are rich in sugars and amino acids that ants can exploit as food (4). Although such interactions are widespread in angiosperms and pteridophytes, and are generally considered a typical example of mutualism, relatively little detailed information is yet available on ant-plant relationships at temperate latitudes (2). This study concerns the relationships between ants (*Crematogaster scutellaris*) and the extrafloral nectar of *Prunus avium* L. (Fig. 1). We investigated whether the presence of ants patrolling the plant induces changes in extrafloral nectaries and nectar. Ten trees in a natural population of *P. avium* were banded with adhesive tape at the base of the stem to exclude ants from climbing the plant, whereas ants had free access to ten other trees of the same population. We sampled nectaries and nectar from ant-attended and ant-excluded plants and compared nectary morphology assessed by scanning electron microscopy, nectar quantity measured by microcapillary sampling and nectar composition determined by high performance liquid chromatography (HPLC). Most nectaries from ant-attended plants had damaged surfaces (Fig. 2) in accordance with occasional field observations of ants “biting” the nectary. The quantity of extrafloral nectar produced by ant-attended plants was about threefold that produced by ant-excluded trees. Total sugar concentration was higher in the extrafloral nectar of ant-excluded plants than that of ant-attended ones whereas there was no difference in total amino acid concentration. The chemical profile of extrafloral nectar differed in sugars and amino acids between ant-attended and ant-excluded plants. The results were consistent in two consecutive years (2015 and 2016). It can be concluded that ants patrolling a plant and foraging nectar indeed had an effect on the activity of extrafloral nectaries and nectar composition.

Fig. 1
An extrafloral nectar of *P. avium* visited by an ant.

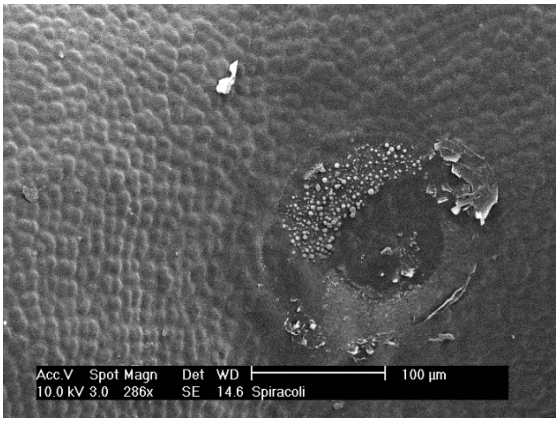

Fig. 2
An extrafloral nectary of *P. avium* after ant visit

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1.2 = PLANT COMMUNITIES AND LANDSCAPE DIVERSITY IN NW SICILY: THE MEMOLA EU FP7 PROJECT CASE STUDY

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The UE FP7 research project MEMOLA (Mediterranean Mountainous Landscapes: an historical approach to cultural heritage based on traditional agrosystems) aims at investigating landscapes through a diachronic study of the relationship between human populations and natural resources. The project analyzes, from an interdisciplinary perspective, the drivers and dynamics that have generated landscapes in four areas of the Mediterranean Region (Sierra Nevada, in Spain; Colli Euganei, in Northern Italy; Monti di Trapani, in Sicily; Vjosa Valley in Albania) with a research group of ten partners (1). The central issue of the Project is the historical use of water, related to traditional and irrigation systems, which has played a key role in the formation and transformations of Mediterranean agricultural landscapes. The presence of traditional and historical irrigation systems was used as one of the criteria for traditional agricultural landscapes identification. These systems are strictly linked to socio-economic structure and organization of the rural populations which have been exploited it since medieval times (2).
The traditional agricultural landscapes of Calatafimi rural district (NW Sicily) are the outcome of the historical relationship between man and nature, resulting from complex interactions between biodiversity (at all levels, including species richness, ecosystem and biotope diversity) and cultural diversity, including material and immaterial aspects (architectural heritage, historical irrigation systems, local traditional agricultural practices, dialectal culture) (3).
The methodological approach of Integrated Phytosociology has been considered a powerful interpretation key to examine this landscape as an integrated whole (4).
The syndynamic study of vegetation (series and geoseries) has been used as a marker to better understand human impact on land mosaic formative processes. Vegetation series are the result of the relationship between landscape natural heterogeneity and diversity produced by humans through historical land use. The existence of a direct causal link between vegetation series and anthropic factors represents a tool for new narratives of the trajectories of Mediterranean land mosaic. Vegetation series have been defined with the purpose of understanding the dynamic relationships between the diverse facets of land mosaic tiles.
Four vegetation series are recognizable: *Cisto cretici-Pino pineae* sigmetum; *Genisto aristate-Querco suberis* sigmetum; *Oleo sylvestris-Querco virgilianae* sigmetum; *Oleo-Euphorbio dendroidis* sigmetum. Agricultural land-uses associated to each vegetation series were then identified for relating traditional agricultural landscapes to ecological factors.

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1.2 = SMALL-SCALE AND HIGH-RESOLUTION IN GEOBOTANICAL MAPPING: THE CASE STUDY OF SICILY

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It is presented the work experience that led to the recent publication of the *Vegetation map of Sicily (scale 1: 250,000)* (1), for a moment of discussion and confrontation in a Congress of the Italian Botanical Society. This is an important synthesis document, useful for both didactic and scientific purposes, whose graphic design was performed through Geographic Information Systems (GIS), applying the methodology of the Physiosociology survey (2). The work was carried out in an extended geographic area – the Sicily region – through an articulated methodology that led to the definition of a small-scale map (1:250,000), starting from a large-scale survey (1:10,000) and maintaining a high-resolution. The surveyed area includes the Sicily – the largest Mediterranean island (25,432 km²) and its smaller surrounding islands (archipelagos of the Aeolian, Aegadian and Pelagie islands, Ustica and Pantelleria islands) for a total area of 25,703 km². The study is the result of years of research on the territory, thanks also to the rich phytosociological-cartographic bibliography produced by several authors especially in the last forty years (1, 3). The same research was partly stimulated by two important legislative measures: 1) the Regional Law 98/1981, which led to the establishment of 6 natural parks (Mount Etna, Madonie Mts., Nebrodi Mts., Sicani Mts., Alcantara River and Pantelleria islands) as well as about 70 natural reserves; 2) the Habitats Directive 92/43/EEC, which led to the identification of 234 Natura 2000 sites [205 Sites of Community Importance (SCIs), 15 Special Protection Areas (SPAs), 14 SCIs and SPAs]. The *Vegetation map of Sicily* defines the vegetation landscape of the territory, whose typological complexity reflects the physiographic, lithological, geomorphological and bioclimatic variability of the ecosystems. The peculiarity of this landscape is also linked to the traditional anthropic activity in an area located in the center of the Mediterranean Region and marked by the historical and cultural events that characterized the same civilization. The realization of the map is the result of several stages of work, carried out over several years: a) preparation of a GIS aimed at creating a 1:10,000 scale graphic (Technical Map of Sicily, published by the Sicilian Region in 2002, and subsequent updates), comprehensive of a database (using the software ESRI's ArcGIS 9.1), implementing the various thematic layers with georeferenced documents (according to the Gauss-Boaga coordinate system in kilometers); b) photo-interpretation of the vegetation through digital color orthophotos (programme "IT 2000™", carried out throughout the country by the "Compagnia Generale di Riprese aeree" of Parma, flight MAE 2007, flight Agea 2007, flight ATA0708 RPA, Perugia, Italy, scale 1:20,000) and satellite images in color (Landsat TM, Google Heart 25 cm pixels), with data digitalization on the Technical Map of Sicily; c) cross-checks of various other informative layers (maps published for Sicily on vegetation, land use, geology, soil science, bioclimatology, etc.); d) validation of the photo-interpretation of the identified phytocoenotic types, through field surveys and verifications; e) digitization of the results and additional data; f) phytosociological characterization of the mapped types and definition of the legend, according to the methodological criteria of the Zurich-Montpellier Sigmatista School; g) final editing of the map on scale 1: 250,000, graphic design and printing (1). The results obtained through photo-interpretation and field validation allowed to identify 36 phytocoenotic types; the syntaxonomic nomenclature at the level of alliance, order and class follow the *Prodrome of the vegetation of Italy* (4). The colors in the legend have been adjusted to obtain a good overall effect, however, to enhance some aspects of the plant landscape as follows: a) the green series has been divided among the natural forest formations (forest of *Quercus ilex, Quercus suber, Castanea sativa, Fagus sylvatica, Quercus pubescens* group, deciduous woods with prevalence of other oaks, coniferous woods, etc.; b) the orange (light and dark) has been attributed to reforestation plants – respectively to *Eucalyptus* sp. pl. and coniferous (*Pinus, Cedrus,* etc.) – to highlight the contrast between these anthropogenic systems with the previous natural aspects; c) the series of purple-fuchsia has been reserved for riparian and dwarf woods of river stretches, watercourses and xeric river terraces; d) the blue and blue sky series has been assigned to humid environments (watercourses, lagoons, etc.); e) other series of colors - white, yellow, red, grey, etc. - were reserved for other phytocenotic categories represented in the territory, predominantly of anthropogenic nature characterized by agroecosystems and built areas.

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1.2 = *EX-SITU* CONSERVATION OF THREATENED ENDEMIC SPECIES, A PROJECT OF THE ASPROMONTE NATIONAL PARK (CALABRIA, ITALY)

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Endemic and threatened plant species are an important component of plant biodiversity, which often requires immediate actions to ensure long-term survival. Strategies for plant conservation include *in situ* approaches in the form of protected areas such as national parks and *ex situ* approaches such as botanical gardens. An efficient approach to plant conservation is possible by combining *in situ* and *ex situ* conservation methods with the primary objective of maintaining a high genetic diversity of the species.
In order to initiate concrete actions to safeguard endemic and threatened vascular flora of southern Italy, a project for the conservation of plants that characterize the flora biodiversity of the Aspromonte National Park has been initiated. The project is part of a collaborative relationship between Aspromonte National Park and the AGRARIA Department of the "Mediterranea" University of Reggio Calabria.
The vascular flora of the Aspromonte consists of about 1,800 taxa, equal to about 70% of the floristic heritage of the Calabria Region (1). A preliminary analysis of vascular flora state of conservation has made it possible to ascertain consistency and distribution of species at risk of extinction of the Aspromonte flora, with particular attention to endemic species, representing 22% of the endangered flora (2).
The project provides the *ex situ* conservation of endemic and endangered species through the creation of habitat useful host them at the Biodiversity Observatory, a centre for the conservation of biodiversity of the Aspromonte National Park. A first phase of the project concerns endemic species of humid habitats, which are currently the most threatened because of their peculiar characteristics linked to ecological conditions and exclusive micro-environmental more susceptible to disturbances that can lead to easy alteration of the habitat and to involve the species extinction. In particular, the realization of some humid habitats of the mountain range has been started: riparian forests, wetlands and streams.
The mountain streams are conservative habitats for many highly specialized and exclusive hygrophilous endangered plants (3), including the endemic: *Alchemilla austroitalica* Brullo, Scelsi & Spamp., *Adenostyles alliariae* (Gouan) A. Kern. *subsp. macrocephala* (Huter & al.) Wagenits & I. Müll., *Chaerophyllum hirsutum* L. subsp. *hirsutum* var. *calabricum* (Guss.) Paoletti, *Epipactis aspromontana* Bartolo, Pulvirenti & Robastsch, *Soldanella calabrella* Kress, *Lereschia thomasii* (Ten.) Boiss., *Senecio ovatus* (G. Gaertn., B. Mey. & Scherb.) Willd. subsp. *stabianus* (Lacaita) Greuter. They are threatened by the water captain to supply the watercolors. Wetlands were widespread in the depressions of the Aspromonte highlands between 1000 and 1300 m, currently occupied by a mosaic of crops, uncultivated land, colonization shrub vegetation and artificial plantations (4). However, some wet depressions still exist today, which inhabit igrophilous and heliophilous habitats such as temporary ponds, peat bogs and meadow. Some hygrophilous endemic species grow in these habitats as *Agrostis canina* L. subsp. *aspromontana* Brullo, Scelsi & Spamp. and *Genista brutia* Brullo, Scelsi & Spamp. The waterways also host some endemic wood species such as *Salix oropotamica* Brullo, Scelsi & Spamp. and *Salix brutia* Brullo & Spamp. that play an important role in the structure of these habitats.
For each species selected to *ex situ* conservation one or more populations has been identified in the Aspromonte National Park area, suitable for the collection of diaspores, such as bulbs, rhizomes, cutting or seeds. The diaspores will then be collected and transferred to the Biodiversity Observatory, in a special nursing already existing, in order to proceed to their multiplication and transfer into the created habitats.
Through this project, the Biodiversity Observatory will better develop the function of a centre for the promotion of naturalistic culture. It will be considered as a true botanical garden for the conservation of *ex situ* biodiversity with breeding function of endangered species in order to be reintroduced into natural environments.

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1.2 = LIFE FUTUREFORCOPPICES - CRITERION 4: “BIOLOGICAL DIVERSITY IN FOREST ECOSYSTEMS”. IS THE USE OF “NEW” INDICATORS TO CORROBORATE GENERIC INFORMATION AS LIKE DIVERSITY OF WOODY SPECIES COMPOSITION JUSTIFIED?

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The FutureForCoppiceS LIFE project (Life 14 ENV/IT/000514) aims at demonstrating, by real datasets collected from a long-term experimental plots, how different management approaches have actually favoured or constrained the overall and the different aspects of Sustainable Forest Management (SFM) of coppice forests in Southern Europe (www.lifefutureforcoppices.it). FutureForCoppiceS compare the sustainability of the different coppice management systems (traditional coppicing, natural evolution, pro-active conversion by selective felling) by means of long-term monitoring trials established over the last fifty by CREA – Research Centre for Forestry and Wood) and making use of indicators dealing with all six SFM criteria. The evaluation is based on some of the traditional SFM indicators and on “new” indicators that are proposed and tested for the first time in this project: Criterion 4, which is referred to the variety of existing life forms and the ecological roles they perform in European forests. Therefore, action B.4 of the project focused on selected SFM indicators and on “new” indicators based on the taxonomic and community levels on three forest types: mountainous beech forests, deciduous thermophilous forests, evergreen broadleaved forests. The “new” indicators, proposed in the action B.4 of the project, are vascular plants, epiphytic lichens, fungi and birds. Aim of this specific study, is to analyze the diversity patterns of vascular plants in the three types of forest under different management histories (i.e. active conversion high forest vs. natural evolution) in order to understand the variation of plant communities in relation to forest and management types. We evaluated plant species cover (Braun-Blanquet) and basal area in 10 m x 10 m plots and species occurrence in the same plots and in nested subplots 0.0001 m^2, 0.001 m^2, 0.01 m^2, 0.1 m^2, 1 m^2, 10 m^2, and the biggest 800 m^2 (1). To explore differences in the main plant functional types in each treatment, they were split into two categories: herbaceous (resulting from the indirect management of forests) and woody species (resulting from the direct management of forests). Species-area relationships (SARs) were fitted using the power-law function $S=kA^z$, where S is the number of species, A is the plot (and subunit) area, and k and z are two fitted parameters. z is the steepness of the species-area curve, and k is the expected number of species in a unit area. The Arrhenius model was fitted by SSarrhenius, “vegan” v2.4-2, (2). All z-values for each 100 m^2 plot were calculated by fitting linear regressions to the data and the exponent z was used as a measure of β-diversity. A Non-Metric Multidimensional Scaling (NMDS) was adopted (2) to represent the specific composition patterns of vascular plants between different management approaches. An additional measure of diversity was calculated: the Shannon index.
Herbaceous plant species richness was affected by the management of woody species. Species diversity patterns at plot scale was affected by the management type, as demonstrated by simple regressions and relation of the z and k values of the SARs on the basal area of the forests. NMDS model shows a distinction between detected communities. For example, treatments in the Is Cannoneris area (evergreen broadleaved forest) differ in specific composition, and in Caselli area (deciduous thermophilous forest) there is a certain overlap between the species communities of the two treatments analyzed. These differences are also confirmed by the results obtained with the Shannon-Wiener index.

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1.2 = THE "DWARF" DAFFODILS OF THE MARITIME AND LIGURIAN ALPS

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Narcissus pseudonarcissus L. [*Sp. Pl.* 1:289 (1753)] (Amaryllidaceae) belongs to the section *Pseudonarcissi* DC., a complex of eight species comprising all the trumpet daffodils; among the most popular spring flowers. These species are characterized by showing large floral coronas, the most prominent feature of *Narcissus* flowers, and uniseriate anthers which are biseriate in the other sections.
The genus *Narcissus* has a complex evolutionary history and, in particular, the trumpet daffodils are the most critical with regard to the discrimination of taxa and the assignment of rank (1). In *N. pseudonarcissus*, for example, a number of species have been recently synonymized in order to accommodate from nine to seven different subspecies (2, 3). However, despite the taxonomic complexity, *N. pseudonarcissus* s.l. is an ideal group for studying species diversification in response to different geographic contexts.
N. pseudonarcissus subsp. *provincialis* (Pugsley) J.-M- Tison was described in 1939 by Pugsley [*J. Bot.* 77: 334 (1939)] starting from living specimens collected from a native population in the Maritime Alps. It has been for long synonymized to *N. pseudonarcissus* subsp. *minor* (L.) Baker (= *N. minor* L.), and more recently, reconsidered by Tison et al. (4) as a subspecies.
Mediterranean mountains are a reservoir of a significantly high number of endemic species and an exceptionally rich flora. In this context, the characterization of morphologically and genetically distinct lineages within a group is pivotal for the identification of biological relevant units of conservation at both local and national scales. According to us and (4), the subsp. *provincialis* would represent a geographically isolated taxon, native to the Maritime Alps and the mountains of Liguria, where its range is contiguous but not overlapping with the range of *N. pseudonarcissus* subsp. *pseudonarcissus* L.
In order to clarify the taxonomic relationships of the subsp. *provincialis* within the nominal species and ascertain its presence and distribution in France and Italy, a biometric and phylogenetic study has been started on a number of native populations ranging in the Maritime and Ligurian Alps. For sake of comparison, morphometric and genetic data were compared with a few populations of *N. pseudonarcissus* living at the Parco delle Capanne di Marcarolo (AL province), where the species was regarded as native (5).

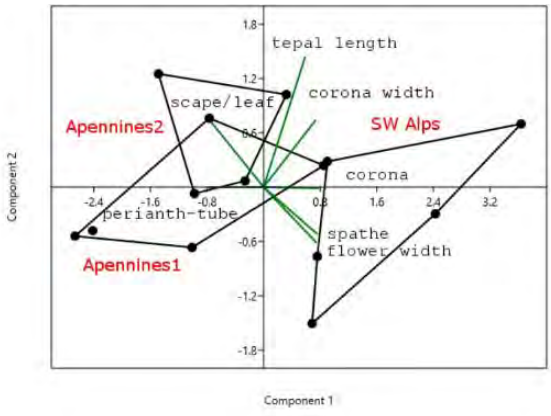

Fig. 1 (left)
Depicts the multivariate analysis of the morphometric data in a population from the Ligurian Alps (SW Alps) and in two populations sampled at Capanne di Marcarolo (Apennines 1 and 2). In the PCA, two axes were designated accounting altogether for 100% of the variance. The first component explained 88.8% variation. The individuals of the SW Alps were characterized by longer coronas, spathes and broader flowers than the individuals of the Apennines, which, on the contrary, were mainly grouped for their longer perianth-tubes and floral scapes.
Fig. 1 (right)
One of the flower of the Apennines.

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1.2 = CONTINENTAL SCALE PHYLOGENETIC DIVERSITY IN MARGINAL POPULATIONS OF COLD ADAPTED PLANTS: IMPLICATIONS FOR CONSERVATION IN A CHANGING CLIMATE

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The extent of species ranges is crucial for determining species extinction risk (1). Nevertheless, we know very little about causal factors shaping geographical location of margin ranges in most plant species. Moreover, understanding processes and factors allowing populations to survive at the edge of a species distribution or migrate is important to trace the effect of past climate changes and to predict the effect of current global warming on future species distribution and evolution (1). This is particularly important for cold-adapted species that are predicted to be affected by climate change more than other species, especially at the southern range edge (2). For these reasons, we intend to emphasize the importance of areas where marginal plant populations group (marginality hotspots) and highlight the role of marginal populations in the study of climate change and conservation management (3, 4). The identification of areas where a high number of arctic-alpine, circumpolar and circumboreal species reach their southernmost distribution represents a unique opportunity to shade light on processes that shaped current biogeographical patterns, persistence and future of these species.
In this study, we identify areas where southernmost populations of 183 cold-adapted (circumpolar, circumboreal and arctic-alpine) species, those predicted to be most affected by climate change, are clustered in north America and Europe. Moreover, we analyze pattern of phylogenetic diversity and their relationships with climatic factors to identify common characteristics of these area that may highlight mechanism and processes of plant persistence in a warmer climate.
We identified 19 and 16 main geographical areas including further 18 and 24 secondary areas in North America and Europe, respectively, based on the number of species reaching their southern range edge in each cell of a 50 × 50 km grid. Although considerable diversity is distributed within areas traditionally recognised as biodiversity hotspots, important areas of diversity are identified only after the application of randomisation-tests and through the phylogenetic metrics. Indeed, while the Pyrenees together with some cells in the Apennines and in the Balkans are confirmed to have significantly high diversity, the Alps exhibited significantly lower diversity than expected.
Phylogenetic signals for climatic predictors were close to zero, meaning that closely related taxa do not exhibit more similar climatic niches than expected by chance, suggesting a degree of casualty in occupying sites or migrating processes. However, while climatic predictors are not phylogenetic clustered they strongly characterize sites with high diversity. Specifically, biodiversity indices are positively associated with annual mean temperature and negatively related with temperature seasonality and precipitation of warmest quarter, indicating that colder sites with stronger continentality and fewer snowfall host a lower number and less diversified cold-adapted flora.
From an applied conservation perspective, we detected areas where studies and monitoring of cold-adapted species are best performed to maximize the knowledge gain and where conservation efforts should be directed to allow marginality hotspots to continue their important function of refugia in a warmer world.

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1.2 = PLANT ADAPTATION TO DROUGHT: EVIDENCE AND MORPHO-ANATOMICAL DIVERSITY IN *KALI* SPECIES (AMARANTHACEAE) FROM INLAND AND COASTAL SITES

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Plants in their natural environments are exposed to a variety of ecological factors that can act as stressors and constraint plant survival and renewal. Adaptation of a plant to different environment conditions, especially when harsh and fluctuating, involves patterns of functional traits (life cycle, growth rate, morpho-anatomical features, reproductive strategies) that may determine the differential success of any species.
Sandy shorelines, foredunes and salt-marshes represent very selective habitats where only specialized plants having particular morpho-anatomical and functional characteristics can cope with extremely detrimental growth factors, including variations in water availability (physical and physiological drought), soil salinity and fertility, intense heat and radiation, highly alternating temperatures.
The genus *Kali* comprises 21 annual species, previously belonging to *Salsola* sect. *Kali* Dumort. (1). Some of them are autochthonous of the sandy coasts of the Mediterranean and Atlantic European territories where they represent true psammophytes [*Kali turgidum* (Dumort.) Guterm. (previously *Salsola kali* L.), *K. tragus* (L.) Scop., *K. ponticum* (Pall.) Sukhor. *et al.*, *K. dodecanesicum* Brullo *et al.*], other *Kali* species are found in the Asiatic steppes and deserts, as well as in natural and ruderal habitats or infesting the crops [*K. tragus*, *K. australis* (R. Br.) Akhani & Roalson], or also linked to synathropic stands [*K. basalticum* Brullo *et al.*, *K. ryanii* (Hrusa & Gaskin) Brullo & Hrusa] (2).
These specie share common features which are distinctive of the genus, stems rigid, not articulate, cortex green to greenish-red, with longitudinal chlorenchymatous striae, leaves linear-cylindrical, broadened at base, provided with apical spine, bracts similar to the leaves, but smaller, membranaceous perianth of 5 free segments, fruiting perianth usually winged, provide with unequal rudimentary abaxial appendices, membranaceous fruits, above flattened.
Notwithstanding, *Kali* species diverge in different combinations of morphological and anatomical characters, mainly regarding gross habit, stem, leaves and bracts, indumentums and salt glands, shape and size of flowers and fruits, which are proved to represent core plant functional traits because of their importance in eco-physiological responses and reproductive strategies.
This study was performed on 6 species of *Kali* from different habitats and bioclimatic conditions in order to determine the structural differences and variability rate between plant populations from maritime and inland areas, particularly morpho-anatomic functional traits of stem and leaf which allow them to survive under specific environmental conditions. Cross sections of stems, mature leaves and bracts were made from living specimens collected in the field then cultivated in the Botanic Garden of Catania University. Morphometric analysis and statistics were carried out on micro-morphological and anatomical characters calculated from 10 samples for each species and the significance of variation for each trait was tested by ANOVA. Data comparison was made by three multivariate approaches (DFA, PCA, UPGMA).
Both stems and leaves showed the main architecture already outlined for other *Salsola* s.l. species and onther Chenopodiaceae (3-4). Stem cross sections revealed a rounded outline with prominent crests. Significant variations among species regarded the number and prominence of the crests, the cuticle thickness and epidermis hairiness, the relative development of the cortex and its components (chlorenchyma, collenchyma, parenchyma and sclerenchyma tissues), as well as the pith and vascular elements. Leaves have a cylindrical to semicylindrical outline and rather reflect the same indumentum as the stem, with no hypodermis, two concentric layers of chlorenchyma, typical of C4 Kranz anatomy (5), and water storage tissue in the central part with the main vein in the middle. Palisade and Kranz cells are interrupted by longitudinal collenchymatic ridges. Differences among species mainly regarded the size of cells and tissue thickness. Plants of coastal arid sites, in particular, showed a combination of halomorphic and xeromorphic morpho-anatomic features.

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1.3 = EFFECT OF DIFFERENT NUTRITIONAL STATUS ON CHLOROPHYLL FLUORESCENCE PARAMETERS IN HAZELNUT SAPLINGS

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Corylus avellana L. (hazelnut) is one of the major world's nut crops (1). In the year 2012, among the worldwide production of tree nuts, hazelnuts represented the sixth most relevant culture and occupied the fourth place, in terms of global trade, just behind pistachios (*Pistacia vera* L.) almonds (*Prunus amygdalus* Batsch.) and cashew (*Anacardium occidentale* L.) Appropriate fertilizing program is necessary in order to obtain high yield and high quality in hazelnut (2). In particular, nitrogen (N), phosphorus (P), and potassium (K) are the most important nutrients in hazelnut. Unfortunately, over fertilization is a common practice to ensure that unknown fertilizer demands are met, often results in environmental pollution (3). The increasing of the public concern about environmental aspects caused by over fertilization renews the interest for the adequate fertilization to maintain crop productivity and fruit quality with less environment impact (4). Several studies investigated the hazelnut response to different environmental factors (5, 6, 7, 8) but to the best of our knowledge, hazelnut response to different nutritional *status* has not been investigated through fluorescence parameters. In this context, we analyzed the effects of different fertilizers supply on both photochemical and physiological leaf traits of *C. avellana* saplings. At the beginning of April 2016 saplings were randomly assigned to three different nutrient treatments (*i.e.* C: control saplings; LN: low nutrient application; HN: high nutrient application). A complete randomized design (CRD) with ten replicates per treatment was established and an organic mineral NPK fertilizer (Nutri ONE Universal liquid fertilizer) was applied twice a month. In particular, the electron transport rates (ETR), the maximal PSII efficiency (F_v/F_M), quantum yield of PSII (Φ_{PSII}) and minimal Chl in dark (F_0) were analyzed. The results showed that Φ_{PSII}, which is linearly related to the carbon fixation efficiency (9) had a large variation among C, LN and HN saplings, resulting 51% and more than 100% higher in HN than in LN and C, respectively. The ratio F_V/F_M provides an estimation of the maximum quantum efficiency of PSII (10). Nevertheless, this parameter seems to be less affected by N and P content than Φ_{PSII}. The larger decrease of F_V/F_0 in C saplings (by 40%, mean value in respect to LN and HN) than in F_V/F_M seems to be a better indicator of the impairment of the PSII photochemistry reflecting more sensitively change in the photosynthetic activity, according to the results of Lima et al. (11). The higher F_0 in C sapling (resulting in the lower F_V/F_0) indicates the lower activity of PSII reaction centers. On the whole, the results showed higher values of Φ_{PSII}, F_v/F_0 in HN and LN saplings compared to C associated to higher net photosynthetic rates. Based on these results we can suggest a lower amount of fertilizer supply in *C. avellana* saplings in order to reduce the environmental footprint of fertilizers.

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1.3 = FUNCTIONAL TRAITS OF LEAF LITTER FUNGAL SPECIES

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Saprotrophic fungi possess a wide range of functional traits associated with biogeochemical cycling processes in leaf litter and soil. Their occurrence and life style usually reflect the availability of different substrates and mainly that of labile carbon (C) and nitrogen (N) (1). Here is proposed a laboratory-based approach for an estimation of some main functional traits of culturable saprotrophic fungi. The method is based on the values of substrate use (respiration) and growth (biomass/turbidity production) obtainable for single fungal isolates in vitro using FF MicroPlates™ (Biolog Inc., Hayward, CA). These are commercial microarrays containing 95 different carbon sources (Fig. 1) that can be used as a Phenotype MicroArray™ (PM) system (2) to test the metabolic performance of microorganisms on different substrates. This method has been used here to characterise the functional traits of a group of Mediterranean leaf litter fungi, achieving a natural succession in distinct phases of cellulose decomposition in a Mediterranean soil system (2).
In our study, we discovered a potential redundancy in the overall exploitation of 95 simple carbon sources between a few species occurring at the start of cellulose decomposition process, and some differences between the strains occurring at later times of the process (three sampling times), thereby showing different strategies in substrate use (Fig. 2). This suggested that the functional traits of single decomposers may determine the chemical composition of organic matter entering the soil, which is ultimately relevant to the homeostasis of the ecosystem. Actually, in some litter decomposition studies initial coloniser identity altered the functional characteristics of the substrate and subsequent community composition (2). It could be hypothesised that even though fungal functional characteristics potentially converge during the decomposition of a given substrate, certain initial fungal decomposers could have an early competitive advantage and possess peculiar attributes, such as a more rapid development and spatial niche differentiation, phenomena which result in different substrate modifications and priority effects.

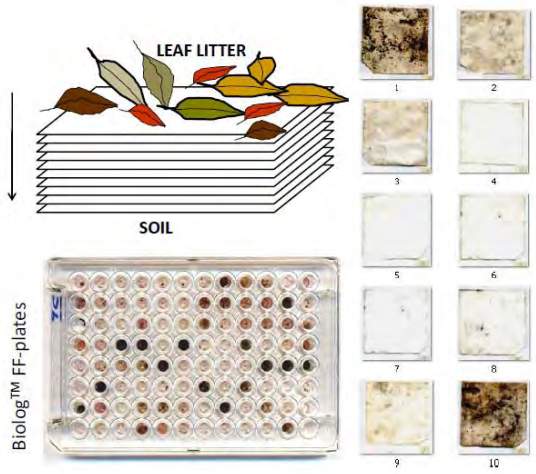

Fig. 1
The trapping system made of cellulose sheets, used for isolating cellulolytic fungi at the soil-litter interface, and the commercial microarrays containing 95 different carbon sources.

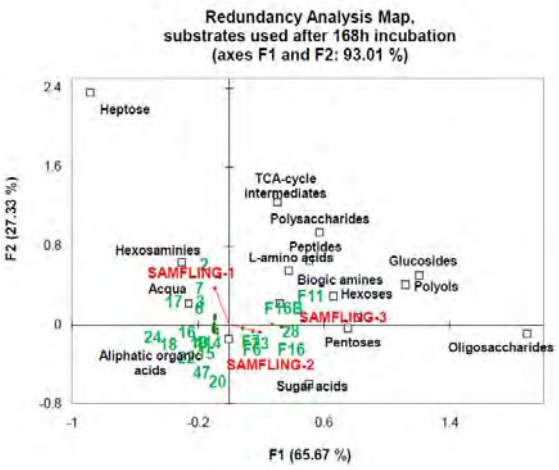

Fig. 2
Redundancy analysis obtained plotting data on fungal species occurrence at the different sampling times and their functional traits in terms of ability in substrates use.

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1.4 = BETWEEN PAST AND FUTURE: LEGACY AND FATE OF AN IMPORTANT CENTRE OF ENDEMISM FOR THE MEDITERRANEAN REGION

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Climate change is one of the greatest threats to biodiversity, particularly in mountain ecosystems, which often show high endemism rate. Studies on past climate changes suggested that current centers of endemism are placed where climatic fluctuations were less pronounced (because of mountainous topography) and that these areas will be less affected by climate change in the future (1). However, this doesn't mean that these areas will not experience drastic climate change effects; in fact, several evidences indicate that mountain endemics are disproportionally exposed to climate change, because usually they are characterized by small range size, narrow ecological tolerance and poor dispersal ability (2).
SW Alps are one the most important centers of endemism of the Mediterranean Basin and are particularly exposed to climate change threat due to the contemporaneous effects of temperature increase and precipitation decrease, as other Southern European mountain systems (3). Taking into account dispersal abilities, we used Species Distribution Models to analyze the distribution of suitable climatic conditions for 100 plant species endemic or subendemic of SW Alps (about 60% of endemisms of this area). We projected predicted suitability in past (i.e.: last interglacial, last glacial maximum, middle Holocene), current and future (i.e: year 2070) time slices. For each time slice we elaborate a map of potential species richness; for each species we also calculated the percentage of range loss and gain in the future, considering both optimistic and pessimistic emission scenarios.
In line with the relationship between climatic stability and centers of endemism, we found a significant positive correlation (r = 0.77) between past climatic stability and current potential endemic species richness. This result suggests that the areas currently showing suitable climatic conditions for several endemic species were less affected by past climatic fluctuations.
Analyzing species response to future climate change, we found that SW Alps endemisms will experience high range loss (average value of 65.5% in optimistic scenario and 82.3% in pessimistic scenario), not counterbalanced by range gain in adjacent areas. Even if only very few species are projected to be extinct in 2070 (less than 5%, only in more pessimistic scenario), roughly 46% of species will experience more than 80% of range loss, giving rise to serious (and urgent) conservation issues.
A significant positive correlation was found between future potential endemic species richness in optimistic scenario and both past stability (r = 0.72) and current potential endemic species richness (r = 0.87). This result suggest that the areas stable in the past are currently richest in endemic species and will be also potentially richest in endemisms in the future. However, when we analyze the climate change effects under more pessimistic future scenario these relationships fall down, because of the very high values of range loss for almost all studied species.
In conclusion, our data substantially confirmed that current micro-hotspots of endemism were stable in the past and show a significant tendency to remain more stable in the future, at least in optimistic scenario. Nevertheless, species loss in the SW Alps may still be large probably because assuming a realistic dispersal rate, the endemisms may not be able to keep up with future climate change.

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1.4 = THE GREEN ROOF'S THERMAL PERFORMANCE IN THE COLD SEASON: FIRST RESULTS FROM DIFFERENT PLANT COVERS

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The mitigation of the urban heat island effect and the reduction of the building energy consumption in summer season is one of the main environmental advantages offered by the green roofs. Green roofs, however, being insulating structures, interposed between the external atmosphere and the indoor of the buildings can have also interesting thermal performance in the cold season (1). This study aims to assess the insulation performances obtained through plant cover made by wild herbaceous species during the cold season. We compared two *Sedum* species (*Sedum lydium* Boiss and *Sedum kamtschaticum* Fisch.), frequently used on the green roofs, and two species such as *Bromus erectus* Huds. (Gramineae) and *Lotus corniculatus* L. (Leguminosae), whose performances during the summer period were already studied and compared with those of the two above mentioned *Sedum* species.
We utilized 5 containers (40 cm x 30 cm x 22 cm height) filled with 1500 g of green roof's substrate (Harpo/SEIC substrate) with the Harpo commercial stratification for extensive green roofs. On September 2016 four containers were planted each with a given species (*B. erectus, L. corniculatus, S. lydium*, *S. kamtschaticum*) at high density to obtain a complete substrate cover. The fifth container was filled only by the bare substrate, as control. Each container, placed outdoor, was equipped with two thermocouples: one buried into the substrate at 7 cm depth to collect the internal temperatures and another one placed on the surface (of plant cover or of the bare substrate) to collect the external superficial temperatures. The experiment lasted from 18th January 2017 to 8th March 2017. The datalogger of each termocouple recorded the temperature values every 30 minutes. We considered the mean value calculated from the values recorded between 4:45 am and 7:45 am, considering it as the coldest period of the day. Then, we analysed the differences between the depth temperatures and the surface temperatures for the five containers under study.
By the surface temperatures, we divided the experiment in two periods: the first one (from 18th January to 30th January) characterized by a minimum average surface temperature of -0.5 °C and the second period (from 31th January to 8th March) with a minimum average surface temperature of 3.5 °C.
During the coldest period, the difference between the depth temperature and the surface temperature was, on average of 0.5 °C for the container with the bare substrate and without plant cover, whereas for the same period the difference between the depth temperature and the surface temperature was, on average of 1.0 °C for all the containers with plant cover.
During the second period, less cold, we observed major differences among the temperatures recorded for the container with *B. erectus* and the temperatures recorded for the containers planted with other species or with the bare substrate. In this second and warmer period, in fact, the differences between the depth temperatures recorded in the container with *B. erectus* were, on average, 3.0 °C higher than the surface temperatures. The differences between the depth temperatures recorded in the containers with the other species were on average 2 °C higher than the surface temperatures. Finally the differences between the depth temperatures and the surface temperatures were on average 1 °C in the container with only the bare substrate.
The better performance of *B. erectus* cover is reasonably due to the higher quantity of biomass produced by this species during the vegetative season, which better acts as insulating layer during the cold season, if all the biomass was maintained during the winter (1, 2). *B. erectus* appears an interesting species with positive effects in summer as well as in winter (3).
From a more general point of view, this study demonstrated that the plant cover of a green roof can give an important positive contribution to the thermal performance of the green roofs itself, not only during the hot season, thanks to the transpiration (3), but also during the winter season, thanks to the quantity of old biomass produced, strictly dependent on the transpiration. An adequate choice of the plant species as well as an equilibrate control of their functions (biomass production, transpiration) during the vegetative season could become a double investment, that improves the thermal performances of the green roofs in the hot season as well as in the cold one.

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1.4 = NURSE PLANT EFFECT ALONG MOUNTAIN VERTICAL GRADIENTS. AN INSIGHT IN CENTRAL APENNINE

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The current trends of climate warming have spurred the scientific community to investigate the effects of climate change on biotic communities. High mountains, being isolated and far from direct human pressure, have been considered as appropriate environments to investigate the effects of climate change on natural biocenoses (1). During the last decades, a shift of subalpine species upslope along with an increase of species richness and a decline of cold-adapted species were registered on several high mountain systems of the world (2). In alpine communities, climate warming and earlier snowmelt are the main climate drivers of change in vegetation composition, and the response of each taxa depends on its specific traits. For instance, recent studies have demonstrated the key role of plant traits on the colonization and establishment processes of thermophilic species upwards (3) and among them the dispersal characteristics seemed particularly important, even if a lot of these models that consider a lot of traits and environmental variables have shown a large variability that is not sufficiently explained. Latent variables have been described as 'interaction currencies' (4) towards novel approaches to modelling biotic interactions in multispecies assemblages at large spatial extents. In other words, quantities that mediate interactions between *taxa* but whose measured values are not included in the model. This is similar to thinking of latent variables as missing predictors, but it puts the emphasis on the idea that correlations across *taxa* are induced by these missing predictors.
This assumption confirms the importance of consider species interaction to have a better view of the effects of global warming on change in plant species composition. Some plants, can mitigate microenvironmental changes and may ensure in the long term the persistence of other alpine species through local migration events and facilitation process (5). Furthermore, empirical studies leave no doubt that the multiple effects of current and predicted climate change affect plant communities either directly, e.g. "thermophilization," and indirectly e.g. modifying the interactions among species.
The main questions of this work are: how species interactions changes along vertical gradient? How this interaction could affect the upward shifting of thermophilic species?
In the context of high mountain environment, it is crucial to understand the interaction among species at different altitudinal ranges, especially, where the stress conditions are extremely variable.
The study was conducted in the two highest massifs of central Apennines: Gran Sasso and Majella. We analyze presence/absence data from NN georeferenced relevés extracted from the VIOLA database (high mountain VegetatIOn of centraL Apennines) (6), collected on altitudes ranging from 1800 to 2700 m. a.s.l. For each relevé, we collected four GIS-derived environmental variables: elevation, surface rockiness, topographic wetness, solar radiation. We prepared 3 main matrices (species per traits, species per site and environmental variables per site) and we analyze them using a model based approach that predict the probability of an observed species in a site as a function of site's environmental characteristics and plant functional traits (fourth-corner model), and the interactions among species were analyzed with residual correlation matrix (7). The results have shown a variation of species correlation along altitudinal gradient suggesting that change in environmental conditions are linked to change in species interaction. Our results suggest the presence of some species as *Silene acaulis*, that are nurseries hosting other less stress tolerant species, in high stress conditions. Nursery effects could offer a stepping stone that promotes the migration at higher elevations of thermophilic species. On the other hand, the absence of efficient nurse plants might slow down upward migration, possibly generating chains of extinction.

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1.4 = FUTURE ADAPTATIONS OF PLANT SPECIES TO ENVIRONMENTAL CHANGES

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The response of organisms to changing environmental conditions is four-fold: they become extinct, migrate, adapt to new sites with suitable environmental conditions or decline into relictual populations, like tertiary endemics did into Mediterranean flora (1).
Quantifying the variation in the ecological and genetic traits of natural plant populations across a species' geographic range is crucial to our understanding the factors that have shaped current distribution patterns (2) and the processes acting in the maintenance and/or expansion of range limits (3). In this project we will explore the relationship between ecology, floral and genetic polymorphism of *Lilium pomponium* L. across its wide altitudinal range, from 100 to 2000 m (spanning from Mediterranean to Alpine habitats). The species is a protected endemism of Maritime and Ligurian Alps.
This study aims to evaluate any difference between central and peripheral populations and among populations at different altitudinal zones and to test the centre-periphery' hypothesis (CPH) predicting that species performance (genetics, physiology, morphology and demography) declines gradually from the centre towards the periphery. The investigations should enable us to define any possible phenological and genetic modifications that might allow the species to thwart future environmental changes (4). To determine if there is any relationship between morphological polymorphisms and environmental gradient we will study: (I) the micro-ecological niche analysing the soil properties and the vegetation coverage; (II) the macro-ecological niche using bioclimatic variables; (III) the floral polymorphism through the determination of (a) the number of flowers per scape, (b) the number of unisexual flowers, and (c) the width and length of the perigonium; (IV) the relative position of sex organs to test herkogamy; (V) the mating system variation to test the possibility of self-compatibility; (VI) the pollen morphology and seed production. Furthermore, we will reconstruct past history of the species to assess how past climate changes have affected the current distributional pattern of morphological diversity. For this purpose, we will also analyse the genetic polymorphism to identify any genetic break (5); the impact of present and past environmental variables on genetics diversity (6) and the consequence of future climate change on genetic diversity (7). Eventually, this study will allow to define the possible modifications that might allow the species to thwart future environmental changes.

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1.4 = MODELLING PRESENT AND FUTURE CLIMATIC NICHE OF MOST COMMON SARDINIAN ORCHIDS

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Orchids are widely distributed, with the exception of the poles. While most of tropical taxa (more than 80%) are epiphytic, temperate taxa are mostly terrestrial (1). Although a few species are achlorophyllous holoparasites, and their life cycle is strictly related to soil fungi through the formation of mycorrhizas, all orchids are myco-heterotrophic during germination and seedling growth. Thus, their relationship with the soil is extremely relevant for their distribution.
This work aims at depicting the climatic niche of the most common orchids which are known to occur in Sardinia, and understanding how it will change in future climate change scenarios. Sardinia, is the second larger island in the Mediterranean basin, and it is classified as one of the 34 most important 'biodiversity hotspot' in the world. From the relevant geomorphological and bioclimatic variability (2) of the island a great floristic richness arises, especially in the family Orchidaceae. In fact, Sardinian orchid flora consists of 68 taxa of which 12 are endemics (3). Since these orchids are geophytic plants, with underground bodies which allow quiescence for unfavorable seasons, they can easily overcome physical stress, such as high temperatures and extensive summer dryness, which are typical of the Mediterranean climate.
Nine *taxa* have been investigated by mean of species distribution modelling (4). An ensemble approach has been applied in order to provide more reliable predictions (5). It averages models from three different algorithms (in this study GLM, MaxEnt, and Random Forest) (6), weighted on their performance score (evaluated by True Skill Statistics metric). The variables used in the models describe temperature, precipitation, soil type and land use. Future projections are based on climate-only data, calculated for year 2070. The nine taxa have a similar distributional pattern. As far as the future scenario is concerned, there is a tendency of widening of the climatic niches. However, such tendency is coupled for some taxa, with a reduction of suitability in the whole niche.

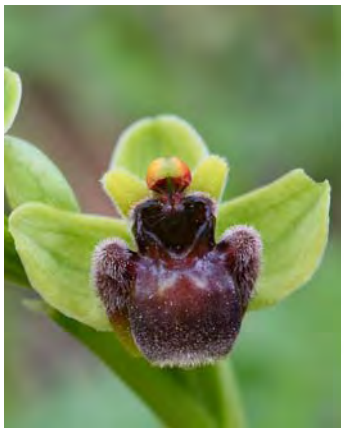

Fig. 1
Ophrys bombyliflora.

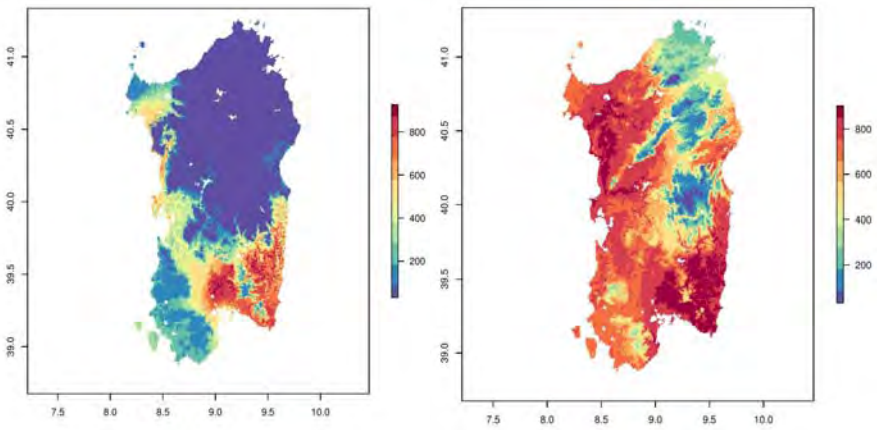

Fig. 2-3
Current and future predicted suitability maps for *Ophrys bombyliflora.*

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1.4 = EFFECT OF GRAFTING ON XYLEM EFFICIENCY/SAFETY MODULATION AND PHOTOSYNTHETIC PERFORMANCE IN *VITIS VINIFERA* 'PIEDIROSSO': CONCERNS IN A CLIMATE CHANGE SCENARIO

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Vitis vinifera L. is the most widely cultivated and economically important fruit crop in the world (1). Italy, one of the world's leading wine-producing countries, shows vineyards distributed all over the mainland. In this scenario, Campania region, due to its peculiar climatic conditions and fertile soils, has always been considered one of the most appreciated districts for wine production (2). Additionally, the presence of several autochthonous grape varieties is a mainstay for this region. Italian legislation does not allow the irrigation of vineyards in order to preserve wine quality in most quality assurance labels. However, the on-going climate changes are determining constraints in viticulture due to rising temperatures, changes in precipitation frequency and occurrence of extreme events, such as heat waves. Grapevine (*Vitis vinifera* L.) yield is expected to suffer from climate change also given that fruit development and ripening occur in the hottest and driest season (3) (4). In the last years, in the sight of climate change, the attention has been rise non wine production sustainability, particularly considering the possibility to adopt cultivation techniques aimed to enhance physiological adaptation to drought (5). In some volcanic areas of the Campania Region, grapevine cultivation is traditionally performed by avoiding the use of rootstocks thanks to the soil type that is inhospitable to Phylloxera pest.
In this context, the aim of the study is to assess how the use of grafting on rootstocks could influence plant adaptation to drought and grape production. This study was conducted on *Vitis vinifera* L. 'Piedirosso' grapevine which is an autochthonous cultivar of the Campania Region. Plant growth, yield, photosynthetic performance and anatomy of water stem-leaf pathway were analysed in plants grown on their own-roots or grafted onto 420A rootstock (*Vitis riparia* x *Vitis berlandieri*). The selected vineyard Vigna Jossa was located within the farm Cantine degli Astroni, in the Campi Flegrei area at 200m above sea level (Naples, Southern Italy). Grafted and non-grafted plants were compared on the basis of several morphological and eco-physiological parameters including: plant architecture, leaf and wood stem anatomical traits, photosynthetic efficiency (by determination of quantum yield of PSII electron transport, maximal photochemical efficiency and pigment content), gas exchanges, etc.
The overall results showed that the expected differences in growth performance and productivity in the two types of plants, were consequent to different eco-physiological and structural properties suggesting a different capability to modulate gas-exchanges and water flow efficiency/safety against embolism under different conditions of water availability. Hence, this study suggested the occurrence of higher plasticity of plant traits involved in the regulation of water flow in the grafted system. Such findings induce to better evaluate the opportunity to move from a traditional cultivation with own-rooted grapevines towards grafted models in the sight of increasing drought. As an alternative, the maintenance of the traditional own-rooted cultivation system should be accompanied by further cultivation trials aimed to optimize training system and plant management.

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1.5 = MONITORING THE EFFECTS OF GRAZING-MANAGEMENT ON PERFORMANCE AND SURVIVAL OF ORCHIDS IN XEROTHERMIC GRASSLANDS

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Xerothermic grasslands are among the most species-rich habitats in the Alpine region as well as in Europe as a whole (1). In the south-western Alpine foothills, where steppic continental elements are found together with sub-Mediterranean and Mediterranean species, these communities are of particular interest. These habitats are currently threatened by the progressive encroachment of trees and shrubs as a result of the abandonment of traditional low-intensity agricultural management. Semi-natural dry grasslands, therefore, constitute a major conservation target and are included as a priority habitat in the Habitats Directive 92/43/CEE (code 6210*). This work focuses on the monitoring activity being performed as part of the European Xero-grazing LIFE project (LIFE12 NAT/IT/000818), which began in 2013 and is being carried out in the Susa Valley (Piedmont, Italy). The purpose of the project is the restoration and long-term conservation of the semi-natural dry grasslands on calcareous substrates located in the SCI IT1110030 "Oasi xerotermiche della Valle di Susa - Orrido di Chianocco e Foresto", an area which is important for its large variety of orchids and Mediterranean species, the latter ones rare in the Alpine region. The habitat restoration is being implemented with a combination of two management strategies - shrub-clearing and sheep grazing. The aim of the monitoring activity is to determine the effects of these measures, in particular on orchids, which are a target species of conservation interest. This group of plants is known to have a complex life history, characterized by a long under-ground recruitment phase before emergence. Periodic phases of vegetative underground dormancy also make it difficult to distinguish dormancy from mortality in data analysis (2). Therefore, studies that investigate effects of management strategies on demography of orchid populations are particularly challenging. In the present study, we are examining the effects of re-introduced sheep grazing on orchids, with a particular focus on the most frequent species found in the study area: *Ophrys fuciflora, Orchis tridentata, Anacamptis pyramidalis*, *Epipactis atrorubens*. A total of 37 permanent plots, each of 25 m^2, were established where there was a relevant density of orchids. To disentangle the effects of management from the effects of other factors - e.g. climate and intrinsic orchid dynamics - 10 out of 37 plots were fenced to exclude sheep grazing, while the remaining plots were alternatively grazed for 4-6 weeks in late spring (May-June) and/or autumn (September-October). During the study period (2014-2017), plots were visited twice per season (before-grazing and after-grazing) with over 2000 ramets being individually assigned *x,y* coordinates using a 1x1 m grid, and then monitored. The following measurements were recorded for each ramet: the phenological status (vegetative, reproductive with flowers and reproductive with fruits), the number of leaves, the length and width of the largest leaf, the height of the flowering stalk and the number of flowers and fruits. GLMs were used to analyse the effects of grazing on the total number of vegetative and reproductive ramets, with respect to both the overall orchid population and each target species.
Preliminary results show a general increase over time of the total number of orchids in grazed plots compared to the control plots, consisting mostly of vegetative ramets. *O. fuciflora*, in particular, reflected this trend, which points to the beneficial effect of sheep-grazing. Further results and analysis will be presented and discussed.

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1.5 = THE LANDSCAPE OF SICILY: POLLEN INSIGHT INTO THE AGRO-PASTORAL SYSTEM OF PHILOSOPHIANA AND VILLA DEL CASALE (UNESCO ROMAN SITE)

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Since the last century, archaeopalynology and archaeobotany have quite substantially contributed to archaeology and to Quaternary science in general, allowing the deeper understanding of both present-day landscapes and past human-environment relationships.
In archaeological sites, plant remains help to identify different types of land use: i) exploitation of plants; ii) cultivation, i.e. the planting and care of useful plants; iii) breeding carrying out the increase of pastures; iv) settlements with spreading of ruderal and nithrophilous plants (1). Pollen and NPPs - Non Pollen Palynomorphs (other microscopical records of biological origin, including fungi and algae) (2) are particularly worthwhile for investigating these kind of actions.
In central Sicily, pollen analyses on two extraordinary rural complexes have been especially useful for palaeo–environmental/economical local reconstructions. The investigated sites are Villa del Casale of Piazza Armerina, a monumental villa included in the UNESCO World Heritage List, and the near 'agro-town' Philosophiana (3). Their long chronology of occupation (from Roman to Medieval periods) offers the opportunity to investigate the land use transformations and the effects of countryside exploitation occurred in the landscape of Sicily. Their palynological investigations may also improve the resolution of vegetational-environmental events recorded from extra-local palynological sequences (4) by giving local information on plant landscape and human influence in the region.
The palynological research here presented has been carried out on samples from Roman-Late Antique contexts, while the comparison between Roman and Medieval samples at Villa del Casale is published elsewhere (5). The pollen spectra are characterized by insignificant forest cover (10%) suggesting that the landscape has been more open than today, with woods distributed quite far from the sites. Conversely, the spectra are dominated by herbs, with Cichorieae (36%) followed by Poaceae-wild grass group (12%), Brassicaceae (4%), Chenopodiaceae (3%) and other anthropogenic pollen indicators. These featured the human-shaped environments producing cereal-type (2%) and grassland pollen grains, both included in the API - Anthropogenic Pollen Indicators (6) sum with a number of wild synanthropic plants.
Along with a high relevance of cereal cultivation, the olive-grapevine Mediterranean economy is evident from pollen spectra. *Olea* is at the centre of the arboreal crop production of both sites, while *Vitis* seems to have a different significance in the two sites: an agrarian product in the Philosophiana farmhouse, and ornamental lianas (growing rampant on the villa walls) with edible fruits at Villa del Casale.
Most of the agrarian landscape should have been also devoted to pasturelands as a number of herb plants common in pastures or in abandoned fields makes great part of the pollen spectra. Moreover, some plants of the legume family (*Trifolium* type, *Medicago*, *Vicia*, *Melilotus*; about 0.8% in both sites) might have been also part of pastures, or were possibly cultivated for fodder and to regenerate soils after cereal cultivation as documented in other Mediterranean Roman contexts (7). In general, pastures looked to have been more expanded than cereal fields.
Pollen data suggest a continuity of the agrarian landscape during the occupation phases of the two sites. Although changes had occurred, subsequent cultures were progressively incorporated in the agrarian-pastoral landscape management. Our data bring evidence and details about the intense land exploitation that contributed to transform the natural environment of this island into the modern agrarian landscape.

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1.5 = CHANGES IN SPECIES ASSEMBLAGES IN THE TRANSITION GRASSLANDS - SHRUBLANDS: DOES PHYLOGENY MATCH THE DIFFERENT CONDITIONS IN TERM OF ECOLOGICAL FACTORS?

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Secondary semi-natural grasslands are important components of European cultural landscapes and resulted from millennia of traditional land use, mainly linked to the grazing by livestock (pastures) or hay-making (meadows) (1, 2). In Europe, these habitats have recently been experiencing a huge loss in surface area due to important changes in the traditional land uses. Indeed secondary grasslands and meadows, no longer maintained under grazing and farming activities, displayed a global tendency to evolve in shrublands and woodlands through natural secondary successions (3, 2), undergoing vegetation dynamics leading to the gradual transition from grasslands to shrublands. While this transition is well documented as to the changing of vegetation structure and floristic assemblages involved, less is known regarding the changes occurring in the communities focusing on phylogenetic and/or functional diversity. Such changes in species composition and co-occurrence may represent a precious chance to test and verify the modern theories in term of species assemblage evolution and development. Within this work, we aimed to study how the ecological differences involved in the natural succession of dry grasslands and hay meadow may drive the processes of species assemblage during the dynamic evolution of such habitats. Particularly, we aimed to: i) verify whether the two habitats are characterized by different trends in species loss and species turnover during their dynamic evolution; ii) verify the links among changes in species richness, composition and phylogenetic diversity and different ecological conditions. Toward these aims, we sampled dry grassland and hay meadow plant communities in the Eastern Tuscan Apennines. In order to reconstruct their dynamic changes, we adopted a chrono-sequential approach. The two habitats showed different features in the species compositional changes during their dynamic evolution. Dry grasslands displayed a trend in species turnover mainly dominated by Nestedness; this indicates that, accordingly with the loss in species richness characterizing the habitat dynamic evolution, poorer sites host a subset of richer ones. On the other hand, the higher values of Richness Difference in hay meadows indicate that in the natural dynamics of these habitats the species turnover yield to a process of higher species replacement. In these habitats, indeed, the loss of species is accompanied by a substitution of species in the more dynamically advanced stages. Following these trends in species turnover during the vegetation dynamics, the indexes of phylogenetic diversity showed valuable differences among the two lines of evolution. A sensible overdispersion of plant composition was detected in the evolution of dry grasslands, while a random process of differentiation characterized hay meadows. Our results appear consistent with the predicted community phylogenetic structure in fig. 1 (according to 4). In dry grasslands the dynamic evolution, constrained by a strong habitat filtering, led to the overdispersion of the community phylogenetic structure since the plant traits are dispersed among the species characterizing the community. On the contrary, hay meadows, probably less stress dominated and not so much subjected to habitat filtering but more to competitive exclusion, are subjected to a random process of differentiation in the community phylogenetic structure.

	Functional traits	
	Conserved	Converged
Dominant ecological force		
Habitat filtering	Clustered	Overdispersed
Competitive exclusion	Overdispersed	Random

Fig. 1
Predicted phylogenetic structure within a community under differing scenarios of functional trait evolution and alternative ecological determinants (Adapted from 4).

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1.5 = THE AGRO-SYLVO-PASTORAL SYSTEM OF 3600-3200 YEARS AGO (TERRAMARE, PO PLAIN; SUCCESSO-TERRA PROJECT)

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An interdisciplinary geoarchaeological and archaeobotanical (pollen and macroremains) investigation is carried out in the framework of the national-funded project SUCCESSO-TERRA (Human societies, climate, environment changes and resource exploitation/sustainability in the Po Plain at the Mid-Holocene times: the Terramara; PRIN-20158KBLNB). The research is providing significant data on the land transformations that occurred at the onset, duration, and end of the Terramare culture in the southern-central Po Plain (Emilia Romagna region). The Terramare are archaeological remains of banked and moated villages, located in the central alluvial plain of the Po River and dated to Middle/Recent Bronze ages (3600-3200 yr. BP).
Pedosedimentary features and biological records from Terramare sites help to shed light on the relationships between Late Holocene regional environmental vicissitudes and land use changes, and allow a detailed comprehension of adaptive strategies of the Terramare people (1).
Pollen samples were collected from trenches excavated within the main structures of the archaeological sites (namely Santa Rosa di Poviglio and Vasca Grande di Noceto sites).
The pollen spectra resulted from both human presence/action and natural vegetation cover in the area. A set of anthropogenic pollen indicators, also common in the spectra from other Italian archaeological sites (2), was considered especially useful to reconstruct the agro-sylvo-pastoral system besides the distribution of wetland plant associations.
The palynological research showed a transformation in flora composition and plant communities, suggesting a complex and dynamic agricultural economy based on wood management, fruit collection on the wild, and crop fields. At the top of the sequence of Santa Rosa di Poviglio, in correspondence with a global, dry climatic episode, a dramatic decrease of fields and woods is recorded. Along with aridity, an intensive land-use might have played a fairly synchronous action on vegetation. Data suggest a scenario of an impoverished plant landscape at the end of the life of the Poviglio Santa Rosa village, and connected with the collapse of the Terramare culture.

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1.5 = MORPHOLOGY OF *VITIS* POLLEN FROM CULTIVARS AND WILD GRAPEVINE AND THE *VITIS* POLLEN FROM THE TERRAMARA SANTA ROSA DI POVIGLIO (PROJECT SUCCESSO-TERRA)

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Currently, grapevine is one of the most economically important fruit crops in the world. Grapevine has been selected by humans in order to ensure a regular and high fruit production, as well as a high sugar content and fermentation yield (1). It is widely accepted that *Vitis vinifera* L. ssp *sylvestris* (wild grapevine) is the ancestor of the present cultivars of *Vitis vinifera* L. The purpose of this research is to identify the parameters that may help in discriminating pollen of *Vitis vinifera* subsp. *vinifera* from pollen of wild grapevine. The increase of morphological knowledge about the pollen of *Vitis* may be useful in the archaeobotanical field, allowing to better understand the past use of this plant. This work is part of a wider project that aims to investigate the relationship between climate and society in the Bronze Age during the Terramare's civilization (SUCCESSO-TERRA project, PRIN-20158KBLNB). The morphometrical analysis was carried out on pollen from 3 different accessions of *V. vinifera* subsp. *sylvestris* (2 male and 1 female) from Italy (Viticultural Centre of Riccagioia - Torrazza Coste - PV) and from 3 different cultivars of *V. vinifera* subsp. *vinifera*: Covra (Modena), Grasparossa e Bianca di Poviglio (Reggio Emilia). The pollen was sampled in May, from 2014 to 2016. Pollen grains were subjected to acetolysis, mounted in glycerine jelly and examined by light microscope. The considered parameters are: polar axis (P), equatorial diameter (E), maximum distance between colpi in mesocolpium, polar and equatorial axis of pore, exine thickness (Ex). In 5 samples, the pollen grains of grapevine are, as expected, 3-zonocolporate. However, pollen grains in the functionally female flowers of wild grapevine are inaperturate (acolporated). Among the 31 archaeobotanical samples coming from the Terramara of Santa Rosa of Poviglio (2), the sample with the highest percentage of *Vitis* pollen was examined in order to carry out measurements on the pollen grains of grapevine dated back to the Bronze Age. Another purpose of the archaeobotanical investigation was to check for the presence of acolporated pollen grains, typical of the functionally feminine flowers of wild grapevine. The principal component analysis (PCA) of the morphometric data reveals that pollen grains of wild *Vitis* (3-zonocolorate and with a finely-reticulated exine) have a higher P/E ratio if compared to the domesticated ones. It was also underlined that the cv Bianca di Poviglio have a pollen more similar to the one of *Vitis vinifera* subsp. *sylvestris* than other cultivars.

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2.1 = DROUGHT AVOIDANCE ADAPTIVE TRAITS IN SEED GERMINATION AND SEEDLING GROWTH OF *CITRULLUS AMARUS* LANDRACES

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Citrullus lanatus (dessert watermelon) cultivation is affected by drought stress (1). *Citrullus* species that grow wild and domesticated in arid areas, are considered potential useful donors of drought tolerance traits (2). Here we characterized drought tolerance in the first stages of plant development in eleven landraces of *C. amarus* (citron watermelon), from most of its worldwide cultivation range and in one *C. lanatus* commercial cultivar ('Sugar Baby'). We evaluated several plant traits: seed mass, seedling establishment and the response of seed germination and seedling growth, in terms of leaf length, shoot length, dry biomass and root/shoot ratio, to different water availabilities. 'Sugar Baby' germinated to lower water potential better than all *C. amarus* landraces, while seedling establishment was much higher in *C. amarus* than in *C. lanatus*. Finally, seedling growth of *C. amarus* landraces and the *C. lanatus* cultivar followed different patterns depending on water availability, showing enhanced biomass growth under wet conditions and no changes between dry and wet growing treatments, respectively. The different water use strategies in seed germination and seedling growth found in the two crops highlight drought avoidance strategies linked to the species growing environment in *C. amarus*, not present in the *C. lanatus* cultivar. The high seedling establishment, the plastic responses to water availabilities and the strong root system, indicate that genetic resources of *C. amarus* may have important applications in breeding programmes and in the selection of water-use efficient rootstock lines. More in general, this study confirms the assumption that landraces show adaptive traits towards marginal conditions and abiotic stresses (3). Moreover, it highlights the potentialities of investigations on functional traits in order to shed a light on habitat-related strategies in useful plants, neglected crops and landraces.

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2.1 = EFFECT OF NICKEL STRESS ON SEED GERMINATION IN HYPERACCUMULATOR PLANTS AND RELATED SPECIES

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High level of available nickel (Ni) in soil exert toxic effects on seed germination of common plants. Most studies are focused on metal accumulation in seedlings and adult plants of crop and hyperaccumulator species under different Ni level, but very few of them on the first stages of seed germination.
As a consequence, the main goal of the present research is to investigate the effect of low-to-high available nickel concentration on seed germination in hyperaccumulator and non-hyperaccumulator species both in native and amended soils.
We investigated the seed germination of the facultative hyperaccumulator *Alyssoides utriculata* (L.) Medik., the obligate hyperaccumulators *Odontarrhena bertolonii* Desv. L. Cecchi&Selvi and *Thlaspi caerulescens* J.Presl & C.Presl and in the non-hyperaccumulator related species *Alyssum montanum* L. and *T. arvense* L. (n=90, each species) on soils spiked with different Ni concentrations (0-1000 mg kg^{-1}). In addition, seeds of *A. utriculata* were collected from 3 populations in NW Italy on native soils (S, serpentine; M, mine site with high Fe and Cu; NS, non-serpentine) and sown on them and on neutral growing substrates used as control. Experiments were replicated in growth chamber in Petri dishes (seeds, n=400), in mesocosm at water holding capacity of WHC=80% and in mesocosm in semi-natural condition with a WHC of 50% (seeds, n=4000). The ecotoxicological and ecophysiological response of the seedlings were investigated as well in terms of root elongation and photosynthetic performance.
We found significant differences in germination rate (G%) between hyperaccumulator and related species up to 100 mg kg^{-1} of available Ni (tolerance). The germination time is longer under semi-natural conditions. Native soils are related to significant lower germination rate. Germination rate is not affected by soil Ni level, and the same for root elongation in hyperaccumulator species. Accumulation is soil-dependent, not population-dependent.
Germination stages are particularly important for successful subsequent development of a crop for phytoremediation.

2.1 = OXIDATIVE STRESS RESPONSES INDUCED BY HEXACHLOROCYCLOHEXANE (HCH) IN SAPROTROPHIC SOIL MICROFUNGI

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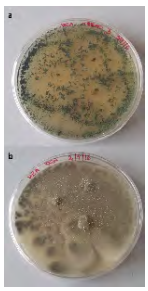
Even if the use of Hexachlorocyclohexane (HCH) was banned or severely restricted ages ago, its widespread adoption between 1950's and 2000 has determined a global and severe environmental contamination. According to estimations, around 1.7 and 4.8 million tons of HCH residues are still present all over the world (1). HCH may cause serious acute and chronic adverse effects to health and, as all persistent organic pollutants, tends to bioaccumulate along the food chain (2). Over the years, the multiple damage caused by exposures to this pesticide has been widely demonstrated. It has been reported to be carcinogenic, teratogenic, genotoxic and an endocrine disrupter; chronic exposure can induce hepatic and renal damages besides adverse effects on reproductive and nervous systems in mammals (3).
Evidences of oxidative stress in organisms exposed to pesticides was reported (4). The study of microorganisms' toxic response to xenobiotics stress allows acquiring more useful information about their tolerance. As reactive oxygen species (ROS) generate damaging effects the investigation of the oxidative stress response, which protects organisms, is key to study these important defence mechanisms. Therefore, the aim of this study was to investigate the oxidative stress responses due to the presence of HCH in two species of saprotrophic soil microfungi: *Trichoderma piluliferum* J. Webster & Rifai and *Penicillium daleae* K.M. Zalessky (Fig.1). The goal was to evaluate the expression linked specifically to the eventual activation, under stress conditions, of antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT) and glutathione transferase (GST).
The studied fungal strains were isolated from environmental matrices collected from sites interested by HCH contamination. Spore suspensions have been prepared and inoculated in liquid medium (*Czapek-Dox) (Fig. 2).* A first cultivation was performed for a predetermined time without the oxidative stress-agent, followed by a second cultivation that was performed with and without the oxidative stress-agent.
The measurements focused on the production of reactive oxygen species, and subsequently on changes in the glutathione transferase (GST) levels, as well as any superoxide dismutase (SOD) and catalase (CAT) activities (Fig.3-4).
The results suggested that high level of HCH promoted the ROS formation. The specific activity of GST and CAT was increased compared to the control cultures in both species, while the SOD activity occurred only in *Penicillium daleae*. Taken together, our results suggested that the growth of the fungal strains in presence of HCH shows signs of increased oxidative stress and antioxidant enzymes responses against the stress condition for scavenging ROS.



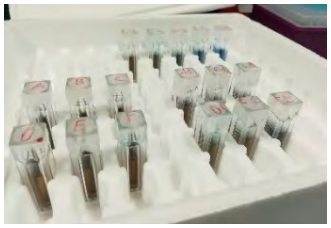



Fig. 1a *Trichoderma piluliferum*; 1b *Penicillium daleae*. Fig. 2 Batch fungal cultures. Fig. 3-4 Enzyme activities assays.

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2.1 = RARE EARTH ELEMENTS AS NEW POLLUTANTS: RESPONSES TO LIGHT AND HEAVY DIFFERENT RARE EARTH ELEMENTS IN DIFFERENT BIOLOGICAL MODELS

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Rare earth elements (REE) are a group of 17 elements: the Lanthanides, Yttrium and Scandium which have similar properties. They are divided in two groups on the basis of their atomic mass: light REEs (from Lanthanum to Samarium) and heavy REEs (from Europium to Lutetium). From the 1980's the use of REEs in human activities, from technology to agriculture and zoothecnics, rouses environmental health concern. REEs show multi-factored aspects from potential benefits in technological, agricultural and medical applications, to adverse effects in ecosystems and human health (1, 2). Many data in the literature pointed to an increase of light REEs in soils and waters occurring in recent years (3), but few data are so far available about the current REE concentrations in the soils. In recent years, the literature concerning REEs and living organisms has increased significantly (1, 4). An extensive body of literature, including also controversial data, is available about the interactions of REEs with living organisms (1). Most of the data concern the use of mixtures of REEs in agriculture and the use of Lanthanum and Cerium in many other fields. Recently, some data reported on Lanthanum and Cerium associated toxic effects at mM concentrations in various plant species (4,5). Many data referred to REE nitrates, chlorides, sulphates and also cerium nanoparticles. The aim of this work was to evaluate the effects of different light and heavy REE chlorides, in different biological systems in order to obtain additional information about their toxicity. The species utilized were *Lemna minor* L. (an aquatic plant), *Vicia faba* L. (a terrestrial plant) and sea urchins, species largely utilized in the toxicity tests. The considered endpoints were growth, morphological alterations, biochemical markers, such antioxidant levels and production of reactive oxygen species (ROS). Toxic effects were evident in all the system analyzed, even if at different concentrations. Sea urchins showed negative effects after treatments at µM concentrations, while *L. minor* and *V. faba* displayed stress symptoms at mM concentrations. In sea urchins REEs application induced alterations in embryo development, fertilization and cytogenetic anomalies. In the two plant species REE chloride treatments resulted in growth inhibition, chlorosis and increase in lipid peroxidation. Alterations of ROS productions and of antioxidant balance were detected in all systems analyzed. The toxicity outcomes were dose-dependent, in some cases with biphasic behaviour and increases with the atomic mass. Generally, heavy REEs are more toxic than the light REEs. Further studies are warranted to elucidate the role of REEs in cell metabolism, the toxicity mechanisms and REE uptake by plants and animals.

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2.2 = SEAGRASSES *POSIDONIA OCEANICA* AND *CYMODOCEA NODOSA*: TRACE ELEMENT ACCUMULATION AND BIOMONITORING OF MARINE POLLUTION

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Trace elements are a category of ubiquitous pollutants that are increasingly spreading across marine environments as a main result of human activities. Trace elements are generally considered as serious pollutants because of their toxicity, their persistence in the environment, and their capacity to accumulate in organisms. The ecological risk of trace elements is also difficult to assess because of their complex behavior and numerous interrelations, especially in aquatic ecosystems.
In coastal waters, marine organisms (e.g. algae, mussels) have been regularly used as bioindicators of trace element pollution. In particular, seagrasses are generally considered as potential indicator species because of their longevity and capacity to integrate biological, physical and chemical parameters. *Posidonia oceanica* (L.) Delile (Fig. 1) is a widely distributed marine plant, endemic to the Mediterranean, which forms dense communities (meadows) with bathymetric range of 0-40 m depth. *P. oceanica* meadows plays a central role in the ecology of the Mediterranean not only as one of the most important contributors to coastal primary production but also as a source of spawning areas, nurseries, and permanent habitats for numerous plant and animal species. *Cymodocea nodosa* (Ucria) Asch. (Fig. 2) is another coastal seagrass of tropical origin, nowadays restricted to the Mediterranean Sea and some locations in the North Atlantic, from southern Portugal and Spain to Senegal. Both *P. oceanica* and *C. nodosa* have several characteristics that make them suitable bioindicators of the environment since they are abundant, widely distributed, long-lived, sensitive to natural and anthropogenic stresses, easy to identify and sample. However, despite previous research, studies comparing the performance of *P. oceanica* and *C. nodosa* as bioindicators of trace element pollution are generally scarce in literature.
This study analyzed the concentrations of the elements As, Cd, Cr, Cu, Hg, Ni, Pb, Zn in the roots, rhizomes and leaves of *P. oceanica* and *C. nodosa*, and in samples of water and sediments. Trace element mobility was also investigated in the various plant organs. The aim of this study was to compare the capacity of both species to accumulate and biomonitor trace elements in seawaters and bottom sediments. This study aimed to shed further lights on the correlations of trace elements between marine plants and the environment.
Results showed that *P. oceanica* has a higher capacity of accumulation, especially from sediment. *P. oceanica* and *C. nodosa* accumulate mainly in roots and leaves, the main organs acting as potential bioindicators. No significant correlation was found between water and both seagrasses. In turn, *P. oceanica* and *C. nodosa* were correlated, to a different extent, with As, Cd, Cu, Ni and Zn in sediments. This study found evidence that such seagrasses may adopt Cr-exclusion mechanisms as a tolerance strategy. *P. oceanica* and *C. nodosa* act as potential bioindicators of trace elements in sediments, thus, their implementation in monitoring protocols should be considered.

Fig. 1
Posidonia oceanica.

Fig. 2
Cymodocea nodosa.

2.2 = STRESS RESPONSE AND TOLERANCE TO DDT: SOIL FUNGAL SPECIES ISOLATED FROM POLLUTED AGRICULTURAL AREAS OF POLAND AND THEIR POTENTIAL IN FUNGAL BIOREMEDIATION

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Since the 1940s, DDT has been widely used all over the world as insecticide for agricultural pests and in the management of mosquito-borne malaria (1, 2). Due to its environmental persistence and health and environmental problems related to its bioaccumulation and biomagnification in ecosystems, DDT was banned in most industrialized countries since 1972 and by Stockholm Convention on Persistent Organic Pollutants in 2000 (1, 2). Despite of this, DDT is still detected in soil and water samples from several countries and long-term, toxic and cancerogenic effects of its occurrence still pose severe environmental risks to ecosystems and humans (2, 3). In the last decades, biodegradation of DDT by fungal species as environmentally friendly, feasible, integrated, cost-effective remediation methods has been investigated, as fungi possess metabolic and enzymatic versatility, leading them to tolerate and transform natural and anthropogenic substrates such as organic persistent pollutants (1, 2, 4). The study of soil microbial community represents an important step to shed further light on the environmental contest in order to select the best candidates for DDT biodegradation. In this research, we have isolated and identified 179 strains belonging to 59 saprotrophic soil fungal species (Ascomycota, Zygomycota and anamorphic fungi) occurring in samples from historically polluted agricultural soils of Poland with high concentrations of DDT. Some of them have been reported in literature for the biotransformation of DDT or other organic pollutants (2). Moreover, we have investigated the tolerance of selected fungal species to 1 mg/L DDT by using tolerance indices (Rt:Rc (%); T.I.(%)). Medium pH after fungal growth was measured and analysed with growth data (diametric extension and dry weight) to study metabolic responses to DDT. The results suggest that the tested fungal strains may provide future applications in environmental management and restoration.

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2.2 = ALLERGENS IN THE PARK AND GARDEN FLORA OF SICILY

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The ornamental flora of parks and gardens, as we know, generally includes high proportions of exotics. In Italy it arose from Renaissance on and increasingly enriched up to include a large proportion of exotics native to countries under Mediterranean and temperate climates. Similarly the garden flora of Sicily has a large number of mostly Mediterranean and subtropical exotics. Even if very similar on the whole, these floras differ in some floristic, biological and ecological features. Indeed, several succulent families and genera that are rare or missing in Italy are frequent in Sicily, but plants from temperate regions are almost absent in the island.
As regards the allergenic patterns referring to pollinosis, there are several aspects to be taken into account: In particular, native ornamental plants in Sicily often have flowering period and pollen emission early than in Italy; besides for alien plants occurring only in Sicilian gardens, the relevant allergenic properties are deduced from literature sources concerning the countries of origin. Therefore, pollen bulletins weekly spread by Italian networks (POLLnet) are not suitable for Sicily which, apart from some old and incomplete data, is presently not covered. Nevertheless, despite the lack of a continuous flow of data, a calendar of the pollen emissions in some urban areas can be programmed. Indeed, taking into account the city of Palermo, where 23 public gardens have been floristically inventoried and mapped, the calendar of each single garden can be obtained using the comprehensive garden floristic list fitted out with the relevant allergenic data now complete for Sicily. In particular, with respect to the sole woody fraction (the whole Sicilian garden flora includes about 740 specific taxa), such list consists of 406 specific taxa, belonging to 191 genera of 83 families. Among these species, 265 - including 182 exotics - are allergenic. The most inclusive group are the conifers, with 23 allergenic species; among the families, the *Oleaceae* with 11.

2.2 = *ASPERGILLUS SYDOWII* IN THE PORT OF GENOA (LIGURIA, NW ITALY)

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Aspergillus sydowii Thom and Church is a cosmopolitan fungus that has been responsible for the mass destruction of coral *(Gorgonia ventalina* Linnaeus*)* over the *last* 15 *years* in the Caribbean Sea (1). In marine environments, this pathogenic invader was also isolated from the West Indian Sea (2) and more recently from Australian coastal waters (3). Nowadays, some studies indicate the presence of live *Aspergillus* spp. spores isolated from recent dust events (4); hence, the hypothesis that *A. sydowii* may have been entered in marine ecosystems by winds. Furthermore little information is available on the environmental factors that may facilitate *A. sydowii* bloom in marine environment (5). The growth of pathogenic *A. sydowii* may be due by environmental stress conditions as, for example, organic pollution, lack of oxygen, high levels of phosphorus and ammoniacal nitrogen (4). Temperature also seems to be a crucial environmental factor for the *A. sydowii* diffusion and pathogenicity since pathogen optima and host optima occur in the same range of temperature.

This study reported the survey of *A. sydowii* in the Mediterranean Sea from marine bottom sediments, water and calcareous shells of bivalve molluscs (*Mytilus galloprovincialis* Lamarck), during a campaign in the Port of Genoa (Liguria, north-western Italy). The area was characterized by severe environmental conditions, mainly represented by organic pollution and high concentration of phosphorus and ammoniacal nitrogen. The port waters are rich in nutrients, faecal coliform, chlorophyll-α and Polycyclic Aromatic Hydrocarbons (PAHs) depending on the input of sewage and industrial discharges. These parameters, associated to rising temperature, could contribute to *A. sydowii* bloom and diffusion. However, the Port of Genoa is subjected to traffic of ferries and cargo to Italian and Mediterranean destinations and periodically, containerships to Mediterranean Sea, West Africa, South and North America, Red Sea, Arabian Gulf, East and South Asia and China. From these data, we can suppose that A. Sydowii may have been imported in Port of Genoa due to transport by the bilge water of the container vessels, or by input from local torrents.This work represents the first step for the implementation of a monitoring study, which may help safeguard of the calcareous sponges and sea fan corals endemic of the Mediterranean and Ligurian seas (e.g. within the protected marine area of Portofino and Bergeggi). The research on this field will continue to check the pathogenicity of the isolated strains following several approaches, including the use of Koch's postulates and population genetic methods to identify possible patterns of genetic diversity and relationships between environmental and disease-causing strains of this fungus.

Fig. A
Biseriate conidial head under optical microscope.

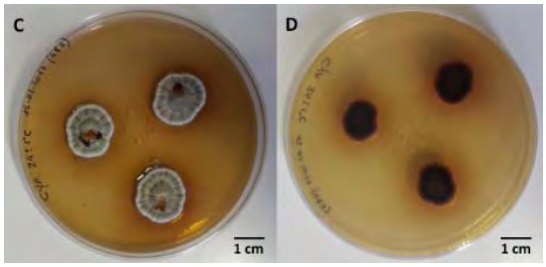

Fig. B
Plate of *A. sydowii* on CYA after 7 days.

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2.2 = ASSISTED PHYTOREMEDIATION OF A POLLUTED SOIL USING AN ELITE CLONE OF *ARUNDO DONAX* L.: HYPOTHESES ON RECLAIM CAPABILITY AND DETOXIFYING MOLECULAR MECHANISMS

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Arsenic (As) toxicity of soil and water is an increasing threat across the entire globe. Erosion of alluvial soil, anthropogenic activities like hydraulic fracturing, and industrial effluent cause mobilization of As in ground-water, inducing disease skin cancer, reproductive disorders, etc. Although the World Health Organization suggested that the maximum permissible limit of As in drinking water is 10 µg L^{-1}, As contamination of groundwater is one of the most environmental problems in countries like Argentina, Bangladesh, China, etc. Generally, As exists in natural water as arsenate As (V) or arsenite As (III) in the ratio of about 70:30, however As(III) is more toxic than the other one. In order to reclaim As soil and water pollution, various conventional methods have been employed. However, these methods show limitations in terms of missing technical expertise and low effectiveness. Recently, As bio-remediation (plant and/or microorganism) has been evolved as a promising technology due to its public acceptance and cost effectiveness. Therefore, we propose the assisted phytoremediation as a valuable green technology to reclaim As contaminated soils and waters, using a widely spread species: the *Arundo donax* L. *A. donax* (giant reed) is a perennial rhizomatous grass reaching 3–10 m high, growing in many-stemmed cane-like clumps, sprouting from rhizomes, which form large colonies many meters large and their roots and rhizomes penetrate deep into the soil up to 1 m. It is native of warm tropical Asia and the Mediterranean region, but it is widespread over the world as few genotype able to live in many different environmental conditions. Moreover, from the molecular point of view, the transcriptome of *A. donax* was sequenced but it is not completely annotated [1]. Some authors hypothesized various pathways of As uptake and plant detoxification, but few information about the gene involved in these pathways has been reported for this plant. For these reasons, our research aimed to i) estimate the remediation capability of *A. donax*, grown in 2L Becker filled with sandy soil, under increasing concentrations of As (2, 10, 20 mg L^{-1} of $NaAsO_2$), even in the presence of a Plant Growth Promoting Bacteria (PGPB) consortium, ii) and to select candidate genes involved in As detoxification. The bacteria consortium added to pots was constituted by two strains of *Stenotrophomonas maltophilia sp*. and one of *Agrobacterium sp*., previously isolated and characterized by Cicatelli [2] for their resistance to a wide spectrum of metals. In the present research, the PGPBs were initially assayed to determine their As MIC (Minimum Inhibitory Concentration). Bacteria showed, as expected, high tolerance, but also a diverse MIC in response to increasing concentrations of the As, even if the two *Stenotrophomonas* strains were similar from genetic point of view (≈ 90% of similarity, Cicatelli et al., 2016). At the end of the trial, biomass production, as well as As content, in different plant organs and sand, were estimated. Interestingly, the plants didn't show any visible effects of toxicity, although some differences in As accumulation were detected in the different organs. However, ≈ 25% of As remained in sand, ≈ 0,15% was accumulated in the plant, whilst the remaining 75% was, probably, volatilized by plants, as previously suggested by Mirza [3] in the case of *A. donax*. In order to investigate the molecular mechanisms involved in the As uptake, translocation and volatilization, gene candidates were *in silico* determined through bioinformatics alignment of genomes and transcriptomes of plant species phylogenetically closer to *A. donax* [4]. In particular, we focused our attention on several transporter gene families (ABC, Phosphate, Phytochelatin, etc.), and also on stress-related genes [5]. The bioinformatics analysis allowed to identify in *A. donax* four genes potentially involved in As detoxification that will be further assayed in RT-PCR.

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2.2 = WASTEWATER MYCOREMEDIATION

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Bioremediation is an eco-friendly technology to remove pollutants from environment and biological wastewater process is an intensive ex situ bioremediation treatment. Many fungi can survive and grow in high concentration of organic pollutants and some of these are used in mycoremediation (1). Recently, there was an attempt to exploit fungi in wastewater treatments which, in this sense, can be defined as a type of mycoremediation in situ for organic pollutant.
Among wastewater treatments, Membrane Biofilm Reactors (MBfRs) are novel systems based on the transfer of a gas through a membrane to a superficial biofilm in contact with a liquid phase containing the substrates (2). A proper choice of the membrane configuration may permit large surfaces of biofilm to grow in a compact reactor volume and therefore they can be a promising treatment method for wastewater.
In this work, a small membrane aerated biological reactor (MABR) was studied with the aim of verifying the adhesion and growth of micro-fungi potentially useful for the degradation of organic substrates in wastewater. Commercial hydrophobic hollow fiber polypropylene membranes are used for biofilm formation while air is flowing in the lumen of the fibers.
Two types of fungi, *Penicillium expansum* Link. and *Trichoderma harzianum* groupe, were inoculated in non-sterile conditions in model waster containing glucose and peptone as organic substrates. The initial adsorption of organic substrates on the polymeric membrane was studied by FT-IR spectroscopy and the biofilm growth was studied by analyzing photos, evaluating weight changes, and analyzing Chemical Oxygen Demand (COD) (3). Finally, the MABR with *P. expansum* was used to check the degradation of gallic acid as a representative of a polyphenol molecule.
The results show that fungi can be used in wastewater treatment, owing to their capability to adhere on the membrane in a MABR, to degrade organic substrate, and to prevail over other microorganisms in a non-sterile condition.

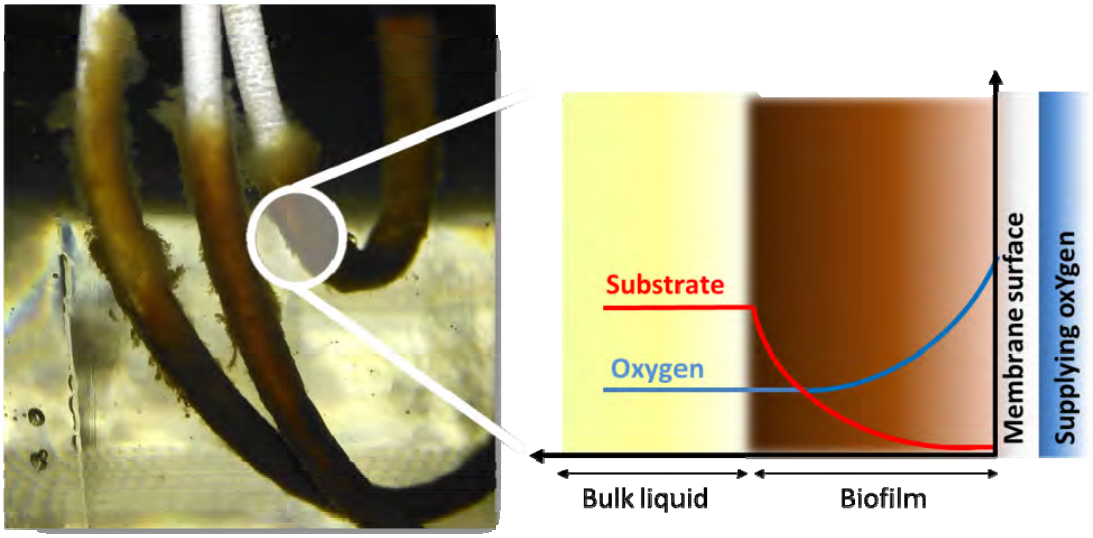

Fig. 1
Penicillium expansum grow on the membrane support and representation schematically of the gas and nutrient exchange nearby biofilm.

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2.2 = TIO_2 NANOPARTICLES AND SLUDGE FROM WASTEWATER TREATMENT PLANTS: A NEW CONCERN FOR CROPS?

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Contaminants of emerging concern are increasing in all the ecosystems, due to the unintentional or intentional release into the environment of new molecules/compounds or to a new employment and disposal of complex and potentially polluted matrices. In this respect, the reuse of sludge from wastewater treatment plants in farming soils is recognized as a cost-effective practice to dispose of a byproduct, that, after specific treatments and maturation, can be provided as a fertilizer, rich in organic matter and nutrients (1). On the other hand, due to the uncertainty of its contents not thoroughly tested for safety, sludge can result a possible sink of unknown priority pollutants as well as of not commonly monitored chemicals, such as nanoparticles (NPs) (2). The latter emerging contaminants are becoming a worldwide problem: nanotechnologies are being gradually more employed in all sectors of technology and innovation, but their effects on living organisms are not yet fully clear and unambiguously interpretable (3). Besides, NPs behavior is poorly estimated in the different environmental matrices, especially in agricultural soils. In such complex matrices, the bioavailability of the different nanomaterials frequently is not predictable, for the tendency of NPs to aggregate, to adsorb/precipitate on solid phase, as well as to be coated by organic molecules (4). In addition, the overall picture of their possible interactions with crop plants and with food chains is not at all clear (5).
In the present work we aimed to investigate the effects of TiO_2 NPs in a sludge amended agricultural soil to evaluate the growth and development of the crop *Pisum sativum* L. at microcosm scale under long term exposure, possibly miming an environmental occurrence. TiO_2 was spiked in two different concentrations in the form of bulk material or of NPs and by using the two main crystal forms (anatase and rutile), applied singularly or in a mixture of the two. Electron microscopy imaging studies allowed us to visualize internalized NPs in the different sub-cellular compartments of the root tissues and their effects on cell ultrastructure. Synchrotron studies demonstrated that both titanium crystal forms, especially anatase, were taken up and moved to the vascular system. Studies dealing with the possible induction of oxidative stress were performed by *in situ* histochemical techniques and by biochemical approach.
The potential fertilized value of the sludge-amended agricultural soil seemed to have no beneficial effects on the plant, but rather to induce disturbance at different levels for the selected crop. The addition of NPs in the sludge-amended soil marked oxidative damages in *P. sativum*. This result was more pronounced at the lowest NPs concentration and this fact could be probably related to the low tendency of the diluted NPs suspension to form homo- and heteroaggregates in a complex matrix, with a consequent greater bioavailability. In our experimental system, the most adverse effects on plants were mainly recorded following exposure with anatase crystal form, alone or mixed with rutile as well as in the presence of the corresponding bulk material.
These findings rise some reflections on fertilization practices of agricultural soils by treated sludge and on the exploitation of nanomaterials, which safety must be carefully evaluated, in order to establish precise regulation over their use, confinement and environmental disposal.

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2.2 = EVALUATION OF MORPHO-PHYSIOLOGICAL ALTERATIONS IN THREE CULTIVARS OF *C. CARDUNCULUS* GROWN ON A POLLUTED SOIL IN VIEW OF ITS USE FOR PHYTOREMEDIATION

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Heavy-metal environmental pollution is one of the most pressing issues affecting the quality of water, air and soil. Nowadays, expensive chemical and physical methods are used to clean up soils (1), often with serious alterations of its natural structure and biological properties. Phytoremediation is a green emerging technology, using the uptake capability of plants to remove or stabilize soil pollutants (2). High biomass yield, root to shoot translocation and high tolerance to heavy-metal toxicity are the plant features useful for phytoremediation. In this study, we investigated the effects of soil pollutants on three cultivars of *Cynara cardunculus* L. (Sardo, Siciliano and Spagnolo), a plant species known for its metal uptake ability (3). The plants were grown in a greenhouse on a soil collected at a car-battery recycling plant, heavily enriched mainly in Cd and Pb. The aim of the study was to test the tolerance of the three cultivars to high concentrations of metals in the soil, based on morphological, physiological and ultrastructural analyses. After 30 d growth on polluted soil, plants were screened for chlorosis and necrosis and ultrastructure of the leaves (Fig. 1); HSP70, Rubisco and D1 proteins were quantified in the leaves as well. Differently from the other two cultivars, *C. cardunculus* cv. Spagnolo, was not affected by necrosis or chlorosis on the leaves, and no chloroplast alterations were observed by TEM (Fig. 2a); this cultivar also showed enhanced production of Rubisco. *C. cardunculus* cv Spagnolo resulted the most tolerant to heavy-metal toxicity; thus, it could be enrolled for a phytoremediation field trial to test its ability to extract/stabilize metals from polluted soils.

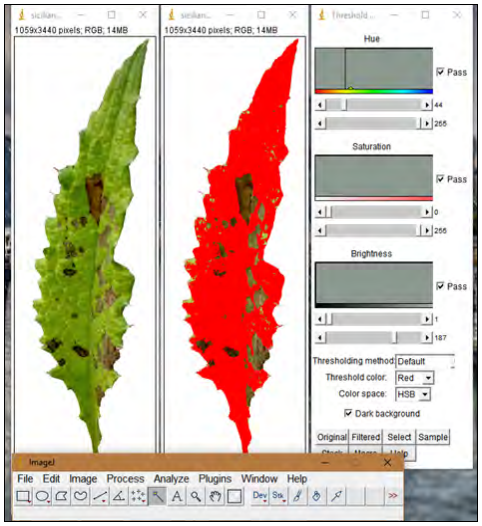

Fig. 1
Analysis of leaf necrotic areas on cv Siciliano by ImageJ software.

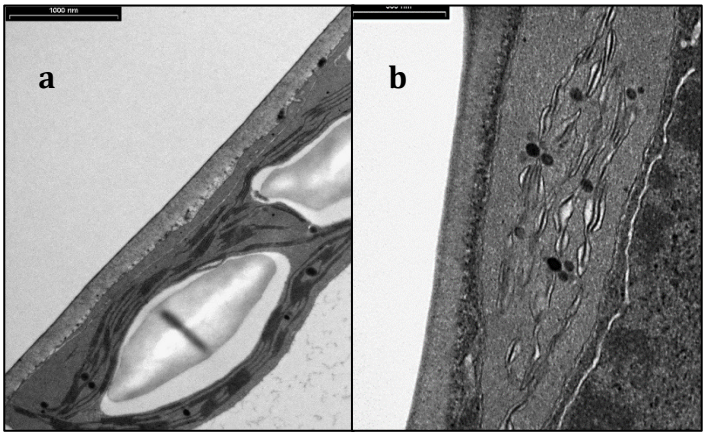

Fig. 2
TEM observations of leaf chloroplasts from cv Spagnolo (a) and cv Sardo (b) grown on polluted soil.

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2.3 = INTERPLAYING BETWEEN EPIGENETIC MECHANISM CONTROLS LEAF DEVELOPMENT IN *ARABIDOPSIS THALIANA*

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Epigenetic mechanisms regulate almost all the genetic functions including transcription, replication, DNA repair and gene transposition thus playing a relevant role in cell differentiation and plant developmental and adapative traits. Tipically, epigenetics include DNA methylation, post-translational modifications of amino acids in histone proteins, chromatin remodelling proteins (1). Small RNAs also play an important role in epigenetic regulation via transcriptional gene silencing through RNA-directed DNA methylation (RdDM). Moreover there is evidence that all these modification are tissue-, species-, organelle- and age-specific (2).
In the present work, some components of the interplaying epigenetic mechanisms underlying leaf development has been detected by using the *drm1 drm2 cmt3 (ddc*) triple mutant of *Arabidopsis thaliana* which is defective in both *de novo* and maintenance methylation activity. Previously, we showed that *ddc* mutant exhibits pleiotropic phenotype abnormalities, from embryogenesis to adult reproductive phase, in line with the role of DNA methylation in plant development. A marked trait of these phenotype abnormalities was the curly shape of the leaf, associated to an altered differentiation pattern dealing with stomatal density and epidermal cell size.
In this context, we planned to monitor in both wild type plant and *ddc* mutant of *Arabidopsis thaliana* the expression pattern of several genes that are components of the complex genetic network involved in the regulation of leaf development. We observed that some of these genes such as KNOTTED1-LIKE HOMEOBOX GENE 6 (*KNAT6)*, *ASIMMETRIC LEAVES 2 (AS2), FAMILY TRANSCRIPTION FACTOR 2 (TCP2)* and *CURLY LEAF* (*CLF)* which encodes a component of POLYCOMB-REPRESSIVE COMPLEX 2 (PRC2) responsible for catalysing H3K27 methylation (3), were differentially expressed in the mutant compared to the WT. In particular*, KNAT6*, *AS2* and *TCP2* resulted downregulated in the mutant *vs* WT*,* while *CLF* was upregulated. This result prompted us to estimate by ChiP analysis the H3K27me3 levels of *KNAT6*, *AS2* and *TCP2* which are target of PRC2 complex. Interestingly*,* we found an increased H3K27me3 levels in the proximal promoter region of *KNAT6*. These results will be discussed in relation to the detected cross-talk between DNA methylation and histone methylation (o H3K27 methylation).

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2.4 = A LOOK INSIDE *LAELIA PURPURATA* SEEDS: FROM SEED MICROMORPHOLOGY TO PLANT-FUNGUS INTERACTIONS

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The loss of orchid habitats has increased the importance of orchid studies and conservation efforts involving *ex situ* propagation approaches. Despite the great ornamental success, the majority of the approximately 28.000 (1) known species are still understudied. In this work, in the framework of an investigation on plant-fungus interactions, we addressed aspects of seed biology and *in vitro* culture of *Laelia purpurata*. Seed morphology was characterized using light and scanning electron microscopy, while *in vitro* germination capability was evaluated both symbiotically and asymbiotically, in comparison with seed viability. Seed micromorphology is comparable to other congeneric species (2), but it was possible to observe for the first time, to the best of our knowledge, spectacular druzes of calcium oxalate inside the cells of a surprisingly long suspensor. Germination was successful on all the six media tested but with lower rates (8-25%) than expected in comparison to the seed viability (approx. 80%). Seeds also germinated with the fungal strain AL13 (*Tulasnella calospora*) and pelotons were observed in root sections.
After seed germination, in many cases, a parasitic interaction was observed, with resulting death of *L. purpurata* protocorms.
The basidiomycete *T. calospora* is generally regarded as an unspecialized soil saprotroph when it is not in association with orchid hosts. However, a recent investigation on the fine-scale spatial distribution of orchid mycorrhizal fungi in two orchid-rich Mediterranean grassland soils investigates this aspect (3). We took a qPCR approach targeting the expression level of different Carbohydrate-Active Enzymes (CAZymes), to address the actual saprotrophic abilities of this fungus under different conditions. The most interesting genes, among the more representative CAZyme families, were selected from a previously obtained transcriptome (4). After RNA extractions, qPCR approach will be performed under different culture conditions using specific primers.

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2.4 = OOMYCETE DIVERSITY IN RELATION TO *PHRAGMITES AUSTRALIS* DIE-BACK

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Phragmites australis [(Cav.) Trin. ex Steudel] die-back is a widely-studied phenomenon characterized by clumping habit, stunted growth, and presence of dead buds. This syndrome, started in northern Europe, has been recently reported in the Mediterranean basin [1, 2], where, in some cases, is leading to an irreversible retreat of reed population. While some of the possible causes of this phenomenon have been investigated, the role of microorganisms living in the soil is still poorly understood. In particular, few studies investigated bacteria and fungi living in reed-beds, and the diversity of oomycetes is unexplored. Culture-independent approaches have enriched the understanding on microbial diversity of soil and deeper knowledge on the role of oomycetes in the die-back is possible using next-generation sequencing techniques. The aims of this study were to analyse the diversity of oomycetes communities associated with reed stands, and to understand whether they could play a role in the decline. Variations in the structure of oomycetes communities were studied by metabarcoding of the internal transcribed spacer (ITS) 1 region of ribosomal DNA from the sediments of five Italian freshwater ecosystems. They were chosen to cover a large variability in terms of surface area, water depth, microclimate and presence of documented reed retreat.
Oomycete communities were found to be structured by several factors, among which the most important was the reed die-back occurrence. We demonstrated also that the abundance of several OTUs assigned to the genus *Pythiogeton* was significantly higher in samples from declining stands compared to samples from healthy stands.
This study represents the first investigation of the diversity of reed-associated oomycetes; additionally, it is the first contribute to assess whether declining reed-beds harbour unique communities of oomycetes that differ from those of the healthy stands, and to understand the factor that influence their distribution.

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2.4 = ANTHROPOGENIC IMPACT IN THE VESUVIUS NATIONAL PARK: EFFECT OF DUST ACCUMULATION ON LEAVES OF *CENTRANTHUS RUBER* (L.) DC. SUBSP. *RUBER*

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In Mediterranean ecosystems, the anthropogenic impact makes the preservation of plant biodiversity critical. Indeed, human pressure can be responsible for the depletion of vascular flora and the reduction in biomass production. Within the Campania region, some areas of the Vesuvius National Park are particularly exposed to the anthropogenic impact due to seasonal tourism. As a matter of fact, during spring and summer, a considerable amount of tourists pass through the Park areas, also with vehicles, thus causing the formation of dust clouds which are deposited over the vegetation close to the paths (Fig. 1).
The main aim of this study is to analyse whether the deposition of dust on the leaf lamina of *Centranthus ruber* (L.) DC. subsp. *ruber* (*Caprifoliaceae*) plants can induce changes in leaf anatomy and in the efficiency of photosynthetic apparatus. The study was conducted on *C. ruber* plants growing at three sites, similar for environmental conditions and species composition. For each site two different sub-areas were considered: 1) the first, located along the side of the road, where a high deposition of dust (HD) was recorded on leaves, and 2) the second, placed at least five meters far from the path, where plants were subjected to a very low deposition of dust as control (LD). The amount of deposited dust was quantified at the study sites, in different periods of the year, characterized by different tourism access (spring-summer-autumn). Being *C. ruber* widespread throughout all the area of Vesuvius National park, it was chosen as an environmental indicator, making possible to evaluate the potential negative effects of the dust deposition on both anatomical and ecophysiological features. Functional traits in leaf lamina (e.g. stomatal size and frequency, percent of intercellular spaces in mesophyll) were quantified through light microscopy on fully-expanded leaves collected in summer (Fig. 2). Ecophysiological traits (chlorophyll *a* emission, photosynthetic pigment concentration and leaf traits) were carried out as well on fully expanded leaves, during spring and summer.
Our results showed that HD leaves enhanced a pool of anatomical and photochemical traits allowing to overcome the effect of dust. Dust-induced changes in photosynthetic pigment content and stomata features were recorded. The overall analysis suggests that the greater amount of dust raised from the paths, shielded the leaf lamina: indeed leaves developing near the paths formed morphoanatomical traits as “shade leaves”. We infer that the phenomenon of dust deposition acts as a screen on leaves thus shading the photosynthetic apparatus from the light excess during summer. Although this phenomenon is positive for the optimization of *C. ruber* growth in these specific sites, the response of other species must be analysed to evaluate possible dust-induced vegetation dynamics consequent to possible species-specific adaptive capability.

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

Fig. 1
Dust raising from the path.

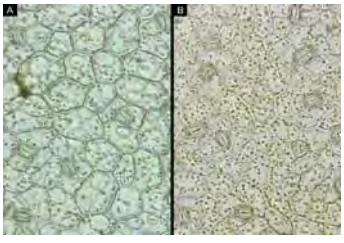

Fig. 2
Light microscopy view of leaf lamina of *C. ruber*. Adaxial (A) and abaxial (B) epidermis.

2.4 = RHIZOSPHERE RESPONSE TO NICKEL IN HYPERACCUMULATOR AND NON-HYPERACCUMULATOR SPECIES

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Rhizosphere, as an important interface of soil and plant, plays a significant role in phytoremediation of contaminated soil by metals, in which, microbial populations are known to enhance phytoremediation processes (1). However, more information on the microbial communities and their functions associated with the majority of hyperaccumulators are required (2).
Bacteria in the rhizosphere are involved in the accumulation of potentially toxic trace elements into plant tissues (1). Additionally, microfungi are essential in colonizing and detoxifying metal-contaminated soil ecosystems and consequently have environmental and economic significance (3).
The aim of this study is to assess the plant response to nickel (Ni) in terms of root biomass and surface development, and to select rhizosphere microbiota with affinity to Ni.
Test species were Ni-hyperaccumulators: *Alyssoides utriculata* (L.) Medik., *Thlaspi caerulescens* J. Presl & C. Presl and non-hyperaccumulators: *Alyssum montanum* L. and *T. arvense* L.. Soils were amended with different concentration of Ni (0-1000 mg kg^{-1}) both in microcosm and in mesocosm (Fig. 1). Root surface development and fresh and dry biomass were evaluated. Various 10-fold dilutions of soil samples were plated on Tryptic Soy Agar added with Ni (0, 1, 5 mM) to determine the number of culturable bacteria (Fig. 2) and on Malt Extract Agar (MEA) and on Rose Bengal agar (RB) to isolate vital fungal strains (Fig. 3) by a dilution plate technique.
Hyperaccumulators do not seem to be affected by nickel stress; on the contrary non-hyperaccumulators exhibit a decrease in terms of root surface area and fresh and dry root and shoot biomass.
In rhizosphere soil of *A. utriculata* bacteria colonies are more abundant than in bare soil but without a clear selectivity by nickel at increasing Ni concentrations. Native soil fungi were screened for Ni tolerance and a strain of *Trichoderma harzianum* group was selected as Ni hyperaccumulator (up to 11000 mg Ni kg^{-1} in dry biomass) suggesting its possible employ in a bioremediation protocol able to provide a sustainable reclamation of broad contaminated areas (4).
The native rhizosphere microbiota can be useful to evaluate possible plant tolerance to Ni stress. Bacteria and microfungi can be inoculated in the hyperaccumulators' rhizosphere to alleviate Ni stress and to increase metal uptake

Fig. 1
Greenhouse mesocosm test.

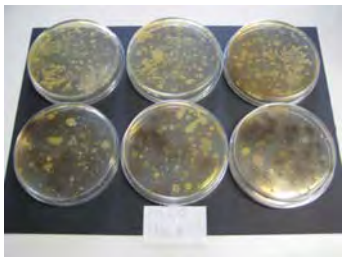

Fig. 2
Bacterial isolation.

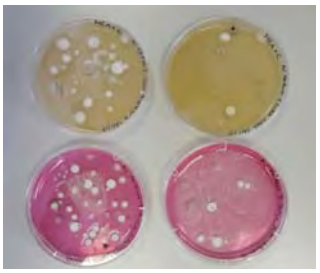

Fig. 3
Microfungal strains isolation.

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3.1 = POTENTIAL APPLICATION OF *BETULA ETNENSIS* RAF (BETULACEAE) ALCOHOLIC EXTRACT AS SINERGIST IN RADIOTHERAPY

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Breast cancer is the most common type of malignant disease in woman and currently represents the second leading cause of death due to cancer in women. The possibility that medicinal plants might help to reduce the risk for various types of cancer raised great interest already in the 1970s, when studies conducted to assess differences in cancer rates and diet among countries, suggested that various dietary factors might have important effects on cancer risk (1). Most natural compounds investigated for their potential usefulness as cancer chemopreventive agents might suppress carcinogenesis mainly during the initiation phase because of their radical scavenger activity. Though surgical intervention, chemotherapy, radiotherapy and endocrine therapy are commonly used as primary treatments, today natural products are an alternative and adjuvant resource widely accepted because of the diversity regarding the structure and the biological mechanism of action. Radiotherapy, in addition to chemotherapy, is currently the primary method for cancer treatment, based on damage of malignant cells by ionizing radiation.

Many natural products, by enhancing selectivity of chemo- and radiotherapy, would allow to reduce their intensities and their side effects as well, alongo with the risk of relapses. Since there is an increasing interest in the *in vivo* protective effects of natural compounds contained in plants against oxidative damage involved in cancer, in this study we investigated the effects of *Betula aetnensis* Rafin bark alcoholic extract on a human breast cancer cell line (MCF-7) after treatment with ionizing radiation.

Betula aetnensis Rafin (Birch Etna, Betulaceae) is a medium-sized deciduous tree, typically reaching 5–20 m tall. It grows on the eastern slopes of Etna, at an altitude between 1200 and 2000 m (2, 3). Many *Betula* species are used in folk medicine to treat skin diseases, infections, inflammations, rheumatism and urinary disorders (4). Nearly all species contain flavonoids, tannins, saponins, sterols and pentacyclic triterpenoids, such as betulin, betulinic acid and ursolic acid which have shown multiple antioxidant, antitumor and antiviral activities (5). In particular, betulinic acid is considered a promising anticancer agent (6).

The present study investigated the effects of *B. aetnensis* Rafin bark alcoholic extract on the viability of MCF7 cells and also on reproductive cell death after treatment with ionizing radiation (2-4-6-9 Gys), proton beams at 62 MeV, thanks the collaboration with the hadrontherapy facility of the Laboratori Nazionali del Sud – Italian National Nuclear Physics Institute (INFN) (7). In addition, in order to elucidate mechanisms of action of this extract, several markers of oxidative stress were also evaluated.

Results obtained showed that alcoholic extract of *B. aetnensis* Rafin significantly reduced cell viability of MCF7, inducing apoptotic and/or necrotic cell death in a concentration-dependent manner. Moreover, clonogenic assay demonstrated that extract of *B. aetnensis* Rafin was able to potentiate the antiproliferative effect of ionizing radiation.

These results suggest that for alcoholic extract of *B. aetnensis* Rafin., *in vivo or ex-vivo* investigations as preventive or adjuvant in antineoplastic radiotherapy are warranted, alongside with a careful phytochemical profiling.

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3.1 = ANTIOXIDANT, ANTIFUNGAL AND CHEMO-PREVENTIVE PROPERTIES OF *FOMITOPSIS PINICOLA* (SW.) P.KARST. EXTRACT

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Among the most important traditional medicinal fungi, *Fomitopsis pinicola* (Sw.) P. Karst. (*Fomitopsidaceae, Polyporales*) has been used as a therapeutic agent for the treatment of headache, nausea, inflammation of the digestive tract, dysentery, excessive urination, liver problems, and as an emetic for purification, anti-inflammatory agents, or as a styptic to treat bleeding wounds (1).
The aim of this study is to investigate the antioxidant, antifungal, and chemo-preventive activities of a *F. pinicola* methanolic extract prepared fromdried fruiting bodies.
The antioxidant activity of the methanolic extracts was tested with DPPH (2). The results showed that the extract activity was 0.35% of that of Trolox.
The chemo-preventive properties of methanolic extract were investigated in a human leukaemia monocytic *cell line (THP-1). The MTT test, used for determination of cell proliferation, showed a considerable inhibition of proliferation in a dose-dependent manner (500 µg/ml – 5 µg/ml). This reduction was coupled with a relevant induction of apoptosis after 24 hours of treatment as evaluated by fluorescence microscope. The genotoxic potential of methanolic extract was studied by single cell gel electrophoresis (SCGE) on normal human leukocytes exposed to 20 µg of extract for 30 min at 37°C and no DNA damage was observed.*
The determination of antifungal activity of mushroom extract against vegetable and human pathogenic fungi [*Aspergillus tubingensis* Mosseray, *A. calidoustus* Varga, Houbraken & Samson, *Fusarium oxysporum* Schltdl., *Fusarium solani* (Mart.) Sacc., *Penicillium chrysogenum* Thom, *Sclerotinia sclerotiorum* (Lib.) de Bary] was investigated by disc diffusion method (6). The fungal extract used in this study was found to have antifungal effects at a variety of degrees against the pathogenic fungi tested.

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3.1 = QUANTITATIVE ANALYSIS AND ANTIOXIDANT ACTIVITY OF THREE ALGERIAN ENDEMIC *GENISTA* SPECIES

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Introduction:
The Algerian flora with its 3000 species offers a 15% of endemic *taxa* butremains poorly investigated from a phytochemical standpoint. In this study, we have evaluated three endemic brooms:*Genista numidica* Sphach, *Genista ferox* Poiret and *Genista tricuspidata* Desf. The objective of this study is to screen the content of flavonoids and polyphenols and to determine *in vitro* the antioxidant activity of the methanolic extracts of the flowers and leaves of these species.

Materials and methods
The aerial parts of the species *G. numidica* and *G. ferox* were harvested during the flowering period in the town of Annaba (Guard Cape) and *G. tricuspidata* in the town of Tipaza.

Preparation of methanol extracts: The flowers and leaves of the previously cleaned and crushed *G. numidica*, *G. ferox* and *G. tricuspidata* species were macerated in methanol (2 g in 200 ml) with gentle stirring for 24 hours at room temperature. The alcoholic extracts were filtered with a filter paper; the methanol was then removed by evaporation in a rotavapor.

Dosage of flavonoids: The determination of the total flavonoid content (TFC) was estimated by the method of aluminum chloride ($AlCl_3$) [1]. The results are expressed in quercetin equivalent in mg / g of extract

Determination of total polyphenols: The content of polyphenols (TPC) was determined with the Folin-Ciocalteu reagent [2]. The results are expressed in Gallic acid equivalents in mg/g of extract

Antioxidant activity of methanolic extracts: To study the antiradical activity of the various extracts, we opted for the method that uses DPPH (diphenyl picryl-hydrazyl) [3]. The results were compared with the values of ascorbic acid and BHT IC50.

Results and discussion

Polyphenol content: The results show that *G. numidica* is the richest in polyphenols for flowers and leaves respectively (113±3.87mg GAE/g and 79.6 ± 3.49mg GAE/g), compared with *G.ferox* (86.66±4.10mg GAE/g and 65±1.74mg GAE/g) and *G. tricuspidata* (53.06±0.64 mg GAE/g and 51±0.72mg GAE/g).

Flavonoid content: *G. numidica* was found to be the richest in flavonoids with respectively (85.47±1.51mg QE/g and 87.31mg QE/g) in flowers and leaves, followed by *G. ferox* (61, 90±1.46mgQE/g and 56.06±1.45mg QE/g) followed by *G. tricuspidata* with the lowest content (44.91±0.32mg QE/g and 44.70±1.14mg QE/g).

Antioxidant activity: The most important antioxidant activity was recorded in *G. ferox* extracts (IC50=0.50±0.006 mg/ml and 0.59±0.02 mg/ml) and in the extract of *G.numidica* leaves (0.55±0.03 mg/ml) followed by extracts of *G. tricuspidata* (0.95 ± 0.13 and 0.93±0.16mg/ml) and *G. numidica* flower extract (0.89±0.06mg/ml).

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3.1 = WOUND HEALING ACTIVITY OF FIVE *SEDUM* SPECIES COLLECTED IN EMILIA ROMAGNA (ITALY)

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Sedum genus (Crassulaceae family) is widely spread in the Northern Hemisphere, Africa and South America. In Italy, several species of this genus are traditionally used to heal haemorrhoids, burns, wounds, warts and abscesses (1). In this work, inspired by these ethnobotanical uses, the *in vitro* wound healing activity of five species of *Sedum* was investigated. In particular, *S. acre*, *S. album*, *S. hispanicum*, *S. sexangulare* and *S. rupestre* were harvested in Emilia Romagna (Italy) within two sites of community importance (SCI), namely: Gessi Bolognesi e Calanchi dell'Abbadessa (SCI IT4050001) and Gessi di Monte Rocca, Monte Capra e Tizzano (SCI IT450027).
Considering the involvement of polyphenols in the wound healing activity showed by several medicinal plants (2, 3), the samples were harvested at the flowering phenophase. In fact, our previous study on *Sedum* species indicated this stage of the plant life as the most rich in this class of metabolites. Thus, aerial parts of each species were freeze-dried and grounded. After this procedure, their water extracts were tested, at different concentrations, in a cell proliferation assay on human keratinocytes (HaCat) and in a scratching wound healing assay on same cell line (Fig. 1). The later test was performed according to the method proposed by Lampugnani (4) with slight modifications.

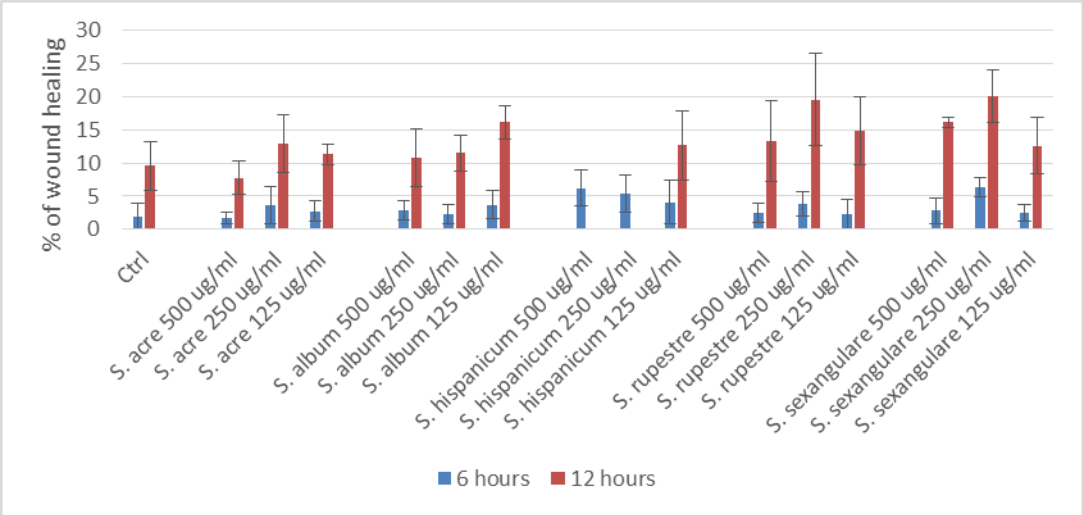

Fig. 1
Percentage of wound healing obtained for the samples tested at different concentrations and measured at 6 and 12 h.

S. sexangulare emerged as the most interesting species in both tests performed. In particular, it was able to significantly increase cell proliferation in a dose dependent manner and it showed significant wound healing activity in the scratching assay. In fact, at a concentration of 250 µg/ml, it was able to decrease the wound size on HaCaT monolayer by 3.27 folds (6 h) and 2.10 folds (12 h) compared to the controls.
In order to individuate the metabolites responsible for the activity of *S. sexangulare*, further phytochemical analyses on this plant are on going.

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3.1 = BIOLOGICAL ACTIVITY OF *CITRULLUS COLOCYNTHIS* (L.) SCHRAD. TEGUMENT AND PLANTULAE EXTRACTS

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Citrullus colocynthis L. Schrader (common names Bitter Apple, Bitter Cucumber, Coloquinte, Donkey Melon, Vine of Sodom) is an annual plant, belonging to the Cucurbitaceae family, which grows in arid and semi-arid regions. Native to tropical Asia and Africa, is also widely distributed in the desert areas of the Mediterranean basin (in Italy the only known population is located in the Aeolian island of Vulcano). Many pharmacological properties (anti-inflammatory, anti-diabetic, analgesic, anti-epileptic) are ascribed to different organs of this plant; extracts and derivatives of *C. colocynthis* are used in folk Berber medicine for the treatment of numerous diseases: the root is used for arthritic pain, breast inflammation, ophthalmia and uterine pain, while leaves are used for treatment of cough, many tumors and as a cholagogue. Recently, biological activities of aqueous extracts from roots, stems, fruits and seeds was reported (1). Many secondary metabolites from *C. colocynthis*, including cucurbitacins, flavonoids, caffeic acid derivatives and terpenoids, have been previously reported (2-4) and could explain the biological activity of this plant.
We developed a combined approach aimed at investigating the biological activities of seeds tegument and hydroponic-cultivated plantulae organic extracts, including: i) classic antioxidant activity *in vitro* tests (DPPH assay); ii) antifungal activity evaluation on human pathogenic species belonging to *Candida* genus; iii) high-throughput fluorescence-based procedure to determinate the inhibitory effect on aflatoxin production in *Aspergillus flavus*. The total phenolic compounds content was evaluated.
Extracts showed a high antioxidant capacity *in vitro*, a significative antifungal activity against *Candida* strains and a promising antimycotoxigenic potential in *A. flavus*.

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3.1 = PHYTOCHEMICAL STUDY AND ANTI-ANGIOGENIC ACTIVITY OF *DAPHNE MUCRONATA* ROYLE SUBSP. *LINEARIFOLIA* (HART) HALDA (THYMELAEACEAE)

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Daphne mucronata Royle subsp. *linearifolia* (Hart) Halda (syn. *Daphne linearifolia* Hart) (Thymelaeaceae) is an evergreen shrub with linear alternate leaves and hermaphrodite flowers, growing in limited areas in southern Jordan, while in Saudi Arabia it is considered a rare and endangered plant (1). *Daphne* genus comprises about 95 species distributed mainly in Europe and Asia (2); the phloem of these plants contains a strong fiber, used as a substitute for string, and the bark is very suitable for the manufacture of high-quality paper. Most species of *Daphne* are poisonous but some are used as cosmetic or medicinal plants (3). In the Jordanian ethnomedicine, *D. mucronata* is well known by Bedouins as "Mitnan" and its stem bark is used to treat inflammation and rheumatism (4). Despite the claimed health benefits that the folk medicine attributes to this species, there is still a lack of scientific data to support this information.
In the present study, a phytochemical investigation of *D. mucronata* root was performed along with the assessment of its anti-angiogenic properties. The dried and powdered plant material was sequentially extracted with *n*-hexane, $CHCl_3$, $CHCl_3$: MeOH (9:1) and MeOH. The anti-angiogenic activity of *D. mucronata* extracts was preliminarily evaluated by the quantitative endogenous alkaline phosphatase and staining assays in zebrafish embryos (5). Results showed that $CHCl_3$: MeOH extract (DLR-CM) exhibited the best inhibitory activity on zebrafish vessel formation (51% of inhibition at 100 µg/ml). In addition, a marked reduction on capillary growth was observed in the microvasculature of chorioallantoic membranes (CAMs) of fertilized chicken eggs after treatment with DLR-CM (56.25% of inhibition at 100 µg/egg).
The bioactive extract (DLR-CM) was separated on Sephadex LH-20 column and the obtained fractions were submitted to RP-HPLC. Siringin, pinoresinol, pinoresinol 4"-*O*-β-D-glucopyranoside, daphnodorin I, lariciresinol 4,4'-bis-*O*-β-D-glucopyranoside, wikstromol, and genkwanol A (6) were characterized by 1D- and 2D-NMR and MS analyses. In conclusion, this study provides additional phytochemical investigations on *Daphne mucronata* root and suggests that its anti-angiogenic activity may account for its use in pathological diseases related to inflammatory conditions.

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3.1 = ANTI-*HELICOBACTER PYLORI* ACTIVITY AND CHARACTERIZATION OF *COPAIFERA LANGSDORFFII* DESF. EXTRACTS

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Brazil has one of the richest biodiversity in the world, with more than 210000 species described (1). Several ethnobotany studies have been conducted, particularly in the North East region (2, 3).
Despite a marked reduction in the mortality rate, infectious diseases still represent a serious problem for public health in Brazil (4).
Copaifera langsdorffii Desf. (Fabaceae) has been used in traditional medicine in Brazil for a wide range of conditions. It is popularly known as *copaiba* and *pau-d'oleo*; its habitat ranges from tropical to subtropical dry forest (5).
Recently, Ribeiro and colleagues reported the use of its oleoresin, fresh or diluted in water, in coffee, or in honey, to treat skin, stomach and intestinal wounds, inflammatory ailments, urinary, lung and throat diseases, as well as against gastric ulcers, in the North Aragiaia microregion, Mato Grosso, Brazil (6). *C. langsdorffii* decoction was also reported for its use as oral intake, bath or wash, in the treatment of general pain and inflammation, stomach pain, gastritis and many other conditions, in the state of Pernambuco, Brazil (7) and in a Cerrado area of Chapada do Araripe, Brazil (8).
Several extracts were obtained in our laboratory from copaiba seeds by using different polarity solvents. Copaiba oleoresin was also used. A preliminary antimicrobial screening was conducted against 5 microbial strains. Samples resulting in a MBC lower than 1000 mg/l on at least two strains were selected for phytochemical characterization. Cytotoxicity was then assessed *in vitro* on PBMC cells and *in vivo* on *Artemia salina* L. Finally, potential anti-*Helicobacter pylori* effects were evaluated by measuring anti-inflammatory activity, through IL-6 release dosing.
C. langsdorffii seed methanolic extract (COME) and *C. langsdorffii* oleoresin (COOL) resulted effective against *Streptococcus pyogenes* and *Staphylococcus aureus* at 1000 mg/l and against *S. aureus* at 500 mg/l. COME contains polyphenols and sesquiterpenes, in particular β-caryophyllene oxide and 14-hydroxy-β-caryophyllene. Diterpenes, such as kaur-16-en-19-oic acid, were also identified.
The polyphenolic fraction of COOL, instead, is mainly composed of flavonoids, whereas the volatile fraction still contains sesquiterpenes, mainly represented by α-humulene. Germacrene D and epi-α-cedrene were also identified. Similarly to COME, kaur-16-en-19-oic acid still represented the main constituent of the diterpenes fraction. Both COME and COOL resulted to be very effective against three different *H. pylori* strains: one standard CCUG strain, one clinical isolated cagA+ and Vac+ strain (10K) and one clinical isolated cagA- and Vac+ (G21) strain. MICs showed by COME ranged from 391 mg/l (G21) to 3125 mg/l (CCUG); MICs showed by COOL ranged from 98 mg/l (G21) to 1563 mg/l (CCUG). Both COME and COOL resulted significantly effective in reducing IL-6 release in LPS-stimulated PBMC from 200 µg/ml.
Overall, given its antimicrobial and anti-inflammatory activity and considering the safety of use (no cellular and *in vivo* toxicity up to 2000 µg/ml), *in vivo* investigations may be suggested in order to better understand the potential of *C. langsdorffii* in the management of *H. pylori* infection. The traditional use of copaiba oleoresin for stomach diseases results strongly supported by our data.

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3.1 = *IN VITRO* EVIDENCE FOR BIOLOGICAL ACITIVITIES OF SICILIAN *OROBANCHE CRENATA* FORSSK. LEAF EXTRACT

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Orobanche crenata Forsk. (Broomrape) is an obligate holoparasitic weed that attacks legume crops, such as faba bean, lentil, pea, and vetch, but also affects a large number of wild legume species (1). Broomrape can cause severe damage to the most important crops in the Mediterranean region and the Middle East (2). As a holoparasite plant, it is able to accumulate special metabolites that enable it to obtain its food from the host plant (3). It lacks in chlorophyll; the host phloem and xylem are its principal sources of carbohydrates and minerals, respectively. It has also some interest as an edible plant because of the high content of antioxidant phenols of its tender shoots, the edible portion of the plant (4). *Orobanche* was used historically by some North American tribes for externally treating ulcers and cancer growth, and internally for treating some bowel disorders. California Broomrape or cancer root (*O. californica*) is used externally to treat skin infections caused by *Streptococcus*, as a poultice or wash for wounds, ulcers and herpes lesions. Gatto *et al.* performed chemical characterization of *O. crenata* phenolic extract: verbascoside and isoverbascoside were quantitatively extracted, purified and characterized:. These compounds proved to be effective in reducing the growth of some important fungi responsible for the decay of fresh fruit and vegetables in postharvest (5). In addition antioxidant capacity of *O. crenata* extract was investigated and it was found that methanolic extract showed the highest activity in DPPH and ABTS free radical scavenging assays (6). Despite the large amount of research on screening for active compounds from plants in different parts of the world, to date little is known about active compounds from *Orobanche.* The current study aimed to investigate antimicrobial and antioxidant activities of *O. crenata* Forsk. acetonic leaf extract. Antibacterial effect on Gram-positive (*Staphylococcus aureus*, *Staphylococcus epidermidis*, *Enterococcus faecium*, *Enterococcus faecalis*) and Gram-negative (*Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*) pathogenic bacteria and antifungal activity against *Candida* spp. clinical isolates were evaluated. Antimicrobial activities were compared to standard drugs (7, 8). Total phenolic and flavonoid contents of the extract were evaluated by spectrophotometric assay; antiradical activity of *O. crenata* Forsk. extract was performed by DPPH test and by superoxide anion scavenging capacity. Moreover *ex vivo* antioxidant assay was performed using percentage of hemolysis induced by APPH in red blood cells (9). Results obtained showed that the extract has both antioxidant *in vitro* activity and antimicrobial ability against Gram-positive strains but with different effectiveness probably due to active compounds present in the extract. *O. crenata* Forsk. extract demonstrated also a good antifungal potential against some tested clinical isolates of yeast. This study allowed us to enhance the knowledge of *O. crenata* Forsk., its chemical composition and biological activities in order to explore other possible applications of this extract.

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3.1 = OPTIMIZATION OF PRODUCTIVE CYCLE OF *CROCUS SATIVUS* FROM ABRUZZO, INVESTIGATING INNOVATIVE USE AND APPLICATION OF DIFFERENT FLOWER PARTS

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Crocus sativus L. (saffron) is the natural source of a spice, represented by stigmata, that is considered the most expensive all over the world.
In the last decades the cultivation of saffron in Italy was subjected to renewed attention and is increasing its diffusion in the traditional growing Italian regions, like Abruzzo, Sardegna, Sicilia and Umbria, as well as in other regions where it could be considered a new crop or a reintroduction of past cultivations.
The traditional production of Abruzzo saffron (Zafferano dell'Aquila) is characterized by an intensive manual work for the selection of bulbs, transplant, collection of flowers and selection of stigmata. These procedures are time-consuming and consequently are considered the main drivers of final cost. On the other hand the manipulation of each flower to separate floral parts allows to produce a very high quality stigmata as well as high quality by-products. In collaboration with local farmer and cosmetic laboratory an experimental research was started to define an innovative product chain based on stigmata, to implement the classical use as food, dye or medicine, and to give new value to byproducts, such as tepals and anthers.
The mean weight of single flower results in 347.2 mg divided as 84, 9 and 7% in tepals, anthers and stigmata, respectively. The loss on drying ranges from 80% in anthers up to 89% tepals. Water, ethanol and olive oil extracts were analyzed by HPLC-PDA and quantitative amounts of phenols and of characteristic compounds, such as crocins and safranal were defined in stigmata and other floral parts. As expected, the highest amount of phenolics were detected in stigmata extracts with highest extraction in water>olive oil>ethanol. The same analysis on tepals and anthers reveal that olive oil was the better extractive solvent, even if quantitative data are lower than those of stigmata. From all floral parts, the most abundant polyphenol resulted catechin that could represent up to 10% of total phenols. The total phenoliccontent was higher in tepals, while stigmata and anthers are the main site of accumulation of proanthocyanidins and carotenoids. Tepals and anthers were also investigated as a potential source of secondary metabolites. Extracts in water and alcohol from all floral parts were tested *in vitro* to evaluate cell viability and their modulatory effects on the oxidative stress. The extracts were tested as protective agents against induced oxidative stress on two cell lines (human carcinoma, HT116 and mouse myoblast C2C12). Results show that all tested extract can modulate the cellular response to exogenous oxidative stimuli. The most active resulted stigmata extracted in hot water, in accordance to literature data and traditional use. Chemical and biological data confirmed that tepals and anthers, that today are considered waste of productive chain can represent a valuable source of phytochemicals with promising applications.

Fig. 1
Saffron flower: Stigma, anthers and tepals.

Fig. 2
Byproducts of saffron, tepals and anthers.

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3.1 = POSSIBLE USE OF *MELILOTUS OFFICINALIS*, *LESPEDEZA CAPITATA* AND *GLYCYRRHIZA GLABRA* IN HUMAN'S HEALTH

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The search for natural principles is attracting much interest in the field of nutraceuticals, pharmaceuticals and cosmetics. A greater interest in the study of bioactive molecules used in traditional medicine is being observed, leading to a demand by industry. The aim of this study is to explore the possible use of three plants (*Melilotus officinalis* (L.) Pall., *Lespedeza capitata* Michx and *Glycyrrhiza glabra* L) belonging to the Fabaceae's family as sources of medicines, pharmaceuticals or food supplements (1). The total polyphenols and total flavonoids content (TPC and TFC, respectively) as well as the antioxidant activity and the effect on viability on intestinal cells were evaluated. In phytomedicine, *M. officinalis* is used to support the treatment of many diseases and its therapeutic activities include anti-inflammatory, antiedematous, phlebotonic, spasmolytic, diuretic or even sedative properties (4). *L. capitata*, in experimental and clinical studies, was reported to exercise positive effects on tissue draining, kidney and cardiovascular diseases as well as diabetes (5, 6). Finally, *G. glabra* is used traditionally to repair skin and to cure gastrointestinal disorders (7).
The TPC and TFC of industrial powder extracts of *M. officinalis*, and *L. capitata* and *G.glabra* were determined spectrophotometrically according to the Folin–Ciocalteu procedure and aluminum chloride colorimetric method, respectively. Antioxidant activity of extracts was quantified by DPPH (2,2-diphenyl-1-picrilidazide) and FRAP (ferric reducing antioxidant power) assays. The effects of extracts on viability of human intestinal cells (Caco-2 and HT29-MTX) were evaluated through a MTS assay. The TPC of the different extracts varied from 12 mg to 23 mg GAE (Gallic acid equivalent) per g of extract. The results show that TFC varied from 3 mg to 12 mg in terms of CAE (Catechin equivalent) per g extract. In what concerns cell studies, extracts did not affect cell viability. Our samples are used also for the hydrogen peroxide and superoxide scavenging: for the hydrogen peroxide scavenging capacity, we observed an inhibition ranging between 55% and 96 %; and for the superoxide scavenging capacity an inhibition ranging between 46% and 56%.
Data indicate that these legumes could be further investigated as potential food supplement ingredients.

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3.1 = *IN VITRO* ANTIOXIDANT AND HYPOGLYCAEMIC ACTIVITIES OF *HYSSOPUS OFFICINALIS* L. SUBSP. *ARISTATUS* (GODR.) BRIQ. (LAMIACEAE) FROM SOUTHERN ITALY

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Hyssopus officinalis L. (Lamiaceae) is a perennial shrub native to southern Europe and temperate Asia and naturalized in the United States. *H. officinalis* has been used as a carminative, diaphoretic, emmenagogue, expectorant, stimulant, stomacher, and tonic (1). Several studies reported the antioxidant, anti-platelet, and anti-fungal activities of essential oil from *H. officinalis* (1).
Hyssopus officinalis L. subsp. *aristatus* (Godr.) Briq. (2, 3) occurs from Marocco to Western Balkan Peninsula on calcareous rocks. Few studies have been conducted on *H. officinalis* subsp. *aristatus*. Particularly investigated is the essential oil (4). In the present study, *Hyssopus officinalis* subsp. *aristatus* extracts were investigated for their potential α-amylase inhibitory activity and antioxidant effects. The aerial parts (360 g) of *H. officinalis* subsp. *aristatus*, collected in 01/10/2013 in Calabria region (Italy), Pollino massif, Timpone Capanna, at 1590 m a.s.l. and authenticated by dr. NG Passalacqua (voucher specimen n. 9801) were subjected to extraction by maceration by using methanol (3 × 1 L) to give a total extract (yield 7.9%). This extract was suspended in methanol and partitioned with *n*-hexane to yield a *n*-hexane-soluble fraction and a methanol-soluble fraction. DPPH, ABTS, FRAP, and β-carotene bleaching tests were used to study the antioxidant properties of total extract and fractions (5). The *in vitro* hypoglycemic potential activity was investigated by the inhibition of α-amylase (6). The involvement of reactive oxygen species (ROS) in the pathogenesis of increasing number of diseases is well demonstrated. Among these, there is diabetes mellitus (DM), a metabolic disease characterized by chronic hyperglycaemia resulting from defects in insulin secretion, insulin action, or both. DM was the direct cause of 1.5 million deaths in 2012 and will be the seventh leading cause of death in 2030 (7). Diabetes can be classified into type 1 diabetes if it is characterized by immune complex mediated attack on insulin producing by pancreatic β cells or type 2 diabetes characterized by insulin-resistance (8). The impairment in glucose control in the long-term leads to dysfunction and failure of different organs and is usually associated with obesity, dyslipidaemia, hypertension, and inadequate lifestyles (9). Natural compounds have become an interesting resource of bioactive agents with a key role in diabetes therapy (10). Several plant extracts and pure compounds demonstrated to inhibit carbohydrates-hydrolysing enzymes. This activity can offer an attractive therapeutic strategy for the management of post-prandial hyperglycaemia (11). A concentration-response relationship was observed for all samples. The methanol-soluble fraction showed an interesting radical scavenging activity with an IC_{50} value of 12.4 μg/ml in ABTS test and was the most activity as α-amylase inhibitor with an IC_{50} value of 169.67 μg/ml. The obtained data underline the potential health benefits of *H. officinalis* subsp. *aristatus* and suggest that it could be used as a potential source of healthy phytochemicals.

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3.1 = TRADITIONAL KNOWLEDGE ON WILD AND CULTIVATED PLANTS IN THE KILOMBERO VALLEY (MOROGORO REGION, TANZANIA)

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This research was performed in four villages adjacent the boundary of Udzungwa Mountains National Park in the Kilombero River plain of Tanzania. The area close to the villages is characterized by self-consumption agriculture, with a population that is on average poor, still very tied to traditions and almost entirely unaffected by modernization and technology. The aim of the present study was to investigate and record local knowledge regarding the use of wild and traditionally cultivated plants used for traditional medicine and for other everyday purposes (e.g., food, fibers and timber).

Ten traditional local healers, three women and seven men, with solid botanical knowledge, were interviewed between June and August 2014 by means of semi-structured questionnaires. For each mentioned plant species, the Swahili folk name, the scientific name, as well as the part of the plant used, the preparation method and the main uses were recorded. Ethnobotanical knowledge was not equally shared between the two genders and the average number of quoted species was 13.7 for men and 7.3 for women.

In total 118 species have been botanically classified: most of the plant species belonged to the family of Fabaceae (24), followed by Lamiaceae (11), Asteraceae (7) and Solanaceae (6). The identified species were chiefly used for food purpose (36.8%), and medicinal use (33.3%), followed by production of raw materials, such as wood and fibers (19.4%) and ritual use (3.5%). The parts of the plants mainly used for the preparation of herbal remedies were leaves (50%), bark (16.7%), roots (13.3%), whole plant (10%), fruits (6.7%) and other, like sap or flowers (3.3%).

Fig.1
The sausages tree, *Kigelia africana*, one of the most cited medicinal species.

In 71.4% of cases, the mentioned medicinal plants were indicated for the treatment of a single disease, 22.5% of the species were used to cure two diseases, while the remaining 6.1% were used in the treatment of three or more diseases (e.g. *Kigelia africana*). Among the most cured diseases were gastrointestinal pathologies (cured by 11 species), weakness and faints (7 species), gynaecological, andrological and urogenital disorders (cured by 7 species), respiratory diseases (6 species), followed by parasites, cardio-circulatory problems and wounds.

In total 53 plants were indicated as having a food use, most of these belong to the Fabaceae family (6 species), followed by Solanaceae, Asteraceae and Cucurbitaceae (3 species each). The parts of the plants mostly consumed as food were fruits and seeds (55.6%), leaves (31.5%), roots (7.4%) and shoots (5.6%). According to the informants, wild food plants do not play a key role in the diet of these communities.

Twenty-eight (19.4%) species were indicated as useful to produce raw materials, of which 21 were woody species used for the production of furniture, house structural elements, firewood and poles. The other seven species were herbs (e.g., Poaceae and Cyperaceae) and palms which are mainly used for the production of fibers and roof covers. Five other species were indicated as having ritual purposes linked to their traditional use within the communities. These species were thought to avoid bad luck (like *Duranta erecta*) or against bad sprits or evil eye. There were also reported 2 plants used to extract dyes and 2 useful for pollen production.

The present study revealed that numerous plant species are still essential in the everyday life of the tribes living in Kilombero Valley. Most of the plants were usually harvested in the wild, however, after the creation of the Udzungwa Mountains National Park, the harvesting pressure has become concentrated on a few unprotected forest patches. Consequently, many useful species are becoming increasingly rare with the risk of losing the connected botanical and traditional knowledge. The present study may, therefore, contribute to record the ethnobotanical knowledge held by these populations, in order to preserve this valuable richness for future generations.

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3.1 = *EUPHORBIA DENDROIDES* L. (EUPHORBIACEAE) LATEX: POLYPHENOLS CHARACTERIZATION AND EVALUATION OF ANTIOXIDANT AND RADICAL SCAVENGING PROPERTIES

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Euphorbia dendroides L. (Euphorbiaceae), is a typical Mediterranean plant. It grows in the wild in coastal areas, up to an altitude of about 700 m above sea level, mainly in Spain (Balearic Islands), France (including Corsica) and Italy (including Sardinia and Sicily). *E. dendroides* takes the form of a rounded shrub or small tree up to 3 m tall (Fig. 1). As all *Euphorbia* species, it produces a white latex that exudes when cut, and it is rich in secondary metabolites. The presence of biologically active compounds in the latex contributes to the pharmacological properties of many *Euphorbia* species [1, 2]. The studies carried out on *E. dendroides* latex, have focused certain components of the latex such as diterpenoids [3,4]. At present, phytochemical and biological studies on poliphenolic compounds of the latex are scarce. The aim of this study was to characterize major phenolic compounds of *E. dendrides* latex by HPLC-DAD-FLU and, in addition, estimate the antioxidant and free radical scavenging properties, by several *in vitro* assays such as DPPH, TEAC and ferric reducing/antioxidant power (FRAP). Total phenolics (TPC) and total flavonoids (TF) content were also determined. The white latex samples from *E. dendroides* were drained into glass tubes by cutting the stem apices of wild plants from Sicily, locality Masse (Messina). The phenolic compounds characterizazion showed that the latex contains high amounts of phenolic acids (80.5%) such as hydroxycinnamic and hydroxybenzoic acids of which major constituents are cumaric and vanillic acid, respectively. Flavonoids represent about 20% of major polyphenolic compounds in *E. dendroides* latex, of which flavanones such as eryodictiol-7-O-glucoside and eriodyctiol as most abundant compounds. A phytochemical analysis performed by GC-MS revealed also that in *E. dendroides* latex the triterpenoids are contained in high amount, following by sesquiterpenes and monoterpenes. The results show that *E. dendroides* latex has significant antioxidative activity, as measured by DPPH assay (2927.01±98.03 µmol of Trolox equivalent (TE)/100g FW). Reactivity towards ABTS radical cation and ferric-reducing antioxidant power (FRAP) values were 7580.95±97.65 µmol of TE/100g FW and 4383.13±95.30 µmol of TE/100g FW, respectively. Furthermore, Brine shrimp (*Artemia salina*) bioassay was carried out to investigate the cytotoxicity of *E. dendroides* latex methanol extract. The larvae viability is significantly affected at concentrations higher than those capable to induce significant antioxidant effects.

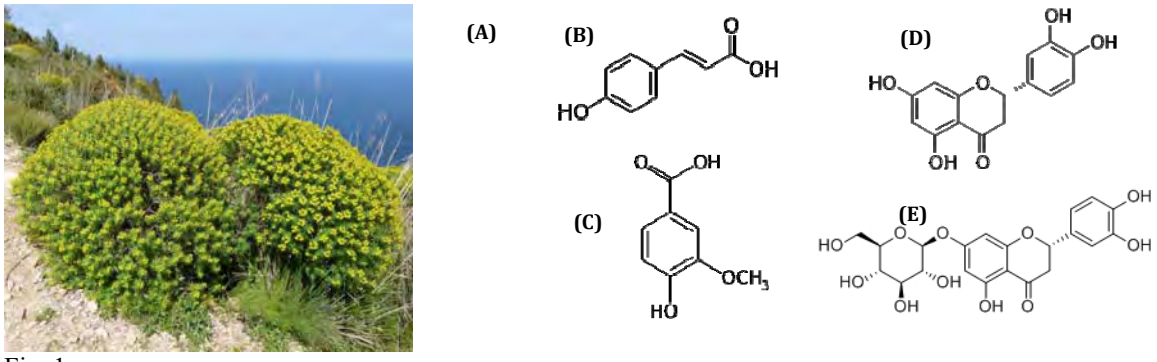

Fig. 1
Euphorbia dendroides L. (A) and main phenolic compounds identified in latex: cumaric (B) and vanillic (C) acid, eriodyctiol (D) and eryodictiol-7-O-glucoside (E).

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3.1 = CHEMICAL COMPOSITION OF THE ESSENTIAL OILS OF SOME TAXA OF THE GENUS *CLINOPODIUM* (LAMIACEAE) FROM SICILY

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The genus *Clinopodium* L. (*Lamiaceae*) is known for its medical uses in folk medicine and as a spice in Italian food. Recently, several taxa previously assigned to *Satureja* L. and *Calamintha* Mill. have been transferred to this genus (1). Pharmacological studies reveal, for instance, that *Calamintha nepeta* (L.) Savi [*Clinopodium nepeta* Kuntze subsp. *nepeta*], commonly known as "nepetella", exhibits cholagogue, expectorant, sedative and antibiotic properties (2); furthermore, the essential oil of its aerial parts showed an antifungal activity (3). The apical flowering parts and leaves of *Clinopodium vulgare* L. are used in popular medicine for their carminative and emmenagogue properties (1); recently, the essential oil of its aerial parts was found to possess remarkable radical-scavenging and antioxidant activities (4). In Madeira Island, the local population uses the leaves of *Clinopodium ascendens* Samp. as a mouth freshener and to alleviate headache and toothache; furthermore, the essential oil of its aerial parts exhibited remarkable antibacterial and antifungal activities (5).
Here, the chemical composition of the essential oils of four taxa of *Clinopodium* growing wild in Sicily is reported. In particular, were investigated *Clinopodium nepeta* Kuntze *subsp glandulosum* (Req.) Govaerts from Cava Grande (Avola, Siracusa), *Clinopodium nepeta* Kuntze subsp. *nepeta* from Cave di Cusa (Campobello di Mazzara, Trapani), *Clinopodium raimondoi* Spadaro, Faqi & Mazzola from Palermo (Fondo Patti, San Gabriele) and *Clinopodium nepeta* Kuntze subsp. *ascendens* B. Bock. from Castelbuono (Madonie, Palermo).
The essential oils, extracted by hydrodistillation according to the *European Pharmacopoeia*, were analysed by GC and CG/MS.
In the four oils, 48 compounds in all were identified: 27 for *Clinopodium nepeta* subsp. *glandulosum* (98,4% of the total oil), 19 for *Clinopodium nepeta* subsp. *nepeta* (98,6% of the oil), 26 for *Clinopodium raimondoi* (96,5% of the oil) and 38 for *Clinopodium nepeta* subsp. *ascendens* (93,5% of the oil).
In *C. nepeta* subsp. *glandulosum*, the most abundant compounds were *trans*-dihydrocarvone (36.5%), carvone (19.2%) and *cis*-dihydrocarvone (13.0%). On the whole, the oil was constituted mainly of monoterpenes (81.5%) and sesquiterpenes (13.4%). In the first fraction, oxygen-containing monoterpenes (73.4%) prevailed over monoterpene hydrocarbons (8.16%).
In *C. nepeta* subsp. *nepeta*, the main components were *cis*-piperitone oxide (39.0%), piperitenone (36.0%) and limonene (7.7%). Monoterpenes constituted the most abundant fraction of the oil (91.0%), with a prevalence of oxygen-containing monoterpenes (81.5%).
Piperitenone oxide (59%) *cis*-piperitone oxide (22.2%) and limonene (6.0%) were the main compounds of *C. raimondoi.* As in the other oils studied, monoterpenes constituted the main fraction and accounted for 90.2% of the total oil with a prevalence of oxygen-containing monoterpenes (82.4%) over monoterpene hydrocarbons (7.8%).
In *C. nepeta* subsp. *ascendens*, the main compounds were carvone (14.4%), *trans*-isopulegone (11.5%) and mint furanone (8.9%). On the whole, the main fraction was constituted of monoterpenes (81.1%). Among these, monoterpene hydrocarbons (73.4%) were the most abundant components of the oil.
In conclusion, all four oils share a high percentage of monoterpenes and a scarce amount of sesquiterpenes. Previous papers on the analysis of the essential oils of *Clinopodium* sp.pl. showed that the monoterpenes group is usually dominant, although the main component may vary.
The results of the present study concur with these findings.

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3.1 = NEW ETHNOBOTANICAL RECORDS ON MEDICINAL PLANTS FOR HUMANS AND ANIMALS IN THE TERRITORY OF PORO AND PRESERRE CALABRESI (VIBO VALENTIA – SOUTH ITALY)

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Ethnobotany is defined as the science which deals with different relationships between people and plants, whose uses are part of the cultural heritage of a people. Humans have always used plants, to feed on, to treat illnesses, to build tools for job in the fields and at home, to make everything which was necessary for everyday life. In the last twenty years, in some Calabria areas, different surveys concerning different uses of plants in the field of popular medicine (1-3) and veterinary medicine (4) have been carried out. The aim of this study is to contribute to the rediscovery and to the recovery of our ethnobotanical heritage connected to different traditional uses of medicinal and veterinary plants, both spontaneous and cultivated (but which have a different aim from that for which the plants are usually cultivated). Furthermore, we want to give a contribution to the knowledge and to a scientific reflection and cultural and economic too. For this study a series of ethnobotanical surveys have been carried out between 2016 and 2017, in different areas of Poro and Preserre Calabresi area (Vibo Valentia). Thyrty-six informers of this area were selected, with whom have been carried out 116 structured interviews in the field, using a paper sheet. These have been integrated with the identification of plants directly picked in full field, some of which have then been dried for preparation of herbarium samples. These interviews have allowed to register medicinal and veterinary uses of 50 plants species belonging to 31 families. Among different original uses of plants in popular medicine, particularly interesting resulted those listed below.
The felt present in the knots of *Arundo donax* L. stems was used to block the blood flow in case of small wounds. *Petroselium crispum* (Mill.) Fuss was used as a laxative to stimulate mechanically children in case of obstinate constipation. To reduce the excessive sweating of feet were used the leaves of *Myrtus communis* L. which were dried, ground and put inside the socks. *Opuntia ficus-indica* (L.) Mill. cladodes were used after cutting a small portion of spongy tissue, taking away epidermis and spines, and which was applied on wounds, ulcers and sores as a cicatrizer. To relieve the stomach ache of the children were used the seeds of *Cannabis sativa* L., cultivated in the past for textile fiber, from which was made a milk which was drank. Besides, data concerning veterinary uses of plants for treatment of pets have been recorded. *Achillea ligustica* All. and *Dittrichia viscosa* (L.) Greuter have been used for their vermifugal power on cattle. *Lupinus albus* L. and *Olea europaea* L. were both used as parasiticidal against louses of chickens, pigs and cattles. Finally, *Spartium junceum* L. was used as containment protection of the cow's uterus prolapse.
Thanks to original research activities carried out in full field as this one, it will be possible avoid losing of local cultural heritage, fundamental for the protection of biodiversity and ecosystem too. Besides, these data will be able to be used to relaunch experiment agricultural and food production chains aimed at the production and marketing of plants and their products destined to the wellness of people and animals, in a natural and sustainable way.

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3.1 = RESEARCH FOR AN ANTIBACTERIAL ACTIVITY OF THE ESSENTIAL OIL OF *CUPRESSUS SEMPERVIRENS* L. FROM ALGERIA, ON SOME BACTERIAL STRAINS

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To investigate the biological properties of *Cupressussempervirens* L. an ethnobotanic study was performed emphasizingits role in algerian herbal medicine.
In particular, we were interested in the identification of essential oilconstituents and in the estimation of its antibacterial activity.
Gas-chromatographic analyses found the presence of 18 constituents, mostly represented by kaurene(37.56%), by monoterpenes, mainly has - alpha Pinene (24.74%), 3-Carene (8.69%), and bysesquiterpenes: D-Germacrene (3.13 %), Cadinene (0.15 %) and monoterpenols: Cedrol (6.23%), Terpinen-4-ol (2.86%).
The evaluation of antibacterial activity of the essential oil of *C.sempervirens*) was performed on bacterial strains frequently isolated in hospitals and acting as human pathogens:*Acinetobactersp, Enterococcus sp, Escherichia coli, Proteus mirabilis, Staphylococcus aureus* and *Streptococcus sp.*All the strains were tested agains four different essential oil dilutions: .The essential oil of the leaf twigs showed an activity on *Acinetobacters*p, *coli E.* and *Enterococcus sp*, but acted weakly against*Staphylococcus aureus*.

Keywords: *Cupressus sempervirens* - leaves - essential oil - CCM-CPG - antimicrobial activity

3.1 = TRADITIONAL USE OF MEDICINAL PLANTS IN THE REGIONS OF EDESSA AND NAUSSA (CENTRAL MACEDONIA, GREECE)

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Medicinal plants have represented for thousands of years the only remedy for various diseases. Phytotherapy still maintain an important role in the treatment of many diseases in Greece. Despite this fact, only few studies explored the use of plants in folk Greek medicine (1, 2).
The present study aimed to collect, analyze and evaluate ethnobotanical and ethnopharmacological information on the use of medicinal plants in Central Macedonia, N Greece. The study was conducted in the area of two small cities, Naoussa and Edessa, and their nearby villages, located at the foothills of Mts Vermio and Voras. The two mountains, which are part of the Natura 2000 Network, are characterized by a rich and diverse flora (>1000 taxa were recorded on Vermio, and >1500 on Voras), including several Greek and Balkan endemic species. The inhabitants are mostly engaged in agriculture and traditional animal husbandry, thus have a strong connection to nature. The population, which has a varied cultural background, consists mainly of four groups, the natives, the descendants of the refugees which came in 1922 from the Black Sea or Asia Minor and the Vlachs. Cultural differences among these groups, though have decreased over the years, are still obvious, and particularly in older people.
The ethnobotanical data were gathered through questionnaires, extensive dialogues and semi-structured interviews with 70 native informants (27 men, 43 women), who had personal experience in self-medication using herbs. In these interviews, the informants were requested to indicate vernacular names of plants, parts of the plant used, folk uses and preparation procedures. In many cases there were included data on specific recipes and their sources. Ethnobotanical investigations were directed from May 2016 to June 2017.
The final database included 75 species belonging to 41 plant families, Lamiaceae (17%) and Asteraceae (12%) being the most represented. Cultivated plants are less frequently used, whereas the information collected is mainly referring to wild taxa, which were used to treat 12 ailment categories. The greatest number of species was used to treat respiratory, skin, gastro-intestinal and genito-urinary diseases.
Most used parts of the plants were flowers and inflorescences, followed by leaves. Principal methods of herbal preparations included infusion, decoction, poultice, maceration and oil.
The highest Relative Frequency Citation was found for *Hypericum perforatum*, *Matricaria recutita*, *Tilia platyphyllos* and *Urtica dioica.*
The results demonstrated that the traditional knowledge of medicinal plants in Edessa and Naussa is well preserved, but a large part of Macedonia remains poorly explored from ethnobotanical point of view.



Figures 1 - 3 Most reported medicinal species: *H. perforatum* (flower Fig. 1) and oil (Fig. 2). *T. platyphyllos* flowers (Fig. 3).

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3.2 = *PUNICA GRANATUM* LEAVES AS A POTENTIAL SOURCE OF BIOACTIVE MOLECULES

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Plants used in traditional medicine are an important source of secondary bioactive compounds responsible for their biological properties. *Punica granatum* L. (Lythraceae family), commonly known as pomegranate is a domesticated tree used from ancient times for its numerous health benefits. All parts of this tree are used in folk medicine. The major class of secondary metabolites of pomegranate tree is polyphenols, including ellagic acid, flavonoids, hydrolysable and condensed tannins. Recent studies have reported several hydrolysable tannins in pomegranate leaves, bark and fruits, in particular gallotannins and ellagitannins as punicalagins, which are pomegranate most powerful antioxidants. The main biological properties of pomegranate are focused on fruit juice and directed towards different cardiovascular risk factors, hypertension, inflammations, hyperlipidemia, diabetes, ageing, Alzheimer's disease, etc. In addition, extracts from different parts of the plant are reported to have antibacterial, antifungal, antiviral, anthelmintic properties.[1] Several studies found that ellagic acid and larger hydrolyzable tannins, such as punicalins and punicalagins, show the highest antimicrobial activities.[2] Literature reports few phytochemical information about pomegranate leaves (PGL) and any studies concerning their antiviral properties. This work aimed i) at obtaining a phytochemical quali-quantitative characterization of the main compounds in PGL from a Sardinian pomegranate cultivar; ii) at evaluating the antiviral activity against Human Immunodeficiency Virus-1 (HIV-1) of PGL ethanolic extract and its enriched fractions. The active fractions were identified via bioassay-guided fractionation procedure and tested on *in vitro* assays to evaluate the HIV-1 reverse transcriptase-associated RNase H activity and integrase (IN) inhibitions. The PGL ethanolic extract and the resulting active fractions were analyzed and characterized using HPLC/DAD/ESI-MS. The main components were quantified through the external calibration methods. The results showed that ellagic acid is one of the main components in PGL. The fact that both PGL and the flavonoids enriched fractions are able to inhibit HIV-1 RNase H and IN activities, demonstrates that the PGL biological activity is not only correlated to the presence of ellagitannins such as ellagic acid, but could also be attributed to flavonoids.

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3.2 = THE ANTIAGING PROPERTIES OF *HELICHRYSUM ITALICUM* (ROTH) G.DON ESSENTIAL OIL: COLLAGENASE AND ELASTASE INHIBITION ACTIVITIES

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Helichrysum italicum (Roth) G. Don is an evergreen shrub of the Asteraceae family whose aerial parts are a source of essential oil. For this study, flowering samples have been harvested and hydrodistilled in July 2016 and the obtained essential oil has been characterized by means of GC/MS. Overall, 68 compounds were identified, of which over 30% are oxygenated monoterpenes: among these compounds, nerol and its esters are the most represented ones, with neryl acetate as the most abundant (15.4%) component of the EO. This pattern is reported in the literature for other *H. italicum* EOs (1, 2). The characteristic helichrysum β-diketones known as italidiones were detected in the composition: 4, 6, 9-trimethyl-8-decene-3, 5-dione (italidione I) and 2, 4, 6, 9-tetramethyl-8-decene-3, 5-dione (italidione II) showed the most relevant relative abundances.
For a long time, the essential oil of *H. italicum* (Roth) G. Don has been used in the preparation of cosmetic products for skin care, including anti-aging products (5). The collagenase activity can be stimulated by several factors as well as UV light: this causes degradation of matrix proteins and skin aging. Elastase are proteolytic enzymes in the dermis that are responsible for the degradation of elastin in the extracellular matrix. The loss of elastin plays a significant role in the development of the visible signs of aging (wrinkles, sagging) in the skin (6). The essential oil of *H. italicum* inhibited the collagenase (Fig. 1) and elastase (Fig. 2) activities with an IC50 (µg/ml) of 36.99±1.52 and 235±6.32, respectively. Values shown represent the mean ± SD from five independent experiments. These inhibition activities have been compared with those of oleanolic acid, which is a skin-protective pentacyclic triterpene.
As evidenced, the elastase inhibition effect is particularly relevant. To the best of our knowledge, there is no other published study dealing with the inhibiting effect of *H. italicum* EO on these enzymes activities.

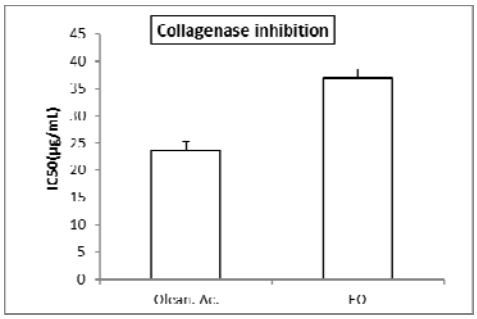

Fig. 1
Collagenase inhibition.

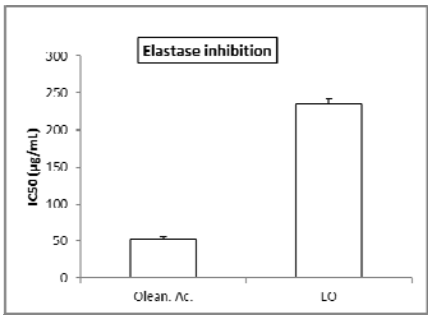

Fig. 2
Elastase inhibition.

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3.2 = ANTIBACTERIAL ACTIVITY OF ROOTS OF *SALVIA CORRUGATA* VAHL.

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Salvia corrugata Vahl. is an American species that is cultivated in the Mediterranean coastal area as an ornamental plant. The major diterpenoids of the exudate of the fresh aerial parts of *S. corrugata*, demethylfruticuline A (1) and fruticuline A (2), showed significant antibacterial activity (1) and inhibited *in vitro* the synthesis of biofilm of multiresistant strains of *Staphylococcus* and *Enterococcus* species (2). Other α-hydroxy-β-isopropyl-benzoquinone diterpenoids, namely fruticulin C, 7α-methoxy-19-acetoxy-royleanone, 7α,19-diacetoxy-royleanone, 7-dehydroxy-conacytone and 7α-O-methyl-conacytone were also isolated (3). *In vitro* biomass, namely regenerated shoots and micropropagated plants showed the presence of compounds 1 and 2 along with 7α,19-diacetoxy-royleanone, conacytone, ursolic and oleanolic acids, α- and β- amyrin, and five compounds previously undescribed (19-acetoxy-7α-hydroxyroyleanone, 7β,20-epoxy-11,12,19-trihydroxyabieta-8,11,13-triene, 7,20-dihydrofruticuline A, 7β-acetoxy-20-hydroxy-19,20-epoxyroyleanone, 7β-ethoxy-6β,20:19,20-diepoxyroyleanone) (4). The regenerated shoots showed the presence of both icetexanes 1 and 2, and the yield of 2 higher than in those of fresh leaves and fresh shoot tips, while micropropagated plants contained only 2. The methanolic extracts of *in vitro* tissues and two compounds, namely 7β-acetoxy-20-hydroxy-19,20-epoxyroyleanone and 7β-ethoxy-6β,20:19,20-diepoxyroyleanone, isolated in suitable amounts, were active at various degrees against multidrug resistant clinical strains of *Staphylococcus aureus*, *S. epidermidis*, *Enterococcus faecalis* and *E. faecium*, showing MIC values ranging from 32, 64 to 128 μg/mL. In order to complete the study of this species, the methanolic extract of the roots and the semi-purified fractions obtained by chromatographic separation were tested against several drug and multidrug resistant clinical strains of various species of Staphylococci (*Staphylococcus aureus*, *S. epidermidis*, *S. capitis, S. haemolyticus, S. hominis, S. lugdunensis, S. saprophyticus, S. simulans, S. warneri)* and Enterococci (*Enterococcus faecalis*, *E. faecium, E. gallolyticus, E. durans, E.avium)*, displaying MIC values ranging from 4 to >128 μg/mL. Two diterpenes, horminone and 7-O-acetylhorminone were isolated from the roots. These two compounds were tested against the same bacterial strains, displaying MIC values ranging from 4 to 64 μg/mL.
Moreover, a protocol for induction of hairy roots was established. Leaves of *S. corrugata*, obtained through *in vitro* culture of nodal segments of wild plants, were transformed with *Agrobacterium rhizogenes* strain (MTCC532) and hairy roots were induced. Quantitative determination of 1 and 2 in the methanolic extract, by means of an HPLC method previously validated (4), showed that this biomass did not produce 1 and 2. The methanolic extract of the hairy roots was moderately active against *E. faecalis* and *E. faecium* displaying MIC values ranging from 64 to 128 μg/mL.

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3.2 = IN DEPTH CHEMICAL INVESTIGATION OF *GLYCYRRHIZA TRIPHYLLA* FISCH & C.A.MEY ROOTS GUIDED BY A PRELIMINARY HPLC-ESIMS^N PROFILING

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Licorice, the dried roots and rhizomes of *Glycyrrhiza* species (Fabaceae), are among the oldest and most extensively used food and medicinal plants in both Eastern and Western countries (1). Chemical investigations on *Glycyrrhiza* spp. have mostly been focused on *G. glabra* L. (typically cultivated in Europe, henceforth called European licorice), *G. uralensis* Fisch and *G. inflata* Batalin (the two species are generally used in the traditional Chinese medicine and are known as Chinese licorice), commonly used as sweetener and as flavor enhancer (1). Furthermore, licorice roots show a variety of pharmacological activities, including antiulcer, anti-inflammatory, antispasmodic, expectorant, antidepressive, antiviral, antioxidant properties (2).

Thus, according to the growing interest in further *Glycyrrhiza* spp. to be used as sweeteners, the roots of the Iranian *G. triphylla* Fisch, explored so far only for its essential oil, (3) have been investigated by liquid chromatography coupled to high resolution mass spectrometry (LC-ESI/LTQOrbitrap/MS) in negative ion mode. The LC-ESI/LTQOrbitrap/MS profile (Fig. 1) and LC-ESI/LTQOrbitrap/MS/MS experiments guided the isolation of 21 compounds among which 6 previously unreported compounds including two isoflavones 7,5'-dihydroxy-6,3'-dimethoxy-isoflavone-7-O-β-D-glucopyranoside (4) and 7,5'-dihydroxy-6,3'-dimethoxy-isoflavone-7-O-(7,8-dihydro-p-hydroxycinnamoyl)-β-D-glucopyranoside (7) and four saponins, named licoricesaponins M_3 (13), N_2 (14), O_2 (16) and P_2 (18), whose structure were elucidated by NMR analysis.

We could observe that the accurate masses of some compounds here reported for the first time corresponded to those of compounds previously described in *Glycyrrhiza* spp. Thus an approach based only on MS analysis would be misleading; only isolation followed by NMR analysis allowed us to unambiguously assign the structures of these previously unreported compounds.

There is a large number of papers in plant metabolomics often describing putative characterization of plant constituents or identification of compounds only by MS analysis. This work highlights once again that the only MS information is not sufficient to determine the complete profile of plant constituents and extends and reinforces the notion that the combination of LC-MS and NMR techniques is a powerful tool to achieve the truly chemical structure of naturally occurring molecules in food plants.

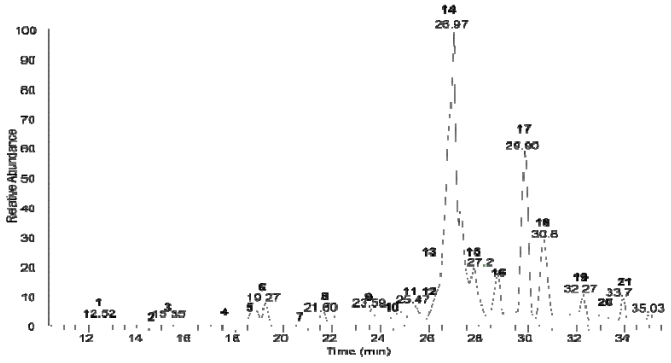

Fig. 1

LC-MS profile in negative ion mode of *Glycyrrhiza triphylla* Fisch methanolic extract.

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3.2 = LINKING VOLATILE PROFILE, GLANDULAR TRICHOMES AND POLLINATORS IN *SALVIA VERTICILLATA* L. (LAMIACEAE)

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For plants, due to their immobility, the release of volatile organic compounds (VOCs) provides a way to interact with organisms across distances, providing a detailed language for communication.
In this context, and as part of a four-year research project (IRIS identification - project 2014-PDF-0363) focused on a selection of *Salvia* species (Lamiaceae) preserved at the Ghirardi Botanic Garden (Toscolano Maderno, Bs), we addressed our attention on *Salvia verticillata* L. (lilac, whorled sage). This is a herbaceous perennial species native to central and eastern Europe and western Asia (1), widely cultivated for ornamental purposes. It is mainly pollinated by bees and flies (2).
The primary goal of this work is gaining the basic information to tentatively decipher the content and meaning of the chemical messages. Therefore, we specifically analysed the phytochemistry of the VOCs spontaneously emitted by leaves and flowers. Since in the Lamiaceae family the biosynthesis of volatiles occurs in glandular trichomes, we also combined a detailed micromorphological investigation on their distribution and histochemistry. In addition, focal plant observations on the pollinators spectrum were performed.
Vegetative and reproductive organs of *S. verticillata* are thickly covered by glandular hairs (3), belonging to two main morphotypes: large peltate and small capitate trichomes. The former are uniformly arranged on the abaxial sides of leaves, sepals and petals, with the latter scattered among them. The capitate types are abundant on the leaf adaxial surface, whereas calyx and corolla adaxial ones are hairless. The histochemical investigation revealed the presence of heterogeneous chemical composition within the storing chambers: both hair types are terpene producers, jointly with muco-polysaccharides in peltate and flavonoids in capitate trichomes. On the leaf, peltate trichome productivity is limited to the early developmental stage of the organ, whereas on the flower, their secretory activity last up to the maturity of the calyx and the corolla. The small capitate trichomes are active during all the stages of development of the organ bearing them.
The VOC emission profiles of leaves and flowers are greatly variable. The flower emission showed a higher complexity, with more constituents than the leaves. Leaf profile exhibits 20 exclusive VOCs, dominated by germacrene D (38.39%). Flower profile is characterized by 37 exclusive VOCs, among which 1,8-cineole (10.39%) is the most abundant one. Sesquiterpene hydrocarbons are the major chemical class in both profiles (83.31% in leaves and 73.66% in flowers) and 19 common VOCs were detected: β-caryophyllene, bicyclogermacrene, δ-elemene, β-bourbonene and γ-muurolene are the dominant constituents, with the former occurring in significant amounts (21.33% in leaves, 15.48% in flowers) and the others in divergent relative abundances, that differ of about 6.0 up to 15.0%.
The target species attracted mostly bees belonging to two functional groups: medium-size bees (mainly honeybees) and large bees (mainly bumblebees). Medium-size bees were three times more frequent than large bees. Pollinators visited on average 29.1 flowers, out of the 56.5 on anthesis (*i.e.* available to pollination), the collected resource being always nectar.
The peltate trichomes are widespread on the whole plant surface and display similar histochemical features regardless of their distribution pattern; however the different duration of their secretory activity on the vegetative and reproductive organs suggest diverse ecological roles: they might function in repelling predators and pathogens on young leaves and in alluring specific pollinators on mature flowers. The co-occurrence of the small capitate trichomes, their predominance on leaves and the productivity in flavonoids support the hypothesis of a synergistic defensive role.
The VOC profiles can be associated to literature data concerning chemical compounds and biotic responses: germacrene D, detected only in the leaves, and β-caryophyllene are common deterrents (4). Conversely, an attractive role towards bees is documented only for 1,8-cineole (4), exclusive of the flowers.
Plant strong attractiveness towards Hymenoptera is certainly confirmed. Experiments are underway to understand if and to what extent the different floral whorls and the floral resources play a role in the strategies of pollinators attraction.

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3.2 = CHEMICAL POLYMORPHISM AND PHYTOTOXIC ACTIVITY OF THE ESSENTIAL OILS OF *THYMUS* TAXA: A CASE STUDY OF SOME WILD SPECIES FROM SICILY AND CALABRIA

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Thymus is one of the most important and complex genera of Lamiaceae family. It includes a large number of species, many of which are characterized by a high morphological, karyological and chemical variability, related to the environmental factors and to genetic variation. About 215 species belong to the genus, as herbaceous perennials or small shrubs, spread across different continents mainly in temperate and cold regions (1). In the Mediterranean Basin, considered the center of differentiation of the genus, around 110 species are present (2). Among the aromatic plants, many *Thymus* species are known as culinary and medical herbs; in folk medicine plant products are used for the treatment of various diseases (bronchitis, asthma, infection). Also, their essential oils are widely used for their biological, medicinal and pharmacological properties. In the present work we propose a chemotaxonomic evaluation, aimed to give a contribution to the taxonomy in clarifying some ambiguous attribution within the genus. A further aim is to evaluate the phytotoxic properties of the essential oils, obtained from different *Thymus* taxa, against some common weeds to understand the allelopathic effect and their potential use as bioherbicide.

The chemical profile of the essential oils, obtained by hydrodistillation, from some wild populations collected during the flowering phases, in various sites of Sicily and Calabria, was analyzed by GC-FID and GC-MS. In particular, different accessions of *Thymus paronychioides* Čelak., *Thymus praecox* Opiz subsp. *parvulus* (Lojac.) Bartolucci, Peruzzi & N.G. Passal., *Thymus spinulosus* Ten. and *Thymus longicaulis* C. Presl. were studied.

The phytotoxic activity of the essential oils was assayed *in vitro*, against seed germination and radical growth of some weeds belonging to *Amaranthus* genus (*A. retroflexus* L., *A graecizans* L. and *A. lividus* L.), and of *Lepidium sativum* L., a sensible species usually used in ecotoxicological bioassays. In this screening phase, different concentrations of the essential oils were tested (from 0.0001 to 1% v/w), by dissolving the oils in acetone (0.5% v/w), or, as a more natural and sustainable alternative, by delivering the oils via colloidal encapsulation systems, formulated with naturally-derived compounds. Also, for each test, a control was investigated to verify the absence of any inhibitory activity of the encapsulation system on seeds germination. These bioassays are currently in progress.

A significant difference was observed in the essential oil yields: *T. paronychioides* gave the lowest amount (0.2% v/w), *T. praecox* subsp. *parvulus* an intermediate value of 0.6%, all others samples gave yields higher than 1%. A total of 164 compounds were identified: 128 from *T. paronychioides,* 45 from *T. longicaulis* from Calabria and Sicily, 62 from *T. praecox* subsp. *parvulus* and 50-60 compounds from the different samples of *T. spinulosus*.

The main constituents in all the samples were monoterpene hydrocarbons (□-terpinene, *p*-cymene and □-terpinene), ranging from 36% for *T. praecox* subsp. *parvulus* to over 64% for one sample of *T. longicaulis* from Sicily. The other class of compounds was represented by oxygenated monoterpenes (the main was thymol), with a percentage between 6% in *T. paronychioides* and more than 50% in *T. spinulosus*. Sesquiterpenes (the main were germacrene-D, *E*-caryophyllene, □-*E*-farnesene, E-nerolidol, eudesma 7,(11)-en-4 ol) were the major components (> 40%) in *T. paronychioides* sample, while they did not reach the 7% in other analyzed samples. Other components (namely non-terpenic compounds) (1-octen-3-ol, 3-octanol, nonanal) ranged from 2% in *T. paronychioides* up to 5% in *T. longicaulis* from Calabria. The bioassays about the capacity of the essential oils to inhibit germination and seedling elongation gave very promising results. In particular, the encapsulation of the essential oils in natural delivery systems enabled the efficient targeting of the weeds, improving the observed phytotoxic activity.

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3.2. = *PTILOSTEMON CASABONAE* (L.) GREUTER: SOME UPDATES ON A LITTLE KNOWN ENDEMIC SPECIES FROM SARDINIA

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Ptilostemon casabonae (L.) Greuter (Compositae) is a Tyrrhenian endemism localized in Sardinia, Corse and Hyères islands (France) (1).
In this work, we collected and analysed *P. casabonae* samples from Sardinia. This is the only species belonging to this genus present in this island, and it is traditionally used for both food and therapeutic purposes (2,3). Since little is known on *P. casabonae*, this work aims to obtain more information on this plant. The hydroalcoholic extract of *P. casabonae* aerial parts was here investigated for the first time, in order to evaluate its phytochemical profile and its potential antioxidant activity. Moreover, nuclear internal transcribed spacer (ITS), 5S-rRNA-NTS, chloroplast ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (RbcL) and photosystem II protein D1 (PsbA) genes were amplified and sequenced. *P. casabonae* RbcL, PsbA and 5s-rRNA-NTS regions were here sequenced for the first time. The comparison with other *Ptilostemon* sequences could provide discriminating molecular markers.
The phytochemical analysis reveals that polyphenols were the most representative class of secondary metabolites found in *P. casabonae* (14% of the total extract). Approximately 20 phenolic compounds were identified or tentatively identified by HPLC-PDA-MS/MS. They include mainly flavonoids and caffeoylquinic acid derivatives.
Results on antioxidant properties show an interesting activity of the extract, in comparison with ascorbic acid (AA) and trolox (T) as positive controls (DPPH: IC_{50} 5.51 µg/mL *vs*. 1 µg/mL of AA and 1.38 µg/mL of T; ABTS: IC_{50} 35.23 µg/mL *vs.* 4.85 µg/mL of AA and 7.94 µg/mL of T).
Sequences obtained in this work were compared with those present in NCBI database, also belonging to other *Ptilostemon* species. ITS and PsbA regions present an higher level of nucleotide variability and could be considered as good molecular markers. Unfortunately, since 5S-rRNA-NTS sequences of closely related species are not available in Genbank, it is not possible to make comparisons. However, this could be an input for further investigation of this gene since it is a documented variable region (4).
These findings reveal that *P. casabonae* contains interesting compounds that may be responsible to the observed antioxidant activity. Moreover, the conducted biomolecular analysis can give powerful molecular markers for the species identification.
Further studies are under way. More extracts have to be prepared in order to evaluate the presence of other secondary metabolites of interest and additional potential biological activities need to be investigated. Moreover, a comparison with *P. casabonae* from Corse have to be studied to emphasize similarities or differences in samples from the two islands where the species is considered endemic.

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3.2 = EFFECTS OF IRRADIATION ON YIELD, PHYTOCHEMICAL PROFILES AND ON THE ANTIOXIDANT ACTIVITY OF *OCIMUM BASILICUM* L. EXTRACTS

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Several studies demonstrated as antioxidant activity and metabolomic composition of plant extracts are strictly dependent from abiotic stress factors, but at the moment nothing is reported on aerial parts of *Ocimum basilicum* L.. Six samples of sweet basil were cultivated in a greenhouse in Campania Region (Southern Italy- 40°34' N; 14°59' E, 40 m a.s.l.) for a month, in different shading conditions, to evaluate the effects given by light and temperature changes. The first span of the greenhouse was shaded through whitening with calcium hydroxide (CHW), the second span using an HDPE white reflective cloth having 36% of shade level installed in position "up" (35-UP) and horizontally, position "down" (35-DOWN) to the greenhouse cover plastic sheet. The third span was shaded using the same HDPE, but with 58% of shade level. Also, this one was put "up" (60-UP) and "down" (60-DOWN) the cover plastic sheet. The last span was left without any shade, so it was considered as a control (FILM). These different factors provoked both morphological changes, concerning the plant height, width, length and the number of leaves, but also changes in phytochemical profile and antioxidant activity of the compounds. After harvesting and drying process, the extraction was carried out through maceration using methanol:water (80: 20) as solvents. To evaluate the differences between the six samples, they were tested by using four *in-vitro* assays: Total Polyphenol Content (TPC) (1), 2,2-diphenyl-1-picrylhydrazyl, DPPH) assay (2), Ferric Reducing Antioxidant Power (FRAP) and *β*-Carotene Bleaching (BCB) (1). To compare data obtained by different chemical methods used to evaluate antioxidant activity of extracts, Relative Antioxidant Capacity Index analysis (RACI) was performed (1). According to obtained results, FILM, (control), is the sample, totally irradiated, that showed the highest RACI value, 0.98, instead sweet basil sample with lowest RACI was 60-DOWN (-0.81). To have a complete picture of the phenolic content in our plant matrices, quali-quantitative analysis of the 6 extracts was carried out using RP-HPLC technique coupled with Diode-Array detector (DAD). The analysis of sweet basil extracts confirmed the presence of 8 phenolic acids (2 benzoic acid and 6 phenylpropanoic acid derivatives) and 2 flavonoids (quercetin glycosides). The highest total content of compounds was found in FILM (22447.47 ± 563.70 mg standard/Kg of extract), while the presence of the net does not affect the quantity of secondary metabolites, such as also whitening calcium hydroxide treatment. In fact, these samples didn't show wide differences on total value of secondary metabolites. So, it is possible to assess that, also in this case, total irradiance and high temperature enhance secondary metabolite content. In conclusion the agronomic and shading conditions during the cultivation in the greenhouse of *Ocimum basilicum*, affected the content of quantified secondary metabolites and their antioxidant activity. FILM was the sample with the highest RACI and the highest content of secondary metabolites. It was, in fact, subjected to highest temperature and incidence of light, that we can conclude are important in phytochemical and metabolomic profile of plants.

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3.2 = *BALLOTA PSEUDODICTAMNUS* (L.) BENTH.: MICROMORPHOLOGICAL DIVERSITY OF TRICHOMES

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Distinctive feature of Lamiaceae species is the presence of glandular and non glandular trichomes distributed, more or less densely, on vegetative and reproductive organs (1). The morphology and distribution of these structures frequently are considered as representative characters at specific and subspecific level (2). Furthermore, glandular trichomes of Lamiaceae produce, store, and release various secondary metabolites important to pharmaceutical, flavouring and fragrance industries (3). *Ballota pseudodictamnus* (L.) Benth. is a small evergreen shrub densely-pubescent with greenish-gray coloration and a large bell-shaped calyx. This species is native to the East Mediterranean region and naturalized in Italy. In some species of *Ballota*, included *B. pseudodictamnus*, biochemical studies have been conducted to identify bioactive molecules (4, 5), however studies concerning micromorphology of glandular and non glandular trichomes are lacking. The main goal of this study was to describe and characterize the *indumentum* located on both the vegetative and reproductive organs of *B. pseudodictamnus*. Samples were prepared according to standard methods for light and scanning electron microscopy (SEM); histochemical tests were also performed, in fresh sections, to localize the secreted within the hairs. Countless large non glandular dendritic trichomes completely cover leaves, stem and calyx of *Ballota*; between them are less numerous uni-bicellular pointed trichomes. Non glandular trichomes are traditionally known as acting in the physical protection of plants against biotic and abiotic stresses, forming a mechanical barrier against high light intensity, temperatures, attacks by insects (6). On the surface of epidermis and covered by the much higher and numerous non glandular trichomes there are two main types of glandular trichomes, i.e. peltate and capitate. The peltate trichomes are constituted by a basal cell immersed in the epidermis, a short stalk cell and a round broad head of eight secretory cells arranged in a circle. Peltate trichomes are generally considered as the site of production and storage of essential oils (3), nevertheless, interestingly, the secreted material of peltate trichomes situated along the connective tissue between the anther lobes contains alkaloids. In capitate trichomes the length and number of stalk cells are quite variable, the secretory head can be formed by one to four cells; essential oils and polysaccharides are present in secretions. Capitate trichomes located at calyx fauce have a very long stalk, sometimes one of the branches of dendritic trichomes transforms in a such type of glandular trichome. This phenomenon reinforces the hypothesis according to which, in some species, glandular trichomes phylogenetically derive from non glandular ones (7).

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3.3 = NEW LIMONOIDS FROM *APHANAMIXIS POLYSTACHYA* (WALL) R.PARKER (MELIACEAE) LEAVES

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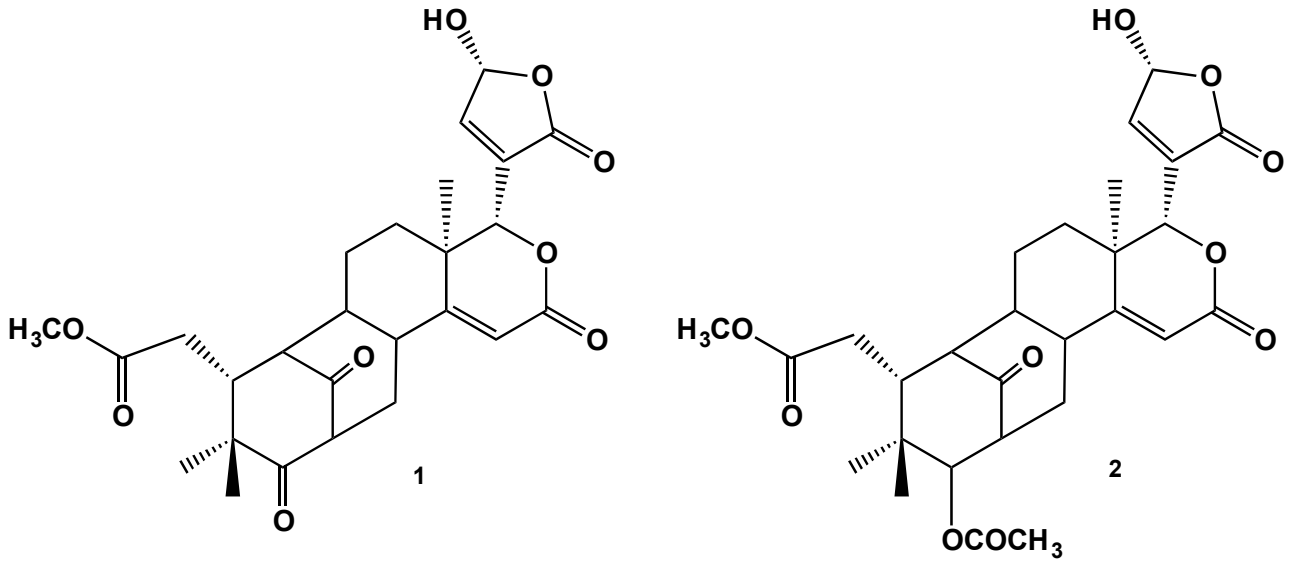
The Meliaceae family, with 50 genera and more than 1400 species, belongs to the Sapindales order, which is one of the richest and most diverse source of secondary metabolites in the Angiospermae. The most abundant secondary metabolites, produced by Sapindales families such as: Meliaceae, Rutaceae, Simaroubaceae, Cneoraceae, are the tetranorditerpenes, known as limonoids, which include different compounds variously substituted on the triterpene core structure. Limonoids are mainly distributed in the Meliaceae with a fascinating structural diversity (1, 2), deriving from a large number of cycles oxidation and rearrangement. These derivatives exhibited a wide range of biological activities on humans such as antibacterial, antifungal, antimalarial, anticancer, antiviral and a number of other pharmacological activities (3). *Aphanamixis* is an important genus in the Meliaceae family. A series of compounds were isolated from plants of this genus, such as triterpenes, limonoids, lignans, flavonoids, and alkaloids (4). In the present study, a phytochemical investigation on *Aphanamixis polystachya* (Wall) R. Parker syn. *A. rohituka* (Roxb.) Pierre leaves was performed. The dried and powdered plant material was sequentially extracted with *n*-hexane, $CHCl_3$, $CHCl_3$:MeOH (9:1) and MeOH. The *n*-hexane extract was partitioned between *n*-hexane and a MeOH:H_2O (3:2) mixture, the aqueous portion was dried and subjected to flash chromatography through Biotage Isolera™. The $CHCl_3$ extract was also chromatographed through Biotage Isolera™, while the $CHCl_3$:MeOH extract was separated on Sephadex LH-20 column. Subsequently, the fractions obtained from both flash chromatography and Sephadex LH-20 column were submitted to RP-HPLC. A lignan and four mexicanolide-type limonoids, including two new natural products (1 and 2) were characterized by 1D- and 2D-NMR and MS analyses.



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3.3 = AROMA PROFILE AND ESSENTIAL OIL CHARACTERIZATION OF FIVE HEALTHY AND INFECTED POLISH HOP CULTIVARS

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Humulus lupulus L., well-known as Hop, is a hardy herbaceous perennial climbing plant, belongs to the Cannabaceae family (1). Its geographic distribution is western Europe and temperate areas of south America and Australia, where it requires a moderate, humid, poorly and ventilated climate (*2*). Hop is a dioic plant with female (cones or strobiles) and male flowers (3). The active organs are cones that secrete a fine yellow resinous powder called lupulin. Hop is known throughout the world as the raw material in the brewing industry where only the female flowers provide a refreshing bitter taste and hoppy aroma to beer (4).
The characteristic hoppy aroma is directly correlated with the chemical composition of its aroma profile and essential oils. The aim of this work was the analysis of essential oils and volatiles of five popular Polish hop cultivars (Sybilla, Lubelski, Pulawski, Magnat and Marynka), healthy and infected by viruses and viroids. In fact, Hop mosaic virus (HpMV), Apple mosaic virus (ApMV), hop latent viroid (HLVd) are commonly found in plants of all crops commercially produced in Poland. The plant material was collected in the Institute of Soil Science and Plant Cultivation (IUNG) - Pulawy (2015), and the analysis were performed by SPME and GC-MS.
Regarding the volatile emission observed in the five healthy and infected hop cultivars, the hydrogenated monoterpenes had higher percentages in the infected cones (82.5 % - 94.3 %) than in the healthy ones (81.1 % - 91.4 %), and increased from the healthy to the infected ones, except for Sybilla (from 90.1 % in infected to 79.9 % in healthy hop). The hydrogenated sesquiterpenes had an opposed trend, except for Sybilla. *Myrcene* is the main constituent in all infected samples (29.9 % - 67 %). Especially in cultivars of Lubelski, Pulawski and Magnat, while α-humulene was present in very lower amount as well as β-caryophyllene and (E)-β-farnesene.
The EO yields related to the five healthy cultivars showed variations between 0.2% in Lubelski up to 1.1% in Marynka, very similar to those observed in the five infected cultivars. A comparison between the five healthy and infected cultivars showed that hydrogenated monoterpenes had higher concentrations in the infected plants (31.1 % - 62.2 %), except for Sybilla (from 56.6 % infected to 64.7 % healthy), while the hydrogenated sesquiterpenes exhibited a lower percentage in the infected cones (Lubelski, Pulawski, Magnat and Marynka) except for Sybilla (from 30.7 % in infected to 29.1 % in healthy plants). Myrcene is always the main chemical constituent in all the infected cultivars, except for Sybilla (from 83.6 % infected to 74.6 % healthy). The second most abundant compound in all the healthy cultivars is α-humulene (from 1.5 % to 5.7 %), while in all the infected samples was observed a decrease in its amount.
This study showed that no significant variation in the aroma profile and essential oil composition were evidenced in the healthy and infected hop cultivars, even though a significant reduction of biomass amount was observed in infected hop cultivars if compared with the healthy ones.

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3.3 = TRACE ELEMENT BIOCONCENTRATION AND TRANSLOCATION IN EDIBLE HERBS: A CASE STUDY FOR *PLANTAGO LANCEOLATA*

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The Mediterranean wild herbs are important foods widely used for the preparation of several traditional dishes, both as raw and cooked ingredients. However, they represent also a potential source of contaminants (e.g. trace elements) from environment to humans through the food chain. Thus, their consumption may be associated to several adverse effects. In this work, we assess possible contamination by trace elements in a common edible wild herb, namely: *Plantago lanceolata*. In particular, we investigated several steps of the contamination process, including the bio-concentration of elements from the soil to the roots and their translocation to the edible aerial parts of the plant.
In particular, we measured the concentrations of As, Cd, Co, Mn, Mo, Ni, P, Pb and Zn by ICP-OES in soils and in samples of roots and leaves of 15 individuals of *P. lanceolata* growing along a gradient of elemental contamination in Liguria (Italy). As a result, the elemental uptake in *P. lanceolata* seems to be controlled at different levels. The bioconcentration in the roots of Cd, Ni, P and Zn was high and it was characterized by an inverse proportionality with the concentration detected in the soil. The translocation of Co, Fe and V to the leaves was inversely proportional to their concentration in the roots, whereas other elements (Mn, As, Mo) were scarcely bio-concentrated in the roots.

3.3 = SYNERGISTIC EFFECTS BETWEEN THE ESSENTIAL OIL OF *THYMUS NUMIDICUS* POIR. AND AMPHOTERICIN B

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The development of fungal infections in patients receiving immunosuppressive therapy requires the search of new antifungal agents, especially as the available drugs such as Amphotericin B cause significant side effects during continuous use.
As part of the study of antifungal properties of algerian flora, it was analyzed the essential oil of *Thymus numidicus* Poir., an endemic from Numidia (north-eastern Algeria) used in traditional medicine in the Mediterranean area. The essential oil showed a high antifungal activity (MIC 80% = 0.695 nL/mL, Kaff = 5.232 nL/mL). GC-MS analysis shows a high rate of thymol (57%) and its precursor, p-cymene (7,55 %). Thymol is well known for its antimicrobial properties.
The concomitant use of this essential oil with Amphotericin B may be useful to decrease the side effects of this antifungal drug.
This study reveals a synergy of action between the essential oil of *Thymus numidicus* Poir. and Amphotericin B for an essential oil concentration of 0.25 µL/mL. Such a combination would be advantageous to develop a less toxic antifungal therapy.
The very small amount needed to obtain this synergy effect let hypnotize that no toxic effects will be observed in vivo, even though proper toxicity tests must be performed before every clinical trial.
Indeed, acute oral toxicity studies of thymol, one of the main constiutants of the essential oil, reveal an LD_{50} value of 2000 mg/kg in rats.

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3.3 = PHYTOCHEMICAL INVESTIGATION OF *SCHINOPSIS BRASILIENSIS*, A BRAZILIAN MEDICINAL PLANT

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Schinopsis brasiliensis Engl. is a plant belonging to the Anacardiaceae family, vernacularly known as "baraúna", "bráuna" or "quebracho". It grows in arboreal habitat and reaches dimensions of about 15 m in height. It can be found in the Brazilian semi-arid regions and it is a native species of the Caatinga and Atlantic forest (1). The "First National Report for the Convention on Biological Diversity" (CBD) has considered this plant as an endangered species, due to the extensive utilisation of its wood in general construction (1). Different parts of *S. brasiliensis*, including leaves, fruits, and mainly bark are used in Brazilian traditional medicine for the treatment of various diseases, such as: inflammation, influenza, cough, diarrhea, dysentery, and as vermifuge (2). *S. brasiliensis* has also been used as a natural antiseptic to treat wounds and superficial mycoses (3). Leaves and bark extracts from *S. brasiliensis* have been reported to possess antioxidant and antimicrobial activities, while a stem bark extract from *S. brasiliensis* and its fractions revealed molluscicidal and larvicidal activities (3).
The phenolic content of the ethanol extract of the stem bark of *S. brasiliensis* has been evaluated together with the antioxidant activity. The good antioxidant activity exhibited in the Trolox Equivalent Antioxidant Capacity (TEAC) assay (TEAC value = 3.04 mg/mL) encouraged us to investigate its constituents. An analytical approach based on LC-ESIMS^n was applied to rapidly obtain a metabolite profile of the ethanol extract of the stem bark of *S. brasiliensis* (Fig. 1). Sixteen phenolic compounds, among which five galloyl derivatives, never reported before, have been isolated and their structures have been unambiguously elucidated by extensive spectroscopic methods, including 1D (^{1}H, ^{13}C, TOCSY) and 2D (DQF-COSY, HMBC, and HSQC) NMR experiments. Moreover, the antioxidant activity of all the isolated compounds was evaluated, along with the cytotoxicity against the cancer cell lines A549 (human alveolar basal carcinoma) and Hela (human epitheloid cervix carcinoma). The new compounds isolated in this work exhibited a high free-radical-scavenging activity, in the range of 1.10-1.86 mM.
None of the tested compounds, in a range of concentrations between 12.5 and 100 □M, caused a significant reduction of the cell number.

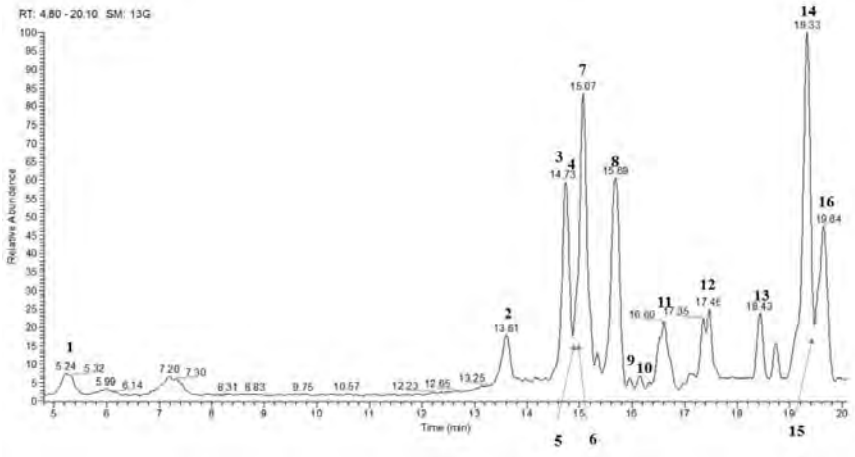

Fig. 1
LC-ESI (Orbitrap) MS profile (negative ion mode) of the ethanol extract of *S. brasiliensis* stem barks.

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3.3 = AROMA PROFILE AND MORPHOLOGICAL ANALYSIS OF *SALVIA BROUSSONETII* BENTH.

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Salvia brousonetti belongs to genus of Salvia, the largest genus of the Lamiaceae family. To the best of our knowledge no reports are present in the literature on its aroma profile and morphological behaviour. However, a few papers were reported in the literature [1-4] on the isolation and characterization of a couple of secondary metabolites.
The spontaneous volatile emission together with the essential oil composition (EO) from aerial parts of *Salvia broussonetii* collected in Sanremo (Italy) were the object of this work. A morphological characterization of the leaf trichomes was also performed. Sesquiterpene hydrocarbons (SH) represented the only chemical group in the volatile emission of leaves (95.7%) while they depicted merely 67.6% in flowers, even though remained the majority class of constituents. EO analyses showed the same behaviour with sesquiterpene hydrocarbons as major class but in lower amount.
The presence of non-glandular and glandular trichomes, such as peltate and capitate, on the aboveground organs of *Salvia brussonetii* is a characteristic feature of Lamiaceae species [5]. However, in the examined species, peltate trichomes had an unusual elongated basal cell acting as a pedestal, whereas glandular head morphology was typical and were never described before.

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3.6 = COMBINED EFFECT OF IONIZING RADIATION AND LIGHT ON MORPHO-ANATOMICAL AND NUTRITIONAL TRAITS OF SOYBEAN SEEDLINGS INTENDED AS INTEGRATORS FOR ASTRONAUTS' DIET

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For Space exploration, the realization of long-term manned missions requires the possibility to grow plants in extra-terrestrial environments. Indeed, life support in Space is based on the *in situ* regeneration of resources (e.g. air, water and food) needed by the crew that can be achieved in plant-based Closed Ecological Life Support Systems (CELSS). In such artificial ecosystems, higher plants, as every photosynthetic organism, utilize CO_2 produced by humans and provide oxygen, can utilize nutrients derived from human wastes and provide not only food, but also non-nutritive benefits as they resemble effective countermeasures against conditions of isolation which elicit behavioural changes in crewmembers (1). The integration of astronauts' diet with fresh food directly produced onboard of Space platforms can also involve the production of sprouts as easy 'vegetal systems' that can be grown in a few days, in small volume, with low energy (2). However, one of the main constraints for the establishment of extra-terrestrial outposts is the presence of high levels of ionizing radiation which affect organisms' growth. Therefore, one of the main challenge is ensuring the productivity of edible plants in CELSS even in such harsh conditions. However, plants are much more resistant than animals to ionizing radiation: doses that are detrimental for animals can be ineffective or induce positive responses in plants (3). The effects of ionizing radiation can be the result of genetic aberrations (4) and their severity depends on radio-resistance that is variable among the species and cultivar as well as among target tissues/organs. Moreover, the possible interaction between ionizing radiation and other environmental factors has been poorly investigated.
The aim of this study was to analyze the combined effect of low-LET (Linear Energy Transfer) ionizing radiation and light on the development of soybean (*Glycine max* (L.) Merr.) seedlings. Irradiation with X-rays was not performed on dry seeds, which are considered one of the most resistant stage, but on germinated seeds when the radicle was at least 1 cm long. Indeed, germinated seeds are considered delicate systems, vulnerable to ionizing radiation because of the occurrence of actively-proliferating meristems. Seedlings were irradiated with three doses of X-rays, namely 0.3, 10 and 20 Gy, and then incubated into two conditions of light, namely absence of light (dark) and presence of white light (w) for a week. Seedlings were then collected and subjected to the preparation for microscopy and biochemical analyses and to detect possible radiation-induced modifications to the structure and nutritional value of fruits under the two light conditions.
Light and epi-fluorescence microscopy observations, coupled with digital image analysis, allowed to quantify anatomical traits such as tissue and cell size, and the amount of phenolic compounds in different organs of the seedlings. Nutritional analyses were focused on anti-oxidant compounds. The overall analysis showed that the seedling-response to increasing doses of X-rays is influenced by light. Results suggested that the possible occurrence of radiation-induced positive outcomes in plants can be severely influenced by other environmental conditions: such a phenomenon should be taken into account in the shielding design of plant-based modules of bioregenerative systems.

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3.6 = CHARACTERIZATION OF BIOACTIVE COMPOUNDS IN NEW SWEET CHERRY (*PRUNUS AVIUM* L.) CULTIVARS MADE IN UNIBO THROUGH A BREEDING PROGRAMME

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Sweet cherry (*Prunus avium* L.) is one of the most popular fruits growing in temperate areas, where it is highly appreciated by consumers not only for its taste, color and sweetness, but also for its nutritional and health-promoting properties ([1]). These are due to the presence of chemical compounds, including polyphenols and antocyanins, which in the last decades have received a considerable attention by virtue of their bioactive properties. In particular, anthocyanins, the class of flavonoids responsible for red skin and flesh color, have been demonstrated to display a wide range of activities such as antinflammatory, anticarcinogenic, cardiovascular disease preventive ([2]). Italy is the fourth largest producer of sweet cherries in the world ([1]), and this can explain the strong development of researches in this field over the last 30 years, aimed at the introduction of new cultivars and orchards.
In this work, the profile of bioactive compounds (phenolic acids, flavonols, antocyanins) and *in vitro* antioxidant activity were evaluated in fruits of seven cultivars of sweet cherries, called SWEET UNIBO cherries, obtained through a program of breeding, and compared with three reference cultivars widely present on the market. Quali-quantitative analysis of antocyanins, phenolic acids and flavonols was carried out by HPLC-DAD on methanolic extracts prepared from freeze-dried fruits. The *in vitro* antioxidant activity of fruit extracts was evaluated through the ORAC assay.
Cyanidin-3-*O*-rutinoside was by far the most abundant antocyanin found in all samples, followed by cyanidin-3-*O*-glucoside, peonidin-3-*O*-rutinoside and peonidin-3-*O*-glucoside, respectively. Significant differences in their content were found among the cultivars, and four out of the seven UNIBO cultivars (Gabriel, Lorenz, Aryana and Marysa) turned out to have a higher antocyanin content compared to the reference ones, ranging from 9 to 15 mg/g DW. Flavonols content also varied among cultivars, with rutin (quercetin-3-*O*-rutinoside) as the major component. As concerns phenolic acids, neochlorogenic, chlorogenic, and coumaroyl-quinic acid were the most abundant compounds present in all cultivars, even though for these class of compounds no significant differences were observed between the Sweet UNIBO cultivars and the reference ones. Overall, total phenolic acid content did not significantly differ among the cultivars. Work is in progress for the evaluation of the antioxidant activity and for correlating the profile of functional compounds with standard qualitative traits and primary metabolites. Results will allow selecting the most promising cultivars to be tested for their health-promoting effects using *in vitro* cell models. Phytochemical analysis will be carried out during a second year of collection, in order to have a more stable picture of the functional features of the Sweet UNIBO cherries.

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3.6 = BIOACTIVE GARDEN IN THE BOTANICAL GARDEN OF FLORENCE

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The Botanical Garden of Florence has been developing, since several time, sustainable systems of plants cultivation applying biological control of parasites and weeds. In parallel a collaboration with Ortobioattivo® has been established in order to practice regenerative and bioactive growing techniques. Considering the increasing interest towards self-production of vegetables and fruits, bioactive gardens by Ortobioattivo® have been installed in the Botanical Garden of Florence with the intention to showing that it possible to grow vegetables in an easy and productive way obtaining high quality food. Bioactive gardens (Ortobioattivo®) are inspired by self regenerating fertility of tropical forests where a synergic combination between microorganisms, organic compounds and minerals in the soils is established. Ortobioattivo® uses layers made of organic material, chopped woods, volcanic sand rich in iron, "starter" layer and permanent mulching. The "starter" layer is made by a mix of fermented plants extracts, rock dust, vermicompost, effective microorganisms, mycorrhizal mushrooms, Tea Compost, biodinamic compound 500, Bokaschi, lactic ferments.
Greens grown with this method have proved to have higher nutritional, bioactive and nutraceutical qualities: in order to measure the improved quality of products a research conducted with the Biological Department of the University of Florence has been started comparing Ortobioattivo® system with two controls substrates.
The installation of an Ortobioattivo® garden in the Botanical Garden of Florence has also a demo function. In fact, it is possible to give information and suggestions to citizens who are willing to apply first hand a sustainable growing technique that can provide multiple advantages: the system is ergonomic because elevated; it is easy to build and easy to maintain because doesn't require mechanical work, not even in larger extension; is applicable on urban areas, both large and small ones like balconies obtaining bio-intensive production; captures CO_2 into the ground; permits to avoid the use of chemicals; reduces nitrites' concentration; promotes the use of local materials; regenerates soil's microorganisms; provides the soil with self-generating fertility; allows to grow vegetables with longer shelf life.
Finally, the same techniques can be used on different collection of a Botanical Garden, reducing numbers of repotting, fertilizations, treatments and obtaining stronger, healthier and luxuriant plants.

Fig. 1
Bioactive garden in the Botanical garden of Florence

3.6 = MULTI-CLASS POLAR LIPID PROFILING OF *CORYLUS AVELLANA* (CULTIVAR "TONDA DI GIFFONI") HAZELNUT KERNEL BY LC-ESI/LTQORBITRAP/MS/MS^N

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Corylus avellana L. (Betulaceae) is the most famous nut tree. The hazelnut world production accounts for about 800,000 tons, with Turkey being the leading producer (64%), followed by Italy (13%) (1). The kernel of the hazelnut seed is edible and has a thin, dark brown skin, which sometimes is removed before eating. Only a small part of hazelnuts are consumed as whole nuts (fresh or roasted); mainly, they are processed by the food industry to obtain a great variety of products, including bakery goods, cereals, snacks, ice creams, various dessert formulations and chocolates.
Several research groups have reported the benefits of inclusion of nuts in the human diet. In particular, hazelnuts (*Corylus avellana* L.) have a high nutritional value due to the presence of lipids (61 % w/w), carbohydrates (16.7 % w/w), proteins (14.9 % w/w), water (5.3 % w/w), sugars (4.3 % w/w), and dietary fiber (9.7 % w/w) (2,3). Thereby, due to their nutritional and nutraceutical properties, Food and Drug Administration (FDA) has recognized hazelnuts as "heart-healthy" foods (4). In particular, the oil extracted from hazelnuts has proved to be able to decrease cholesterol levels in blood and to control adverse effects of hypertension. This may be due to the favourable hazelnut oil lipid profile, highly rich in MUFA (primarily oleic acid), PUFA (primarily linoleic acid), tocopherols and sterols (5). In Italy there are two hazelnuts registered with the mark of Protected Geographical Indication (PGI): "Nocciola del Piemonte" and "Nocciola di Giffoni". The latter is a cultivar of the Campania region (Tonda di Giffoni, 'TG'), which contributes largely to the production of national hazelnut, of which it accounts for about a third.
Considering that until now no comprehensive analysis is available about polar lipids of 'TG' *C. avellana* hazelnut kernel, in the present work a multi-class lipid profiling of the *n*-butanol extract of fresh 'TG' hazelnut kernels (without skin) was performed by using an analytical approach based on high-performance liquid chromatography coupled to positive and negative multiple-stage linear ion-trap and orbitrap high-resolution mass spectrometry (LC-ESI/LTQOrbitrap/MS/MS^n). This methodological approach enabled the analysis of a wide range of compounds from oxylipins to intact high molecular weight lipids, such as sphingolipids and glycerolipids (i.e. phospholipids, diglycosylated monoglycerides, and sulfoquinovosyldiacylglycerols).

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3.6. = SEEDS FOR EVERYONE'S TASTE

Alberta Cascini[1], Simona Pappalardo[2], Federica Grillo[2], Luca Mastracci[2], Laura Cornara[1]
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The last decade or so has seen an exponential rise in vegetarian and vegan diets, with increased consumption of fruit and vegetables, particularly berries and others small fruits full of antioxidants. Concurrently, the use of seeds, both cooked and raw, such as sesame, millet, poppy seeds, quinoa, hemp, etc. has also become fashionable in order to flavour breakfast, salads or soups. All these dietary changes have probably led to an increase in involvement of seeds, as foreign bodies, in different gastrointestinal diseases such as appendicitis and diverticulitis; indeed seeds have been reported as the third most frequent foreign object found in appendicectomies (1).
The identification and the distinction between edible seeds and other possible etiologies, such as gastrointestinal parasites, may be a problem as the size and the anatomical structure of these two different foreign bodies can be, at first sight, similar. Distinction between seeds and helminths is mandatory (2), as intestinal parasites require treatment.
In particular small sized seeds, found in histological sections of appendixes surgically removed due to appendicitis, characterized by having a curved embryo, are reminiscent of the structure of intestinal parasites such as *Anisakis* and *Enterobius vermicularis*. A comparison between a tomato seed (*Lycopersicum esculentum*) in the appendiceal lumen and an encysted *E. vermicularis* (parasite in its quiescent stadium), is shown in figures 1 and 2, respectively. A quinoa seed section (*Chenopodium quinoa*) and a section of an *E. vermicularis* (structure of a live parasite shown) are shown instead in figures 3 and 4, respectively.
In conclusion, appendicitis and its complications are a common problem affecting patients of all ages and ethnicities and the possibility of obstructive appendicitis secondary to seed ingestion should always be considered, especially in patients with large consumption of fruits, vegetables and cereals. Nevertheless, the increasing consumption of raw or undercooked fish and meat means that patients are also more exposed to parasitic infections (3) and this differential diagnosis is becoming ever more important.

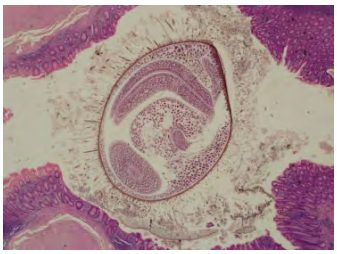
Fig. 1

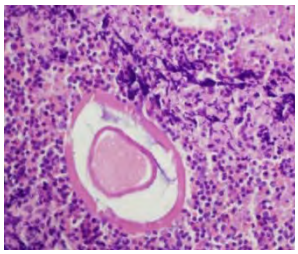
Fig. 2

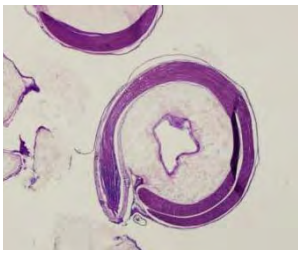
Fig. 3

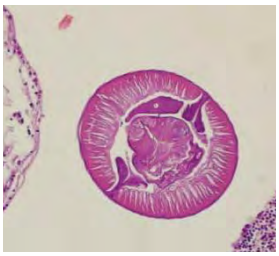
Fig. 4

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3.6 = THE PROBLEM OF MISIDENTIFICATION BETWEEN A WILD EDIBLE PLANT AND A POISONOUS ONE: THE CASE OF BORAGE

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At present, in many European countries people is looking for wild edible plants to experience new tastes and flavors, by following the new trend of being green and environmentally friendly (1). *Borago officinalis* L. (borage) is an annual herb originating in the Mediterranean region but naturalized and widely cultivated throughout most of Europe, traditionally used for culinary and medicinal purposes. Its leaves are mixed in salads and used as a vegetable in different European countries, such as Germany, Spain, Greece, and Italy (2), especially in Liguria as a stuff of traditional *ravioli* and *pansoti* (3). However, young borage leaves can be easily confused by inexpert pickers with those of other plants, including poisonous ones, such as *Mandragora autumnalis* (mandrake), common in Southern Italy and Sicily, or *Digitalis purpurea* (foxglove), in Northern Italy. Patients who turn to Italian Poison Control Centers or Hospital Emergency Rooms after accidental ingestion of these plants, show anticholinergic symptoms due to unintentional ingestion of the leaves of mandrake (most commonly) or foxglove (less frequently). In the period 1995-2007, 50 cases of intoxication by accidental ingestion of mandrake and 6 cases after ingestion of foxglove have been reported in Italy (1). In the present work we show the pharmacognostic characterization of young leaves from *B. officinalis* (Boraginaceae), *M. autumnalis* (Solanaceae), and *D. purpurea* (Scrophulariaceae). Micro-morphological, phytochemical and molecular identification techniques were used. Fresh leaf samples were analyzed by optical and scanning electron microscopy, highlighting main anatomical and histological features, such as stomata and trichome types and distribution (Fig 1, A-C). DNA barcoding sequences (using a region of the plastidial RbcL as DNA marker), analyzed using a bioinformatics tool (MEGA v. 7.0), allowed to determine the intra- and inter-genetic variability (K2P distance) among the three taxa. No genetic variation was detected within species, while consistent genetic distances were observed among species: borage vs foxglove K2P = 7.46%, foxglove vs. mandrake K2P = 7.46% and borage vs mandrake K2P = 7.46%. GC-MS analysis of fresh leaves pentane/hexane extract (5:1, v/v) revealed a typical chemical fingerprint of each plant analyzed and a particularly interesting difference between poisonous and non-poisonous plants. In fact, *M. autumnalis* and *D. purpurea* leaf extract contain vitamin E, while in *B. officinalis* this metabolite is absent. Therefore, the authors conclude that, in this case, the presence of this metabolite is discriminant among poisonous and edible plants and could be used as a phytochemical marker, while among the poisonous ones becomes useful analyze the discriminant wax alkanes to differentiate them.
This study provides a multi-disciplinary approach to the problem of misidentification between wild edible plants and poisonous species. The reported protocols provide fast and reliable determination of species causing poisoning, allowing a quick management of poisoned patients.

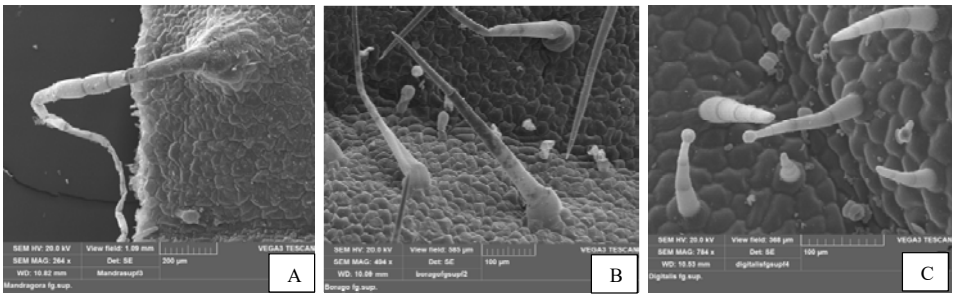

Fig. 1 A-C
Comparison among covering and glandular trichomes of the upper leaf surface of mandrake (A), borage (B) and foxglove (C).

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3.6 = FIRST RESULT OF MORPHOMETRIC ANALYSIS ON DIFFERENT POPULATIONS OF SOLINA GRAIN (*TRITICUM AESTIVUM* 'SOLINA')

Valter Di Cecco[1], Michele Di Musciano[1], Andrea Ianieri[2], Marco Di Santo[3], Luciano Di Martino[3], Simona Casavecchia[2], Donato Silveri[4], Anna Rita Frattaroli[1]
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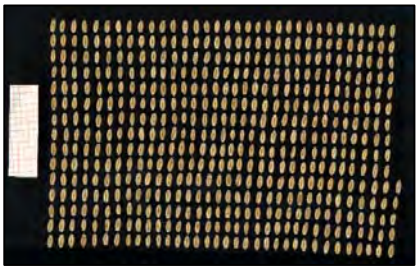
Solina is a common wheat variety cultivated in mountain areas of central Italy (mainly in the Abruzzo region). Cultivation has been documented since the 16th century (1) and it is adapted to sustainable agriculture carried out in marginal areas with cold climates; therefore Solina-based products are of interest for consumers, territorial sustainment, and biodiversity (2).
Aim of this study was (I) analyze the variability of morpho-colorimetric seed features, (II) implement statistical classifiers able to discriminate among the studied population, (III) verify whether from a morpho-colorimetric point of view the different sites studied can be attributed to one or more varieties, and (IV) increase the knowledge about the variation of the current extant Solina seed collections.
They have been examined 500 seeds from nine different Abruzzo locality: Barisciano, Castel del Monte, Sant'Elia, Rocca Pia and Castelvecchio Subequo in the Aquila province; Decontra, La Canale and San Nicolao in the Pescara province and Roccaspinalveti in the Chieti province.
The images were acquired through the application of computerized analysis techniques, using a flat scanner and Image J software (3, 4). On each single caryopsis, 30 descriptive parameters of shape, size and color were measured. Of these, the following were selected: Area, Perimeter, Mean gray value [Average gray value within the caryopsis], Width, Height, Circularity, Aspect ratio, Roundness, Solidity and Weight [10 replication of 20 seeds each].
For the statistical analysis we used the main features analyzed by ANOVA followed by post-hoc Tukey test. The morpho-colorimetric differentiation among localities were analyzed by Linear Discriminat Analysis using R software.
The results have shown a separation of seeds that came from different localities. This first characterization of the variety in central Apennines, cannot confirm the existence of different types of Solina seeds. This work want make the first step to investigation the variability inside of the Solina variety, follow the historical knowledge that confirm the presence of different local ecotypes (5).
For the future studies we want to consider other localities along all Apennine farm communities.



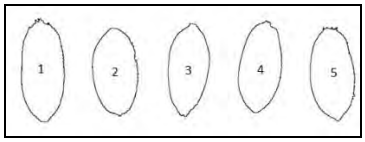


Fig. 1, 2, 3
Image of Solina caryopses acquired with the flat scanner; Digitized caryopses through ImageJ software; Detail of the caryopsis.

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3.6 = CULTIVATION OF MUSHROOM ON FOOD WASTE: A POSSIBLE SOLUTION TO IMPROVE THE LIFE SUPPORT SYSTEMS (LSS) DURING LONG-TERM MISSION AND SPACEFLIGHT

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The past 50 years were marked by numerous and critical advancements in space exploration. Progress has been such that the goals of space agencies include long-term spaceflight and planetary missions (such as a mission to Mars). One of the challenges is the management of life-support systems (LSS). For many years, scientists have been focusing on the optimization of on-board LSS efficiency. Concerns to be faced include, for instance, the difficulties of food stock management and waste recycling. One way of addressing those could be combining several organisms to create a balanced biological system on-board. In such a system, inedible parts of plants are among the most difficult elements to recycle and make "closing the recycling loop" difficult. A solution could be using edible white rot fungi: those can decompose cell-wall-associated macromolecules and digest lignin polymers more efficiently than bacterial organisms. The cultivation of saprotrophic species of mushrooms may consequently be helpful to recycle a considerable part of organic waste. Even though industrial cultivation of several edible species of mushrooms is a standardized process, the same operation may be complicated during a space mission by constraints on resources such as expertise, crew time, space, and volume.
In light of this, we tested the cultivation of the edible mushroom *Pleurotus ostreatus* (oyster mushroom) in long-term spaceflight analogous conditions, during the fourth Hawaii Space Exploration Analog and Simulation (HI-SEAS IV). We performed a preliminary experiment whose goal was to set up a low-technology protocol for edible mushroom cultivation in LSS. We tested fungal substrates added with three different concentrations of food waste (0%, 20% and 30% by weight). Six boxes containing inoculated substrate (straw) were sent to the HI-SEAS habitat, where three of them were mixed with food waste. All boxes were then cultivated for a period of 20 days. Replicate cultures were performed in a laboratory at the University of Genoa.
Basidiomata were produced at each concentration of food waste, thus supporting the possibility to exploit fungi to recycle food waste during long-term space missions. This preliminary experiment also highlighted several issues related to cultivation management, contamination, and integration of fungi in a LSS. Future experiments will aim at i) testing the cultivation of fungi in LSS using specific devices to better control culture conditions, and ii) further assessing the growth rates and basidioma production, on substrates supplemented with different types of food waste, in the conditions of a long-term mission.

3.6 = SAFFRON (*CROCUS SATIVUS* L.) QUALITY OF THE ALPINE AREAS

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Saffron is the most expensive food spice used in the food industry and is obtained by drying the pistils of *Crocus sativus* L. flowers (1). The quality of saffron is determined according to ISO 3632 1,2:2010-2011 which classifies the spice in three different categories (I, II, and III) depending on the amount of crocins (colouring strength), picrocrocin (flavour or bitterness strength) and safranal (aroma strength) (2).
In recent years, there has been a greater interest in this spice and consequently an increase in its production, especially in mountain regions like the Alps (3). This has been mostly evident where land was abandoned due to emigration to the cities. This new trend might have a strong impact on the valorization and economical recovery of mountain areas.
In the present study we investigate the quality of saffron produced in the Italian Alps (Fig. 1) by using a spectrophotometric analysis according to ISO 3632 1, 2:2010-2011. All saffron samples (n=29) were produced between November 2015 and March 2016 in different areas of the central Italian Alps at an altitude up to 1143 m a.s.l.
The results confirmed a high quality of the spice as most of the samples belong to the "I Category of quality" (Fig. 2) according to the ISO classification. Thus, our study provides valid information about the quality of saffron produced in the Alpine areas and suggests this crop as a powerful and strategic resource for the mountain economy. Moreover, it brings sustainable economic value to multifunctional farms in mountain areas such as the Alps.

Fig. 1
Origin of saffron (*Crocus sativus* L.) samples.

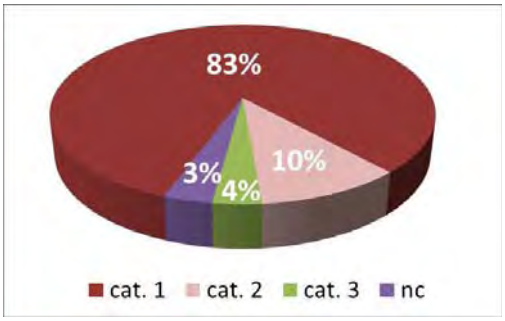

Fig. 2
Representation of saffron samples under the corresponding quality category.

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3.6. = LIGHT QUALITY ENHANCES ANTHOCYANINS MODULATION IN RED AND GREEN CULTIVARS OF *ATRIPLEX HORTENSIS* L.

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Red leaf plants have aroused intriguing scientific debates over the last few years, not only to investigate their ecological role but especially to assess and improve their nutritional value as food plants. In most species, red colour in leaves is due to the accumulation of anthocyanins, the red pigment known for its antioxidant properties and therefore considered as an additional high nutritional value of red-leaf food plants.
New research projects aimed at producing high quality food plants are supported by both Italian (ASI) and European Space Agencies (ESA) because plants represent a key element in bioregenerative life support systems, such as MELiSSA (Micro-Ecological Life Support System Alternative), not only for the water/air/waste recycling but also for the production of high quality plant food. Increasing attention is paid at such features in the selection of crop species aimed to become fresh food for astronauts in Space environment.
Previous studies indicate that anthocyanins carry out several functions in plants, representing the nature's Swiss army knife. A role in photoprotection is widely accepted, although this hypothesis is not always applicable and several exceptions are mostly explained ecologically by considering plant/herbivores interactions or various stress responses (1, 2). From a chemical and physical point of view, these water-soluble pigments, generally located within cellular vacuoles of epidermal and/or external mesophyll cells (Fig. 1), strongly absorb light in ultraviolet, blue and green wavelengths. The hypothesis was that high-energy wavelengths stimulate anthocyanins production to protect photosynthetic tissues, thus chloroplasts, from energizing wavelengths that commonly induce inhibition phenomena of the photosynthetic apparatus.
Interestingly, green crop species grown under different light spectra are reported to modulate the synthesis of various compounds, including anthocyanins (3, 4). The general aim of our work was to apply the LED-based plant cultivation systems to maximise nutritional value of red-leaf plant food. More specifically, we grew green and red cultivars of *Atriplex hortensis* L. under different combination of light wavelengths (Fig. 2) to evaluate *whether* and *how* leaf anthocyanins content varies depending on light spectra. Our purpose was to understand plant's response to different light wavelengths, especially in this valuable microgreens species intended for human diet. Our results showed that there is an increase in the foliar anthocyanins amount related to the amount of blue light within the incident light spectrum, especially in red leaf cultivar. Moreover, the combination of red and blue wavelengths stimulates biomass production and photochemical efficiency in both varieties of *Atriplex hortensis* L. These results provide useful informations to improve quality of plant food, without neglecting plant productivity, by using specific light spectra. Considering that anthocyanins content is significantly influenced by light quality, red leaf plants grown with adjustable lighting systems represent a promising biological system for high quality food production.

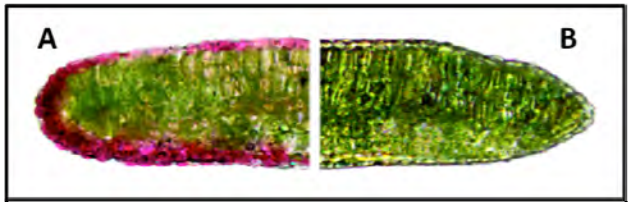

Fig. 1
Leaf section of *A. hortensis* L., red (A) and green (B) cultivars.

Fig. 2
Red and green cultivars of *A. hortensis* L. grown under four light treatments.

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3.6 = NEW ETHNOBOTANICAL RECORDS ON FOOD PLANTS IN THE TERRITORY OF SOUTHERN ASPROMONTE (REGGIO CALABRIA – SOUTHERN ITALY)

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Ethnobotany is the science that studies the plants of a territory and their popular use through the traditional knowledge handed down by local culture. From the past, the use of spontaneous and cultivated plants was an irreplaceable factor for the survival of populations. Various were the use, including: phytoalimurgy, handicraft, phytotherapy, cosmetic, ornamental, magical and religious. In recent years several studies in the Mediterranean territory, have argued that the use of spontaneous plants plays an important role in local food traditions (1, 2, 3, 4, 5 6). The main purpose of this work was to collect and analyze the traditional uses of plants in the food field so as not to miss what is the great value of many of them. The ethnobotanical survey was carried out on the Southern Aspromonte (Calabria). The information was collected between 2012 and 2016, with the help of structured interviews in the field, using a paper sheet divided into five parts: generality of the interviewee, plant name, uses and purposes, information on spontaneous species and information on the cultivated species. In addition to food uses, other types of uses were recorded but they are not reported here. The collected data were subsequently stored in a database created through "Microsoft Office Access"©. They were considered the interviews of 51 people. From these, 62 species of vascular plants, both spontaneous and cultivated, have been identified for which 157 different types of food have been recorded. Of the various species, the following uses were very interesting and unusual in other territories.
Fruit peel and cladodes of *Opuntia ficus-indica* (L.) Mill. were coated with breadcrumbs and fried after having removed the spines. Fruits of *Fragaria vesca* L. was cut, put into the pan and sauted, then added the rice: everything everything was stirred and nuanced with red wine. The leaves of the nettles (*Urtica membranacaea* Poir. ex Savigny) were boiled and cooked together with rice. In addition, this species was used in the filling of tortelloni instead of spinach. The latex produced from fig branches (*Ficus carica* L.) was used as a rennet in the preparation of ricotta. The flowers of *Hibiscus rosa-sinensis* L. were added to the batter of the pancakes or in the cooking sauce. The capitula of *Cynara cardunculus* L. were deprived of spines and boiled, then joined to eggs and cheese to prepare an omelette. There are also many species whose young sprouts are used as salads or soup preparations. *Crepis vesicaria* L. and *Hyoseris radiata* L. were boiled with other vegetables such as tomatoes, carrots, celery, to prepare soups. The tuber of *Helianthus tuberosus* L. was cut into slices and cooked together with water, oil, pepper, salt and parsley and chopped to make a sauce. The leaves of *Hypochoeris achyrophorus* L. are seasoned in salad. Another important group of species are used to flavor foods and beverages. *Mentha aquatica* L. was used for the preparation of a liqueur: 85 leaves immersed in ethyl alcohol then added to a decoction of sugar and water and finally we have to wait another 10 days before the consumption. The inflorescences of *Thymbra capitata* (L.) Cav. were dried and used to flavor both meat and fish. *Foeniculum vulgare* Mill. subsp. *piperitum* (Ucrìa) Beg., however, has several uses: dried fruits were used to flavor bread dough, the olives in brine, while new sprouts were added to flavor soups, sauces and risottos.
This ethnobotanical investigation has made it possible to highlight the close link between plants and people in this area. The whole survey had as its point of reference the phrase "as it was once", so that it can return to the present, making it a resource for greater sustainability of the territory. From this information, we can draw for the future, so that the natural resources of our planet are preserved and valued at the local as well as globally.

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3.6 = GENETIC AND METABOLIC PROFILE OF WILD CARDOON POPULATIONS (*CYNARA CARDUNCULUS* L. SUBSP. *CARDUNCULUS*) IN CALABRIA REGION

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Cynara cardunculus L. belongs to the genus *Cynara*, - (Asteraceae) and is a diploid form (2n = 34), predominantly cross-pollinated (1-2). This species includes three different taxa: the perennial wild cardoon, variety *sylvestris* Lam., which, only recently, has been recognized as the ancestor of both cultivated forms, leafy or cultivated cardoon, variety *altilis* DC., and the artichoke variety *scolymus* (L.) Hegi (globe artichoke) (2-5).
The interest in this species also derives from its enormous potential both for nutraceutical and culinary applications. In fact, the plants of *C. cardunculus* L. are rich in antioxidant compounds, including both polyphenols and flavonoids, namely caffeic acid, chlorogenic acid and its derivatives, dicaffeolchinic acids including cinarine (6-9). More than 80% of the approximately 130.000 ha of artichoke grown in 2009 is found in the countries bordering the Mediterranean (10). Currently, artichoke cultivation is mostly based on the use of local populations (11), often characterized by wide genetic variability (12-13) and a high degree of heterozygosity, so recourse to agamic (vegetative) propagation is obligatory.
The wild variety, is well represented in the Calabria region – (Southern Italy) and still widely used as a traditional food. However not much data regarding the genetic structure of these populations are present in the literature. Starting from this *scenario*, the main aim of this work was to characterize at both genetic and metabolic level some natural populations of *C. cardunculus* L., putatively referred to as a wild cardoon, in the oriental region of the National Park of Pollino. The long term aim is to preserve biodiversity and promote ecotypes which exhibit major nutraceutical proprieties. To achieve this goal microsatellite (SSR) markers combined with HPLC analysis and with the *in-vitro* DPPH (2,2-diphenyl-1-picrylhydrazyl) assay and *β*-carotene bleaching test was applied.
The preliminary results deal with the genetic variation present at SSR loci assessed in three Calabrian populations of wild cardoon, compared to one cultivated and one wild Sardinian genotype, provided by the Department of Heart Science, Botanic section, from University of Cagliari (Sardinia, Italy). Five to thirty individuals, randomly sampled for each population were genotyped at 6 SSR loci. Genetic distance was estimated both within and between populations. Obtained results showed that most of genetic variation was partitioned within rather than amongst wild populations. Moreover, the examined Calabrian populations seem to cluster around the wild taxa. These results are in line with data related to phytochemical characterization, conducted through HPLC, which allowed us to estimate the level of several polyphenols (gallic acid, chlorogenic, vanillic, siringic, *p*-coumaric and ferulic acid) and flavonoids (epicatechin, mirycetin, lutein, quercetin, esperetine and kaempferol) usually present in *C. cardunculus* L. (14). Finally, the phytochemical variations, among the three wild Calabrian populations, was well linked to differences in antioxidant activity observed during the *in-vitro* experiments.

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3.6 = CRENATE BROOMRAPE (*OROBANCHE CRENATA* FORSSK.): FROM PARASITIC PLANT TO TRADITIONAL AGRI-FOOD PRODUCT OF PUGLIA (SOUTHERN ITALY) WITH INTERESTING NUTRITIONAL TRAITS

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Crenate broomrape (*Orobanche crenata* Forskal), also known as bean broomrape, is a root parasite plant that produces devastating effects on many crop legumes and has become a limiting factor for faba bean production in the Mediterranean region. However, some authors have reported its use as a food or in folk medicine, but the only information available on crenate broomrape potential as a valuable resource for human nutrition and not just as a pest is scarce and fragmented. For example, crenate broomrape is interesting for its content of phenols that are both flavour components and healthy compounds as antioxidants[1]. Nevertheless, to our best knowledge the literature lacks information with regard to the characterization of the crenate broomrape.
In some areas of Puglia (Southern Italy), crenate broomrape is considered a wild edible plant used to prepare several traditional dishes. This thanks to the quick wit of farmer that has transformed this parasite "from an enemy of the fields to a friend of the dining table". Traditionally, the stems of crenate broomrape are usually cleaned, washed (Fig. 1) and boiled in water and salt. After boiling, the stems are soaked in water for about 12–24 h to reduce the bitter taste before being used as an ingredient in several dishes (Fig. 2).
The culinary use of crenate broomrape in Puglia has ancient origins and is linked to food scarcity as well as to the poverty to which the ancestors of the current inhabitants were exposed. Indeed, the parasite was a real disaster for the main legume crops, which were often the only source of protein for human nutrition. Therefore, eliminating crenate broomrape from cultivated fields enabled farmers both to preserve a significant crop and to gain an extra food source for their families. It is likely that the similarity of the young stems of *O. crenata* to large asparagus was a decisive factor in harvesting and consuming this species.
Starting from these considerations, in the present study a compositional analysis of crenate bromrape was performed. The general goal was to assess some nutritional traits, such as lipids, proteins, carbohydrates, fiber and mineral content, in order to furnish an overview and evaluate the potential of this foodstuff as a traditional agri-food product for human nutrition.

Fig. 1
Stems of crenate broomrape cleaned and washed before cooking.

Fig. 2
Salad of crenate broomrape with virgin olive oil, vinegar, mint and fresh garlic.

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4.1 = AN ASSESSMENT OF GEOGRAPHIC SAMPLING IN WIKIPLANTBASE #TOSCANA

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Wikiplantbase #Toscana is an online database of georeferenced floristic data for Tuscany (1, 2), currently storing more than 150K occurrence records. This growing body of data is used to support biodiversity-related researches (3, 4). In the perspective of further studies on the distribution of the Tuscan flora, we started a work to assess the completeness of geographic sampling in the database.
To this aim, we calculated species richness (SR) and the number of occurrence records (OR) in a regular grid of 431 cells with approximate 10 × 10 km size; then, we calculated the upper (95^{th}) and the lower (5^{th}) percentiles of SR and OR from a) an empirical density function, and b) a fitted distribution. Finally, we used these values as thresholds to rank grid cells accordingly. In addition, we compared the identified thresholds with the expected values by fitting an asymptotic regression to taxa as response and records as predictor and mapped the cells exceeding the thresholds.
SR ranged from 0 to 1172, OR from 0 to 6320. Threshold values are consistent across the different methods (empirical density function: for SR, lower threshold = 4, upper threshold = 687, for OR 4–1350; fitted distribution: for SR 3–665, for OR 2–1127), however the values derived from the fitted distribution are lower than those derived from the empirical density function and expected SR values according to the asymptotic regression are lower than the observed ones. On the other hand, the maximum SR value observed lies approximately 20% below the predicted maximum SR (= 1392) according to the asymptotic regression.
As regards the geographic pattern of SR and OR, cells with high OR tend to cluster around sites traditionally known to be highly rich in species: on mountain ranges (e.g. Apuan Alps and Tuscan-Emilian Apennines), along the coast, and in the Tuscan Archipelago. High OR values generally correspond to high SR; however, in Monte Pisano, Monterufoli-Caselli, Monte Leoni, Val di Merse, Tuscan-Emilian Apennine, and hills north of Florence, high SR is matched by an intermediate OR value, i.e. SR exceeds the upper threshold while OR lies between the lower and upper thresholds. In part of the Tuscan Archipelago and part of the Apuan Alps, OR lies above the upper threshold while SR falls below it.
Cells with low SR and low or intermediate OR are located mostly along the regional inner boundaries and in the southern provinces.
This pattern mostly reflects incomplete data entry and inadequate sampling (the so-called "botanist effect"; 5), but to some extent it also depend on niches diversity matching real floristic knowledge. Further steps must be taken to discriminate the possible drivers of this pattern by taking into account appropriate environmental predictors. In any case, much work (data entry, field work, or both) is needed to provide floristic records for cells with insufficient coverage, so as to lessen the wide gap between the upper and lower thresholds of OR and SR.

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4.1 = A FIRST CONTRIBUTION TO THE KNOWLEDGE OF THE URBAN FLORA OF MODENA

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Apart from some sporadic observation, mainly performed during the floristic exploration of the entire provincial territory, the sole investigation published concerning the urban flora of Modena is a list of 69 species, mostly ruderal (1). In this study, carried out from February to December 2014, only the northern part of the historic town centre was considered. All the roads and public squares were sampled, excluding parks, gardens and private courtyards (2). Only vascular plants growing in sidewalks, bicycle paths, roads, pavements and their fissures, flowerbeds, house walls up to a height of 50 cm above the ground were recorded. Cultivated ornamental species were recorded only in case of ascertained propagation by seed. During the study period, 8 complete samplings of the whole study area were performed, in order to cover the entire vegetative period. Globally, 267 specific and subspecific taxa were recorded. Most of them were ruderal synanthropic species, typical of trampled areas (*Polycarpon tetraphyllum* L., *Polygonum aviculare* L., *Portulaca oleracea* L., etc.), others were species typical of grasslands or cultivated fields (*Bellis perennis* L., *Cynodon dactylon* L., *Cynosurus cristatus* L., *Festuca rubra* L., *Setaria viridis* L., *Stellaria media* L., *Taraxacum* F.H.Wigg. sect. *Taraxacum*, *Trifolium*, etc.), others were typical rupiculous species (*Cymbalaria muralis* G.Gaertn., B.Mey. & Scherb., *Parietaria judaica* L.). It is worth noting that 5 species resulted new for the provincial flora: *Berberis aquifolium* Pursh, *Lobelia erinus* L., *Mollugo verticillata* L., *Solanum pseudocapsicum* L., and *Viburnum tinus* L. All of them are alien species for the provincial flora and, in many cases, they are alien also for the flora of Italy (3, 4). Apart from *M. verticillata*, they are ornamental plants whose presence can be considered as casual, likely escaped from culture in parks and gardens. Further interesting species were *Dianthus armeria* L., protected at a regional level (Emilia-Romagna L.R. 2/77), *Fumaria capreolata* L., last found in 1883, and *Sonchus arvensis* subsp. *uliginosus* (M.Bieb.) Nyman, infrequent if compared to the nominal subspecies (4). Among all the recorded taxa, 19% of them are allochthonous neophytes, mainly coming from the New World (*Galinsoga quadriradiata* Ruiz & Pav., *Gleditsia triacanthos* L., *Phytolacca americana* L., *Symphyotrichum squamatum* (Spreng.) G.L.Nesom, etc.). The percentage of alien flora is not so elevated, if compared to other European cities where alien species can reach up to 50%. Probably, the reason for such a low percentage can be the climate of Modena, that shows some sub-Mediterranean features allowing Mediterranean species to establish and grow: this fact perhaps subtracts space to alien and invasive species. Among aliens, 6 are invasive (3): *Ailanthus altissima* (Mill.) Swingle, *Ambrosia artemisiifolia* L., *Buddleja davidii* Franch., *Erigeron canadensis* L., *Panicum dichotomiflorum* Michx., and *Robinia pseudoacacia* L. It is worth noting that species richness, due to the great variety of environments typical of the cities, can reach notable values (up to 145 species in the same road). Thanks to a comparison with herbarium samples preserved at MOD, we noticed that certain species (e.g., *Amaranthus deflexus* L., *Broussonnetia papyrifera* (L.) Vent., *Diplotaxis tenuifolia* (L.) DC.) are present in Modena since the late 1800: at that time, they were collected on the city walls. This study demonstrates the utility of such investigations, to increase the floristic knowledge of the territory and to discover and monitor the presence of new alien species that is becoming more and more common in a globalized world. The southern half of the historic centre of Modena will be analyzed in the next years, so to have a more complete picture of the urban flora.

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4.1 = FIRST REPORT OF *CYCLAMEN AFRICANUM* BOISS. & REUT. IN ITALY

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During field investigations in the north-western part of Sicily some peculiar populations of *Cyclamen* with autumnal flowering were identified. Actually, in Sicily are known only two species of the genus *Cyclamen*: *C. repandum* Sm. with spring blooming and *C. hederifolium* Aiton with autumnal blooming (1). Inside the last species some authors (2, 3) described several taxa with uncertain taxonomic value, such as *C. hederifolium* subsp. *confusum* (Grey-Wilson) Grey-Wilson, *C. hederifolium* subsp. *crassifolium* (Hildebr.) Culham, Denney & P.Moore, etc. The surveyed populations differs from the typical *Cyclamen hederifolium* for several features, such as the size and the morphology of tuber, leaves and flowers, which allow to refer them to *Cyclamen africanum* Boiss. & Reut. This species is reported only for the northern part of Algeria and Tunisia (4, 5) and shows very close relationships with *C. hederifolium*, so much so that Ietswaart (6) treats it only at subspecies rank. In particular, the most important distinctive characters of *C. africanum* concern the tuber, which is more rounded and rooting all over its surface (while in *C. hederifolium* is flatter and rooting mainly on the top and the shoulders, Fig. 1), the leaves which are larger in size and fleshy, the corolla which have very long lobes (Fig. 2) in comparison with *C. hederifolium* (2). Also from the ecological point of view, the two species are well differentiated, since *C. hederifolium* grows mainly in the undergrowth of oak forests, while *C. africanum* prefers more arid environments such as cliffs and sunny slopes. The Sicilian specimens were compared with the Tunisian populations and result morphologically very similar, while the chromosome number remains to be verified. This species in Sicily is currently known only for three stands in the Trapani province, where it is mainly found in rocky areas and sometimes also in the underlying scrub of coastal or inland areas. The populations are quite consistent from a numerical point of view, but further investigations are needed to clarify its effective distribution in the region.

Fig. 1
Tubers of *Cyclamen hederifolium* (left) and *C. africanum* (right).

Fig. 2
Flowers of *Cyclamen africanum* (left) and *C. hederifolium* (right).

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4.1 = HISTORICAL AND RECENT INVESTIGATION OF VAL GRANDE NATIONAL PARK'S VASCULAR FLORA (PIEMONTE, NW ITALY)

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Val Grande National Park (PNVG), located in the Lepontine Alps (NE Piedmont), is notoriously known as the largest wilderness area in Italy, established in 1992. As a consequence of the dramatic depopulation occurred after the Second World War, the traditional management of the territory changed, with abandonment of alpine summer pastures and spontaneous reforestation. An area of difficult access, Val Grande National Park appears to be scarcely investigated from a botanical point of view, even though floristic surveys occurred during the past centuries (e.g., 1, 2, 3, 4). After the institution of the protected area, floristic investigation increased, although concentrated in limited areas (5, 6) or on specific topics (7, 8). Since an updated checklist regarding the whole park's territory is still lacking, in 2016 the National Park funded and supported new research activities on this subject. We checked herbarium specimens collected from the study area, with regards to Emilio Chiovenda's and Oliviero Boggiani's collections respectively preserved at BOLO and FI. New material has been collected in the field, specifically from unexplored or lesser known areas. Particular attention was paid to neglected critical groups (e.g., *Alchemilla*, *Festuca* and allied genera, *Hieracium* and allied genera, *Rubus*), with the aid of experts' opinion. Resulting data have been stored into IPLA's database and georeferenced, highlighting changes in the local flora occurred since 1850. Approximately 16,000 records have been archived, among these 1,000 herbarium data (historical and recent), about 3,000 bibliographic records and at least 12,000 original field data. Currently, the number of taxa amounts to about 1,400, corresponding to the 40% of Piedmont flora (9); with respect to the latest study on the PNVG's flora (10), a more than double increase has been recorded. Our research, still in progress, is expected to produce an updated checklist by the end of 2018.

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4.1 = FLORISTIC HERITAGE OF THE MT. CAVALLO PATH (OROBIE BERGAMASCHE REGIONAL PARK)

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The identification of elements of special environmental interest occurring in protected areas is indispensable for a full valorisation of their resources. This is even more important in protected areas that have only recently been established, such as the Orobie Bergamasche Regional Park (Lombardy, Italy) (L.R. 15/9/1989 n. 56). This research reports the results of the analysis of the flora of the Mount Cavallo path (Fig. 1), a little-known and little-used area of the Orobie Bergamasche Park. The floristic census conducted, which led to the identification of 241 species, showed that the study area has a high number of endemic plants, some of which are endemic of Italy, including *Linaria tonzigii* Lona, *Allium insubricum* Boiss. & Reut. ex Reut., *Campanula raineri* Perp., *Saxifraga vandellii* Sternb., *Galium baldense* Spreng., and *Saxifraga hostii* Tausch subsp. *rhaetica* (A.Kern. ex Engl.) Braun-Blanq. *Linaria tonzigii* occurs only in the province of Bergamo where it has been reported in few locations. Moreover, it is also a species of Community interest included in Annex II of the Habitat Directive (92/43/EEC), but the study area is not part of a Site of Community Importance. In addition, some of the plant species identified in the study area are also rare (8%) and very rare (2%) in Central and Eastern Lombardy. The ecological spectrum of the floristic list shows that most of the species surveyed are heliophilous microthermal species living on basic, dry and nutrient-poor substrates. In fact, most of them are typical of rocky environment communities, limestone screes, and basophilous grasslands, although there are various species of *Festucion variae* localized in the short stretch in which the path is on acid substrate.
Given the wealth and significance of the floristic heritage of the study area, as well as its unique geological and environmental characteristics, a proposal is presented for its valorisation, involving the construction of a nature trail: the "Mount Cavallo Botanical Path" (1). This research, presenting the distribution maps of the various endemic species along the path and the results of the chorological and ecological analyses of the local flora, provides the scientific background knowledge for the development of a project for the promotion and enhancement of one of the most interesting and beautiful areas of the Orobie Bergamasche Regional Park.

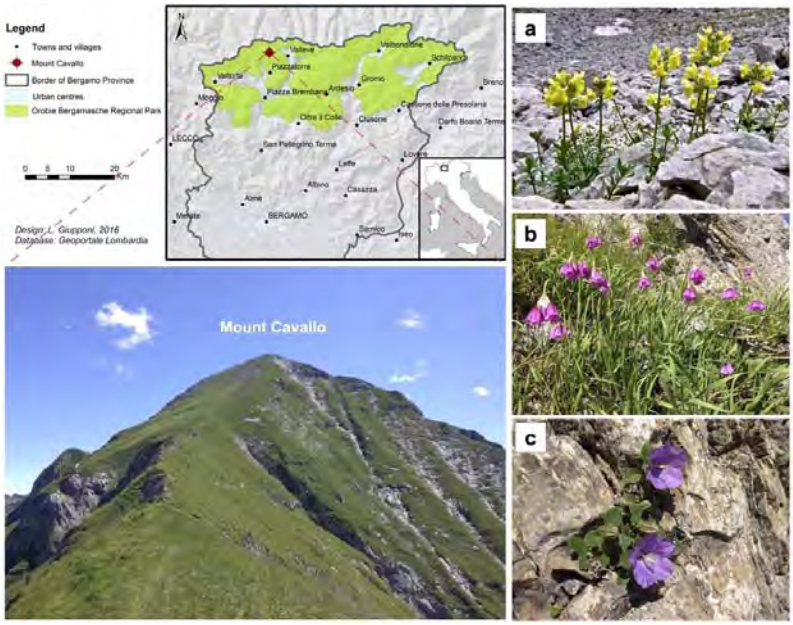

Fig. 1
Location of the study area and some endemic species along the Mt. Cavallo path: *Linaria tonzigii* (a), *Allium insubricum* (b), *Campanula raineri* (c).

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4.1 = ANTIQUE FLORISTICS: THE STATUS OF KNOWLEDGE OF TUSCAN FLORA (CENTRAL ITALY) AT THE BEGINNING OF TWENTIETH CENTURY

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With the exception of the very first volume (out of eight scheduled) of "Flora analitica della Toscana", published some months ago (1), the only comprehensive flora of Tuscany (Central Italy) is that published by T. Caruel in 1860 (2), complemented in the following decades by a series of Supplements published by E. Baroni between 1897 and 1908 (3). These works assembled all the floristic information available at that time. A long and complex digitalization process of these seminal works started on September 5th, 2013 and came to completion on May, 25th, 2017. Floristic records have been georeferenced and stored in the free online resource Wikiplantbase #Toscana (4, 5) with updated taxonomic nomenclature (6, 7). Accordingly, after more than a century, we are finally able to summarize the status of Tuscan floristic knowledge at that time and to compare it with the current knowledge of this regional flora (6, 7, 8). Taken together, the floristic records contributing to the flora of Tuscany at the beginning of twentieth century resulted 39,109, for 3,834 distinct toponyms. They cover most of the Tuscan territory, albeit with marked difference in different portions of the region: the Tuscan Archipelago and the continental portion of the northern provinces are more densely covered than the continental part of the southern provinces (Fig. 1). These records refer to 2,751 different specific and subspecific currently accepted taxa, pertaining to 901 genera and 150 families. The most represented families are Asteraceae (4,832 floristic records), Fabaceae (4,166), and Poaceae (2,698). The five plants showing the highest number of records are *Sedum monregalense* Balb. (Crassulaceae) and *Trifolium campestre* Schreb. (Fabaceae) (both with 52 records), followed by *Orlaya grandiflora* (L.) Hoffm., *O. platycarpos* W.D.J.Koch (Apiaceae), *Narcissus tazetta* L. subsp. *tazetta* (Amaryllidaceae), and *Sagina subulata* (Sw.) C.Presl (Caryophyllaceae) (all represented by 51 records).
Compared with the current knowledge, these taxa amount to about 70% of the Tuscan flora. This striking difference is due to (a) hundreds of neophytes recorded since the second half of twentieth century, (b) a limited number of floristic novelties discovered in the last 110 years, and (c) different taxonomic concepts in several groups, whose species were circumscribed more comprehensively than today.

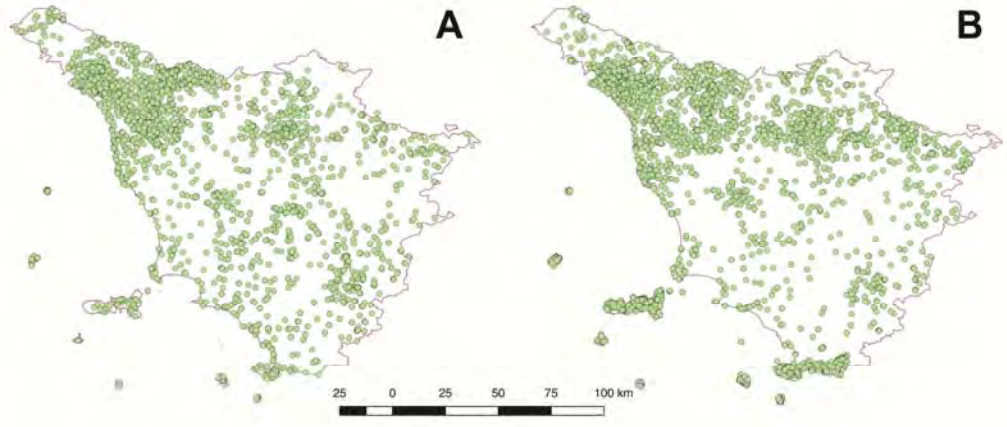

Fig. 1
Georeferenced floristic records derived from (2) (A: 19,892) and (3) (B: 19,217).

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4.1 = TAXONOMIC, ECOLOGICAL AND CHOROLOGICAL REMARKS ON *CLINOPODIUM MINAE* (LAMIACEAE), CRITICAL AND RARE PLANT OF THE SICILIAN FLORA

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In a previous contribution (1) the finding in the wild of *Clinopodium minae* (Lojac.) Peruzzi & F.Conti [Bas. *Calamintha minae* Lojac.], a critical species close to *Clinopodium corsicum* (Pers.) Govaerts and well distinct from *Clinopodium alpinum* (L.) Kuntze subsp. *nebrodense* (A.Kern. & Strobl) Bartolucci & F.Conti was reported. It is a critical taxon, described by Lojacono, author of the most extensive work on the Sicilian flora (2) based on the examination of the collections of the Herbarium of Palermo (PAL!) (3). In the above cited note, the specific characters as well as the taxonomic relationships with other taxa were highlighted. At the same time, the location of a small population of *C. minae* on the northern slopes of the calcareous system of the Carbonara, the highest mountain of the Madonie (N Sicily), was reported.
Starting from this first population, field surveys have been extended to other localities of the Madonie. These have also enabled us to find C. *minae* also on the western slopes of Monte Quacella that, named the "Sicilian Alps" by the same Lojacono, is the richest area in mostly endemic plant species, with respect to Sicily (4).
In this context, *C. minae* occurs on consolidated dolomite debris, in semi-shaded places, from 1450 to 1700 m, at margins of larger communities at *Juniperus communis* subsp. *hemisphaerica* (J.Presl & C.Presl) Arcang. and *Daphne oleoides* Schreb., together with *Odontarrhena nebrodensis* (Tineo) L. Cecchi & Selvi subsp. *nebrodensis*, *Clinopodium alpinum* subsp. *nebrodense*, *Anthemis arvensis* subsp. *sphacelata* (C.Presl) R.Fern., *Carlina nebrodensis* DC., *Centaurea parlatoris* Heldr., *Helianthemum cinereum* subsp. *rotundifolium* (Dunal) Greuter & Burdet, *H. oelandicum* subsp. *incanum* (Willk.) G.López, *Hypochoeris radicata* L., *Jurinea bocconei* Guss., *Laserpitium siler* subsp. *siculum* (Spreng.) Santangelo, F.Conti & Gubellini, *Linum punctatum* C.Presl, *Onosma echioides* subsp. *canescens* (C.Presl) Peruzzi & N.G. Passal., *Paeonia mascula* (L.) Mill., *Pilosella hoppeana* subsp. *sicula* Di Grist., Gottschl. & Raimondo, *Pimpinella tragium* subsp. *lithophila* (Schischk.) Tutin, *Orchis anthropophora* (L) All., *Sesleria nitida* subsp. *sicula* Brullo & Giusso, *Silene italica* subsp. *sicula* (Ucria) Jeanm., *Teucrium montanum* L., *Thymus striatus* Vahl, and some grasses.
Compared with the plants from the Carbonara Mount, the population of Quacella is much richer in individuals and shows an accentuated variability, not remarked in the taxonomic analysis by Lojacono.
At present, however, based on the recently collected materials, *C. minae* occurs on both calcareous and dolomite substrates, above 1,450 m a.s.l. Based on the collections by A. Todaro housed in PAL! and in the course of field surveys, the authors do not exclude the possible occurrence of *C. minae* in other carbonatic reliefs in the province of Palermo.

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4.2 = DISTRIBUTION OF REPETITIVE DNA SITES IN *CYNODON* (POACEAE)

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Cynodon Rich. (Poaceae) is a genus typical of both tropical and subtropical regions and is widespread around the world. It is also important because of its agricultural and economical role such as forage, soil stabilizer and soccer field grass (1). A wide morphological and genetic variability can be found in this genus (2), due to a large number of different cytotypes, including polyploids (3). For this reason, misidentifications occur frequently. Cytotaxonomic studies in *Cynodon* can be useful for discriminating species and better understanding their taxonomic relationships. Thus, this study aimed at studying CMA/DAPI banding patterns and at locating 45S and 5S rDNA sites by fluorescent *in situ* hybridization (FISH) in *Cynodon dactylon* (L.) Pers., *C. incompletus* Nees, *C. nlemfuensis* Vanderyst, and *C. transvaalensis* Burtt Davy. *Cynodon dactylon* and *C. transvaalensis* are tetraploid ($2n = 4x = 36$), while *C. incompletus* and *C. nlemfuensis* are diploid ($2n = 2x = 18$) (4).
In all species, we observed two interstitial 5S rDNA sites (Fig. 1). *Cynodon dactylon* showed four pericentromeric 45S rDNA sites (Fig. 1D), while in *C. transvaalensis* three pericentromeric 45S rDNA sites were found (Fig. 1C). The diploid species presented two pericentromeric sites 45S rDNA with equal signal intensities (Fig. 1A-B). In *Cynodon incompletus* there were two DAPI terminal bands for all chromosomes (Fig. 1A); by contrast, in *C. lemfuensis* we observed two DAPI terminal bands in six chromosomes (Fig. 1B). CMA bands are co-located with 45S rDNA sites in the studied species (Fig. 1). In the tetraploid species, 45S rDNA sites can discriminate between the two species, while DAPI bands are useful to discriminate the diploid species.

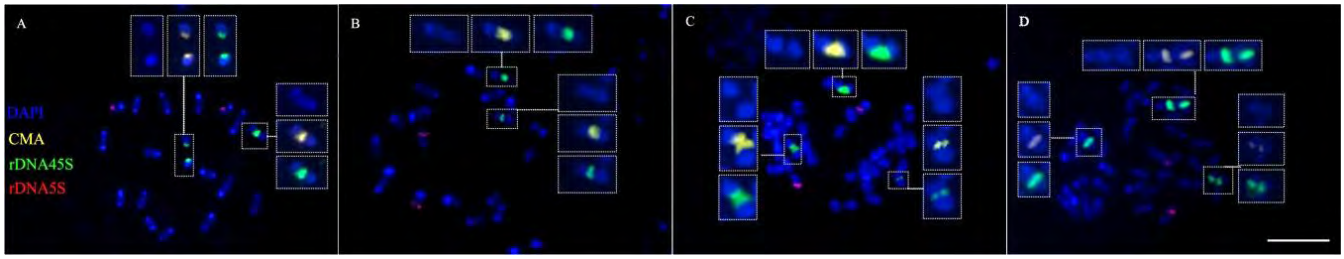

Fig. 1
Metaphase with 5S rDNA interstitial bands (red) and 45S rDNA pericentromeric bands (green), located along with CMA^+. A) *Cynodon incompletus* ($2n = 2x = 18$). B) *C. lemfuensis* ($2n = 2x = 18$). C) *C. transvaalensis* ($2n = 4x = 36$). D) *C. dactylon* ($2n = 4x = 36$).

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4.2 = DISENTANGLING INFRASPECIFIC TAXONOMY OF *CIRSIUM VULGARE* (SAVI) TEN. (ASTERACEAE). A FIRST CONTRIBUTION FROM TUSCANY

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The genus *Cirsium* Mill., one of the largest genera in Asteraceae, contains about 250 species distributed worldwide; one of them is *Cirsium vulgare* (Savi) Ten., originally described by Gaetano Savi (1) as *Carduus vulgaris*, and later recombined by Tenore (2). Concerning the infraspecific classification of this species, currently 3 subspecies are recognized: *C. vulgare* subsp. *critinum* (DC.) Arènes, *C. vulgare* subsp. *silvaticum* (Tausch) Arènes, and *C. vulgare* subsp. *vulgare*. The taxonomic value and circumscription of these subspecies have been widely discussed in the past, causing different treatments by various authors, and leading to scarcely reliable floristic records, despite an increasing standardization in the usage of subspecific ranks would be desirable (3).
In Tuscany, currently two subspecies are recorded; the autonymic one was described for the nearby of Pisa (Tuscany, Italy), with the following diagnosis: "*Carduus foliis semi-decurrentibus, bifariam pinnatifidis, calycibus solitariis ovatis, sublanatis*". The second subspecies, namely *C. vulgare* subsp. *silvaticum*, corresponds to *C. silvaticum*, described (4) from Hořovice (Bohemia, Czech Republic), with the diagnosis "*foliis decurrentibus oblongo lanceolatis sinuato-pinnatifidis, supra strigosis, subtus cano-tomentosis, laciniis sub 3-fidis ciliato-spinulosis, pudunculis 1-floris, squamis anthodii arachnoidei bracteati recurvatis patulis*", later recombined by Arènes (5). In order to investigate the taxonomic value of these two taxa, we performed a quantitative morphological analysis of herbarium specimens topotypical at regional scale. We obtained specimens from Tuscany from PI, FI, and SIENA, whereas those from Bohemia were obtained from BNRL, CB, CBFS, HOMP, OL, PL, and LIT. We considered only those specimens falling within a radius of about 100 Km from each *locus classicus*, and bearing adult well-developed plants. All the measurements were performed on middle cauline leaves and fully developed capitula. According to the main features reported in the protologues and in analytical keys (e.g. 6, 7) we choose to calculate the following ratios: (i) spine length / terminal lobe length, (ii) maximum involucre length / maximum involucre width, and (iii) abaxial / adaxial leaf RGB triplet values. We also recorded other categorical binomial data: presence/absence of hairs on the abaxial leaf surface and of involucral arachnoid hairs, habitat type (open or shaded). Our observations suggest that there is no significant distinction between the two topotypical specimen groups. For this reason, we propose to put *Cirsium silvaticum* Tausch among the heterotypic synonyms of *Cirsium vulgare* (Savi) Ten., holding nomenclatural priority (Art. 11.4 of the ICN; 8). The putative differences previously recorded in literature likely originated from a misinterpretation of the huge variability in some phenotypic traits occurring among individuals of this species (e.g., concerning leaf morphology and colour, length of spines, hairs indumentum density). Hence, the observed patterns of diversity are not worthy of taxonomic recognition, representing just morphotypes.

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4.2 = NICHE DIVERGENCE BETWEEN PUTATIVE TAXA: ECOLOGICAL NICHE MODELS IN TAXONOMIC RESEARCHES

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For over a century, species delimitation was a critical task for biologists, concerning both species concept and operational methods necessary for the individuation of taxa boundaries. According to the recent approach of integrative taxonomy, species have to be considered as hypotheses that should be tested with different methods. Ecological interchangeability can be an element that can corroborate the separation of two putative taxa, but ecological data are often difficult to use. Ecological Niche Models (ENMs) provide a quantification of niche parameters and could be a useful tool for investigating taxa boundaries. For this purpose, we used ENMs for testing ecological (climatic) differentiation in three pairs of related taxa recently subdued to taxonomical revisions without explicitly considering ecological evidences.
The selected taxa pairs are: *Rhaponticum bicknellii* (Briq.) Banfi, Galasso & Soldano and *R. heleniifolium* Godr. & Gren.; *Gentiana burseri* subsp. *actinocalyx* Polidori and *Gentiana burseri* subsp. *villarsii* (Griseb.) Rouy *Fritillaria tubaeformis* Gren. & Godr. subsp. *moggridgei* (Baker) Rix and *Fritillaria tubaeformis* subsp. *tubaeformis*. For each pair, we assessed niche overlap, niche equivalency and niche similarity in both multidimensional environmental space (E-space) and geographical space (G-space).
We found a moderate-low degree of niche overlap in both E- and G-space (lower in *Rhaponticum*, intermediate in *Gentiana* and higher in *Fritillaria*), suggesting that in our study taxa isolation by ecology may have occurred. In line with niche overlap, the results of niche equivalency test both in E- and G-space clearly indicate that these niches are not interchangeable, suggesting a certain degree of divergence. However, equivalency test investigates niche interchangeability in the strictest sense, because two niches are considered equivalent only if they share both optimal position and breadth. Hence, if a population occupies a marginal position of the niche of another group, their niches are not interchangeable. Moreover, the evidence of niche differentiation underlined by niche overlap does not allow to understand whether this difference is due to a different habitat selection (i.e.: habitat suitability) or simply to a different habitat availability in the background areas.
Similarity test can help to discern between this two different scenarios. We found opposite results of similarity test in E- and G-space: in E-space the niches are generally more similar to each other than expected, while in G-space they are generally less similar to each other than expected. The two tests use contrasting approaches to reduce the dimensions of an environmental dataset, potentially leading to biased overlap estimation in G-space: for this reason, results of similarity test in G-space should be considered with caution. The results of similarity test in E-space suggest that the observed divergence could not be the result of an actual difference in habitat selection between taxa. Nevertheless, moderate niche overlap values suggest that taxa differ in their optimal niche position. Congruently with this result, in background analyses of *Rhaponticum* and *Fritillaria* the niche of one taxon is more similar to the other one, while the vice-versa is not significant. This suggests that, despite the two taxa share the climatic niche, one taxon might occupy a marginal portion of the niche of its partner, the so-called “niche included” pattern. Contrary to previous analyses (i.e., equivalency test and niche overlap), similarity test does not provide further evidences supporting taxonomic separation for the studied taxa, despite the observed niche divergence may have had important consequences for local adaptation and in generating phenotypic diversity.
The different analyses widely disagree on their conclusions about ecological separation of putative taxa, probably as a result of the variety of niche patterns that may occur. Furthermore, several limitations in analysis methods still persist when ENMs are used for taxonomic purposes. Our results underline that analysis in E-space may reduce bias associated with geographical space and that similarity test should be preferred to equivalency test in order to correctly investigate niche conservatism or divergence between putative taxa. Evidences of niche divergence (as inferred by niche overlap assessment) are not enough to corroborate taxa separation, unless similarity test does not explain such divergence as result of different habitat selection made by putative taxa. A tool aimed to explore the role of ecological differentiation in speciation mechanisms may help taxonomists in reaching a decision about species boundaries, albeit ENMs cannot probably provide conclusive evidences.

4.2 = CONTRIBUTION TO THE TAXONOMY OF *POLYGALA FLAVESCENS* GROUP (POLYGALACEAE): KARYOLOGICAL AND MORPHO-COLORIMETRIC DATA

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The genus *Polygala* L. is the largest of the family Polygalaceae, showing a cosmopolitan distribution, except for the Arctic, Antarctica and New Zealand, and comprising between 325 and 725 species (1, 2). One of the most taxonomically controversial groups in Italy is that of *Polygala flavescens* DC., a taxon endemic to the country, currently reported from Emilia-Romagna to Basilicata (4).
Besides *P. flavescens* DC. s.str., originally described from central Italy, this group includes two other taxa, treated by previous authors at different taxonomic ranks (5, 6). According to the most recent monograph of the genus in Italy (6), these two taxa are recognised as subspecies of *P. flavescens*, i.e. *P. flavescens* subsp. *maremmana* (Fiori) Arrigoni and *P. flavescens* subsp. *pisaurensis* (Caldesi) Arcang. *Polygala flavescens* subsp. *maremmana* was originally described as a form, based on plants from Mt. Argentario (7). According to Arrigoni (6), all the plants occurring on the coasts of southern Tuscany, from San Vincenzo (Leghorn) to Capalbio (Grosseto), should be assigned to this taxon. *Polygala flavescens* subsp. *pisaurensis*, originally described as a species from Pesaro, is currently restricted to coastal hills of Emilia-Romagna and Marche.
All the changes in the group's taxonomic setting were made without any quantitative support. In addition, karyological information is completely lacking (8). To fill this gap of knowledge, karyological and morpho-colorimetric studies have been performed. Five populations were selected, including the topotypical populations of *P. flavescens* subsp. *maremmana* and *P. flavescens* subsp. *pisaurensis*.
For the morpho-colorimetric analysis, ten characters of leaves, flowers and stems were measured on 20 plants for each population. Furthermore, for the same populations, five characters on 50 fruits, and three characters on 20 seeds, were measured. To evaluate differences in the flower colour, pictures of 20 flowers for each population were taken in standard light condition, and the relative contribution of Red, Green and Blue (RGB) was measured using the image analysis software Gimp 2.8.14 (9).
Concerning karyology, all the five populations share the same chromosome number $2n = 2x = 22$, showing very small chromosomes (0.6–1.0 mm).
The length of the elaiosome was confirmed as a character useful to separate *P. flavescens* subsp. *pisaurensis* (2.5 ± 0.3 mm) from the other two taxa (1.9 ± 0.2 mm in *P. flavescens* subsp. *maremmana*, and 1.8 ± 0.2 mm in *P. flavescens* s.str.). Concerning the distinction between *P. flavescens* s.str. and *P. flavescens* subsp. *maremmana*, among the studied characters, the length of the flower wings and the length of the flower bracts resulted significantly different (ANOVA and/or Kruskal-Wallis, $p < 0.01$). Both these features are larger in *P. flavescens* subsp. *maremmana* (10.9 ± 0.9 and 5 ± 0.5 mm) than in *P. flavescens* s.str. (8.2 ± 0.8 and 3.9 ± 0.5 mm, respectively). The flower colorimetric analysis highlighted that *P. flavescens* subsp. *maremmana* is characterized by wings and fringed keel with significantly higher contribution of Red and lower contribution of Blue, resulting in flowers more markedly yellow-orange, whereas in *P. flavescens* s.str. and *P. flavescens* subsp. *pisaurensis* the flowers range from yellow-whitish to yellow.
Our karyological and morpho-colorimetric results seemingly support the current taxonomic treatment at subspecific level (6). However, a thoroughly revision of herbarium specimens, by checking the characters which resulted most significant in separating the three taxa, will be useful to clarify their distribution in Italy.

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4.2 = A MOLECULAR-ASSISTED INVESTIGATION OF THE HALYMENIALES (RHODOPHYTA) COLLECTED IN TWO RECENT EXPEDITIONS IN MADAGASCAR AND GUADELOUPE REVEAL HIDDEN TAXONOMIC DIVERSITY

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The Atimo Vatae Expedition (1) was the joint effort of the Muséum National d'Histoire Naturelle (MNHN, Paris), the Institut d'Halieutique et des Sciences Marines, University of Toliara (IH.SM), and the Wildlife Conservation Society (WCS) Madagascar Programme. The expedition explored the coastal marine fauna and flora of southern Madagascar, from Androka to Fort Dauphin, from April to June 2010.
The Karubenthos Expedition (2) was coordinated by the Parc National de Guadeloupe, along with the Muséum National d´Histoire Naturelle, the Université des Antilles-Guyane, and the Université Pierre et Marie Curie. The aim of this marine expedition was to overcome the lack of knowledge on the algae and more generally on the diversity of benthic organisms. This expedition explored the coastal waters around Guadeloupe during May and December 2012.
In both expeditions, a comprehensive sampling of the marine benthic algal flora was conducted. Specimens of macroalgae were pressed onto herbarium sheets and subsamples were dehydrated in silica gel for subsequent molecular analyses.
Among the collected material, those specimens morphologically identified as halymeniacean taxa have been sequenced for the DNA barcode gene (COI5') and their sequences were compared on public online databases (BOLD, GenBank) (3, 4). Subsequently those recognized as putative new taxa were thoroughly observed to document their vegetative and reproductive anatomical structures. Additional genetic markers (*rbc*L, LSU) of those putative new taxa were sequenced and phylogenetic analyses were conducted to assess the phylogenetic relationships of the Malagasy and Guadeloupian samples with their worldwide relatives.
The first published result was a report of two novel species of the genus *Yonagunia* from Madagascar (5), moreover here we examine additional new halymeniacean taxa that were uncovered and will be formally described.

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4.2. = CRYPTIC DIVERSITY OF BANGIALES (RHODOPHYTA) ALONG THE ITALIAN COASTS REVEALED BY DNA BARCODING

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The order Bangiales (1) includes red algae exhibiting morphological similarity and anatomical simplicity, geographically ubiquitous, occurring from tropical to polar seas; most species are intertidal, growing on rocks, shells, or other algae, but some are found solely in subtidal habitats, and some are obligate epiphytes (2). A single family, Bangiaceae (3), is defined within the order of Bangiales, traditionallyincluding two basing on the gametophyte morphology: species with filamentous gametophyte have been included in the genus *Bangia* Lyngbye (4), while species with laminar gametophyte in the genus *Porphyra* C.Agardh (5). According to Yoon et al. (6) and most previous authors, Bangiaceae include twelve genera with ca. 130 species. Instead, a comprehensive revision of the order of Bangiales (2) recognized fifteen genera, seven filamentous and eight foliose, and three further lineages including filamentous entities still to be defined nomenclaturally. Due to their simple and highly variable morphology, one of the major challenges for taxonomy of the Bangiales is the difficulty in identifying taxa based solely on morphological and anatomical characteristics (7). A comprehensive review of the taxonomic status of Bangiales in the Mediterranean basin has not completed yet (8, 9, 10, 11, 12). The aim of the present paper is to report on a DNA barcoding survey of Bangiales along Italian coasts.
Sampling of representative of the Bangiales was conducted in various localities along Italian coasts. Collected specimens were pressed onto herbarium sheets (hold at PhL and PC) and subsamples were dehydrated in silica gel for subsequent molecular analyses. The specimens have been sequenced for the DNA barcode gene (COI5') and obtained sequences were contrasted to public online databases (BOLD, GenBank) (13, 14). Furthermore, an alignment including also all publicly available COI5' sequences of Bangiales was built and a distance tree generated. Among specimens identified in the field basing on morphological features as *Pyropia leucosticta*, DNA barcodes showed the presence of *P. yezoensis*, *P. koreana*, and *P. elongata*. DNA barcoding data obtained from collected specimens of *Bangia* s.l. suggested the presence of three cryptic species, two of which already reported in north Atlantic. The need of comparison with type and historical specimens, the verification of old names and, last but not least, the definition of diagnostic characters for such taxa prove to be a challenge.

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4.3 = PHYTOPLANKTON ALIEN SPECIES IN MARINE AND FRESHWATER ECOSYSTEMS FROM LATIUM REGION (MIDDLE TYRRHENIAN SEA, ITALY)

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Alien species are considered one of the main drivers of biodiversity changes. The Marine Strategy (MS) Framework Directive (2008) considers the Non-Indigenous Species (NIS) one of the 11 qualitative descriptors of good environmental status; the EU Biodiversity Strategy (2011) highlights the need to identify alien species and to assess their pathways of invasions in order to control and prevent introduction of new alien species. Long time series and refined taxonomy are necessary to achieve a sound assessment of biological diversity and alien species for a specific geographical area. In particular, invasions by phytoplanktonic organisms are poorly recorded due to biological and ecological characteristics of microalgae, such as ephemeral and patchy distribution, short time occurrence and difficulties in their sampling (e.g. resolution of sampling program, preservation).
This work represents a contribution to the knowledge of regional phytoplankton assemblages and to the reporting of microalgal invaders. Records of alien species were extracted from phytoplankton datasets collected in the framework of different national monitoring programmes in Latium inland and marine coastal waters. The freshwater phytoplankton database was obtained from monitoring activity at four lakes (Bracciano, Martignano, Nemi and Albano) from 2013 to 2016, for a total of 185 samples analysed. The marine database includes data from more than 700 samples collected at 16 stations within 500 m from the coastline from 2001 to 2006 and from 2013 to 2016. In addition, in the frame of the Marine Strategy monitoring for NIS descriptor, species composition was assessed bimonthly in samples collected at two stations located in the Civitavecchia harbour. For the MS Biodiversity descriptor, samples were collected along 4 transects, each including 3 localities (located at 3, 6 and 12 NM from coastline), at 0.5 m and DCM (Deep Chlorophyll Maximum) depths. In this ongoing programme, started in July 2015, over 180 samples have been so far analysed.
Freshwater alien species were found in Lakes Albano and Nemi. Particularly, the presence of *Cylindrospermopsis raciborskii* (Woloszyńska) Seenayya & Subba Raju, a tropical toxic filamentous cyanobacterium was recorded in Albano, from late spring to autumn, in 2013 and 2014, with peaks of 8×10^7 cells/l. A seasonal succession of *C. raciborskii* and *Planktothotrix rubescens* (DC. ex Gomont) Anagnostidis & Komárek, was recorded in Lake Albano. *Planktothrix rubescens* is a toxic species responsible of recurrent winter blooms (up to 10^9 cells/l) in the lake since '50s, although its original distribution is holarctic. This species was detected also in Lake Nemi but with lower abundances. The subtropical heterocystous cyanobacterium *Rhaphidiopsis mediterranea* Skuja was also detected in Lake Albano, in September 2013, with density up to 1.5×10^6 cells/l.
In marine samples, the toxic diatom species *Pseudo-nitzschia multistriata* (H.Takano) H.Takano was occasionally recorded from 2001 with abundances up to 1.5×10^6 cells/l. The dinoflagellates *Alexandrium insuetum* Balech and *Lingulodinium polyedrum* (F.Stein) J.D.Dodge were also sporadically observed. These species are reported as "casual" for the Mediterranean Sea, but they were frequently recorded in our area. During the blooms of the benthic dinoflagellate *Ostreopsis* cf. *ovata* Fukuyo, recurrently recorded in summer along Latium coasts from 1999, the species was also detected in phytoplankton samples. Actually, the presence of *O.* cf. *ovata* in water samples is used, in the monitoring activity for the assessment of Bathing Water Quality, as a proxy of its massive proliferation in the microphytobenthic assemblages.

4.3 = HOW MUCH CLOSE IS THE CONNECTION BETWEEN NON-INDIGENOUS SPECIES AND HUMAN ACTIVITIES? AN EXAMPLE FROM A MEDITERRANEAN COASTAL ZONE

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The introduction of non-indigenous species (NIS) is a pressing issue in the marine environment throughout the world, nowadays. Indeed, several of them, named invasive, can pose real risks to biodiversity, productive activities and human health (1).
It is said that among the marine environments, transitional water systems and port areas are the most exposed to the risk of this kind of introduction, due to the economic activities here performed, that is on the one hand aquaculture and mussel importation, and on the other shipping activities (2). For this reason, Taranto (southern Italy, Mediterranean Sea) is a paradigmatic case for this issue. Indeed, the Taranto seas, that is the transitional system Mar Piccolo, and the adjacent Mar Grande (Fig. 1), have always been the seat of those economic activities, which heavily exposed the surrounding environments to all the known factors of risk (3).
According to published literature, the mollusc *Bursatella leachii* (de Blainville, 1817) was the first NIS observed in the Taranto seas, in 1973 (4). However, the presence of NIS can be furtherly backdated to the Twenties. Indeed, in Pierpaoli's Herbarium, a sheet dated 1922 was found with a sample of an Atlantic macroalgal species, the Rhodophyta *Solieria filiformis* (Kützing) Gabrielson, misidentified as the native *Gracilaria confervoides* Greville (5). Over the course of less than one hundred years, and with a rising trend in the last twenty years, a total number of 48 NIS was recorded so far, among them 15 are seaweeds.
Due to the urgency of the problems, starting from 2001, several research projects were carried out, to detect any possible new NIS, the likely activities and vectors of introduction, and the possible suggestions to avoid new entries in the Taranto seas. Therefore, the analysis of ballast waters, collected from ships entering the port, and the surveillance of coastal areas, especially along the Mar Piccolo where intense mussel farming and import-export activities are commonly performed, became usual activities.

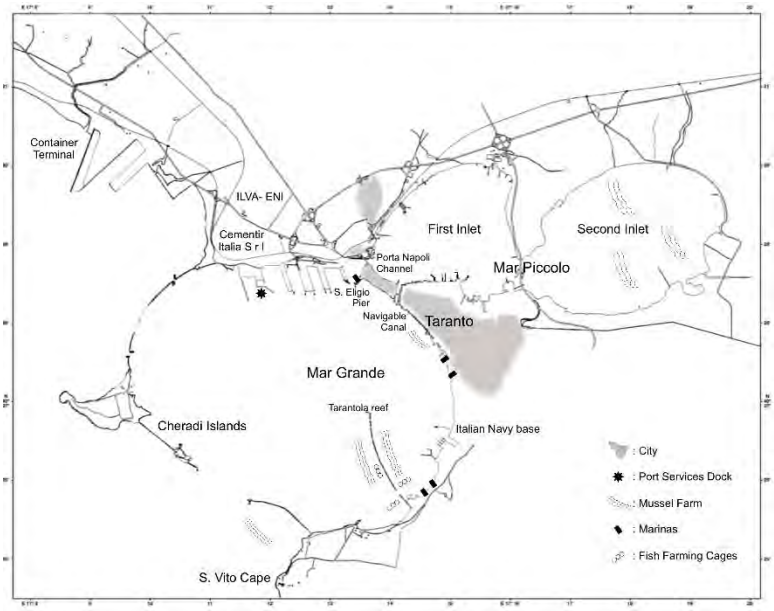

Fig. 1
Map of the Taranto seas showing the main activities here performed (from (3)).

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4.3 = LIFE ASAP – INCREASING AWARENESS ON ALIEN SPECIES IN ITALY

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In the EU alone, social and economic impacts of invasive alien species (IAS) are estimated at over 12 billion euros annually: of the 12,000 alien species reported today in Europe, more than 3,000 are present in Italy, of which over 15% are invasive. This is a growing phenomenon: at global level the annual rate of first records dramatically increased in the last 200 years, with 37% of all first records reported most recently (1), while in Europe, the number of alien species increased by 76 percent over the past 30 years.
The project LIFE ASAP (Alien Species Awareness Program, LIFE15 GIE/IT/PROTECTED), co-funded by the European Commission, started in October 2016 and will end in March 2020. The coordinating beneficiary is ISPRA - Istituto Superiore per la Protezione e la Ricerca Ambientale and beneficiaries partners are the University of Cagliari, Legambiente, Federparchi, Regione Lazio, Nemo s.r.l. and Unicity s.r.l.
The general aim of the project is to reduce the rate of introduction of IAS and limit the impacts on the Italian territory through i) the increase of public awareness of the risks associated with biological invasions and by promoting their active participation and the adoption of responsible behaviours; ii) supporting the national implementation of the Regulation (EU) no. 1143/2014, which entered into force on the 1st January 2015, "on the prevention and management of the introduction and spread of invasive alien species". The LIFE ASAP project envisages various and diverse education, information and communication campaigns addressing citizens and all major stakeholders and professionals directly or indirectly involved in the management of invasive alien species. ASAP target audiences range from public administration personnel, protected natural areas staffs, zoos, botanical gardens and scientific museums operators to professionals such as veterinarians, biologists, landscape planners, foresters, and agronomists; it also includes the hunting and sport fishing sector and the trader of pets, plants and seeds. Since Botanical Gardens, together with zoos, scientific museums and protected areas, interact with society as a whole, organizing dissemination activities for schoolchildren and the general public, hosting cultural events of social interest, they represent ideal venues where training and information coexist.
In this framework, the Botanical Section of the Department of Life and Environmental Sciences of the University of Cagliari, in collaboration with the Botanical Garden Services Centre (HBK) of the University of Cagliari will be the “botanical heart” of a national network that will involve many supporting partners: the Italian Botanical Society, the Botanical Gardens of Catania, Rome and Siena, CREA (Consiglio per Ricerca in Agricoltura e l'analisi dell'Economia Agraria, Bagheria, Palermo), CIRBFEP (Centro di Ricerca “Biodiversità, Fitosociologia ed Ecologia del Paesaggio”, Rome), and the floriculture company “Sgaravattiland” (Capoterra, Cagliari).
In the first year of activity, alongside the first training sessions for botanical gardens, scientific museum and zoo operators, an educational path on alien species will be established in the Botanical Garden of Cagliari. This path will guide visitors through the observation of a selection of invasive alien plants, and will be integrated with the description of the alien animal species associated with these plants. Short questionnaires will be distributed to visitors to evaluate the awareness acquired.
During the life-span of this LIFE project, thematic days on particular categories of alien species and various activities dedicated to schools of every order and degree will be proposed, in order to raise awareness and involve different sectors of the general public and the future citizens.
Hopefully, in the long period, the dissemination of information on good practices and the implementation of responsible behaviours by every sector of the society will lead to a more effective policy of contrasting biological invasions in Italy.

Fig. 1
LIFE ASAP Logo; http://www.lifeasap.eu/it/.

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4.3 = ROLE OF SICILY AND CIRCUM-SICILIAN ISLANDS AS RECIPIENT AND DONOR AREA FOR ALIEN MARINE MACROPHYTES IN THE CENTRAL MEDITERRANEAN SEA

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The spread of alien species is an ongoing phenomenon which is widely recognized as a major threat to biodiversity at all levels. The particularly high rate of alien introductions to the Mediterranean Sea has been mainly fuelled by the opening of the Suez Canal, by shipping, aquaculture and by a rising trend in seawater temperature (1). As far as marine macrophytes are concerned, a total of 134 species have been listed as possible aliens in the Mediterranean Sea (2, 3). Among the possible pathways of introduction, shipping is considered the dominant vector of unintentional species introduction in coastal marine systems worldwide (4). Traversing the Strait of Sicily, the chief passageway from south to north and from east to west, is considered crucial for extending the range from west to east or *vice versa* of alien species introduced into the Mediterranean Sea. Sicily and the circum-Sicilian Islands, as a consequence of their strategic position at the crossroads between the western and eastern Mediterranean and by virtue of the intense maritime traffic volumes skirting the region, are particularly congenial for and vulnerable to biological marine invasions (5, 6). In particular, the southern and south-eastern coasts, bordering the Strait of Sicily, deserve particular attention. The fact that many marine areas colonized by communities of alien species are located in the immediate vicinity of harbours, supports this hypothesis. The analysis of the first Mediterranean and/or Italian records made in the waters around Sicily, or in waters contiguous to those of Sicily (e.g. southern Italy, Malta, North Africa) and the fraction of aliens entering through the pathway of shipping (e.g. 63% of the alien macrophytes entering the Mediterranean Sea), led us to conclude that Sicily and the circum-Sicilian Islands are an important recipient area for marine non-indigenous species. Moreover, the intense maritime traffic in the area would produce a constant spillover of newcomers into surrounding areas, making this area a crucial transit zone and source for secondary dispersal, highlighting the key role it can play in the circulation of alien species within the Mediterranean Sea.
It has been already suggested that the invasion of the Mediterranean region by *Caulerpa cylindracea* Sonder and by *C. taxifolia* var. *distichophylla* (Sonder) Verlaque, Huisman & Procaccini might have originated along the southern coast of Sicily (7, 8). Moreover, Sicily would have been the source of the Maltese populations of *C. taxifolia* var. *distichophylla*, as a consequence of regular traffic between the two islands (9). The assessment of marine alien species distribution, of the areas at high risk of introduction, of the main pathways and of the vectors of introduction are all essential elements in designing an effective management and conservation program. Therefore, this area, due to its crucial position within the Mediterranean Sea, could be an important transboundary station for monitoring the entry and spread of marine alien species, as solicited in (10).

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4.3 = FIRST ASSESSMENT OF THE BOX TREE MOTH (*CYDALIMA PERSPECTALIS*) INVASION IN NAT2000 HABITAT 5110 IN LIGURIA

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The Box tree moth, *Cydalima perspectalis* (Walker, 1859) (Lepidoptera: Crambidae), is an alien invasive species accidentally introduced in Europe from China, out of its primary range which extends from India to the Russian Far East, Corea and Japan. Several species and varieties of Box (*Buxus sempervirens* L.), including cultivars and hybrids (1), are the most important hostplants for its larvae. The first record of *C. perspectalis* from Europe (Germany, Baden-Württemburg) dates back to 2007; since then the species underwent fast spreading into several other European countries: The Netherlands, Great Britain, Switzerland, France, Austria, Italy, Belgium, Hungary, Romania, Czech Republic, Slovakia, Turkey, Croatia, Spain and Greece.
In Italy, it was firstly recorded in October 2010 in a garden nearby Vicenza (Veneto) (2), and thereafter in Lombardia, Emilia Romagna, Toscana, Friuli Venezia Giulia, Liguria, and Piemonte. In Liguria, the Box three moth has been recorded for the first time in summer 2013 in Hanbury Botanical Garden (Ventimiglia, Imperia), probably arriving from France, but reputedly it was present in the surroundings of Genoa before that time. Records from this region, and from Italy as a whole, were at first exclusively from parks and gardens. Following observations carried out on 22nd July 2016 in Vara Vallley (Eastern Liguria), one of us (FB), working for the State Forestry Corp, informed the Regional Agency for Environmental Protection (ARPAL) about a severe attack suffered by wild *Buxus* plants.
In the framework of the project «ALIEM - Action pour Limiter les risques de diffusion des espèces Introduites Envahissantes en Méditerranée» (EU Program Italie/France "Maritime" 2014-2020, Axe 1), we started to monitor immediately the development of the infestation in the Habitat "5110. Stable xerothermophilous formations with *Buxus sempervirens* on rock slopes (*Berberidion*)", protected after the EU 92/43 Directive. Monitoring surveys were carried in Special Areas of Conservation (SACs), above all in SAC "IT1342806 Monte Verruga - Monte Zenone - Roccagrande - Monte Pu", where Habitat 5110 has a major role in vegetation and landscape. Observations were nevertheless carried out outside the SACs, throughout the region (3).
Due to the lack of a standardised protocol, during surveys visual inspections were carried out on plant crowns, with particular attention to youngest leaves and twigs, recording the various developmental stages of the pest and estimating both average damage on individual plants and land cover by the vegetation formation. Attack intensity was evaluated as damaged phytomass for each plant and based also on population density of *C. perspectalis* larvae. Damage levels were then represented after a value scale subdivided into three grades. Visual inspections following also the so-called "walking census method" and attention was dedicated also to spotting eggs and other juvenile instars of the pest on Box plants (4).
Approximately 70% of the 516 hectares monitored were severely damaged and 22% were moderately damaged.
On 13rd October 2016, Parks and Biodiversity Office of Regione Liguria sent a request to the Ministry of Environment asking for the registration of *C. perspectalis* in the Official Italian List of Invasive Alien Species. This action was considered necessary because the Box tree moth is not listed at present in the "Executive Regulation (EU) 2016/1141 of the Commission of 2016 July 13rd," that provides a list of alien invasive species of relevance for the Union in application of the 1143/2014 (EU) regulation by the European Parliament and Council.
The resilience of natural Box tree formations will also have to be assessed, so for the capacity by *C. perspectalis* larvae to shift to other hostplants "*in situ*" in case Box tree would be missing.

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4.3 = INVENTORY OF THE DISTRIBUTION AND ABUNDANCE OF ALIEN MACROALGAE IN THE VENICE LAGOON: *GRACILARIA VERMICULOPHYLLA*, AN EMBLEMATIC CASE

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A revision of the macroalgal non-indigenous species (NIS) in the Venice lagoon, the Italian hotspot of NIS introductions, shows that the number of valid taxa, i.e. only those with unequivocal identity and non-indigeneous status is 31, until now. During the last three years, their distribution was monitored in different areas of the lagoon: a) 88 localities over the soft substrata of the whole lagoon in 2014, b) 150 localities of soft substrata spread in confined areas of the central lagoon in 2015 and, c) many localities of hard substrata along the island docks recorded in 2014-2017. On the basis of these surveys, we estimated that their total fresh standing crop is approximately 147 ktons, i.e. 32% of the total lagoon macroalgal standing crop measured in soft and hard substrata in May-June 2014 (about 459 ktons).
Among these non-indigenous macroalgae the most abundant species is *Gracilaria vermiculophylla* (1, 2), with a biomass which is estimated to be approximately 66 ktons. *Agardhiella subulata* and *Hypnea flexicaulis* (3) follow with about 38 and 28 ktons, respectively. These three species grow mainly free-floating and colonize mostly the soft substrata of the lagoon. *Sargassum muticum* and *Undaria pinnatifida* (4), instead, grow only attached to hard substrata; therefore their distribution is significantly lower than that of the previous species, despite their abundant presence in the docks of the city of Chioggia and the historical centre of Venice.
Recent studies on the relative growth rate and biomass yield of *Gracilaria vermiculophylla* (5) show that, in clear and confined areas rich of nutrients, this species produces about 24 kg fw m^{-2} and can be a promising species for the extraction of sulphated polysaccharides or for the production of pharmaceutical, cosmetic or food products (6). In addition, in confined and very turbid areas, this species replaces *Ulva* reducing or avoiding anoxic crises. In fact, *G. vermiculophylla* is able to grow in areas where even the growth of Ulvaceae is hampered by water turbidity.

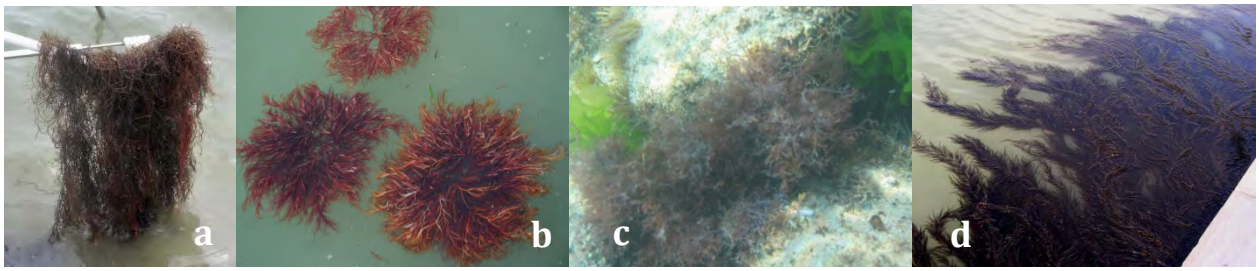

Fig. 1
The four most abundant macroalgal NIS in the Venice Lagoon: a) *Gracilaria vermiculophylla*, b) *Agardhiella subulata*, c) *Hypnea flexicaulis*, d) *Sargassum muticum.*

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4.3 = THE INCREASE OF PLANT INVASION IN THE MEDITERRANEAN AREA: THE ALLOCHTHONOUS VASCULAR FLORA OF PUGLIA (SE-ITALY) AS A CASE STUDY

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Biotic invasions represent a more and more increasing part of ecology, biology, and conservation biology (1, 2, 3, 4). National, regional or subregional comprehensive data sets of alien species are recognized as providing a crucial source of information and an important tool for invasion research and management (5, 6, 7). This is important especially in protected areas and generally in highly biodiverse regions (4). The knowledge on the alien flora provides important information on the naturality of the flora of a given region, and the distinction in invasive, naturalized-non-invasive, and casual aliens gives significant indications for management, especially in protected areas.
An update of the information available in Puglia (SE-Italy) shows a great increase of alien plant species in the last years. To date, the non-native flora of this highly biodiverse Mediterranean region is composed by 338 currently occurring taxa (including 27 taxa that are alien in Puglia, but native or doubtfully native in Italy). This represents more than 13% of the whole flora of this region. Considering their alien status in Puglia, 199 are casual (58.9%), 118 are naturalized-non-invasive (34.9%), and 21 are invasive (6.2%); considering their residence time, 266 are neophytes (78.7%), 45 are archaeophytes (13.3%), 22 are native elsewhere in Italy (6.5%), and 5 are doubtfully native in Italy (1.5%). The inventory comprises 393 taxa, including 26 no longer confirmed taxa, 9 doubtfully occurring taxa, and 20 taxa currently occurring but doubtfully native in Puglia.
Less than 10 years ago, the amount of alien plant species in Puglia was estimated in 170-172 taxa (8, 9). The two-fold increase in alien species (+97%) with respect to previous knowledge is certainly due to an increased research effort aimed at the better knowledge of the non-native flora in Puglia. Nevertheless, a more or less important part of the new alien species arrived in the last years, showing an alarming trend, that could negatively affect the conservation of the autochthonous flora and consequently of the whole ecosystems. Especially invasive species need a constant survey, aimed at the mitigation of their impact on the autochthonous flora and vegetation. In particular, 4 of the 21 invasive vascular plant taxa occurring in Puglia are reported as worldwide invaders, as they are widespread, best studied and most influential around the world, i.e. *Acacia saligna*, *Ailanthus altissima*, *Arundo donax*, and *Opuntia ficus-indica* (4).

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4.4 = SEAVE – SEAWEEDS OF VENICE LAGOON: A BARCODE OF LIFE DATA SYSTEM PROJECT REVEALING HIDDEN MACROALGAL DIVERSITY

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The biodiversity concept involves the knowledge of the variety of life that can be studied at genetic level, species and ecosystem levels, both on time and spatial scales. To avoid underestimating and/or overestimating the biodiversity of an area, a correct identification of species is essential. DNA barcoding is considered the preferred approach in this task, for species identification and to monitor the biodiversity a highly productive biological areas like coastal lagoons, which play an important ecological role by providing a mosaic of habitats for economically important species. DNA barcoding is an appealing tool for biodiversity investigations, able to identify specimens during all life stages, from fresh or preserved material, and to detect potential cryptic species, which are very common among macroalgae.

Within the Mediterranean transitional environments, Venice Lagoon is a peculiar environment, with both high biodiversity and a significant human influence; it is particularly rich in macroalgal species, and numerous reports of discoveries and of new introductions have been published so far. This richness depends both on the peculiar environmental characteristics of the basin and some anthropic activities, such as aquaculture and maritime traffic, which encourage the introduction and dispersal of new species as well as the decrease and/or removal of the native flora.

At present, only a few studies of molecular systematics focus on the macrophytes of Venice Lagoon, dealing with specific taxa (1-4), while a checklist based on molecular methods is still sorely missing.

Within the program of census of marine flora of Italian coastal lagoons (5), we are aiming to compile a DNA barcode inventory and eventually a taxonomic revision of most critical taxa occurring on hard substrata of the historical centre of Venice and surrounding islands.

Inside this framework, we present new molecular data obtained for Ulvacean samples and for cryptic taxa of the Bangiales and Ceramiales, including alien species.

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4.4 = EPIZOIC ALGAE ON LOGGERHEAD TURTLE (*CARETTA CARETTA* LINNAEUS) FROM AEOLIAN ARCHIPELAGO (SOUTHERN TYRRHENIAN SEA, ITALY)

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Algae are common epibionts on the carapace of the aquatic turtles (1, 2). Despite this, most of the papers on marine species concern to the animal component (3, 4, 5). The flora of the loggerhead turtle, *Caretta caretta*, is one of the best studied (5, 6). However, the data on Mediterranean individuals of this species are limited in number and are prevalently restricted to few areas (6, 7, 8, 9). Here we report the results obtained studying 41 individuals of *Caretta caretta* from the Aeolian Archipelago (Sicily, Italy). Yearly boat-based surveys were conducted in an area of about 280 Km^2 around Filicudi Island and specimens were obtained from October 2015 to January 2017. A total number of 17 species of algae were sampled and identified. Some of these are reported for the first time as epibionts on *C. caretta* carapace. Moreover, a morphological and molecular analysis was conducted on individuals of a red alga with a four pericentral "*Polysiphonia*" habitus, occurring on 94% of the studied turtles. A comparison with other four "*Polysiphonia*-like" pericentral species is here reported. The main morphological characters used are habit, number of pericentral cells, cortication, origin of branches, origin and the morphology of the rhizoids, arrangement of tetrasporangia, origin of spermatangial axes, and the size and the shape of cystocarps (10, 11, 12, 13, 15). Our specimens presented several morphological similarities with *P. carettia* Hollenberg and *Neosiphonia cheloniae* (Hollenberg & J.N.Norris) J.N.Norris. Both species were collected on the carapaces of Californian individuals of *Caretta caretta* and *Chelonia mydas*, respectively, and described as new taxa (16, 17). *Polysiphonia carettia* has been reported also from the Atlantic Ocean and recently from some Mediterranean areas on the carapace of *C. caretta* individuals (6, 18, 19). Plastidial (*rbc*L) and mitochondrial (COI) markers were sequenced from our specimens. The sequences, contrasted on public online databases (BOLD and GenBank), revealed that the specimens from Filicudi Island should be ascribed to the genus *Neosiphonia* M.S.Kim & I.K.Lee. Unfortunately, no other DNA sequence is presently available for the epizoic *Polysiphonia*-like species, including those taxa described exclusively on the carapace of marine turtles (*P. carettia*, *P. tsudana* Hollenberg and *N. cheloniae*).

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4.4 = KARYOMORPHOLOGY AND DNA QUANTIFICATION IN THE MARINE ANGIOSPERM *HALOPHILA STIPULACEA* (FORSSK.) ASCH. (ALISMATALES, HYDROCHARITACEAE) FROM RED SEA

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Cytogenetic studies for systematic and evolutionary purposes, utilizing basic karyological features, are lacking on marine phanerogams (1). The genus *Halophila* is widely distributed along tropical and subtropical coasts (2, 3). According to Guiry's AlgaeBase (4), it includes about nineteen species of which only one, *H. stipulacea* has been reported in the Mediterranean Sea (from Egypt to southern Italy), and it is possibly an early introduction through the Suez Canal. In this work we described, for the first time, the karyotype and genome size of a *Halophila stipulacea* population from type area (Golf of Aqaba, Red Sea, Israel). Karyotype analysis were performed on the following indices: long arm length (L), short arm length (S), length of each chromosome (C), arm ratio (r), centromeric index (CI), total length of the haploid set (THL), total relative length [TRL= (length of whole chromosome/total length of all chromosomes in the haploid set including the one being measured)·100]. To estimate karyotype asymmetry, the Coefficient of Variation of Chromosome Length (CV_{CL}) and the Mean Centromeric Asymmetry (M_{CA}) values were calculated according to Peruzzi and Eroglu (5). The estimations of DNA amounts were carried out by Image Cytometry (IC) on material previously treated for Feulgen-densitometric measurements, using the interphase-peak method (6). The results obtained have been compared with those reported for the Mediterranean populations (7). No infra-populations differences were found in the karyotypes. All had similar karyologycal features with $2n$= 18 chromosomes and a karyotypic formula $2n$= 18= 10m + 2sm + 6st and presented a slightly bimodal karyotype with four large and five smaller chromosomes. Associated with this constant karyotype, only small differences in chromosome size between populations were detected. The similarity in chromosome length was also supported by genome size data. Moreover, attempts to infer patterns of chromosome evolution by comparative karyotype analysis with other species in the genus are also presented. Aneuploidy and polyploidy seemed not involved in the speciation of this genus. Within the order Alismatales, only two families (Alismataceae and Araceae) possess genomes larger than 1C= 5pg (8). Although polyploidy has played a role in generating these larger genomes, the increase in chromosome size seems the main mechanism behind these results, with the largest chromosomes so far reported in species with low chromosome numbers in Alismataceae, Hydrocharitaceae, and Araceae (8, 9).

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4.4 = SHORT TERM INTERACTIVE EFFECTS OF MUCILAGE AND SEDIMENTATION ON ENCRUSTING CORALLINE ALGAE

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Crustose coralline algae are among the most abundant and widespread occupants of hard marine substrata in the photic zone worldwide (1). (2) asserted that crusts are better than other algal forms at withstanding disturbance (both biotic and abiotic) and they are more stress-tolerant than most other algal forms. However, (3) and (4) have recently demonstrated that they can be particularly damaged by both mucilage and sediment deposition but no information on the interactive effects of the two above-mentioned factors of crustose coralline algae are available in literature yet.
To deepen the knowledge on this topic, a manipulative field experiment was performed in the summer of 2015 in Tavolara Punta Coda Cavallo Marine Protected Area, along the north-eastern coast of Sardinia (western Mediterranean) during a bloom of the allochtonous, mucilage-forming microalga *Chrysophaeum taylorii* Lewis & Bryan. In a C zone of the MPA, 12 randomly located plots (15 × 15 cm) were prepared in the upper subtidal zone (about 1.5 m of depth). Two treatments were randomly assigned to the plots following a completely orthogonal design (n = 3) (Fig. 1). Plots were attributed to 4 different treatments: in some plots mucilage was manually removed from half of the units once a week during the bloom (M+ *vs* M-) and sediment deposition was increased (S vs. S+) doubling the amount of sediment in each (estimated using sediment traps). The percent cover of dead (white) and live (pink) coralline algae (Fig. 2) was assessed in each plot at the beginning and at the end of the study on photographs taken by a digital camera, superimposing onto each image a grid of twenty-five sub-quadrats. Each sub-quadrat was scored from 0 to 4% and the total % cover value was obtained by adding up the 25 resulting values. Finally, A 2-way ANOVA was performed to test for the effect of 'Mucilage' (and "Sedimentation" (2 levels, fixed and orthogonal) on live corallines. Cochran's test was used prior to the ANOVA to check for homogeneity of variance, while SNK tests were used for posteriori comparisons of means.
At the beginning of the experiment (July), the percent cover of live crusts was included between 40 and 50% in all plots while at the end of the experiment (September) notable differences were observed among treatments. In particular, where mucilage was not present, higher percent covers were observed in control plots where no sediment was added (SM-: 23%; S+M-: 7%). On the contrary, in plots where mucilage was not removed, surprisingly, live corallines were more abundant where sediment was added (S+M+: 19%; SM+: 6%). These results were confirmed also by the statistical analysis and suggest that the percent cover of live coralline algae on the substratum is regulated by the interaction of both the considered stressors. In particular, their effect seems to be antagonistic because their final impact is smaller than the one obtained evaluating each single stressor (5).

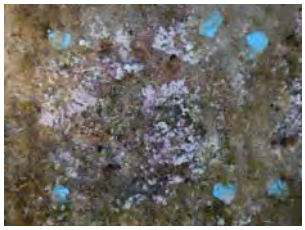

Fig. 1
Live (pink) and dead (white) coralline algae in one of the experimental units.

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4.4 = THE GENUS *ANTITHAMNION* (CERAMIALES, RHODOPHYTA) IN THE MEDITERRANEAN SEA: NOMENCLATURAL AND TAXONOMICAL PROBLEMATICS

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The genus *Antithamnion* Nägeli 1847 (1), as currently defined, is restricted to those filamentous red algae with axes completely lacking rhizoidal cortication, determinate "branchlets" arranged in opposite pairs, presence of a small quadrate cell at the base of each "branchlets", presence or absence of gland cells, cruciate tetrasporangia, and carpogonial branches borne singly on the basal cells of the "branchlets" (2, 3, 4). According to Guiry's AlgaeBase (5), the genus includes 41 species widely distributed between the tropics and temperate regions worldwide. Several authors have suggested that some of these species have extended their distribution range from the Pacific to the Atlantic and Mediterranean coasts (6, 7, 8, 9, 10, 11, 12). The branching pattern of the determinate "branchlets", the origin of new indeterminate lateral axes, and the position of gland cells are useful characters for discriminating between species in *Antithamnion* (3, 13, 14). No molecular data are available on Mediterranean populations. On this basis, seven species are presently reported in the Mediterranean Sea (5, 6, 8, 15): *A. amphigeneum* Millar, *A. decipiens* (J.Agardh) Athanasiadis, *A. cruciatum* (C.Agardh) Nägeli, *A. heterocladum* Funk, *A. hubbsii* Dawson, *A. piliferum* Cormaci & G. Furnari and *A. tenuissimum* (Hauck) Schiffner.
In this work, we present, for the first time, a molecular and morphological study on an *Antithamnion* population from the Straits of Messina (Sicily, Italy). Our result highlighted a discrepancy between molecular and morphological data. Specifically, the studied population could be attributed to *A. amphigeneum* using the morphological characters, while its *rbc*L sequence is determined as *A. nipponicum* (*sensu* 3) in BOLD and GenBank online databases. This discrepancy has led us to a careful analysis of the morphological and molecular data reported in the literature on the *Antithaminon* species. A number of imprecisions, producing a confusing state on both taxonomic and nomenclature aspects, were detected and are here discussed. In conclusion, we can say that although the genus *Antithamnion* is one of the most well-known marine taxon and several articles on its taxonomy and systematics have been published, yet attribution of samples at species level is problematic. This uncertainty is surely due in part to the high variability of the morphology of some species, but also to the lack of care of some authors in comparing the morphological characteristics of their specimens with those reported in the literature. This is further aggravated by the fact that type specimens are hardly used as reference for identifications and names with uncertain application are commonly adopted. It is advisable that more morphological and molecular data on the type material of each species of *Antithamnion* are done to assess its taxonomy. Therefore, the starting point would be the characterization of *A. cruciatum,* the generitype, from the type locality (Trieste, Italy).

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4.4 = GEOGRAPHIC PROFILING: HOW A CRIMINOLOGICAL METHOD CAN HELP TO FIND THE ORIGIN OF A BIOLOGICAL INVASION BY A GREEN ALGA (*CAULERPA CYLINDRACEA* SONDER) IN COMBINATION WITH OTHER ANALYTICAL TECHNIQUES

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Geographic profiling is an analytical method used for investigating a series of crimes in order to identify the origin (the home of the offender) of them (1). This method uses data given by the position (x, y) of the investigated facts on a map. The analysis is based on a function that reduces the probability of the fact increasing the distance from the origin and another function that introduces an exclusion zone around the origin where the probability of the fact would be very low. The attempts of using the method to find the origin of biological events on a map is related to the presence and the meaning of the exclusion zone, that should be evaluated case by case. The use of this method for the identification of the origin of a biological invasion is justified by the hypothesis that the exclusion zone may be related to the increase in efficiency of the dispersal mechanisms of the organisms spreading in the territory and, as a matter of fact, the method proved to be useful in various cases of biological invasions, for instance for the spreading of two species of *Caulerpa* (Chlorophyta) in the Mediterranean (1) and of a pest (2). Enhancements of the method are based on the use of other analytical tools that can deal with 1) multiple origins for an invasion (Fig. 1); 2) non-centrifugal patterns of dispersal; 3) very different quantitative meaning of each observations on the territory; 4) the proposal of robustness indexes for the obtained results.
The first point was faced with the use of Kmeans, Voronoi tessellation and Silhouette for finding the most probable number of subclusters (3); of the Dbscan method for not centrifugal dispersal (4); of a weighted method for cases in which the observations have a very different importance point by point (5), while the robustness of the results may be assessed with Jackknife (6).

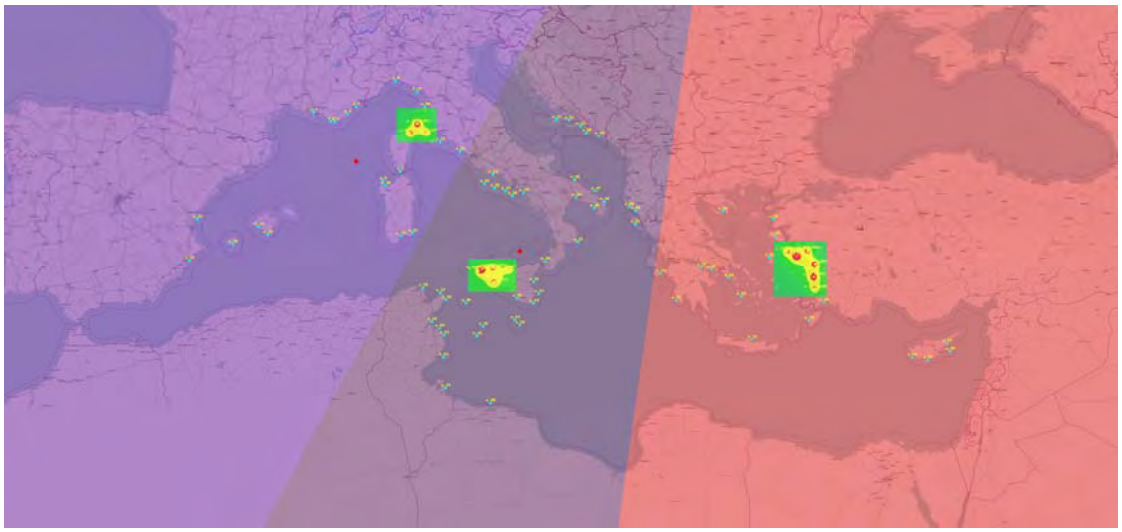

Fig. 1
Centres of origin in the three subclusters of *Caulerpa cylindracea* Sonder in the Mediterranean.

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4.4 = BALLAST WATERS AS A PROMINENT VECTOR FOR THE TRANSFER OF HARMFUL AQUATIC ORGANISMS: THE STUDY OF PHYTOPLANKTON AND DINOFLAGELLATE CYSTS IN THE ITALIAN PORT OF BARI (BALMAS PROJECT)

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Ballast water (BW) transferred by vessels has been recognized as a prominent vector of harmful aquatic organisms and pathogens (HAOP), causing global environmental changes and posing a threat to human health, property and resources. Transport by BW is an increasing problem in the Adriatic Sea and the volume of BW discharged into its ports is above 10 million tons per year. Due to a foreseeable growth in traffic, BW transfer could soon increase considerably, as the Adriatic Sea is a seaway used mainly by international shipping transporting goods to and from Europe (as hinterland), with intense local shipping as well.
The general aim of BALMAS project was to establish a common cross-border system among Adriatic countries in order to avoid unwanted risks to the environment from the transfer of HAOP, through control and management of ships' ballast waters and sediments. One of the objectives of the BALMAS project was the realization of port baseline surveys (PBS) in the biggest ports of the Adriatic Sea, including the one of Bari (Italy) (1). PBSs are used to develop an overview of native species and non-indigenous species (NIS) present in the ports, which is the basis for developing a list of HAOP to be used as a baseline for tracking new introductions.
A wide range of sampling techniques was used to collect marine organisms from the different habitats within the port area (2). As for phytoplankton, samples in the Italian port of Bari were collected during five sampling campaigns at six fixed stations, located inside and outside the port. The total list of phytoplankton taxa found during PBS in the port of Bari comprises about 300 entries. More than half of the taxa belonged to dinoflagellates, one third to diatoms, including 1 potentially toxic diatom and several toxic dinoflagellates. Some microalgae have remained unidentified (e.g., *Alexandrium* sp., cf. *Azadinium* sp., *Karenia* sp., *Ostreopsis* sp.) or with uncertain taxonomic identification (e.g., *Karenia* cf. *mikimotoi*, *Karenia* cf. *selliformis*), thus the number of potentially harmful organisms could increase.
Sediments for analysis of dinoflagellate cysts analysis were sampled in May and November 2014 at three fixed localities inside the port of Bari. Cysts were extracted from the sediment samples and analyzed to determine the concentration of living cysts. In particular, cysts of the potentially toxic species *Alexandrium* sp., *Gonyaulax spinifera* and *Lingulodinium polyedrum* were observed. These species were detected also as vegetative cells in phytoplankton samples collected inside the port. Some cysts were singularly isolated and incubated under controlled environmental conditions in growth chambers; the aim was to induce the excystment of the vegetative cells and achieve a correct identification of the corresponding dinoflagellate species. A total of 32 cysts were isolated from the sediment samples collected in spring 2014 while 42 were obtained from the survey performed in winter. All the cells were periodically monitored and two cultures were finally obtained which corresponded to the species *Scrippsiella* cf. *trochoidea*.

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4.5 = COMPARATIVE REPRODUCTIVE BIOLOGY OF *TULIPA PUMILA* MOENCH AND *T. SYLVESTRIS* L. (LILIACEAE)

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Preliminary observations conducted in 2010 revealed that Tuscan population of the only two tulips native to Italy, i.e. *Tulipa sylvestris* L. and the systematically closely related *T. pumila* Moench (= *T. australis* Link), hosted several individuals with male flowers. This can be considered as a case of morphological androdioecy (1, 2), which in the genus was reported before for a population of *T. sylvestris* around Modena (3). According to (2), morphological androdioecy can be caused by: i) cryptic dioecy, ii) functional androdioecy and. iii) resource-dependent sexual allocation. We investigated the breeding system of these two uniflorous bulbous geophytes, to provide an explanation for the occurrence of male and hermaphroditic flowers.
Demographic surveys on 6 populations (2 for *T. pumila* and 4 for *T. sylvestris*), morphometric analyses on scape diameter, leaf width and perigone length on 3 populations (2 for *T. pumila* and 1 for *T. sylvestris*), along with pollination (1 population each species) and seed germination (2 for *T. pumila* and 1 for *T. sylvestris*) experiments have been carried out from 2010 to 2012. Pollen viability (MTT staining test) and germinability (4), along with stigma receptivity (Perex Test), have been also evaluated. Pollination experiments were performed according to the following treatments: i) emasculation and bagging of hermaphroditic flowers (iE), ii) self-pollination of hermaphroditic flowers (iA), iii) cross-pollination with pollen of hermaphroditic flowers (ixE), and iv) cross-pollination with pollen of male individuals (ixE). For each of these treatments, seed germination was carried out using incubators and outdoor nurseries (quasi-natural conditions).
Generally, we found a low number of flowering individuals in all the surveyed populations, suggesting that in both species vegetative propagation plays an important role. Indeed, clonality is favoured by the presence of droppers, i.e. tubular hypogeal axial organs carrying buds inside their tips. We found droppers in both species, and directly observed their formation during plantlet development. No seeds were produced after iE experiment, pointing to the absence of apomixis, whereas after iA experiments a few seeds were obtained in *T. pumila*, which is probably partially self-compatible.
An extremely variable (from 5 to 65%) occurrence of male individuals (i.e. with aborted gynaeceum), fluctuating among years, was found in all the surveyed populations. Male individuals showed scape diameter, leaf width and perigone length lower with respect to hermaphroditic ones and their fitness was not increased, neither in terms of pollen viability nor in terms of germinability, as well as of production, size and seed germinability. The lack of an increased fitness of male individuals and the extremely variable sex ratio do not support cryptic dioecy. Similarly, functional androdioecy does not fit with smaller male individuals, not showing an increased fitness (5). On the other hand, the occurrence of genetically-based male individuals is not consistent with the fluctuation of frequency seen across reproductive seasons (6). Taken altogether, our findings suggest that unisexual flowers could represent the extremization of a resource-dependent sex allocation strategy, with the possibility of a sexual phenotype switch during lifetime (gender diphasy), as already reported in other Liliaceae (6, 7, 8). Hence, in accordance with what found in *Lilium apertum* Franch (8), both the studied tulip species show an andromonoecious sexual system, where female fitness of hermaphroditic individuals is increased by male flowers, through an enhancement of both the attractiveness for pollinators and/or the availability of pollen (8, 9, 10).

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4.5 = POLLEN TRANSFER EFFICIENCY IS A CHARACTERISTIC OF SPECIES RATHER THAN POPULATIONS IN THE MEDITERRANEAN FOOD-DECEPTIVE ORCHID *ORCHIS ANTHROPOPHORA*

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Pollen transfer efficiency (PTE), i.e. the proportion of exported pollen reaching conspecific stigmas, is a key factor for the evolution of pollination systems in flowering plants. The success of entomophilous pollination systems is largely dependent on the increasing of PTE with respect to anemophilous systems (1). In this context, orchids have pollen packed in peculiar structures called pollinaria that allow an insect to carry all or half of the total amount of the pollen produced by a flower in a single visit thus further increasing PTE. Recent studies (2, 3) showed that in deceptive pollination strategies PTE differs in generalised and specialised systems being higher in the latters and likely representing one of the reasons that lay at the basis of the multiple independent transitions occurred among pollination strategies in this plant group (2). This finding suggests that PTE can be linked to the specific pollination system employed by a species rather than being influenced by local environmental and pollinator community differences as occurs for male (MRS) and female (FRS) reproductive success. However, the levels of variability of this parameter in multiple conspecific populations are still unclear.
Here we surveyed seven different populations of the Mediterranean food-deceptive orchid species *Orchis anthropophora*. We estimated PTE as well as MRS and FRS by observing the amount of pollen exported and deposited.
Overall, our results show that although MRS and FRS vary among populations, PTE, that is a combination of both, is less variable (Fig. 1). These results strongly suggest that PTE is mostly characteristic of species rather than populations and likely depends on pollinator behavior being thus less influenced by ecological factors that can alter the pollinator local abundance and composition.

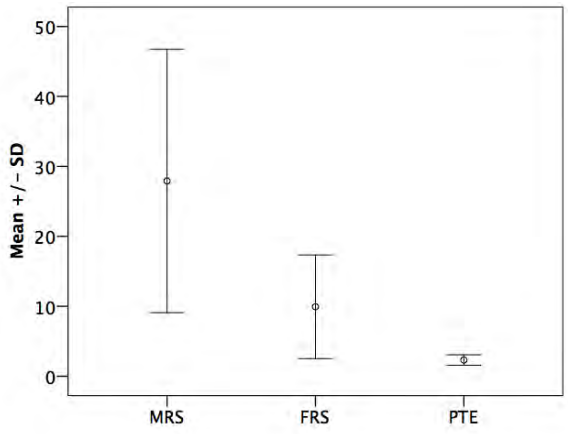

Fig. 1
Mean +/- Standard Deviation for MRS, FRS and PTE.

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4.5 = A COMPARATIVE STUDY ON FLORAL TRAITS IN TWO MEDITERRANEAN ORCHIDS WITH A DIFFERENT POLLINATION STRATEGIES

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The extraordinary diversity of living forms is the direct consequence of the rising of reproductive barriers which interrupt genic flow between populations causing their differentiation and so an independent evolution. Most species of flowering plants offer rewards to their pollinators, but some base their pollination on deception (1). Deception is typical of Orchidaceae and some authors define this strategy as the key element of the diversification of this family (2). Using a morphological and functional approach on eight orchids species as sample of food (3) and sex (4) deception strategies, we predicted that stigmatic cavity and pollinia will covary, since they are both involved at different levels as a pre-zygotic barrier; their relation should be stronger in sexual deceptive orchids than in those that use food deception. We also investigated (i) the variation of each floral part within each species and each deception strategy; (ii) if pollinia and stigmatic cavity are interrelated and finally (iii) if a precise morphology of stigmatic cavity and pollinia is promoted by natural selection. In Sardinia there are 16 endemic species among 68 taxa of orchids, within the subtribe Orchidinae, the genera *Anacamptis, Himantoglossum* and *Orchis* are used examples of food deception and *Ophrys* as an example of sexual deception.
We examined 160 flowers over the duration of this study. Means and standard deviations of floral traits were run. Correlations between pollinia length and height and width of stigmatic cavities are performed (Fig. 1). We correlated these values both at species and strategy levels. Positive correlations are generally expressed in both strategies. We found that phylogentically closely related species have pollinia length very similar. Hence, we suggest that this is due to adaptation to the similar morphology of strictly phylogenetically related pollinator or because the studied species can be affected by phylogenetic constraints.
The comparative analysis of the various floral traits underlines how the each sterile portion is variable within in each species. This confirms the idea that in deceptive orchids the variation of sterile floral traits can be relevant in the deception process. In contrast, a stabilizing selection is proposed to explain the low variability in fertile traits.

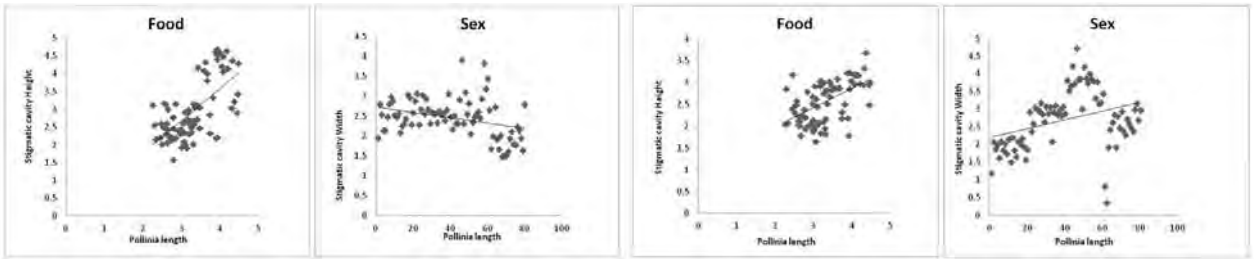

Fig. 1
Correlation between stigmatic cavity height/weidth and pollinia length in the two strategies.

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4.5 = GENOMIC SCAN REVEALS CLADOGENETIC AND BIOGEOGRAPHIC PATTERNS IN AN ORCHID BIODIVERSITY HOTSPOT

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Understanding why some geographic regions harbour an exceptionally high species diversity is a key topic in evolutionary and conservation biology. Overall, two scenarios are generally invoked: 1) speciation/extinction rates due to the local availability of ecological opportunities shaping the so called "ecological limits" on diversity (1) and/or 2) secondary contacts of formerly geographically separated lineages that increase the genetic diversity of taxa so offering the opportunity to natural selection for creating new and unique combinations (2).
Several phylogeographic studies have suggested that Gargano promontory represents a meeting point of eastern and western plant lineages, as a consequence of a putative Pliocenic land bridge between the two banks of the Adriatic Sea (3). The promontory has been repeatedly connected and disconnected from Greece and Italy (4). Nowadays, despite its connection with Italy, it is considered an “ecological island”, because the surrounding landscape is characterised by very different ecological conditions.
The genus *Ophrys* is renowned to be the orchid clade experiencing the faster evolutionary radiation in the Mediterranean basin (5), thus representing a suitable model to study the reasons of local biodiversity. This plant group is particularly species-rich on the Gargano region with several local taxa with a high morphological diversity that is not mirrored in a corresponding genetic differentiation.
However, recent advances in genomic tools allow making cladogenetic and biogeographic inferences even among closely related taxa thus shading light on species delimitations and, at the same time, on the reasons that lay at the basis of biodiversity hotspots. With this aim, we investigated genomic variation in four closely related species in the *Ophrys sphegodes* group by Genotyping By Sequencing (GBS) method.
We gained different outcomes using different criteria of SNPs selection. By using SNPs shared by at least 10% of species accessions, we obtained a tree supporting a cladogenetic signal clearly separating the four species otherwise indistinguishable with traditional molecular tools. Complementary, when using highly conserved and shared SNPs across accessions (mainly including organellar DNA), we identify two main biogeographic lineages grouping individuals independently from their species categorization. This suggests that Gargano may represents the meeting point of two *Ophrys* lineages likely differentiated in the opposite side of the Adriatic Sea. To confirm this biogeographic pattern, further studies including accessions from the eastern and western populations are in progress.
Overall, our results suggest that species divergence in this group is extremely recent whilst biogeographic lineages are older. Also, our findings support a scenario in which the Gargano hotspot of orchid biodiversity is a consequence of the meeting among different lineages of taxa that, once in secondary contact, increased their genetic variability and provided the fertile background for further diversification. This study highlights the potential of genomic data not only for the characterization of cladogenetic patterns in complex plant groups but also for the identification of recent biogeographic routes.

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5 = A REVIEW OF THE SUCCESS IN WETLAND PLANT TRANSLOCATIONS

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Translocation (*sensu* IUCN) is largely applied as a technique to halt the loss of biodiversity.
To be effective in the long-term perspective, translocations should be applied with the supporting science. Recent reviews have shown that translocation can only be partially effective (1). In fact, the success of translocation can be compromised with even minor gaps in knowledge of the target species biology, ecology, interactions with other species, threats, etc. leading to failure (2). The analysis of several translocation cases is very useful to improve our understanding of causes of success and failure. The aim of this work is to analyze several cases of wetland plant translocation to find out best practices for improving the success of translocation. Wetland plants are often grown *ex situ* but, unlike terrestrial plants, the interplay of different ecological factors such as the climate, the water quality, the soil and the water circulating in the soil, make acquiring a real understanding of the target species ecology, the identification of threats and their removal, the selection of suitable release site, etc. more challenging. A worldwide review of the rate of success in wetland plants translocation is presented. The review takes into account how translocations were carried out, how threats were identified and removed, reasons for success or failure, the types and aims of pre-translocation studies performed and the how the success is perceived and evaluated.

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5 = THE GENUS *CORTINARIUS* IN ITALY: MOLECULAR STUDIES FOR TAXONOMIC AND NUTRACEUTICAL REASSESMENT

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Cortinarius is the most species rich genus of the Agaricales with a worldwide distribution. *Cortinarius* species are important ectomycorrhizal fungi associated with different trees and shrubs belonging e.g. to the order Fagales and families Pinaceae and Salicaceae.
The high variability of the existing species makes it very difficult to achieve their clear and complete taxonomic cataloguing. Nevertheless, in recent years, many studies concerned their taxonomy and the advent of new molecular strategies has given new impetus to the research by providing an important contribution to taxonomic reorganization.
The experimental work presented here - which is part of a larger project - has focused in particular on *Cortinarius* species found in the Alps and the Apennines of Marche, with the aim of widening knowledge on this genus and providing important information for accurate taxonomic reassessment.
In addition, the polyphenolic content and the antioxidant activity of the ethanol extract of the *Cortinarius glaucopus* species, one of the most common cortinaries (edible but not yet on the official list of edible fungi and not much appreciated) was analyzed by biochemical studies.
All experiments were carried out on both fresh samples and samples of personal collections or public herbaria. All the samples received were subjected to morphological and microscopic observations and sequence analysis of the ITS region.
The morphological and microscopic characterization of old herbarium specimens was based on original diagnosis, while expert mycologists described the fresh samples.
The ITS sequences of all the samples were compared with Databases sequences by BLAST algorithm and phylogenetic analyses.
The analyses of the results obtained highlight some critical issues in the definition of the identification parameters used in the current taxonomy of *Cortinarius* spp, confirming that the morphological identification alone is not sufficient. Moreover, this demonstrates the importance of an integrated molecular analysis in order to recognize the various species and their real diversity.
Finally, given the increasing interest in the nutraceutical content of (many) mushrooms we have evaluated the antioxidant activity of *Cortinarius glaucopus*; specifically, the presence of polyphenols and the antioxidant activity of the ethanol extract of the *Cortinarius glaucopus* have been evaluated. This investigation is based on the initial preparation of alcohol extracts and their subsequent analysis of the total content of polyphenols.
The results obtained from biochemical investigations revealed a significant antioxidant activity of *C. glaucopus* harvested in Italy. Such evidence could give this mushroom a value in the pharmacological-nutritional field.

5 = WHEN ART MEETS BOTANY: FULGENZIO VITMAN'S HERBARIUM IN PAVIA (PAV)

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The *Herbarium Universitatis Ticinensis* (PAV), currently housed at the Botanical Garden of the University of Pavia (Department of Earth and Environmental Sciences), was probably constituted in the late 18th century, under the direction of Giovanni Antonio Scopoli (1723−1788), between 1777 and 1788 (1). A key figure in the development of the institution, he was likely responsible of acquiring the herbarium of the Swiss naturalist Albrecht von Haller (1708−1777), subsequently stolen during Napoleon's occupation in 1796 and now stored at the Muséum National d'Histoire Naturelle (P) (2−5). Unfortunately, no traces of Scopoli's collection exist today in Pavia; it probably shared the same fate of Haller's herbarium (3).
Despite these historical and dramatic losses, a relevant amount of specimens from the 18th century still persist at PAV, specifically represented by the herbarium of Fulgenzio Vitman (1728−1806). Italian abbot and botanist, Vitman is best remembered today as the founder of the botanical gardens of Pavia (1773) and Brera in Milan (1777) under the Austrian Empire (5). Since his education period at the Vallombrosa Abbey (Tuscany), he organized a private herbarium, which reached an amount of 6,000 specimens (6). In 1785, a large part of the herbarium (40 out of 60 folders) was sold by him to the Austrian government and then transferred to the University of Pavia, where it was employed for teaching purposes by Scopoli, by the will of the Emperor Joseph II (5). Currently, 1,450 specimens are still present at PAV, while 315 (probably derived from the 20 unsold folders) have been found at the herbarium of the Natural History Museum of Verona (VER) (6−7).
Being mostly without localities and dates of collection, Vitman's specimens are mainly of historical and artistic value. Many of these actually feature dried parts completed with watercolour drawings, usually depicting plant structures, like flowers, fruits and fleshy leaves, difficult or impossible to be dried with satisfactory results. This "mixed technique" was elaborated by Vitman himself for learning purpose, allowing his students to examine morphological characters otherwise impossible to be preserved at that time; he defined his collection as an "Orto Secco" (i.e., "dried garden") for serving as a support to the winter lectures, in place of the fresh specimens employed during spring and summer (8). Vitman's illustrations are highly realistic with respect to shapes, proportions and coloration; details, such as hairs and seeds, have been delineated with relevant scientific care. Colors have been tastefully chosen, they appear lively and pleasantly in contrast with the duller desiccated organs. The combination of painted and dried plant features is highly peculiar on both scientific and aesthetic grounds, making this collection, as already stated (6), a unique example among the Italian herbaria.
Vitman's herbarium at PAV is under study by the authors, in view of its possible connection with both the history of the Pavia Botanical Garden and some of the taxa he described within his main work, *Summa plantarum* (9).

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5 = COMPARISON OF DUNE HABITATS OF NORTH MEDITERRANEAN COASTS - TUSCANY (IT) WITH THOSE OF BLACK SEA COASTS -DOBROGEA (RO, BG): SIMILARITIES AND DIFFERENCES

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Data related to the floristic and phytocoenotic features of dune areas located in two European coastal areas are reported. These areas are very different by the biogeographical point of view: the coast of Tuscany (Italy, Mediterranean Region, Italo-Thyrrenian province), and the coast of Dobrogea, a cross-border coastal strip between Romania and Bulgaria on the Black Sea (Eurosiberian Region, Escitian province). Both coasts are about 300 km long, and include main habitats (sensu Directive 92/43/EEC) of European coastal dune environments (habitats 1210, 2110, 2120, 2130, 2160, 2210, 2230, 2240, 2250) (Tab. 1). Some habitats have been identified exclusively in a specific geographical area (H2210, H2230, H2240, H2250 only in Tuscan coast (T), H2130, H2160 only in Dobrogea (D), some common to both (H1210, H2110, H2120) (Fig.1) (1, 2).
A gradual increase of stenomediterranean species (Tuscany) or eurimediterranean species (Dobrogea) proceeding from north to south was found; as well as a high floristic diversity between the two compared bioregions even within the same habitats. Finally the floristic difference tends to increase from foredune habitats to fixed dunes. This is related mainly to phytoclimatic, phytogeographical, ecological and geomorphological factors.
In both study areas a discontinue presence of some foredune habitats (eg. H1210, H2110, H2120) due to anthropic alterations was found.

Tab. 1
Dune habitats of the reported areas.

N°	Habitats Directive 92/43/EEC	Natura 2000 code	
1	Annual vegetation of drift lines	1210	T, D
2	Embryonic shifting dunes	2110	T, D
3	Shifting dunes along the shoreline with *Ammophila arenaria* (white dunes)	2120	T, D
4	Fixed coastal dunes with herbaceous vegetation (grey dunes)	2130*	D
5	Dunes with *Hippophaë rhamnoides*	2160	D
6	*Crucianellion maritimae* fixed beach dunes	2210	T
7	*Malcolmietalia* dune grasslands	2230	T
8	*Brachypodietalia* dune grasslands with annuals	2240	T
9	Coastal dunes with *Juniperus* sp. pl.	2250*	T

Fig. 1
Vegetation landscape of dune of Tuscany (M.di Vecchiano) (left) and Dobrogea (Durankulak) (right).

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5 = PHYSIOLOGICAL ALTERATIONS IN OLIVE TREES AFFECTED BY CO.DI.R.O.: PRELIMINARY STUDIES

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The Olive rapid decline complex ("Complesso del disseccamento rapido dell'olivo"; Co.Di.R.O.) is a disease characterized by the presence of leaf scorch and scattered desiccation of twigs and small branches which, in the early stages of the infection, prevail on the upper part of the olive (*Olea europaea* L.) canopy (Fig. 1A-D). Diseased trees show the concomitant presence of: i) extensive galleries drilled by *Zeuzera pyrina* L. larvae; ii) necrosis of the sapwood, which is invaded by different fungal species of *Phaeoacremonium*, *Phaemoniella*, *Pleumostomophora* and *Neofusicoccum* (1-3); and iii) *Xylella fastidiosa* (Wells et al.) subsp. *pauca* (4). Co.Di.R.O. abbreviation identifies the strain of *X. fastidiosa* observed in the southern of Apulia (Salento, Italy), whereas the disease name is changed into "Olive quick decline syndrome" (O.Q.D.S.), considering the bacterium as the only aetiological agent (5). In the present investigation we analyze some physiological features involved in Co.Di.R.O.-affected trees. Leaves were collected from trees of the cultivar *Ogliarola* in the province of Lecce (Apulia, Italy). 50 Co.Di.R.O. affected plants were selected and for each plant 150 asymptomatic leaves were collected. The levels of total phenols, antioxidant capacity, lipid peroxidation and electrolyte leakage were determined in the collected leaves according to methods described by Buono et al. (6), Miller and Rice-Evans (7), Heath and Packer (8), and Bajji et al. (9), respectively. Leaves collected from healthy trees in not-infected area were utilized as control. Data were statistically analyzed by ANOVA test; comparisons of means were performed according to Dunnet's LSD test. Leaves of infected plants showed a 48 % decrease of the total phenolic content (Fig. 1E), a three-fold increase of antioxidant capacity (Fig. 1F) and a six-fold increase of lipid peroxidation (Fig 1G). Damages on membrane components are confirmed by the increase of electrolyte leakage (Fig. 1H) data. In conclusions, Co.Di.R.O.-affected plants show structural and metabolic alterations of the asymptomatic leaves which could be utilized as precocious markers of the disease development.

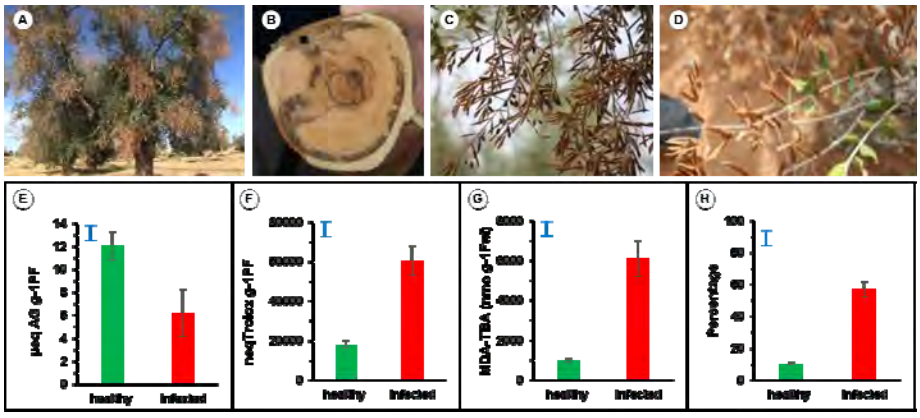

Fig. 1
Olive tree symptoms (A-D). Total phenols content (E), antioxidant capacity (F), Malondialdehyde (MDA) concentration (G) and electrolyte leakage (H) of *Olea europaea* cv Ogliarola leaves. Values are the average ± sd. LSD (P=0.05) is presented as vertical bar.

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5 = IMPROVEMENT OF *IN VITRO* DEVELOPMENT FOR EPIPHYTIC ORCHIDS: COMPARISON BETWEEN CULTURE *MEDIA*

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The Orchidaceae family is the largest of angiosperms, with about 28.000 species known (1). Many of these plants are protected by national and international laws, and most of them feature a great ornamental value. *Cymbidium*, *Phalaenopsis*, *Dendrobium* and *Paphiopedilum* are the main genera for the ornamental sector. In this study we tested different culture media for asymbiotic *in vitro* sowing of species native to Eastern Asia. Germination results from three "traditional" *media* (MS, MM and KC) (2, 3, 4) were comparable, with > 95% germination rates for fresh seeds of *Dendrobium wardianum,* > 65% for *Cymbidium tracyanum* seeds. By contrast, germination rates were significantly higher for *Laelia purpurata* old seeds, stored for 1 year at 4°C, by using 1/4 MS (25.1 ± 9.1%). Morphological data were also collected for *Cymbidium tracyanum*. Statistically significant differences were observed in the production and length of leaves and roots. Indeed, after four months 1/4 MS enriched with coconut water induced the production of a higher number of leaves than those obtained with MM and KC, with a higher average length, a higher number of roots and a higher average root length. 1/4 MS also improved the chlorophyll content extracted from leaf tissues.
The comparison of these three "traditional" *media* suggest the utilization of Murashige & Skoog basal salts for the *in vitro* propagation of these Asian epiphytic orchids.

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5 = SEED HETEROMORPHY INFLUENCES SEED LONGEVITY IN *AEGILOPS*

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The genus *Aegilops* L. had a great role in wheat domestication and evolution and needs to be conserved, in order to preserve wheat wild relatives genetic resources for crop improvement (1, 2, 3). Some *Aegilops* species produce dimorphic pairs of seeds, within dispersal unit, different both in size and in colour (one seed is bigger and lighter-coloured while the other is smaller and darker) but also in the germination behaviour. Hypothesis from previous studies (4, 5) suggest that this strategy is probably due to bet-hedging strategies to avoid the competition among sibling seedlings. Preliminary observations in *Aegilops* seed pairs, suggest that the bigger seed is ready to germinate soon after the dispersion (at the end of summer) while the smaller one remain dormant in the soil until the next year. To clarify this hypothesis a comparative seed longevity tests has been carried out for different seed morphs of six species of the genus (*Ae. biuncialis*, *Ae. cylindrica*, *Ae. geniculata*, *Ae. neglecta*, *Ae. peregrina*, *Ae. uniaristata*), growing in the Mediterranean Basin. Comparative aging tests allow to evaluate differences in seed longevity among seed lots, thereby inferring differences of soil seed bank persistence and *ex situ* storage life span (6, 7). Our results show that bigger seeds are shorter-lived and germinate faster than smaller ones. This supports the hypothesis of different germination timing and soil seed bank persistence among seed morphs. Differences among species, in terms of seed longevity, highlight dissimilar dispersal strategy and germination ecology among *Aegilops* species. The significant differences in seed longevity between dimorphic seed pairs in most of the *Aegilops* species tested, suggest that seed morphs may have different longevities also under *ex situ* long-term seed conservation. Biomolecular analysis investigating biochemical and genetic basis of the differential longevity found here are in progress. This study highlights the potential of comparative longevity test as a fast method to investigate seed longevity in species showing seed heteromorphy.

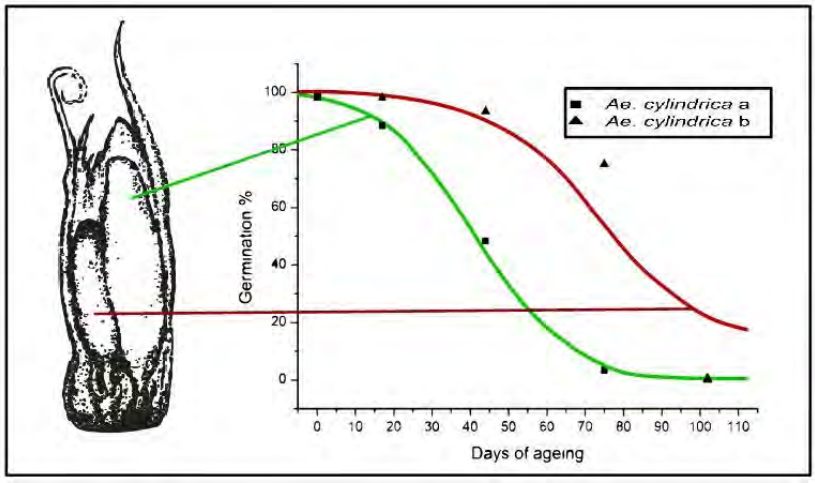

Fig. 1
Spikelet of *Ae. cylindrica* with bigger (a) and smaller (b) seeds and related survival curves fitted by probit analysis.

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5 = POLLEN MORPHOLOGY OF TWO FASCIATED PLANTS OF THE FAMILY OF *ASTERACEAE*

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Fasciation is an abnormal development in plants in which meristems become elongated and produce wide banded stems, distorted flowers, fruits, roots, as well as alterated development of axillary buds. Fasciation seems to be the result of internal influences such as hormonal imbalance, genetic mutation, genetic predisposition, or extrinsic factors such as bacteria, fungi, viruses, insects, mites, frost, herbicides and/or physical damage (1). It is found frequently occurring in many cultivated plants. In agriculture and horticulture, fasciation is a problem because could reduce crop production and then the economic value of plants (2). Fasciation has been recorded in over 100 plant families and is common in the Asteraceae, Cactaceae, Rosaceae, Onagraceae, Fabaceae (3). We have observed this phenomenon in two Asteraceae: *Artemisia umbelliformis* Lam. ssp. *eriantha* (Ten.) Vallès-Xirau & Branas (alpine wormwood) and *Bellis perennis* L. The first is an endangered, herbaceous aromatic plant growing wild at an altitude over 1600 m asl on rock crevices and screes. This plant is used to prepare "genepì" a strong-flavoured liquor, its fasciation was unknown before our study. *Bellis perennis* is an evergreen perennial plant forming rosettes of dark green, spoon-shaped leaves, with a single white daisy-like flower in late spring and summer. This daisy may be used as a potherb. Fresh flowers and leaves are employed in decoctions, ointments and poultices.
B. perennis is well known for fasciation. Twelve fasciated plants of *A. umbelliformis* ssp. *eriantha* have been obtained in an experimental field, located in the Gran Sasso and mountains Laga National Park, at 1170 m asl, where we transplanted about 10,000 clones obtained by *in vitro* propagation. Furthemore we have collected five fasciated plants of *B. perennis* in garden settings. Both *A. umbelliformis* ssp. *eriantha* and *B. perennis* have showed linear fasciation, the stems and flowerheads appeared flattened and elongated. We have compared their pollen grains with Scanning Electron Microscopy (SEM) to assess whether there was a difference between fasciated and not-fasciated plants. Pollen dissected has been gold-coated using Balzer's SCD 040 sputtering, and was viewed using a Philips 505 scanning electron microscope.
In the present study we have observed at SEM about one hundred pollen grains of fasciated and not-fasciated plants. They not have exhibited morphological differences (Fig. 1; Fig. 2). We will continue our research to evaluate other features, like development and increased pollen sterility that could occur in fasciation (4) e (5).

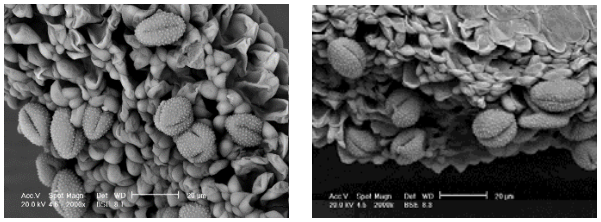

Fig. 1a-1b
Pollen grains of *A. umbelliformis* ssp. *eriantha*: not-fasciated and fascinated.

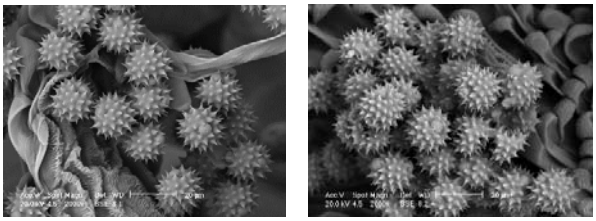

Fig. 2a-2b
Pollen grains of *B. perennis*: not- fasciated and fasciated.

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5 = THE PALEOLITHIC SITE OF POGGETTI VECCHI (GROSSETO): A PALEOPALYNOLOGY RESEARCH

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In 2012, during excavation of a small basin, a prehistoric site was discovered at Poggetti Vecchi, Grosseto (south Tuscany, Fig. 1). The excavation revealed a long stratigraphic sequence with numerous vertebrate bones (*Palaeoloxodon antiquus* and other mammals), wooden and stone tools in the lower units (1): indeed, archeological investigations suggest an iterated occupancy starting at Lower/Middle Paleolithic. Wooden tools were mainly made of *Buxus sempervirens*. Palynological analysis were carried out to gather information about the environmental transformation of the surrounding area in the period of human frequentation and to evidence exploitation of the plant resources. Samples were taken along two stratigraphic sequences: PV1 out-site, and PV2 on-site. A cushion moss picked up near the study area made possible to compare the present vegetation with that of the past. Pollen samples were treated with the routine methodologies and observed at light microscope. The analysis revealed a high pollen content and a good grain preservation in all samples: this made possible an accurate reconstruction of the flora compiling a list of taxa of nearly 100 different morphotypes. The results indicate the study area as an extensive grassland dominated by herbaceous formations typical of wet and moist substrates. The most represented family is Poaceae. The percentage variations of hygro-hydrophilous taxa along the sequence suggest repeated rises and falls of the water table in the alluvial plain. Hygrophilous trees of riparian vegetation like *Alnus* sp., *Salix* sp. and *Populus* sp. were also recorded. Low pollen percentages suggested the occurrence of mixed oak woods probably widespread on the hillside surrounding the site: the pollen of these plants could have been transported to the plain by air and by watercourses. Interestingly, low values of *Buxus* pollen grains were found along all the sequence, despite its short dispersal distances (2) and preservation in the sediments. The presence of *Buxus*, which usually grows on well-drained calcareous substrates as it is the case of the Poggetti Vecchi site, hints human exploitation of the local timber.

Fig. 1

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5 = CAN AN OPTICAL SENSOR DETECT THE SYMPTOMS OF FLAVESCENCE DORÉE AND ESCA DISEASE IN *VITIS VINIFERA*?

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Vitis vinifera cultivation is one among the most popular and profitable activities in Italy. Viticulture represents history, income and landscape. Every year many vines are affected by pathogen attacks that compromise their health state and often cause plant death, leading to important production decreases. Flavescence Dorée (FD) and Esca disease (ED) are among the most common and frequent diseases that affect V. *vinifera*. FD is widely present in the vineyards of the Northern Italy, it is trasmitted by a vector insect (*Scaphoideus titanus* Ball) and it is caused by phytoplasmas, wall-less bacteria belonging to the order *Mollicutes*. The main FD symptoms are: growth reduction, leaf yellowing or reddening and downward rolling of leaves [1]. ED is associated with a group of fungal trunk diseases. The ED complex includes grapevine leaf stripe disease (GLSD) and wood decay that are both wood vascular infections. The vines affected by ED show mainly foliar interval necrosis and choloris [2].
The aim of this work was to evaluate the ability of an optical sensor to detect plants affected by FD and ED in field conditions. In order to test the optical sensor, three vineyards were selected and handly mapped directly into the field. During the first survey the positon of each plant, pole, cutting and fault was recorded on a sheet paper and then reported on a Microsoft Excel digital sheet. From April until the end of July three field surveys were carried out to assess plant health status: the position of each plant that showed symptoms of FD or ED was recorded. During the last survey (July 2015 and 2016) symptomatic plants have been georeferenced by a portable GNNS – MobileMapper® 120 (Spectra precision, Beijing China) and, at the same time, the detection with optical sensors (OptRx™ - Ag Leader, South Riverside Drive Ames, Iowa) on all vineyards surface was performed. The OptRx ™ sensors are part of a "mobile lab" comprising: mean of transport, mobile Pc, software G.I.S., precision G.P.S. They emit light and exploit the principle of reflectance (ratio of reflected light and emitted light) calculated at three known wavelength (VIS 670 nm, RedEdge 730 nm and NIR 775 nm). They record the reflectance every fifty centimeters and, for each point, vegetation indices (NDVI and NDRE) are calculated. Over 18000 points and 10000 plants were considered. Data were processed following two different approaches: the first one based on the statistical analysis of raw data in order to find differences between treatments and compare the two years of experimentation; the second one based on the use of filters to evaluate sensitivity and sensibility of the sensor to detect the diseases and create prediction indices (Fig. 1).
Concluding, the obtained results were very good: the instrument, consisting of a low cost commercial sensor, was able to detect the presence of problems within the vineyard. The “mobile lab” can be improved by increasing the number and improving the quality of the sensors.

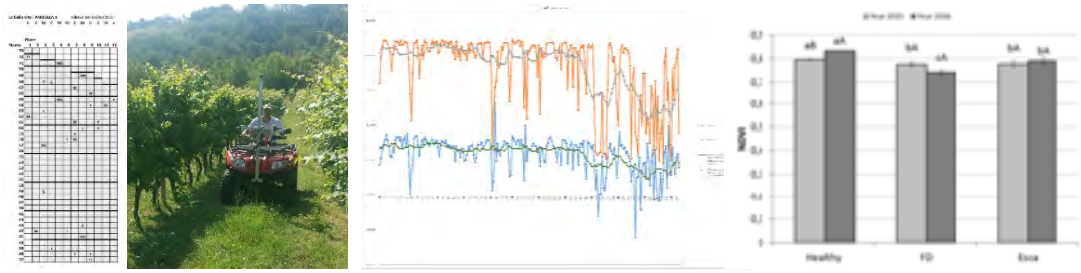

Fig. 1
Schematic representation of methods used in this work

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5. = MICROCOSM EXPERIMENT AND POLYPHASIC APPROACH FOR THE ASSESSMENT OF *FUSARIUM SOLANI* MYCELIAL GROWTH ON A STONE SUBSTRATUM

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Fusarium is a genus of filamentous fungi (Ascomycota, Hypocreales) commonly retrieved as soil plant pathogen; *(1)*. *Fusarium* is also frequently found in the biofilm deteriorating stone monuments (2; 3; 4). Fungi can be a serious threat for cultural heritage and artworks and are among the major agents of microbial deterioration of building stones (5). Besides the spoiling due to the color change and patina formation, they can deeply colonize cracks and fissurations because of the extraordinary penetrating power of their hyphae into the substratum. A better understanding of biodeterioration mechanisms and their effects on materials is needed in order to preserve monuments from fungal colonisation; to this aim, in vitro experiments are useful to assess bioreceptivity of different lithotypes (6) or biodeterioration due to phototrophs colonisation (7) or fungal colonisation (8).
Here we present a microcosm laboratory test to study the early steps of the colonisation above Neapolitan yellow tuff tiles by a *Fusarium solani* strain isolated from ancient Herculaneum suburban *thermae*.
Fungal growth has been followed in a 20 days experiment in which a microcosm was created with a single carbon source and high relative humidity in glass petri dishes containing the stone tiles. Because of its thin and translucent hyphae, *Fusarium* growth can be barely observed by naked eye on a rock substratum: a surface overlay documentation of the spreading colonies and their hyphal branching was observed both by metallurgical microscopy and fluorescence microscopy with the use of a fluorescent chitin-binding dye during the whole experiment, in order to assess mycelial architecture and its development on a stone substratum. Thickness of the biofilm was also measured in central, medial and distal areas of the colony for each tuff tile, and data were compared to a CLS-microscopy stack analysis. All the photographic documentation was used for a segmentation image analisys on Fiji software to calculate the overlay and the volume of the mycelium.
Our findings confirm that microcosm experiments coupled with microscopic observations are useful tools to evaluate and quantify fungal biomass on a stone substratum, especially in the early steps of fungal colonisation.

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5 = BIOLOGICAL ANALYSIS, FOR CONSERVATION PURPOSES, OF MATILDIC PARISH OF SANTA MARIA ASSUNTA (XI CENTURY) LOCATED IN SASSO DI NEVIANO ARDUINI (PARMA APPENNINES)

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The Parish of S. Maria Assunta (Fig. 1), mentioned for the first time in a 11th-century document, stands isolated on top of a hill on the watershed between Parma and Enza streams valleys (Fig. 2); traces of prehistoric, Roman and Lombard periods were found in the same area. There are no certain traces of the first church dating back to the 10th century, except perhaps in the left apse whose design appears simpler than the others. The basilica-like structure and the architectural and decorative elements meet the building canons of early-medieval churches and lead to a dating of the building in the 11th century. The Parish was rebuilt by the Grandcountess Matilde of Canossa, forming the present building with a three-naves plant concluded by three east-facing apses, timber ceilings and slate roof. The walls are made of square sandstone blocks, of various shapes and sizes; the exterior is decorated on each side and on apses with hanging archlets. The façade is divided in three parts by two pilasters that underline the central nave. Alongside the left apse there is the sacresty which incorporates a tough ruined basement, maybe the remains of the ancient bell-tower (1).

Fig. 1
Parish façade.

Fig. 2
Site location.

The building, which have undergone restoration at the end of last century, today shows again traces of deterioration due to the growth of vegetation, with greater evidence in the lower zone until about 2 meter from the ground (2). The southern side and the eastern apsidal side present the wider biodeteriogen coverage, obviously due to the proximity of spontaneous arboreal vegetation arriving a few meters from the monument and constantly shading it, while the northern side seems less colonized. The west-facing façade doesn't show vegetation growth, but only a modest lichen colonization.

The chromatic stains spreaded on the sandstone and slate stones are due to the massive presence of lichens (*Caloplaca flavescens*, *Lecanora muralis*), while between the cracks and on the stones vegetate mosses (*Brium murorum*) and ferns (*Asplenium tricomanes, Asplenium ruta-muraria*). A massive development of higher plants has been detected in the spaces between the stones, where the crumbling mortar and the organic material deposit form a favourable substrate (*Corilus avellana, Quercus pubescens, Silene nutans, Arabis hirsuta, Sedum album, Rubus ulmifolius, Fragaria vesca, Vicia cracca, Acer campestre, Hedera helix, Plantago major, Salvia pratensis, Crepis vesicaria, Dactylis glomerata, Triticum aestivum)* (4).

A future intervention on this Parish shall provide the recovery of mortar in the critical zones and the removal of biodeteriogens by a biocide treatment according to the provisions of the NORMAL 37/92 and 38/93 Recommendations (3).

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5 = ROLE OF BOTANIC GARDEN OF UNIVERSITY OF URBINO IN THE APPLICATION OF INQUIRY-BASED EDUCATION

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The aims of modern education and of inquiry-based education in particular require students to become more independent learners. This means teachers developing new relationships with students and having the confidence to allow students to develop their own ideas.
In the Botanic Garden Center of the University of Urbino, researchers utilize, for the activities aimed at school students, a pedagogical approach which conducts investigations using the scientific method.
This approach interests students and its distinctive features compared to other pedagogical approaches, are a switching between active and passive learning and an inductive and active teaching.
The innovative elements are an authentic research experience and an active involvement of the student.
In this way skills are developed through a botanical-based learning on the investigation. In the Botanic Garden, as a laboratory are then planned activities directed to the discovery of something. It then follows a path of linked steps which are not fixed arbitrarily.
Inquiry-learning is characterized as a multifaceted activity that involves making observations, posing questions, examining all sources of information to see what is already understood, planning investigations and already reviewing what is known in the light of experimental evidence (1).
The Scientific inquiry requires identification of assumptions, use of critical and logical thinking, and the consideration of alternative explanations by finding answers to questions. Inquiry-learning is not about memorising facts: it is about working with living organisms (mainly plants), observing natural phenomena, formulating questions, linking evidence to explanations and finding appropriate solutions to explain observations and address questions and problems (2).
In this type of approach, used for botanic education and developed in the Botanic Garden of Urbino, there are very important elements such as the sharing of time, results of group discussion and argumentation to support students ideas and reflection on their own learning and the significance of what they learned.
Science education based on investigation has proven effective both in primary and secondary schools and the studies show that science education serves to increase the interest and the performance of pupils and to stimulate the motivation of teachers. The role of Botanic Gardens as centers of education, in this framework, is of primary importance, as this kind of teaching cannot always be done inside the classroom.

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5. = BRYOPHYTE DIVERSITY IN SOME SICILIAN PROTECTED AREAS

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The results of some investigations carried out in two Sicilian protected areas characterized by chalky reliefs are reported. These are the Serre di Ciminna and Monte Conca reserves.
The Oriented Nature Reserve of Serre di Ciminna is located in northwestern Sicily, in the province of Palermo. Established in 1997 to protect the interesting karst phenomena in a vast macro-crystalline gypsum outcrop of the Messinian age, the series of "collapse valleys" and a swallowtail, the reserve falls within the Rocche di Ciminna, Site of Community Importance (SIC) (code ITA 020024). It includes some habitats of Community interest: pseudo-steppes with grasses and annuals of the Thero-Brachypodietea (code 6220), western Mediterranean and thermophilous screes (code 8130) and thermo-Mediterranean and pre-desert shrublands (code 5330).
In order to contribute to the knowledge of biodiversity within the reserve, collections of bryophytic material were carried out in the grasslands, garigues, maquis and small fragments of *Quercus virgiliana* (Ten.) Ten., forest repeatedly burned, situated in the localities of Balzo di Canalotto, Annunziata, Stretta di Carcaci, Cerami, Santa Caterina, Balzi della Chiusa e Cozzo Ginestra.
The determination of specimens allowed to recognize 32 taxa (30 mosses and 2 liverworts), belonging to 23 genera of 13 families. This is a rather large contingent of species, given the dryness characterizing the area in general. Overall, saxicolous, photophylous, markedly xerophylous and calcicolous species prevail. From a chorological point of view the temperate, Mediterranean and oceanic-Mediterranean taxa are highly represented.
The Integral Natural Reserve of Monte Conca is located in central Sicily, in the province of Caltanissetta. It was established in 1995 to protect the numerous karst environments in the area, extending to 2.45 Kmq. It is constituted by the homonymous chalk relief and it is crossed, from east to west, by the river Gallo d'Oro. The site is mostly characterized by farming systems and in the most natural areas there are riparian communities with *Populus*, *Tamarix* and *Salix* species, as well as some aspects of maquis and garigue.
11 taxa, of which 10 mosses belonging to 5 families, and one liverwort were found. They mainly include Mediterranean and widely spread in temperate zones species.
Particularly interesting is the occurrence of *Tortula revolvens* (Sw. ex anon.) Rubers. moss, rare in Europe, linked to the chalky substrates.

5 = PROTECTING BIODIVERSITY ON PRIVATE PROPERTY: AN EXPERIENCE IN CORLEONE (SICILY)

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Bona Furtuna is an organic farm located 8 km far from Corleone closed to Campofiorito (Palermo). The study area includes the slopes of Castro, Giardinello and Valle Fredda which goes up to Barraù Mt. included in the Special Areas of Conservation (ITA020037) according to the Natura 2000 European Birds and Habitat Directives.
The high level of biodiversity is supported by over 502 taxa of vascular flora, 38 of which are endemic to Sicily (1). The study area is quite diversified from a bioclimatic point of view as well, presenting different gradients from the Lower Mesomediterranean (Upper dry) and the Lower Supramediterranean (Lower subhumid) (2).
The landscape has been shaped by frequent changes in geological strata with the alternation of clayey or marly hills and calcareous reliefs of the Mesozoic period (Sicana facies) (3). This has resulted in a sequence of hills with gentle slopes, irregularly interrupted by isolated mountains with steep, if not abrupt, slopes, which reach the considerable height of 1,420 m a.s.l. (Monte Barraù).
Two different vegetation series were identified:

1. The Holm oak woods series (*Ampelodesmo mauritanici-Querco ilicis* sigmetum)
2. The Downy oak series (*Oleo oleaster-Querco virgilianae* sigmetum)

The mature stage of the first series consists of a coppiced holm oak wood (*Ampelodesmo mauritanici-Quercetum ilicis viburnetosum tini*). The degradation of this wood is indicated by shrubberies with *Prunus spinosa* (*Pruno-Rubion ulmifolii*).
The presence of pasture and farming practices is the main cause of the passage to a further stage of degradation of the series, leading to the formation of secondary *Ampelodesmos mauritanicus* grasslands (*Helictotricho convoluti-Ampelodesmetum mauritanici*).
The second series is widespread over clayey substrates (sometimes with a rocky matrix originating from limestone) with hilly morphologies, in stations with a certain environmental xericity (annual average rainfall from 500 mm to 800 mm). The mature formation consists of small remnants of oak woods (*Oleo oleaster-Quercetum virgilianae*) near waterways. In marginal areas (escarpments and steep slopes) hardly exploitable for agricultural uses, fires and grazing have triggered degradation processes that normally lead to the establishment of *Ampelodesmos mauritanicus* grasslands or Mediterranean grasslands, often dominated by *Arundo collina*. During the last fifty years, abandoned areas have been re-colonized by shrubs of Sicilian sumac (*Rhus coriaria*) whose abundance testifies to the ancient and widespread cultivation of this species in the area.
The farm since its founding (year 2013) was distinguished by several voluntary self-protection strategies adopting the following actions: removal of alien species that threaten biodiversity, grazing control, restoring habitat corridors, hunting activity control, fire prevention, preserving wetlands and spring waters, microhabitats protection (e.g. heaps stone, stonewalls and terraces), protection against soil erosion, removal of electrical lines to preserve birds and landscape, restoring polluted sites, organic farming, demolition of buildings with negative visual impact.
The company in accordance with University of Palermo founded different projects including floristic, phytosociological, mycological, zoological and archaeological studies.
Moreover, a new project for a Botanical Garden is coming soon; it will include existing habitats and wild species of native flora and ethno-varieties. This project aims to protect biodiversity *in situ* not only to maintain plant collections but entire plant communities for display education, research, conservation and enjoyment.

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5 = DEVELOPING LIPID-BASED NANODEVICES FROM *PYROCYSTIS LUNULA* AND *ARTHROSPIRA PLATENSIS* FOR BIOMEDICAL APPLICATIONS

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Photosynthetic microorganisms represent an important source of biological material to develop innovative and biocompatible nanodevices for different medical applications. In particular, liposomes and solid lipid nanoparticles are considered promising drug carriers due to their versatility of formulations, scalable production and superior performance. In this project, we aim to prepare nanovectors starting from two different microorganisms.
The bioluminescent dinoflagellate *Pyrocystis lunula* is provided with scintillons, responsible for the bioluminescence[1]. The insertion of the scintillons' content[2] into lipid-based vectors could deliver light into human body through non-invasive systems, thus opening up interesting perspectives in both photodynamic therapy and imaging.
The cyanobacteria *Arthrospira platensis* is well known as a food supplement and its easiness of cultivation leads to a large biomass availability. An efficient delivery of antibiotics by lipid nanovectors obtained from this source could improve their effectiveness and decrease microbial resistance[3]. For this reason, we created *A. platensis*-derived lipid vectors loaded with three commonly used antibiotics (ampicillin, kanamycin, nalidixic acid). We aim to test their effect on *Burkholderia cepacia*, an opportunistic human pathogen dangerous for immunocompromised individuals.
Structural characterization of our biocompatible nanovectors will be performed by dynamic light scattering and small-angle scattering techniques (SAXS, SANS).

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5 = A CENSUS OF THE MYCOLOGICAL *HERBARIUM* CAG: MACROMYCETES FROM THE MEDITERRANEAN AREA

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Sardinia is considered one of the hotspots for biodiversity in the Mediterranean basin and it is included in EU and IUCN conservation policies (1). This high level of Biodiversity is possibly found also for what concerns Kingdom *Fungi*. From the foundation of *Hortus Botanicus Karalitanus* in 1866 Patrizio Gennari has undertaken the collection and exchanges of *exsiccata* of plants, bryophytes, lichens, algae and fungi. Moreover P. Gennari himself writes in one of his works dated 1874 (2) that the dry collections of the Botanical Garden comprehends also specimens coming from the "Erbario Crittogamico Europeo" by Rabenhorst and from the "Erbario Crittogamico Italiano" by the "Società Crittogamologica Italiana". So inside the *Herbarium* CAG various historical collections was achieved: apart from Raberhorst - *Fungi europaei* and Erbario crittogamico italiano also *Fungi Longobardiae exsiccati* by Cavara (3) was acquired. At the present time the *Herbarium Mycologicum Calaritanum* was further enriched by over 3000 *exsiccata* of Macromycetes, some of which have been reported as treated species on several published papers (4)(5)(6)(7)(8)(9). Mostly are coming from donations by Marco Contu, a Sardinian Mycologist of international fame who deserves the credit for the description of most of the various type species. A preliminary census that concerns the most recent collections is ongoing and this lead to a new organization of the data. The most represented *phylum* is *Basidiomycota* with roughly 3000 specimens and the most featured families are *Tricholomataceae*, *Cortinariaceae*, *Agaricaceae* and *Amanitaceae* and the most represented *genus* is *Amanita*. A major part of the collection, over 85%, comes from Sardinia. Is therefore of the uttermost importance to preserve this patrimony.

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5 = LONG TERM MONITORING OF AIRBORNE POLLEN CONCENTRATION AND PHENOLOGY OF HAZEL

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The airborne pollen trend and the hazel phenology are monitored in several hazel orchard located in different cultivated areas: in Italy and Georgia, from winter 2012-2013 to 2016-2017, in Chile, from 2013 to 2016 and in South Africa, from 2012 to 2016. The monitoring is currently in progress.
The pollen monitoring is carried out by using spore traps (Hirst, 1952) (Fig. 1a) placed in Baldissero d'Alba (CN, Italy), in Georgia, near Chitatskari city, in Chile, near Los Niches, Caracas, Camarico and San Sebastian, and in South Africa, near Spring Valley and Hogsback town. The laboratory framework is carried out following the classical method described by Mandrioli (1990) and currently adopted in aerobiological monitoring. Strips are weekly collected from spore-traps and observed under microscope (400x) in order to obtain an estimation of daily pollen concentration, expressed as number of pollen grains per m^3 of air. Moreover, besides the presence of normal hazel pollen grains, also the presence of smaller and empty pollen grains is monitored (Fig. 1b). These grains are considered "anomalous" and they are always dead, as results from the viability tests.
The airborne pollen concentration can be compared with the phenology observed in field in order to obtain a more accurate and comparable interpretation of data, in particular regarding the overlapping of flowering between cultivars and pollen donors. The phenological monitoring is carried out following the method of the Monitoring of Italian phenological gardens (Puppi and Zanotti, 1998), adapted to hazel.
The vegetative development of hazel is recorded from the budburst to the leaf fall and the male and female flowering is observed until catkins drop and fructification. The fruit ripening is monitored from the enlargement of the ovaries to the nut fall.
The results show clearly differences depending on meteorological conditions of cultivated areas and the age of the hazel orchard. The main peak appearance was registered in Caracas, Chile, during winter 2015 (1969,8 gr/m^3 at July 7th) and in Georgia during the last winter 2016-2017 (1411.2 gr/m^3 at February 9th), while the lower values were scored in South Africa (0 gr/m^3) depending on extreme environmental conditions for wind, solar radiation and low relative air humidity.

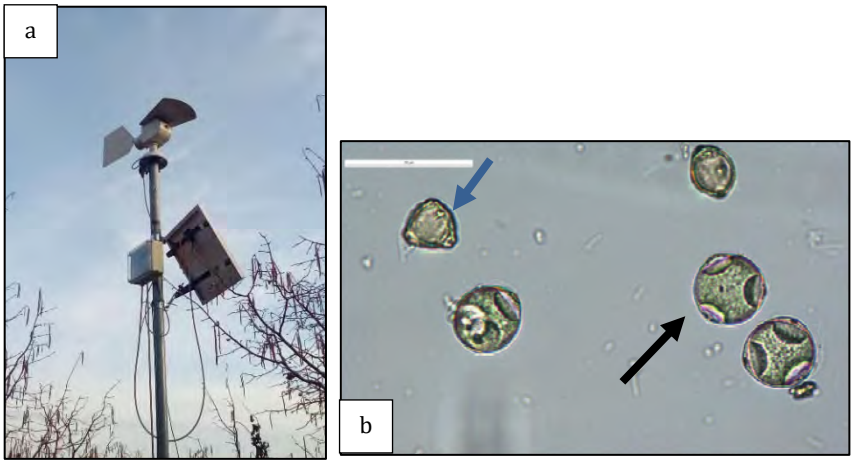

Fig. 1
a) spore trap located in Baldissero d'Alba (CN, Italy) study site; b) normal (black arrow) and anomalous (blue arrow) hazel pollen grains observed under microscope (400x).

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5. = ECTOMYCORRHIZAL FUNGAL COMMUNITY OF A *TUBER BORCHII* ORCHARD ESTABLISHED IN A NON-TYPICAL ENVIRONMENT

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Tuber borchii Vittad. is one of the species in *Tuber* genus with the highest ecological adaptability and its cultivation is becoming popular in several countries. Only two studies on EM community in *T. borchii* orchards (natural and cultivated) are available, both sited in the same habitat type suited to this truffle species. In 2016, Iotti and colleagues reported the first production of *T. borchii* ascomata from plants (*Pinus pinea*, *Quercus pubescens*, and *Quercus robur)* inoculated with pure cultures. Guard rows of non-inoculated *Corylus avellana* seedlings were used to separate the six *T. borchii* strains introduced into the plantation. This truffiere is located in an intensive farming area, in the province of Bologna, where *T. borchii* doesn't naturally grow. The root samplings were carried out during the *T. borchii* fruiting period (February and March 2016) in order to characterize and compare the ectomycorrhizal fungal communities on the different host plants and to evaluate the state of *T. borchii* colonization 8 years after planting. Twenty six and 13 root samples were collected in fruiting areas (under *P. pinea* and *Quercus* spp.) and non-fruiting areas (under non inoculated *C. avellana*), respectively. Molecular identification of ectomycorrhizas from each morphotype was performed by amplifying and sequencing the ITS region with a direct PCR approach.
The total number of ECM fungal species found in the truffiere was 22 (5 ascomycetes, 16 basidiomycetes and one undetermined species). The genus with the highest number of species was *Tomentella* (7), followed by *Scleroderma*, *Inocybe* and *Tuber* (including *T. borchii*) with 3 species each. The species richness in *Quercus* spp. was significantly higher than in *C. avellana* and *P. pinea*. In particular, only 5 ECM fungal species (including *T. borchii*) were found on *P. pinea*, the unique tree species out of its native range. *T. borchii*-inoculated plants were dominated by *Tomentella coerulea* and *T. borchii*, even if the structure of the ECM communities differed slightly between oak and pine. *Tuber maculatum* was the most abundant and frequent species found on non-inoculated hazel trees.
After 8 years from planting, root colonization with the target species *T. borchii* still exceeded 15% in the fruiting areas but it was not able to spread in truffle ground and to colonize the non-inoculated hazels. Even if the cultivation has been successful, *T. borchii* seems to be poorly competitive in this area and it could be replaced on roots by other ECM species such as the native truffle *T. maculatum*. Noteworthy, the diversity of ECM fungal inoculum found in the study area, which is dominated by arable lands and orchards and the ECM tree species are rare and scattered, is very high.

5 = PLANT PRODUCTS TO CONTROL BIODETERIORATION OF CULTURAL ASSETS

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Fungi and bacteria are known as major biodeteriogens of cultural heritage, able to colonize, altering and degrading a wide range of materials, such as metals, paints, paper, paperboard, rocks, photos, textiles, leather, plastics, etc. (1, 2).
The use of traditional chemical biocides, to control microbial growth on cultural assets, has become a serious threat to public health and environment.
The aim of this study has been to develop biocompatible antimicrobial compounds testing on specific *taxa*, that were isolated from biodeteriorated artifacts (books, papers, stones, woods, canvases) or environmental aerosols (museums, archives, libraries) and characterized by microscopy, *in vitro* culture and molecular analysis (3).
Previously extracted (4) and new plant products, *Tea Tree* essential oil and *Calamintha nepeta* L., *Allium sativum L.*, *Crithmum maritimum*, *Ferula communis L.*, have been tested against *Bacillus subtilis*, *Micrococcus luteus*, *Staphylococcus aureus*, *Penicillium chrysogenum*, *Aspergillus versicolor* and *Alternaria* spp.
The susceptibility of microbial strains to different natural compounds was tested by *agar disc diffusion*, *well plate diffusion* and *microdilution* methods.
Our results evidenced the efficacy of same plant products, such as *Tea Tree* oil, *Calamintha nepeta* L., *Allium sativum L.* and *Crithmum maritimum* exstracts, to control microbial growth, suggesting a potential application as natural biocides in cultural heritage field.
Further studies are required to develop appropriate methods to apply products of botanical origin as valid alternatives in sustainable conservation of cultural heritage.

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5 = THE BIZARRE CARNIVOROUS PLANT *GENLISEA*: *IN VITRO* PROPAGATION AND MICROSCOPICAL INVESTIGATION OF GLANDULAR STRUCTURES

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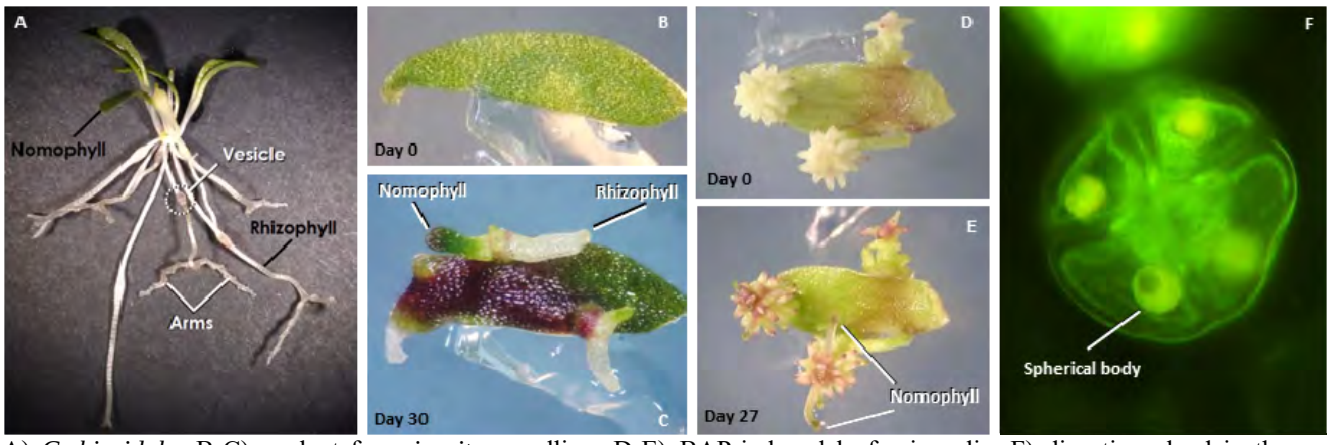
Genlisea is a genus of small tropical carnivorous plants distributed throughout Africa and central-south America, growing in wet terrestrial to semi-aquatic environments (1). The main feature of *Genlisea* is the absence of a true root and a remarkable heterophylly: the plant possesses aerial photosynthetic leaves, or "nomophylls", and subterranean tubular achlorophyllous leaves called "rhizophylls", which are and specialized for trapping preys.
Genlisea possesses a trapping mechanism comparable to an "eel trap" or a "lobster pot", that capture a wide range of soil organisms, ranging from unicellular organism as protozoa and algae, to metazoans as crustaceans, mites and nematodes. Preys enters the rhizophylls through openings of the arms and then they can move in a single direction due to the presence of detentive hairs orientated towards the trap vesicle. The vesicle, also called "stomach", is a swelling of the rhizophyll where the digestion and uptake of released nutrients occur. On interior surface of the vesicle there are glands, which are supposed to be responsible for the secretion of digestive enzymes and the absorption nutritional elements.
Several species coming from Africa (ie *G. hispidula*, *G. subglabra* and *G. margaretae*) and Central and South America (ie *G. pygmaea* and *G. oxycentron*) are currently grown in pots in a climatic chamber. The first objective of this research is the *in vitro* propagation of *Genlisea* spp. Preliminary data showed that leaf explants do not survive the sterilization procedure. Consequently, fresh seeds have been harvested from pots-grown plants. The seeds showed resistance to 10% sodium hypochlorite, whereas they have lose germinability after pretreatment whit 70% ethanol. Preliminary results obtained with *G. hispidula* showed a rate of germination ranging from 8% to 17%, depending on whether the sterilization treatment were done directly on seeds or on the entire capsule. The germination were observed 10 days after the inoculum on hormone-free culture medium. In the early stages of seedling growth it has been observed a peculiar developmental pattern of the shoot apical meristem, which generated alternatively nomophyll and rhizophyll primordia. This pattern is abolished by the presence of cytokinin (BAP) that, even at relatively low concentrations, prevent the development of the rhizophylls and induce the formation of several primordia similar to nomophylls. The next step of this research will consist in the evaluation of the role of auxin in the apical meristem development, and of the combined effect of auxin/cytokinin. Another objective of the research is the study of the different secretory structures present in the rhizophyll, that are currently poorly investigated. Microscopic analysis shown a spherical large body inside the glandular head of digestive glands. The existence of these bodies has been reported only in one paper (2), however no hypothesis has been formulated on its chemical nature and function. In the fresh sections, after 24-48 h, we observed a gradual disappearance of the spherical bodies, that suggests a role in nutrient storage.



A) *G. hispidula*; B-C) explant from *in vitro* seedling; D-E) BAP-induced leaf primordia; F) digestive gland in the vesicle.

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5 = BRYOPHYTIC COMPONENT FROM MEDITERRANEAN TEMPORARY PONDS IN UMBRIA (CENTRAL ITALY): STATE OF KNOWLEDGE ON BIODIVERSITY AND VEGETATION

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The Bryophytes are a very important component of the Mediterranean temporary ponds and with the present study we try enhancing their knowledge focusing on bryophytic biodiversity and vegetation in thes e rare and peculiar ecosystems. The study was carried out in Umbria region, inland Central Italy (Piana di Ferretto), near the Trasimeno Lake, in a biotope of biodiversity conservation interest and a Natura 2000 site (IT5210020) hosting a huge system of scattered mediterranean temporary ponds classified as a priority natural habitat (3170*) under the Habitats Directive 92/43/EEC (1). The conservation efforts of this biotope are supported by the "SUN LIFE" project (13 NAT / IT / 000371), thanks to which a strategic plan for the management of the Natura 2000 network in Umbria is under development. The area belongs to the Mesomediterranean belt of the Mediterranean Bioclimate, with clear transitional traits to the Temperate Bioclimate affecting its floristic and ecologic features. The Mediterranean temporary ponds in Mediterranean Europe are mostly distributed in costal and subcoastal areas, for this reason the site of study is very peculiar and biogeographically interesting.
This site hosts rare bryophytic *taxa* whose survival is strictly dependent on the occurrence of precise ecological and hydrogeological conditions. Particularly interesting is the finding of liverworts included in the "*Checklist and country status of European bryophytes – towards a new Red List for Europe*" (2). They present different levels of threat, according to the IUCN categories and criteria (3). Some of this are *Fossombronia caespitiformis* De Not. ex Rabenh. subsp. *multispira* (Schiffn.) J.R.Bray & D.C.Cargill, *F. wondraczekii* (Corda) Dumort. ex Lindb, *Riccia beyrichiana* Hampe*, R. canaliculata* Hoffm. and *R. crozalsii* Levier (4). Other significant mosses are: *Archidium alternifolium* (Hedw.) Mitt., *Imbribryum alpinu*m (Huds. ex With.) N. Pedersen, *Ptychostomum pseudotriquetrum* (Hedw.) J.R. Spence & H.P. Ramsay var. *pseudotriquetrum*, *Entosthodon fascicularis* (Hedw.) Müll.Hal, *Tortula truncata* (Hedw.) Mitt., *Campylopus introflexus* (Hedw.) Brid. Phytosociological investigations allowed the identification of eleven vegetation types, referred to the following alliances: *Fossombronio-Pohlion annotinae* von Hübschmann 1986; *Dicranellion heteromallae* Philippi 1963*; Funarion hygrometricae* von Hübschmann 1957; *Phascion cuspidati* Waldheim 1944 nom. inval. ad interim*; Mannion androgynae* Ros et Guerra 1987; *Pleurozion schreberi* von Krusenstjerna 1945*; Campylopodion polytrichoidis* Giacomini 1951. All of them are here reported for the first time for the Umbria region.
This study provides basic floristic, chorological and syntaxonomic information and offers an updated overview of the bryological flora and vegetation of the Mediterranean temporary pond systems in inland central Italy, contributing significantly to improve the knowledge of a floristically rich territory with special attention to a frequently neglected taxonomic field. From the bryophytic point of view, it is necessary to promote the study of these habitats and preserve them from a series of human pressures that threaten their conservation.

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5 = IMPORTANCE OF COVER CROPS FOR WEED CONTROL IN A SUSTAINABLE MEDITERRANEAN AGROECOSYSTEM

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From some decades, the usefulness of the cover crop was confirmed by farmers who are increasingly aware that the soil should be kept and that his over-exploitation involves not only a drop-in productivity over the medium to long term, but also the 'pollution of environment and desertification' (1; 2). Cover crops are perfectly integrated in the perspective of sustainable and environmentally friendly agriculture that can be used in different contexts and in many different areas of production (3).
To evaluate the use of *Trifolium subterraneum* L. (subterranean clover) as cover crop to control weeds, in a Mediterranean 3-years apricot orchard, cvs Pinkcot®, Wonder cot e Big Red®, with a planting density of 400 plant ha^{-1} and 5x3 m tree spacing, a factorial experiment with 3 factors and 4 replications, in a randomized complete block design, was done in 2015/2016 in Southern Sicily, in province of Caltanissetta (CL) (37°13'36.0"N 14°05'02.4"E, 290 m a.s.l.). The following treatments were compared to the control (conventional management): cover cropping with subterranean clover and weed flora (by hand weeding and residues incorporation). To evaluate the effects of cover crop on weeds, in the central part of each elementary plot, we identified, randomly, permanent squares of 1 m to the side; these plots were used to identify and separate, from subterranean clover, the occurring weed species; the fresh and dry weight of phythomass was calculated after a treatment in a drying thermo-ventilated oven at 105 ° C to a constant weight. Species were identified using Pignatti (1982); the nomenclature of species followed Conti et al. (2005). Before sowing, settlement and during the development of cover crop, a amoA gene-specific quantitative PCR in soil samples was done. Quality and integrity of DNA extracted from soil was verified by electrophoresis on 1% agarose gel and it was subsequently quantified with standard concentrations of calf thymus.
Results of the study showed that the number of species and their botanical families were strongly contained by subterranean clover, regardless of its management. This confirms that the positive effects of crop cover on the weeds can be attributed to the sharp reduction in the number of plant species as well as the number of seeds per plant (4). This study represents an initial contribution of knowledge on the role of cover crop in the management of Mediterranean orchards. In the field conditions, the subterranean clover showed very good settlement ability and a good growth and development. In particular, *T. subterraneum* has always proved able to considerably reduce the soil seed bank that represents the potential flora. This means that this cover crop can significantly reduce the real flora and vegetation that grows in orchards. The highest amount of nucleic acids, which corresponds to the lowest levels of *Ct*, was constantly higher in presence of cover crop. This would allow, therefore, a reduced use of inputs such as tillage, mowing, herbicides, making the agro-ecosystem most sustainable. The excellent settlement and growth of *T. subterraneum*, which has substantially increased the activity of the bacteria associated with the nitrogen cycle, are also a guarantee of release of crop residues in the soil, with a high nitrogen content, given the nitrogen-tacker peculiarities of this species.

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5 = THE RED LIST OF ITALIAN ENDEMIC VASCULAR PLANTS

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The Italian vascular flora is one of the richest in the European Union and the Italian peninsula is located at the centre of the Mediterranean Basin, one of the 25 world biodiversity hotspots with an exceptional loss of habitats and species (1). Preliminary to any conservation initiative concerning plant diversity, a prioritization of the most endangered species is needed. IUCN Red List protocol is widely recognized and used to evaluate the conservation status of a species, according to its estimated extinction risk (2). Assessing the conservation status of plants endemic to an entire nation is a key challenge, because of the huge amount of data, knowledge and information required. Such a result can be achieved only through the collaboration of many specialists and an adequate financial base. "The Red List of the Italian Flora" project, promoted by the Ministry for Environment and Protection of Land and Sea General, Directorate for Protection of Nature and Sea, in collaboration with the Italian Botanical Society, started in 2013 with the aim to update the conservation status of Italian plants. In 2016 the assessment of all the vascular plants strictly endemics to Italy was completed. Considering all the Italian endemics, with the exclusion of taxonomically critical genera (i.e. *Alchemilla, Hieracium, Ophrys, Pilosella, Ranunculus, Rubus* and *Taraxacum*), a total of 1088 taxa was assessed. Three taxa are considered extinct (EX), one extinct in the wild (EW) and six are possibly extinct (CR[PE]). Around 27% of the Italian endemics are included in one of the major threat categories (CR, EN and VU), while c. 20% may become threatened with extinction in the near future (NT). Around half of the Italian endemic taxa (506) are widespread and abundant taxa (LC). Finally, 80 species (7%) were categorized as Data Deficient since the available data did not allow a robust assessment, indicating that further taxonomic and field studies should be undertaken in the next future.

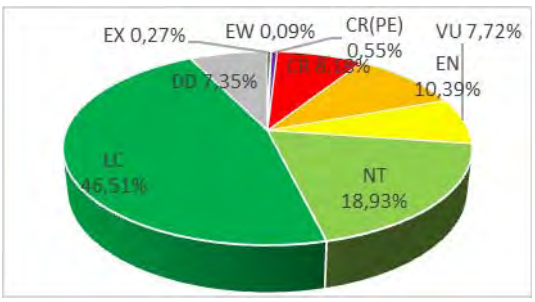

Fig. 1
Percentage of Italian endemic species in each IUCN risk category.

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5 = AN ARBUSCULAR MYCORRHIZAL FUNGUS POSSESSES THE POTENTIAL FOR THE BIOSYNTHESIS OF SECONDARY METABOLISM COMPOUNDS

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Arbuscular Mycorrhizal Fungi (AMF) are among the most widespread group of fungi, since they associate to more than 80% of land plants, with which they establish the so called Arbuscular Mycorrhizal (AM) symbiosis. AMF are obligate biotrophs belonging to the Glomeromycotina clade (1), which is an ancient group of basal fungi, strictly related to the Mucoromycotina. Glomeromycotina co-evolved with plants for at least 450 million years, following land plant evolution (2). AMF contribute to the uptake of soil nutrients in plants, thus increasing their productivity and conferring resistance to stresses. In turn, they obtain organic carbon from their photosynthetic hosts.
It is acknowledged that basal fungi are not able to produce secondary metabolites, such as those synthesized by polyketide synthases (PKSs) and non-ribosomal peptide synthases (NRPSs), although this view has been challenged by the finding that some Zygomycetes possess the genomic potential to produce secondary metabolites (3). As far AMF are concerned, the genome of the only sequenced AMF, Rhizophagus irregularis, confirms the absence of genes encoding for NRPS and PKS (4). In some fungi this lack is compensated by the presence of endosymbiotic bacteria, that can synthesize bioactive compounds (5). Since AMF belonging to the Gigasporaceae can host a population of rod-shaped, vertically transmitted endobacteria named Candidatus Glomeribacter gigasporarum (6), we wondered whether we could detect evidences for similar systems.
The genome sequencing of the endobacterium thriving inside Gigaspora margarita isolate BEG34 (7) provided evidence of two large ORFs encoding for mixed NRPS-PKS whose expression was verified in all the stages of the fungal life cycle by using Real time qPCR. Surprisingly, the transcriptome sequencing of G. margarita retrieved NRPS-PKS-related transcripts also when the fungus was cultured in the absence of the endobacterium. Indeed, the sequence resulted to belong to the fungal genome, but deeply re-edited following the eukaryotic codon usage. The predicted protein matches at 33% the NRPS-PKS of the Candidatus endobacterium. The occurrence of the NRPS-PKS was searched among different AMF isolates, but its presence was exclusively detected within the Gigasporaceae family, including species that have never been reported to host the endobacterium (6, 8). The phylogenetic tree built on the fungal NRPS-PKS sequences nicely mirrors the one inferred from ITS and rRNA gene sequences (8).
Our data strongly suggest that a lateral gene transfer event occurred from the endobacterium to its fungal host; this might have represented one of the strategy for the acquisition of the ability to synthetize secondary metabolites in the fungal kingdom.

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5 = EARLIEST EVIDENCE OF *PRUNUS DOMESTICA* L. IN ITALY DURING THE PHOENICIAN AND PUNIC PERIODS (6TH-2ND CENTURY BC)

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During the archaeological excavation in the Phoenician and Punic context of Santa Giusta (Oristano, Sardinia), dated to the 6th - 2nd century BC, several *Prunus* spp. endocarps were recovered in an exceptional state of preservation.
In order to identify the species, and investigate the domestication level of the waterlogged remains, morphometric analysis of the endocarps was performed by computer vision techniques. Currently, digital image analysis represents an accurate, reliable and repeatable alternative to distinguish wild species from cultivated ones (1,2) also at varietal level (3).
Archaeological endocarps were compared with 11 modern populations of *Prunus spinosa* L. and 22 varieties of *Prunus domestica* L. collected from the field catalog of CNR-ISPA (Nuraxinieddu, OR) in Sardinia.
Digital images of both modern and archaeological *Prunus* endocarps were acquired with a flatbed scanner and analysed by the open source software ImageJ v.49, applying a specific plugin (Particles8) (4) able to compute 26 morphometric features.
Applying the stepwise Linear Discriminant Analysis (LDA), a morphometric comparison between the archaeological endocarps and the modern ones was performed.
These analyses allowed to identify 53 and 11 endocarps of *Prunus spinosa* and *Prunus domestica*, respectively, showing that the archaeological endocarps of *P. spinosa* have similarities, in the 92.5 % of the cases, with a wild population that at present grows near the Phoenician and Punic site of Santa Giusta. In addition, the archaeological endocarps identified as *P. domestica* show similarity (81.8 %) with a modern variety of red-violet plum called 'Sanguigna di Bosa', currently cultivated in the village of Bosa (Oristano).
These results, as far as we know, provide the first evidence of *P. domestica* in Italy during the Phoenician and Punic periods.
This study provides an important piece to understand the origin of plum introduction and diffusion in Italy with the aim to produce a similar research to that carried on peach (5).

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5 = PLANT CONSERVATION CENTRES IN SICILY: THE 'VALLE MARIA' REGIONAL CENTRE (GODRANO, PALERMO) AND THE CASE STUDY OF THE RARE *CENTAUREA ERYCINA* (ASTERACEAE)

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Ex situ conservation of wild plants in Sicily started thanks the research activities of the Department of Botany Sciences of Palermo University, climaxing in 1992 with the institution of the Seed Bank at the Botanic Garden. This actually represents the oldest scientific structure specifically devoted to wild plant conservation and propagation in our country (1). Soon afterwards, many other seed banks for wild plants spread in Italy, similarly linked to the scientific support of botanists and university Botanic Gardens.
Another Seed Bank in Sicily, distinctively involved in the conservation of wild plants with special target on rare and endangered species, also stands at the Botanic Garden of Catania University, while two big conservation centres belong to the main Regional Authority and are currently headed by the Regional Department of Rural and Territorial Development.
The Sicilian Plant Conservation Centres are located in two far away high-nature-value areas, '*Marianelli*' in South-East Sicily (district of Siracusa), inside the "Vendicari" Oriented Natural Reserve and '*Valle Maria*' (Godrano) in North-West Sicily (district of Palermo), inside the "Bosco di Ficuzza, Rocca Busambra, Bosco del Cappelliere e Gorgo del Drago" Oriented Natural Reserve. Both Centres have started active collaborations with the geographically related University that provided scientific support and expertise in planning conservation activities and running labs and collection fields.
As far as the conservation activities on wild rare plant species are concerned, the '*Valle Maria*' Centre in particular cooperates with the Section of Botany and Plant Ecology - at the Palermo University Department of Biological, Chemical and Pharmaceutical Sciences and Technologies (STEBICEF) – that has always undertaken to technically manage and scientifically improve the Seed Bank collections.
For the purposes of a recently renewed Convention between the two Institutions, research activities were planned and shared with a special focus on *in vitro* and *in vivo* propagation and conservation of endangered endemic species of the Sicilian vascular flora. Among the targeted wild plants there is *Centaurea erycina* Raimondo & Bancheva. This is a very rare graceful species belonging to the daisy family (Asteraceae), up to now only known from its *locus classicus* on Mount Erice (near Trapani). The species is represented by just a single natural population with no more than 100 individuals. Hence, it has been included in the Red List of the Italian flora (2) where it is classified as "critically endangered" (CR) based on the latest IUCN criteria (3). Main threats for this species depend on: 1) habitat alteration and reduction due to the increasing anthropization linked to the touristic relevance of Erice, the historic town located on top of the homonymous mount, 2) reduced seed viability, often dramatically infested by the Fruit Fly *Trupanea amoena* (Frauenfeld, 1857) [Diptera: *Tephritidae*] (4).
Mature seeds were collected in the field and treated following the international protocols for the management of wild plant germplasm, regarding seed cleaning, dehydration and medium-long term storage, germination tests and related pretreatment or direct seeding (5).
First results from these activities allowed plant propagation and *ex situ* conservation of *C. erycina* in the nursery of '*Valle Maria*' Centre and provided more than 200 fertile individuals available for enhancing the native population and promoting the horticultural use of this species.

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5 = RED LIST OF ASCOMYCOTA (PEZIZOMYCOTINA) IN UMBRIA

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A Red List of all 108 Pezizomycotina (Ascomycota) species recorded in Umbria Region (central Italy) (1) is provided. According to the IUCN categories and criteria (2, 3), 60.18% of the assessed species are classified as threatened, whereas 12.96% are Near Threatened (NT), 1.86% are Least Concern (LC), and a noteworthy amount of 25% are Data Deficient (DD).
As a consequence of the downlisting applied to the majority of the assessed taxa, according to the guidelines for application of IUCN red list criteria at Regional level (3), only 1.54% of the threatened species is Critically Endangered (CR), while 46.15% are Endangered (EN), and 52.31% are Vulnerable (VU).
In Umbrian forests all types of substrate host threatened species but, in absolute values, the largest number of them is terrestrial, such as saprotroph or symbiont. A surprising number of threatened taxa belongs to saprotrophic fungi living on sandy and humus soils (St) suffering, like the mycorrhizies, the adverse effects of harmful atmospheric deposits (fertilizers, pollutants). The proportion of vulnerable species among the lignicolous saprotrophs (Sh) is also considerable.
Intensification and change in land-use and management, particularly in forestry and agriculture, is the major cause of change and decline of Pezizomycotina diversity in Umbria.
The IUCN protocol for assessing the conservation status of species has no legal implications. However, the scientific community has endorsed its use, even despite possible errors that have been identified (4, 5). We agree with Anton et al. (6) that this approach may represent a starting point for a legal protection of species, also in Umbria, as has occurred in many regions and countries. Nevertheless, as pointed out by Rossi et al. (7), legal protection alone is not sufficient to guarantee a good conservation status of the protected species.
Given that the present work represents the first complete regional red list of Pezizomycotina in Italy, and that a national, as well as a European red list does not exist to date, it could be considered as a case study for other Italian Regions as well as for other European countries, aiming at the compilation of a national and European red list of this fungal group mostly overlooked in conservation strategies.

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