SEASONAL VARIATION IN ROOT INFECTION AND POPULATION LEVELS OF FUSARIUM spp. IN CITRUS NURSERIES IN EGYPT. Y.M. Ahmed1, H. El-Shimy1, A. Ippolito2, T. Yaseen3, A.M. D’Onghia2. 1Plant Pathology Research Institute, Agricultural Research Center, Giza, Egypt. 2Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi “Aldo Moro”, Via Amendola 163/A, 70126 Bari, Italy. 3Centre International de Hautes Etudes Agronomiques Méditerranéennes, Mediterranean Agronomic Institute of Bari, Via Ceglie 9, 70010 Valenzano (BA), Italy. E-mail: ippolito@agr.uniba.it

Fusarium species are commonly associated with different citrus diseases, such as dry root rot, root rot, feeder root rot, wilt, twig dieback, and citrus decline. Fusaria are ubiquitous in citrus groves and nurseries, attacking feeder roots under stress conditions. This study aimed at monitoring the seasonal variation of Fusarium spp. in soil and feeders roots in Egyptian citrus nurseries with the purpose of detecting the most common species associated with diseases in the nursery plots. The study was conducted in two nurseries, located in different regions (Delta and Desert area). Soil and root samples were collected monthly from March to July from sour orange and Volkmanniana lemon rootstocks. The inoculum density of Fusarium spp., expressed as colony forming unit (CFU) per gram, was estimated by soil dilution using semi selective media. The percentage of infected feeder root was assessed by culturing feeder root pieces on the same selective media. Fusarium isolates were grouped according to their morphological characteristics and classified by amplifying and sequencing a regions of the beta-tubulin gene (benA). In both nurseries Fusarium solani was the prevailing species, followed by F. oxysporum. The inoculum density of Fusarium and the percentage of root infection varied according to the rootstock, the environmental conditions and the nursery management, reaching the highest values during high temperature periods.

PRELIMINARY SURVEY OF COMMON FUNGAL DISEASES OF MANGO IN SICILY. Y. Ahmed1, A. Ismail Mahmoud2, T. Yaseen3, A.M. D’Onghia2, G. Cirvilleri1. 1Dipartimento di Scienze e Tecnologie Fitosanitarie, Università degli Studi, Via S. Sofia 100, 95123 Catania, Italy. 2Centre International de Hautes Etudes Agronomiques Méditerranéennes, Mediterranean Agronomic Institute of Bari, Via Ceglie 9, 70010 Valenzano (BA), Italy. 3Centro di Competenza per l’Innovazione in Campo Agro-Ambientale (AGROINNOVA), Università degli Studi, Via Leonardo da Vinci 44, 10095 Grugliasco (TO), Italy. E-mail: gcirvill@unito.it

Mango (Mangifera indica Linn.), is a promising crop in Sicily due to the favorable climatic and soil conditions. Mango cultivation was introduced a few years ago on a limited surface, mainly in the provinces of Palermo, Ragusa, Messina and Catania. Kensington was the main variety obtained by locally produced seeds, probably coming from Australia. In the near future, it is expected that commercial and backyard plantings of mango trees will increase, being a profitable crop. Being the phytosanitary state of this crop in Italy unknown, surveys were conducted in spring and in autumn in seven commercial groves located in different areas, to assess the occurrence of diseases and associated causal agents. Typical symptoms of die-back, gray leaf spot, anthracnose on twigs, branches, leaves and fruits, Phytophthora crown rot and root rot, and Armillaria root rot were observed. Samples were collected from symptomatic plant tissues and plated on different media. Pure fungal cultures were identified based on their morphological and microscopic features. Preliminary results indicated that foliar, fruit and soil-borne diseases were present in all the investigated areas. Pestalotiopsis mangiferae, Botrytis dahliae, Colletotrichum spp., and Phytophthora nicotianae were the most prevalent pathogens isolated from leaves, branches, soil and roots. Other fungi were identified, including Pythium spp., Rhizoctonia solani, Fusarium spp. and Armillaria mellea.

VARIABILITY OF SCLEROTIUM ROLFSII ISOLATES FROM ORNAMENTAL PLANTS AND TURFS IN ITALY. D. Aiello1, I. Castello1, V. Guarannaci1, R. Catiazzo2, A. Carella2, E. Lahoz2, G. Polizzi1. 1Dipartimento di Scienze e Tecnologie Fitosanitarie, Università degli Studi, Via S. Sofia 100, 95123 Catania, Italy. 2CRA, Unità di Ricerca per le Colture Alternative al Tabacco, Via Pasquale Vittolo 106, 84018 Scafati (SA), Italy. E-mail: gpolizz@unict.it

Southern blight, caused by the soilborne fungus Sclerotium rolfsii is a serious disease of a wide variety of plants, including field, vegetable, fruit, ornamental crops and turf. In recent years, southern blight was particularly damaging to ornamentals and turfs in eastern Sicily, where 37 isolates were recovered over a 2-year period. Variability of these isolates was investigated based on morphology (mycelium growth rate and sclerotium formation), teleomorph formation on three different media, and mycelial compatibility. Fungal isolates varied considerably in growth rate at different temperatures, in the number of sclerotia per plate and in their dry and fresh weight. Only one isolate formed hymenia and basidial of Athelia rolfsii on potato dextrose agar (PDA) containing 2% activated charcoal. Five vegetative compatibility groups (VCGs) resulted from isolate pairings. In compatible reactions mycelia of the two isolates intermingled at the zone of interaction. Thirty-six out of 37 isolates showed compatibility with at least one isolates and were grouped into four groups, whereas one isolate was self-compatible and constituted another VCG. The pathogenic variability of five isolates belonging to different VCGs was assessed on Laurus nobilis seedlings. In addition, the variability in esterase and polygalacturonase electrophoretic patterns was considered in relation to VCGs and the main characteristics of the isolates. Studies on the variability of S. rolfsii strains from a geographical region are important as they can help documenting their origin and the changes that might be occurring in the population.

A PCR-BASED METHOD FOR THE IDENTIFICATION OF FUSARIUM FUJIKUROI AND F. PROLIFERATUM AND DETECTION IN RICE SEEDS. M.T. Amatulli1, D. Spadaro1,2, M.L. Gullino1, A. Garibaldi1. 1Centro di Competenza per l’Innovazione in Campo Agro-Ambientale (AGROINNOVA), Università degli Studi di Torino, 10095 Grugliasco (TO), Italy. 2Dipartimento di Valorizzazione e Protezione delle Risorse Agroforestali, Università degli Studi, Via Leonardo da Vinci 44, 10095 Grugliasco (TO), Italy. E-mail: mariateresa.amatulli@unito.it

Northern Italy represents the largest production area (232,900 ha in 2007) of rice in Europe. Several fungal diseases affect this crop. Among these, Fusarium spp. are important agents of plant and seed diseases. In the last decades, bakanae has emerged in Italian rice fields, becoming a serious problem for seed production and for seed companies. The causal agent of this disease is Fusarium fujikuroi, which is the most abundant, but other species can be isolated from rice, among which F. proliferatum, phylogenetically closely related to F. fujikuroi and morphologically indistinguishable from it. Multiple alignments of translation elongation factor gene sequences of various Fusarium spp., showed a deletion of six nucleotides in F. fujikuroi and two nucleotide polymorphisms in the same region in F. proliferatum. These sequence variants...
were used to set up a PCR-based diagnostic protocol. The species-specific primer pairs FUJI1F/1R and PROLI1F/1R gave products of 179 and 188 bp, respectively. Primer specificity was confirmed by assaying DNA from different species in the GPC, as well as from 298 isolates present in our *Fusarium* spp. collection, found on rice plants and seeds. Primer sensitivity was tested on 10 pg of pure fungal DNA. Primers successfully detected fungal DNA from infected rice tissues and seeds. The developed PCR assay can be used for the rapid identification of these species and may represent a powerful tool for their detection in rice seeds.

**IDENTIFICATION AND CHARACTERIZATION OF BACILLUS SUBTILIS ET-1, A STRAIN WITH ANTIFUNGAL ACTIVITY AGAINST FRUIT ROT PATHOGENS. A. Ambrico, M. Trupò, L. Lopez. Unità Tecnica Tecnologie Trisaia, Laboratorio Biotecnologie, Centro Ricerca ENEA Trisaia, ss 106 Jonica km 419.3, 75026 Rotondella (MT), Italy. E-mail: alfredo.ambrico@enea.it**

A bacterial strain (ET-1) isolated from soil and used in these studies, was identified as *Bacillus subtilis* based on morphological and physiological tests, Biolog and the 16S rDNA sequence. This species is commonly regarded as a biological control agent. *Botrytis cinerea* and *Penicillium digitatum* are known to cause severe rotting of strawberries and citrus fruits during storage and shelf life. Inhibition of mycelial growth and conidial germination of the two fungal pathogens by a strain ET-1 cell-free supernatant was tested on agar substrate. Preliminary assays were also conducted on strawberry and lemon fruits to assess the ability of ET-1 bacterial suspension and ET-1 cell-free supernatant to inhibit the growth of fungal agent rots in post-harvest. *In vitro*, isolated ET-1 significantly reduced mycelial growth of both fungal pathogens forming a clear-cut inhibition zone. Cell-free supernatant diluted 1:32 and 1:128 caused over 95% inhibition on conidial germination of *B. cinerea* and *P. digitatum*, respectively. Promising results were obtained in vivo trials. The more efficient protection was observed on strawberry and lemon fruits treated with cell-free supernatant. Our results are in agreement with those of other studies and confirm the ability of strain ET-1 to secrete secondary metabolites with antifungal activity. Further investigation is needed to verify the effectiveness of ET-1 under real conditions of fruits storage and to identify the molecules involved in fungal inhibition.

**THE EXPRESSION OF THE GENE CODING FOR CERATO-PLATANIN IS MODULATED BY BIOTIC AND ABIOTIC FACTORS. I. Baccelli1, R. Bernardi2, L. Carresi1, C. Comparinii, L. Pazzagli3, A. Scalii. 1Dipartimento di Biotecnologie Agrarie, Sezione di Protezione delle Piante, Università degli Studi, Via della Lstruccia 10, 50019 Sesto Fiorentino (FI), Italy. 2Dipartimento di Biologia delle Piante Agrarie, Sezione di Genetica, Università degli Studi, Via Matteotti 1/5, 56124 Pisa, Italy. 3Dipartimento di Scienze Biochimiche, Università degli Studi, Viale Morgagni 30, 50134 Firenze, Italy. E-mail: ivan.baccelli@unifi.it**

Ceratoctysis platani is the causal agent of canker stain, the most serious disease of plane trees. The fungus produces cerato-platanin (CP), a protein of about 12.4 kDa acting as a PAMP in host and non-host plants. On plane leaves, CP elicits the transcription of defence-related genes earlier than *C. platani*. The amino acid sequence 1-119 of CP is a new protein domain, called “cerato-platanin domain”; thus, CP is the founding member of the cerato-platanin family (pfam PF07249). To date, a number of highly conserved proteins produced by Ascomycetes and Basidiomycetes proved to contain this domain. They have been reported to interact with plants and humans, but very little is known about the regulation of the genes coding for these proteins and about their primary role in the lifestyle of the producing fungi. With the present work we show that CP is released by the fungus during the interaction with the host plant, and the expression of the cp gene is highly modulated. This gene is expressed more rapidly when the fungus is inoculated on the plane leaves than when it is grown in axenic culture. Some other potential abiotic and biotic stressors have been investigated: temperature, H$_2$O$_2$, umbelliferone phytoalexin, matrix water stress, light, growth on sawdust of susceptible and resistant plane or elm trees, and co-culture with *Trichoderma atroviride* P1 and *T. harzianum* T22. Gene expression was evaluated by qRT-PCR using TaqMan probes. The highest effect on the modulation of the cp gene was caused by temperature and matrix water stress.

**EVOLUTION OF KIWFUIT BACTERIAL CANKER. G.M. Balestra, A. Rossetti, L. Ricci, M. Renzi, A. Quattrucci, M.C. Taratufolo, A. Mazzaglia. Dipartimento di Protezione delle Piante, Università degli Studi della Tuscia, Via S. Camillo de Lellis, 01100 Viterbo, Italy. E-mail: balestra@unitus.it**

Italy is the first kiwifruit world exporter but this crop is economically important also for many other countries. Hayward (*Actinidia deliciosa*) is by far the most common green cultivar, whilst over the last ten years there has been a considerable increase of yellow cultivars (*Actinidia chinensis*), such as Hort 16 A-Zespri Gold and Jin Tao-Kiwi Gold. Among diseases affecting kiwifruit, the bacterial canker caused by *Pseudomonas syringae pv. actinidiae* (PSA) proved to be the most dangerous during the last years. PSA is able to infect quickly host plants causing severe damages on different plant organs and inducing typical symptoms such as abundant exudate production. PSA affects different *Actinidia* spp. and cultivars, survives within host tissues in winter, and its spreading during the vegetative season is favoured by heavy rains, late frosts and hail storms followed by mild temperatures. Pruning and harvest, as well as nutritional strategies, are crucial for PSA disease development. Different molecular approaches were used to assess the genetic relatedness among strains from several countries. Genetic fingerprints showed few but significant differences between Italian, Japanese and Korean bacterial populations, suggesting a separate origin for the disease. The implementation of effective control strategies by sustainable approaches such as natural compounds/antagonists, induced resistance and reduced amount of cupric salts, coupled with proper nutritional supports, is in progress. Recent findings are also reported on PSA biological cycle and worldwide spread.

**FUSARIUM GUTTIFORME, THE INCITANT OF PINEAPPLE FUSARIOsis, CAUSES MYCOTIC INFECTION TO HUMAN HOSTS. V. Balmas1, B. Schern2, A. Marcello3, F. Spanu4, H. Harak2, K. O'Donnell2, T. Aoiki4, Q. Migheli2. 1Dipartimento di Protezione delle Piante, Unità di Ricerca Istituto Nazionale Biostrutture e Biosistemi, Università degli Studi, Via E. De Nicola 9, 07100 Sassari, Italy. 2Ospedale di Sesto San Giovanni, Milano, Italy. 3National Institute of Agrobiological Sciences, Genebank Unit, Tsukuba, Japan. 4Microbial Genomics Research Unit, Agricultural Research Service, U.S. Department of Agriculture, Peoria, Illinois, USA. E-mail: balmas@unis.in.it**
During an extensive survey of clinically relevant Fusarium species (Mighelli et al., 2010, Journal of Clinical Microbiology 48: 1076-1084), fifty-eight fusarias isolated in Northern and Central Italy from 50 Italian patients between 2004 and 2007 were subjected to multilocus DNA sequence typing to characterize the spectrum of species and circulating sequence types associated with dermatological infections, especially onychomycoses and paronychia, and other fusarioses. Sequence typing revealed that isolates were nearly evenly divided among the Fusarium solani (FFSC, N = 18), the F. oxysporum (FSC, N = 20) and the Gibberella (Fusarium) fujikuroi (GFSC, N = 20) species complexes. Among the GFSC, a two-locus typing scheme was used to successfully identify 17 isolates as F. verticillioides, two as F. sacchari and one as F. guttiforme (the latter from paronychia on a 65-year-old female in good health). Since the only known host for F. guttiforme is pineapple (Ananas comosus), we wanted to determine whether the patient had come in contact with fresh pineapple fruits possibly harbouring this pathogen and to further characterize this isolate to see if it was pathogenic to pineapple. A follow-up interview revealed that the patient frequently consumed fresh pineapple after peeling it by hand. Results of a pathogenicity experiment revealed that the human pathogenic F. guttiforme isolate NRRL 53131 was able to induce tissue necrosis and to sporulate profusely after 14 days incubation at 25°C when inoculated on artificially wounded pineapple leaves. The same symptoms were induced by the ex-holotype positive control strain of F. guttiforme (NRRL 25295).

FLUORESCENT AFLP ANALYSIS OF ALTERNARIA ALTERTANA ISOLATES CAUSING BROWN SPOT OF CITRUS. P. Bella¹, G. Ialacci¹, M. Russo², A. Catara¹,2, V. Catara¹.
¹Dipartimento di Scienze e Tecnologie Fitosanitarie, Università degli Studi, Via Santa Sofia 100, 95123 Catania, Italy. ²Plesso Didattico G. Scarabelli, Università degli Studi di Bologna, Viale G. Ascani 15, 40026 Imola (BO), Italy. E-mail: mariagrazia.bellardi@unibo.it

Alternaria brown spot, caused by the tangerine pathotype of Alternaria alternata, seriously affects yield and fruit quality of citrus tangerines and their hybrids. After the first report in Italy in 2000, the disease has become a real threat, especially for the more susceptible citrus cultivars, which require an adequate schedule of treatments. Since no information on the population structure of A. alternata in Italy is available, a collection of isolates already characterized by conidial morphology, pathogenicity tests and endoPG sequence analysis, was further compared by fluorescent amplified fragment length polymorphism analysis (fAFLP), in order to assess intra-population diversity. Pre-amp Primer Mix I (Invitrogen™), containing adapter complementary AFLP primers for EcoRI and MseI sites, each with one selective nucleotide, was used in the pre-amplification reaction. To choose the most performing selective primer combination, selective amplification was preliminarily performed on few isolates using four different selective primer pairs, bearing at 3’ends two selective nucleotides. Three primer pairs were discarded since the number of picks they generated neither allowed a clear comparison of fingerprint patterns nor detected genetic variability. The primer combination, Ecosel2 (+GA)/Msesel(+CT), produced an adequate number of picks and was chosen for the analysis of the collection of A. alternata isolates. UPGMA-based cluster analysis from the similarity matrix obtained using Jaccard coefficient, revealed the presence of at least two sub-populations. Isolates obtained from different groves either clustered together or into two distinct clusters, regardless of the cultivar they came from.

FIRST REPORT OF IMPATIENS NECROTIC SPOT VIRUS INFECTING ONCIDIUM IN ITALY. M.G. Bellardi¹ and C. Cavicchi².
¹Dipartimento di Scienze e Tecnologie Agroambientali, Sezione di Patologia Vegetale, Università degli Studi, Viale Fanin 42, 40127 Bologna, Italy. ²Plesso Didattico G. Scarabelli, Università degli Studi di Bologna, Viale G. Ascani 15, 90026 Imola (BO), Italy. E-mail: mariagrazia.bellardi@unibo.it

Oncidium is a large genus of orchids that includes over 600 species. The quality of Ligurian Oncidium potted plants and cut flowers is highly appreciated in the Italian floral auction market, and the number of producers has increased in the Sanremo area in the last decade. During spring 2008, Impatiens necrotic spot virus (INSV) was found by DAS-ELISA in greenhouse-grown Oncidium orchids displaying leaf symptoms that ranged from necrotic concentric rings to necrotic lesions 1-2 cm in diameter. Subsequently, symptomatic leaves exhibited yellowing and necrosis along the veins but no flower symptoms. Oncidium plants were growing adjacent to Ranunculus hybrids infected by this tospovirus. To confirm the presence of INSV, total RNA was extracted from symptomatic Oncidium leaves and used for RT-PCR amplification of the nonstructural protein gene of INSV. The resulting primers directed the amplification of a PCR product of approximately 1300 nt. To verify that the amplified products were derived from INSV RNA, the PCR fragment was cloned and two independent clones were sequenced in both directions. Sequence analyses of the cloned PCR product showed 98.6% nucleotide sequence identity, and 98.2 and 99.2% amino acid identity and similarity, respectively with a previously published INSV-Ns sequence (Gene Bank accession No. NC_003624). Methods for reducing the risk of this disease include the use of healthy plant material, removal of weeds acting as hosts for the insect vector (Frankliniella occidentalis) or the use of insecticides to prevent virus transmission. This is thought to be the first report of INSV infecting Oncidium in Italy.

STUDY OF TWO DIFFERENT HYDROPHOBINS IN GEOSMITHIA spp. P. Bettini¹, L. Carresi², C. Comparini², G. Tomai², R. Viganò², L. Pazzaglia², A.L. Pepori³, A. Santini³, G. Cappugi², F. Scala², A. Scala².
¹Dipartimento di Biologia Evoluzionistica “Leo Pardi”, Università degli Studi, Via Madonna del Piano 17, 50125 Firenze, Italy. ²Dipartimento di Biotecnologie Agrarie, Sezione di Protezione delle Piante, Università degli Studi, Via della Lastruccia 10, 50019 Settimo Fiorentino (FI), Italy. ³Dipartimento di Scienze Biochimiche, Università degli Studi, Firenze, Italy. ⁴Istituto per la Protezione delle Piante del CNR, Via Madonna del Piano 10, 50019 Settimo Fiorentino (FI), Italy. ⁵Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università degli Studi “Federico II”, Via Università 100, 80055 Portici (NA), Italy. E-mail: aniello.scala@unifi.it

The genus Geosmithia includes several fungal species associated with phloeum-feeding bark beetles. In previous studies, we showed that the gene encoding cerato-ulmin (cu) in Ophiostoma novo-ulmi was also present in a Geosmithia isolate obtained from an elm tree affected by Dutch Elm Disease (DED). To explain this result, we hypothesized a horizontal gene transfer (HGT). Thirty-six isolates of Geosmithia, collected from elm trees with DED symptoms, were used in this work to verify if other Geosmithia species contained the cu gene sequence. Sequencing of the PCR products obtained with different primer pairs designed on the cu gene sequence revealed that fragments highly homologous to the cu gene were present in all the analyzed Geosmithia isolates. Culture filtrates of 16 Geosmithia isolates gave a positive response in ELISA assays using anti-CU antibodies; however, Western blotting and
mass spectrometry analyses showed that the MW of the protein was higher than that expected for CU. This protein was purified and characterized. Applying Edmann sequencing and Genome Walking techniques, we obtained a partial nucleotide sequence of the gene and an amino acid sequence of the protein, that were different from those known for CU. Our results show that isolates of Geosmithia spp. harbour two different hydrophobin genes: one, homologous to the cu gene from O. novo-ulmi, could have been acquired by HGT between the two fungal species, as they occupy the same habitat in elm trees. The other gene codes for a new hydrophobin, named Geo1, with a good homology level to the O. novo-ulmi CU protein.


1Dipartimento di Scienze e Tecnologie Agroambientali, Sezione di Patologia Vegetale, Università degli Studi, Viale Fannin 40, 40127 Bologna, Italy. 2Intrachem Bio Italia, Servizio Tecnico, Ricerca e Sviluppo, Via Calzino 2085, 47023 Cesena, Italy. 3Parco delle Acque della Versilia e Idroenergia, Università degli Studi, Via TOLARA di Sopra 50, 40064 Ozzano del’Emilia (BO), Italy. E-mail: umberto.mazzucchit@unibo.it

Bacterial canker of kiwifruit, caused by Pseudomonas syringae pv. actinidiae (Psa), became relevant, especially in 2008 and 2009. Epidemics occurred in Lazio and Emilia Romagna (central and northern Italy) and the causal organisms were identified on the basis of phenotypic and genomic characteristics. In Italy, the control of bacterial canker relies mainly on agronomic prophylactic measures and on the use of copper-based products. In this study, we investigated the activity of Bacillus subtilis strain QST 713 (Serenade Max) to inhibit the growth of two different Psa strains in vitro, and its ability to survive and reduce the population of two rifampinc resistant pathogen strains on female flowers of Actinia chinenis and A. delicosa. The microbial control agent was able to survive on female flowers of both Actinidia species, reaching a population of ca. 10^5 CFU/flower 48 and 96 h after application. Moreover, the antagonist reduced the Psa population on A. chinenis flowers by more than one order of magnitude 48 h after its application. These preliminary results indicate that B. subtilis strain QST 713 may be a promising tool for the biological control of bacterial canker of kiwifruit, and provide insights into the interaction between the microbial control agent and Psa.

**ANTIFUNGAL ACTIVITY OF THREE NOVEL SAPONINS FROM ALLIUM CEP A.** G. Bonanomi1, A. Gargiulo1, V. Antignani2, L. Zannetti2, F. Scala2.

1Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università degli Studi di Napoli “Federico II”, Via Università 100, 80055 Portici (NA), Italy. 2Dipartimento di Scienze degli Alimenti, Università degli Studi di Napoli “Federico II”, Via Università 100, 80055 Portici (NA), Italy. E-mail: giuliano.bonanomi@unina.it

During their life cycle plants interact with a wide range of different microbial species, including pathogens. Thousands of diverse natural products are produced by plants and many of these are involved in plant defence. The phytochemical diversity of antimicrobial compounds include terpenoids, phenolics, phenylpropanoids, stilbens, alkaloids, glucosinolates, indole and saponins. In this study, the effects of three novel saponins (ACE15G3, ACE15E, ACE15D4) isolated from Allium cepa were tested on soil-borne pathogens (Fusarium oxysporum f. sp. lycopersici, Rhizoctonia solani and Sclerotium cepivorum), air-borne pathogens (Alternaria alternata, Aspergillus niger, Botrytis cinerea, Mucor sp., Phomopsis sp.) and two antagonistic fungi (Trichoderma atrovirens and T. hamatum). Antifungal activity of all three saponins increases with their concentration and varied with the following rank: ACE15G3 > ACE15E = ACE15D4. F. oxysporum f. sp. lycopersici, S. cepivorum and R. solani were very little affected by saponins. Among the other fungi, B. cinerea and the two Trichoderma species were the most sensitive. We found a significant synergism in the antifungal activity of the three saponins against B. cinerea. Growth of this fungus was strongly inhibited when the three saponins were applied in combination.

**PLANT LITTER PHYTOTOXICITY AND SOIL-BORNE PATHOGENS CAN EXPLAIN TREE DIVERSITY GRADIENT AT GLOBAL SCALE.** G. Bonanomi1, F. Giannino2, G. Incerti3, C. C. Dekker3, M. Rietkerk3, S. Mazzoleni1.

1Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università degli Studi di Napoli “Federico II”, Via Università 100, 80055 Portici (NA), Italy. 2Dipartimento di Ingegneria Agraria e Agronomia del Territorio, Università degli Studi di Napoli “Federico II”, Via Università 100, 80055 Portici (NA), Italy. 3Department of Environmental Sciences, Copernicus Institute, Utrecht University, PO Box 80115, 3508 TC Utrecht, The Netherlands. 4Dipartimento di Scienze della Vita, Università degli Studi, Via Giorgieri 10, 34127 Trieste, Italy. E-mail: giuliano.bonanomi@unina.it

The diversity of plant species increases from the poles to the equator ranging from almost monospecific forests at high latitude, to intermediate species richness in temperate climates, to the hyper-diverse tropical forests. For plants in aquatic ecosystems, however, this does not occur. Current theories based on resources competition cannot explain such latitudinal patterns of plant diversity. Moreover, the co-occurrence of hyper-diverse stands in lowland terra firma forests and almost monospecific stands in mangroves and gallery riparian vegetation within the tropics remains enigmatic. Here we present a new mathematical model in which, besides the positive feedback of plant growth by nutrients release, litter decomposition and associated changes in soil microbial communities build up a species-specific negative feedback due to soil-borne pathogens and phytotoxicity released by the decaying plant litter. We validated the model by comparing it with extensive published data sets collected both across and within latitudinal or climatic zones. The model predicts correctly the number of tree species, their relative abundance as well as their biomass production in all environmental conditions providing a putative explanation also for the diversity variations observed within the tropics. The model advances in the direction of a unifying ecosystem theory and demonstrates a mechanistic link between the carbon cycle, the soil microbial communities and the tree diversity patterns.

**DISCOVERY OF NEW PHLOMIS SPECIES NATURALLY INFECTED WITH PHLOMIS MOTTLE VIRUS.** D. Bosca1, P. Saldarelli1, A. De Stradis1, C. Vovlas2.

1Istituto di Virologia Vegetale del CNR, UO Bari, Via Amendola 165/A, 70126 Bari, Italy. 2Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi “Aldo Moro”, Via Amendola 165/A, 70126 Bari, Italy. E-mail: d.bosca@ba.ivv.cnr.it

Phlomis mottle virus (PhMV), a putative member of the family Flexiviridae, was isolated in 2008 from Phlomis fruticosa L.

Grapevine yellows (GY) are an important threat to grapevine industry in Europe. In Italy, GY are mostly represented by Flavescence dorée (FD) and Bois noir (BN) associated with infection by 16SrV and 16SrXII phytoplasma ribosomal groups, respectively. In the frame of a regional strategy aimed at monitoring GY presence, an extensive survey was carried out covering the seven provinces of Tuscany (central Italy), and collecting 385 leaf samples from commercial vineyards inspected in summer 2009. Preference was given to samples showing symptoms referable to GY diseases. To allow tracking of FD-positive samples for uprooting, all vines were marked and localised by “Global Positioning System-GPS”. Nested-PCR and Real-time PCR confirmed that BN is largely the most important GY in Tuscany with an ubiquitous distribution. In fact, it was present in almost all surveyed vineyard with an overall incidence of 70% (418 out of 585 samples tested). Alarming presence of FD was recorded in 23 samples distributed in five provinces, i.e. Arezzo, Massa-Carrara, Florence, Siena and Lucca. Molecular characterization performed with PCR/RFLP analysis on 165 ribosomal and on SecY genes confirmed the presence of either FD-C and FD-D subgroups. FD-D is reported for the first time in Tuscany and is identical to the strain described earlier in France and in Veneto (northern Italy). Further investigations on insect vectors, natural reservoirs and molecular characterization of grapevine-infecting phytoplasmas are needed to monitor and understand the epidemiology GY diseases in Tuscany.

EXTEMPORARY SURVEY OF VIRUSES INFECTING AN OLIVE VARIETAL COLLECTION IN TUSCANY. H. Bouyahia, D. Rizzo, M. Della Bartola and A. Materazzi.

The occurrence was investigated of olive-infecting viruses in the regional varietal collection at the experimental station “Santa Paolina” (CNR-IVALSA, Follonica-Grosseto). Starting from 2004, a total of 245 mother plants belonging to 26 different Tuscan olive varieties were sampled and tested for the presence of nine olive viruses. Largely grown varieties like Leccino, Frantoio and Moraiolo and the less known Correggiolo, Grappolo, Leccio del Corno, etc. were included. Hardwood cuttings taken from different part of the canopy were sampled twice a year (spring and autumn). Cortical scrapings were powdered in liquid nitrogen and total RNA was extracted. During the first years, cDNA synthesis followed by PCR was employed and successively replaced by one-Step RT-PCR for the detection of Arabis mosaic virus (ArMV), Cherry leafroll virus (CLRv) Cucumber mosaic virus (CMV), Olive latent virus 1 (OLV-1), Olive latent virus 2 (OLV-2), Olive ringspot virus (OLRSV), Olive leaf yellowing-associated virus (OLYaV), Strawberry latent ringspot virus (SLRSV) and Tobacco necrosis virus (TNV). Seventeen of 245 samples (6.9%) were infected. Of the nine virus tested for only ArMV, CLRv, OLYaV and SLRSV were detected and no cases of mixed infections were found. Specifically, six mother plants of cvs Lecino, Frantoio and San Fancisco were infected with OLYaV. ArMV was found in cvs Correggiolo, Oliavastra and Ornellaia and four mother plants of cvs Moraiolo and Maurino were positive to CLRv. Two positive samples to SLRSV were detected in cv Maurino and Grappolo. As previously reported, we confirmed a satisfactory sanitary status of olive germplasm in Tuscany.

The spread of bois noir was monitored from 2005 to 2009 in seven vineyards of cv. Sangiovese established in 2001 in Tuscany (central Italy). In each season the number and position of the vines showing typical symptoms (leaf discoloration, downward rolling of leaves, incomplete lignification of canes, shrivelled bunches) were recorded. Only the presence of 16SrXII-A phytoplasmas was ascertained by PCR analysis on bulk samples of symptomatic leaves. With the purpose of determining the spatial pattern of the disease (random vs. aggregated or clustered), for each year of assessment, field data on annual incidence were analyzed at three spatial levels (hierarchies) including: (i) adjacent vines within the row and across the rows (ordinary runs analysis), (ii) within vines grouped into sampling units (distribution analysis) and (iii) among groups of plants at some distance from one another (SADIE-Spatial Analysis by Distance Indices). As a whole, the pattern of temporal disease incidence showed a similar trend in six of the seven study sites: annual incidence rate progressively decreased from year to year after the first or second
season of survey. Among the other epidemiological phenomena observed the most important were: (i) a sharp decrease in the frequency of new disease records in previously unaffected grapevines; (ii) a random distribution of disease incidence for the majority of the plots and assessment periods at all spatial levels of analysis, suggesting the lack of disease spread between adjacent vines. Studies designed to characterize the role of insect vectors in disease spread are in progress.

MYCORRHIZAL SYMBIOSES AND PLANT PATHOLOGY. S. Burraruo1, E. Corda2, A. Franceschini2, G. Innocenti2, M. Iot-ti3, E. Lancellotti2, L. Montecchio1, S. Mutto Accordi1, F. Pia-toni2, L. Scattolin1, L. Torta1, A. Vanni2, A.M. Vettraino3, A. Zambonellii, 1Dipartimento di Scienze Entomologiche, Fitopatologiche, Microbiologiche, Agrarie e Zootecniche, Università degli Studi, Viale delle Scienze 2, 90128 Palermo, Italy. 2Dipartimento di Protezione delle Piante, Università degli Studi, Via De Nicola, 07100 Sassari, Italy. 3Dipartimento di Protezione e Valorizzazione Agroalimentare, Università degli Studi, Viale dell’Università, 35020 Legnaro, Italy. 4Dipartimento di Protezione delle Piante, Università degli Studi della Tuscia, Via S. Camillo de Lellis, 01100 Viterbo, Italy. 5Dipartimento di Protezione e Valorizzazione Agroalimentare, Università degli Studi, Via Fanin, 40127 Bologna, Italy. E-mail montecchio@unipd.it

The first detailed morphological description of a mycorrhiza was by Giuseppe Gibelli in 1883, in a paper on chestnut ink disease. Since then, much of the published literature reported the importance of mutualistic mycorrhizal symbioses for the improvement of the vegetative and health status of plants, mainly by means of an increase in water and mineral nutrition, and by protection from fungal parasites. Rare examples of parasitic mycorrhizal symbioses are also known. In natural ecosystems, almost all terrestrial plants are associated with a wide community of mycorrhizal fungal species, whose composition is the result of quick dynamic interactions involving vegetative, nutritional, physiological and pathological features. Since a significant correlation between plant health and mycorrhizal community composition is well established, a synthetic evaluation of the plant or plantation fitness, status. An in-depth examination of the role of mycorrhiza, the majority of which are still undescribed, and of the dynamics regulating their presence and frequency in any given ecosystem, are therefore of main importance in plants health management. Thanks to the availability of modern microscopic, enzymatic, molecular and biostatistics methods, the Italian research on this topic is in constant growth at the international level, also in applied sectors, i.e. production of artificially mycorrhized plants to be employed in unfavourable sites, or production of high-priced sporocarps.

HIGH SUSCEPTIBILITY OF THE TRIPLOID HYBRID LEMOX TO MAL SECCO DISEASE CAUSED BY PHOMA TRACHEIPHILA. S.O. Cacciola1, A. De Patrizio2, F. Raudino3, A. Pane1, V. Lo Giudice3, G. Magnano di San Lio5. 1Dipartimento di Chimica Biologica, Chimica Medica e Biologia Molecolare, Università degli Studi, Viale Andrea Doria 6, 95125 Catania, Italy. 2Dipartimento di Scienze Entomologiche, Fitopatologiche, Microbiologiche, Agrarie e Zootecniche, Università degli Studi, Viale delle Scienze 2, 90128 Palermo, Italy. 3Dipartimento di Scienze Entomologiche, Fitopatologiche, Microbiologiche, Agrarie e Zootecniche, Università degli Studi, Viale delle Scienze 2, 90128 Palermo, Italy. 5Dipartimento di Scienze Entomologiche, Fitopatologiche, Microbiologiche, Agrarie e Zootecniche, Università degli Studi, Via P. Sofia 100, 95123 Catania, Italy. 4Via Calvario 45, 95030 Mascalucia (CT), Italy. 5Dipartimento di Gestione dei Sistemi Agrari e Forestali, Università Mediterranea degli Studi, Località Feo di Vito, 89122 Reggio Calabria, Italy. E-mail: olga.cacciola@unicat.it

‘Lemox’, a triploid obtained by crossing the tetraploid lemon ‘Doppio Lentini’ and the spontaneous lemon hybrid ‘Femminel-lo’ x ‘Pera del Commendatore’, is a seedless and early bearing new lemon cultivar, with a good fruit size and high yields. In this study, the susceptibility of ‘Lemox’ to mal secco disease was tested in comparison with commercial lemon cultivars, including nucellar and old clones of ‘Monachello’, ‘Femminello’, ‘Femminello Zagara bianca’, ‘Cerza VCR’, ‘Lunario’, ‘Femminello Siracusano 2K’, as well as three triploid hybrids of ‘Femminello’ x allotetraploid somatic hybrids of ‘Valencia’ sweet orange x ‘Femminel-lo’(VF), ‘Milam’ lemon x ‘Femminello’(MF), and Key lime x ‘Va-lencia’sweet orange (KIV), respectively. In autumn 2008, 1-year-old potted trees on sour orange rootstock were stem-inoculated with a conidal suspension (10° conidia/ml) of a virulent isolate of Phoma tracheiphila. In spring 2009, symptom severity was rated visually and the extent of xylem colonization by the fungus was assessed by both isolation on potato-dextrose-agar and a PCR-based assay with specific primers (PtFOR2 and PtREV2). ‘Lemox’ and ‘Femminello Siracusano 2K’ proved to be the most susceptible cultivars, followed by ‘Lunario’ and ‘Femminello’, whereas both nucellar and old clones of ‘Monachello’ were tolerant. ‘Cerza VCR’ and ‘Femminello Zagara bianca’ were moderately tolerant. The MF hybrid proved to be very susceptible whereas the VF and KLV hybrids were moderately tolerant. However, the tolerance to mal secco of these two hybrids was not comparable to that of ‘Monachello’. The test was repeated in 2009-2010, yielding similar results.

EFFECT OF DOWNY AND POWDERY MILDEWS ON GRAPEVINE BERRY JUICE COMPOSITION. T. Caffi, S.E. Legler, V. Rossi. Istituto di Entomologia e Patologia Vegetale, Università Cattolica del Sacro Cuore, Via E. Parmentenese 84, 29122 Piacenza, Italy. E-mail: tito.caffi@unicatt.it

Plasmodora viticola and Erisyphe necator are the causal agents of downy and powdery mildew of grapevine, respectively. They can cause severe yield losses but their effect on the quality components is little investigated. Clusters of different red- and white-berried cultivars of Vitis vinifera were hand-harvested from untreated plots in commercial vineyards of Emilia-Romagna, Piedmont, and Lombardy. Individual berries were removed from clusters and classified as follows: (i) healthy from healthy clusters; (ii) healthy from affected clusters; (iii) with a single downy mildew lesion, or powdery mildew colony; (iv) with multiple lesions or colonies; (v) totally affected or cracked. Sub-samples of the berries belonging to the same category were crushed, de-stemmed, and pressed. Juice yields were determined, and chemical and physical measurements were performed on the juice (i.e., °Brix, pH, treatable acidity, malic and tartaric acids, and polyphenols). Both pathogens negatively affected juice yield and average weight of berries, and modified quality components of the juice. For instance, powdery mildew increased °Brix from 18.3 to 23.3, while downy mildew increased treatable acidity by 77% in average. Equations were developed for estimating the impact of downy and powdery mildews on quality of bunches.

EPIDEMIC DEVELOPMENT OF RHIZOCTONIA SOLANI ON BRASSICA OLERACEA var. ACEPHALA, AND CHARAC-TERIZATION OF ISOLATES. R. Caiazzo, A. Carella, R. Nicoletti, F. Raimo, F.A. Porrone, E. Lahoz. CRA, Unità CAT, Via P.
Brassica oleracea L. var. acephala known as ‘torzella’ was in the past largely cultivated in southern Italy, then abandoned, and reevaluated in recent years. In a farm in Scafati, where torzella was cropped for the first time, collar rot symptoms were observed on about 20% of the plants. Isolates of Rhizoctonia solani Kühn, showing the typical hyphal branching and multinucleate cells, were recovered from affected tissues. Determination of anastomosis groups was carried out by pairing isolates with tester strains on 2% water agar (WA) in Petri plates. Hyphal anastomosis was only observed with tester isolates of AG-4, producing both C2 and C3 reactions. Moreover, typical AG-4 isozyme patterns were obtained after polygalacturonase gel electrophoresis. The clonal condition of the isolates was also assessed by examining tuft formation in pairings on PDA supplemented with 0.5% activated charcoal. Finally, a biomolecular analysis was carried out by means of RFLPs of rDNA-ITS by using HincII and HpaI as restriction enzymes to assign torzella isolates to one of the three homogeneous groups (HG-1, -II and -III) so far characterized within R. solani AG-4. Pathogenicity test confirmed the ability of R. solani AG-4 isolates to induce collar rot. These isolates were recovered from symptomatic tissues, thus fulfilling Koch’s postulates.

TRANS-RESVERATROL AND LEAF SYMPTOM EXPRESSION IN ESCA OF GRAPEVINE. F. Calzarano1, V. D’Agostino1, F. Osti2, S. Di Marco2. 1 Dipartimento di Scienze degli Alimenti, Università degli Studi, Via C.R. Lerici 1, 64023 Mosciano Sant’Angelo (TE), Italy. 2 Istituto di Biometeorologia del CNR, Via P. Gobetti 101, 40129 Bologna, Italy. E-mail: felice.scala@unina.it

Esca of grapevine is a serious disease some aspects of which, including the nature of foliar symptoms, are still poorly understood. Leaves collected at different phenological growth stages from symptomatic grapevines cv. Trebbiano d’Abruzzo showed the presence of higher concentrations of trans-resveratrol, the major stilbene produced in grapevine, compared to leaves from healthy plants. Increased levels of trans-resveratrol are supposed to be associated with the host-plant defence response, but this condition raises questions on the possible role of the stilbene in the foliar symptom development. Symptomatic leaves collected in 2009 were divided into 4 classes estimating symptom severity as the percentage of chlorosis and necrosis on the total leaf area. For each class, leaves were collected at pre-bunch closure, post-veraison and harvest, and the level of trans-resveratrol was determined. The results showed higher concentrations of trans-resveratrol in symptomatic leaves collected at pre-bunch closure compared to what observed at the other growth stages. These results are in agreement with our previous findings, i.e. the stronger response of the leaves might be correlated with leaf functioning at the different phenological growth stages. Moreover, the increase of trans-resveratrol concentration with increased severity of leaf symptoms seems to exclude an involvement of the stilbene in symptom development. Further investigations on cut leaves inoculated with culture filtrates of the pathogen and/or trans-resveratrol may lead to a better understanding of the relationship between the stilbene and foliar symptom expression.

THREE BASIDIOMYCETES CONTAMINATING COMPOST FOR CULTIVATION OF PLEUROTUS ERYNGII. I. Camele, L. Altieri, G.L. Rana. Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali, Università degli Studi della Basilicata, Via Ateneo Lucano 10, 83100, Potenza, Italy. E-mail: ippolito.camele@unibas.it

The basidiomycetes Oxyporus latemarginatus, Schizophyllum commune and Bjerkandera adusta were found contaminating substrate commonly used to grow Pleurotus eryngii in southern Italy in 2009. More specifically, basidiomes of O. latemarginatus and S. commune appeared on the compost surface at beginning of a cultivation in Sardinia and in an incubation tunnel of ITALMICO agricultural farm in Basilicata, respectively. B. adusta formed sporophores after the first production flush in a P. eryngii autumn culture in Apulia. Identification of the three contaminants was first achieved on the basis of their macro- and microscopic features. Molecular analysis, done by PCR and nucleotide sequencing, confirmed the identity of the first and second contaminant as O. latemarginatus and B. adusta. DNA was extracted from pure cultures of both contaminants and amplified with primers ITS4/ITS5. Nucleotide sequences of O. latemarginatus (isolates n. 947) and B. adusta (isolate n. 1147) were 606 and 609 bp in size and showed a 99% similarity with GenBank sequences of the two fungi (accession numbers AF 232721 and AF 006672). Two nucleotide sequences of the above isolates of O. latemarginatus and B. adusta were deposited at the European Molecular Biology Laboratory (UK) with access codes FN 232852 and FN995241, respectively. This is the first report of O. latemarginatus and S. commune as contaminants and competitors of P. eryngii in exploiting cultivation compost. By contrast, B. adusta was already known as contaminant of the same substrate.

EFFECT OF ORGANIC AMENDMENTS AND MICROBIAL DIVERSITY ON SOIL FUNGISTASIS. M. Capodilupo, G. Bonanomi, M. Ceniccola, V. Antignani, F. Scala. Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università degli Studi di Napoli “Federico II”, Via Università 100, 80055 Portici (NA), Italy. E-mail: felice.scala@unina.it

Soil fungistasis is defined as the inhibition of fungal propagule germination under favourable conditions. In this study, we analyzed the effect on fungistasis of four different carbon sources (glucose, starch, PDB and Medicago sativa hay) at different times of decomposition (0, 2, 4, 7, 28 and 46 days) applied at three concentration levels (3%, 0.3% and 0.03%). Fungistasis was assessed by germination tests carried out with four fungi: Arxergillus flavus, Botrytis cinerea, Mucor sp. and Trichoderma harzianum. Microcosms were characterized for pH, electrical conductivity and fluoresceine diacetate hydrolytic activity (FDA). We also tested the influence of microbial diversity on fungistasis. Microbial consortia with three levels of diversity (1, 3 and 9) were assembled from a pool of known microorganisms selected among different functional groups. In this case, fungistasis was assessed by using Rhizoctonia solani as a target fungus. We found that fungistasis was rapidly lost after the application of all organic amendments but it was also restored, usually within 7-28 days, according to the type and the amount of amendment. Mucor sp. was the most sensitive fungus while A. flavus was the less affected. Fungistasis towards R. solani was enhanced and much less variable in the presence of microcosms of increasing microbial diversity. We conclude that soil fungistasis is strictly dependent on decomposition of organic matter and diversity of the microbial communities.

OCCURRENCE OF LECANICILLIUM MUSCARIUM AS AN ANTAGONIST OF SOIL-BORNE FUNGAL
PATHOGENS OF PERENNIAL WALLROCKET. A. Carella and R. Nicoletti. CRA, Unità di Ricerca per le Colture Alternative al Tabacco, Via P. Vitiello 108, 84018 Scafati (SA), Italy. E-mail: rosario.nicoletti@entecra.it

The role of fungal antagonists in plant protection may be quite relevant in short-cycle crops where the use of fungicides is restricted. During an investigation on crown and root rot of perennial wallrocket (Diploptaxis tenuifolia) in the Piana del Sele area, the occurrence of fungal antagonists was observed in the isolation plates of the causal agents, Rhizoctonia solani and Sclerotinia sclerotiorum. Besides some well-known mycoparasites, a fungus producing Verticillium-like conidiophores was recovered from subcultures of both pathogens which was identified as Lecanicillium muscarium (Petch) Zare et Gams. While its antagonistic ability against several rusts and powdery mildews is already documented, to our knowledge this is the first report in relation to the above-mentioned soil-borne pathogens. Their mycelial mat was slowly overgrown by L. muscarium isolates in PDA cultures, and no colonies originated as mycelial plugs were transferred into new plates. S. sclerotiorum sclerotia harvested as soon as they had developed were colonized by the mycoparasite, as they assumed a soft consistency and failed to germinate when placed on fresh medium a month later. Mycoparasitic interactions in dual cultures were not obvious since coiling or growth inside hyphae of the suscept did not occur. However, a depressed growth of the hyphae was indicative of the likely establishment of a trophic relationship, as we have previously ascertained electron microscopically for other R. solani antagonists. Mycoparasitic aptitude by isolates of L. muscarium was also supported by the production of chitinases and glucanases in liquid cultures.

DIFFERENTIAL COLONIZATION OF RESISTANT AND SUSCEPTIBLE MELON GENOTYPES BY FUSARIUM OXYSPORUM f. sp. MELONIS. V. Catalano1, A. Haegi12, L Luongo1, N. Ficcadenti2, A. Belisario1. 1CRA, Centro di Ricerca per la Patologia Vegetale (CRA-PAV), Via C.G. Bertero 22, 00156 Roma, Italy. 2CRA, Unità di Ricerca per il Vivaismo e la Gestione del Verde Ambientale ed Ornementale (CRA-VIV), Via dei Fiori 8, 51012 Pescia (PT), Italy. 1CRA, Unità di Ricerca per l’Orticoltura (CRA-ORA), Via Salaria 1, 63030 Monsampolo del Tronto (AP), Italy. E-mail: alessandra.belisario@entecra.it

Fusarium wilt of melon, caused by the soil-borne fungus Fusarium oxysporum f. sp. melonis (FOM), is the most important and least controllable disease. The most effective control is the use of resistant host genotypes. Investigations were conducted to assess the qualitative and quantitative traits of FOM colonization in the compatible and incompatible interactions. The absence of symptoms on susceptible scions was considered of particular interest. To this purpose, FOM race 1 (ISPaVe1070) and race 1,2 (ISPaVe1018) were used to inoculate grafted melon plants. The resistant DH line NAD-1 and the susceptible Charentais-T were used in all possible combinations. Pathogen colonization of the stem at different heights and at 2, 4, 14 and 18 days post inoculation (dpi) was evaluated. Assays were performed both by direct isolations from stem fragments and by PCR with a selective primer pair. The results showed that in plants with resistant rootstock no disease symptoms appeared at 18 dpi, and the two fungal strains were reisolated mainly from the rootstocks and rarely from the susceptible scions. Conversely, in plants with susceptible rootstock disease symptoms became obvious at 7 dpi and the plants died at 18 dpi. The two fungal strains were always reisolated from the whole stem. PCR confirmed the outcome of direct reisolations and showed to be more sensitive. Plants with resistant rootstock inoculated with either FOM race 1 or race 1,2 remained symptomless until the end of the experiment, notwithstanding the presence of the fungus.

CHARACTERIZATION OF A NEW ILARVIRUS SPECIES FROM VIOLA TRICOLOR. M. Ciuffo1, R. Lenzi, V. Masenga, M. Turina. Istituto di Virologia Vegetale del CNR, Strada delle Cacce 73, 10135 Torino, Italy. E-mail: m.turina@ive.cnr.it

In 2009, a number of samples of Viola tricolor from Lombardy (northern Italy) showing white mosaic and deformation of younger leaves were mechanically inoculated on a range of host plants. Electron microscopy observation failed to detect virus particles in the original sample, but inoculated Nicotiana benthamiana plants showed leaf deformations that could be easily mechanically transmitted. Numerous attempts to purify the virus using standard procedures failed. We therefore proceeded to isolate dsRNA from infected N. benthamiana. A random primer cDNA library from dsRNA yielded clones specific to infected plants. Sequence analysis showed some similarity with Prune dwarf virus (Harvisor, Bromoviridae) RNA-3 coding region. From sequence later on from the initial clone, specific oligonucleotides were designed to extend cloning and sequencing to other RNA-3. Region. The full length coding sequence is now available of the putative movement protein (MP) and coat protein (CP). Phylogenetic analysis of a number of Harvisor sequences suggests that the virus under study is a new Ilarvirus species, for which the name Viola white mosaic virus (VWMV) is proposed. Specific oligonucleotides were used in RT-PCR for diagnostic purposes in a number of viola samples.

WIDESPREAD OCCURRENCE OF RANUNCULUS LATENT VIRUS (MACLUARVIRUS, POTYVIＲＩＤＥＡ) IN ARTICHoke CROPS IN ITALY. M. Ciuﬀo1, R. Lenzi 1, M. Testa2, M. Turina1. 1Istituto di Virologia Vegetale del CNR, Strada delle Cacce 73, 10135 Torino, Italy. 2Dipartimento per la Ricerca nelle Produzioni Vegetali, AGRIS Sardegna, Cagliari, Italy. E-mail: m.turina@ive.cnr.it

In 2004 four new virus species belonging to the family Potyviridae, were found in Ranunculus asiaticus in Liguria (Northern Italy). One of these, the macluravirus Ranunculus latent virus (RaLV) was detected by DAS ELISA in symptomatic and symptomless artichoke plants. Several other samples from Liguria, Sardinia and Latium were tested in the following years and most of them were positive for RaLV. The presence of this virus in the samples was also confirmed by specific western blot analysis. A fragment of the sequence corresponding to the 3’ proximal region of the genome of about 500 bp was amplified cloned and sequenced using RaLV specific primers. Comparison of the artichoke isolates from Liguria, Sardinia and Latium, with the ranunculus isolates of RaLV showed 98% identity at the nucleotide level. The sequence of the artichoke isolate was than extended upstream to cover a part of the Nla coding sequence. The Nla fragment from RaLV showed 80% identity with Artichoke latent virus (ArLV), a virus known in artichoke since the early sixties. Due to the similarity with ArLV we tried to detect RaLV infected samples with an ArLV commercial probe, but no reaction was obtained. Based on our results RaLV is distinct from ArLV, its presence in artichoke germplasm is cause of concern, particularly because it seems to exacerbate symptoms expression of other viruses when present in mixed infections.
ANTIFUNGAL ACTIVITY OF ALCOHOLIC EXTRACT FROM \textit{SPARTIUM JUNCEUM} : PRELIMINARY SCREENING STUDIES. F. Clematis, C. Pasini, P. Curir. CRA, Unità di Ricerca per la Floricoltura e le Specie Ornamentali (CRA-FSO), Corso Inglesi 08, 18038 Sanremo (IM), Italy. E-mail: f.clematis@isflori.it

The antifungal properties of \textit{Spartium junceum}, a perennial shrubs native to the Mediterranean area and widespread throughout Italy, were tested against eight selected fungal pathogens. Plant material (stem and leaves) were extracted with ethanol (1:1) in a soxhlet apparatus under refluxing conditions for 3 h. The efficacy of plant extracts was tested \textit{in vitro} by poisoned food technique at different concentrations (2, 1.6, and 1%). The best inhibition values ranged between 21.3 and 46%. The highest concentration of plant extract (2%) showed significant reduction on the growth of \textit{Fusarium oxysporum} isolated from \textit{Danae racemosa} and \textit{F. oxysporum} f. sp. \textit{dianthi}, while the lowest concentration (1%) was already effective in suppressing \textit{Alternaria dianthi} and \textit{Botrytis cinerea}. A. \textit{dianthi} was inhibited by 46.0% when 2% of plant extract was applied, \textit{B. cinerea} and both \textit{F. oxysporum} strains were inhibited by 45.3 and 37.3%, respectively. This preliminary screening showed significant inhibition activity of \textit{S. junceum} extracts at low concentrations. Preliminary TLC and spectrophotometer analysis showed the presence of phenolic compounds in the extracts using UV absorbance at 275 nm. Investigations are in progress to study the phytochemical characteristics of plant extract by HPLC and TLC.

AN APPROACH TO GRAPEVINE SANITARY SELECTION IN SARDINIA: FIELD STUDY AND SEROLOGICAL DIAGNOSIS. L. Cogotzi, V.A. Prota, R. Garau. Dipartimento di Protezione delle Piante, Sezione di Patologia Vegetale, Università degli Studi, Via Enrico De Nicola 1, 07100 Sassari, Italy. E-mail: l.cogotzi@uniss.it

Strategies to control viruses are essentially preventive and based on sanitary selection programs. Within this project, the creation of a germplasm collection will aim at progressive sanitary selection. Field studies, carried out over two years (2008-2009), focused on eleven local grapevine varieties: Vermentino, Canonau, Monica, Bovale, Malvasia, Carignano, Nuragus, Nebbiolo, Moscato, Caricajola, and Nasco. Selected vineyards were inspected in spring, summer and autumn. Symptoms of virus and phytosanitary infections were common in these vineyards. Almost 5000 essentially symptomless vines were selected in the course of numerous field surveys carried out throughout the entire region and were tested serologically (ELISA). We focused on the presence of two types of viruses associated with leafroll disease (GLRaV-2, GLRaV-3) that cause severe losses, reduce yield and affect negatively fruit quality.

\textit{Grapevine fanleaf virus} (GFLV) and \textit{Grapevine virus A} (GVA), an agent associated with Kober stem grooving were also looked for on a lower number of plants. Results showed that, in Sardinia, infection rates of GLRaV-2, GLRaV-3, GFLV and GVA range between 3 and 70%, 5 and 80%, 0 and 40% and 5 and 60%, respectively. ‘Carignano’ from the Sulcis area (south-west Sardinia) had the highest percentage of virus-free plants while ‘Nuragus’ and ‘Malvasia’, both from the south of Sardinia (Parteolla area) had the lowest. Selected plants will undergo further sanitary selection.

\textit{Work funded by the Con.Vi.Sar. consortium of Sardinia}

PLANT VIRUSES AS POSSIBLE PLATFORMS FOR MOLECULAR FARMING. V. Condelli, A. Vitti, M. Nuzzaci, M.T. Lanorte, P. Piazzolla. Dipartimento di Biologia Difesa e Biotecnologie Agroforestali, Università degli Studi della Basilicata, Campus di Macchia Romana, Via dell’Ateneo Lucano 10, 85100 Potenza, Italy. E-mail: valentina.condelli@unibas.it

Plant viruses are emerging as an attractive system for the expression of foreign epitopes used as immunogens for the development of innovative vaccination strategies. In such a way, plant viruses carrying on their coat protein (CP) peptides of medical interest can be considered, in association with their hosts, right partners of biological systems devoted to pursue the goal of functioning as medical molecular farming. \textit{Cucumber mosaic virus} (CMV) is one of the plant viruses used as a carrier of foreign epitopes because of its wide host range, which comprises edible plants (celery, lettuce, cucumber, tomato, carrot, pepper, banana, etc.). The results of immunological experiments, obtained by using CMV as a presentation system for the \textit{Hepatitis C virus} and \textit{Alzheimer’s disease}-derived epitopes, in association with the proven stability of CMV in gastrointestinal environment, seem to open a new prospect for the development of effective vaccine candidates. These data could be considered of paramount importance to health and medicine, thus suggesting to extend this strategy to other human and animal diseases, such as Influenza. Alternatively, another plant virus, \textit{Potato virus A} (PVA) was selected as a carrier because the filamentous shape of its particles offers no packaging limitation for rather large genome insertion. A molecular modeling approach has been used to identify the possible insertion points of foreign determinants, in order to get the best conditions of stability and infectivity of chimeric virus particles.

MYCOTOXICIGENIC \textit{FUSARIUM} SPECIES AND MYCOTOXINS IN WHEAT GRAIN IN 2008 IN UMBRIA. L. Covarelli, G. Beccari, M. Tinelli, G. Santoponte. Dipartimento di Scienze Agrarie e Ambientali, Università degli Studi, Borgo XX Giugno 74, 06121 Perugia, Italy. E-mail: lorenzo.covarelli@unipg.it

In 2008, a study was conducted on 53 durum wheat (DW) and 36 soft wheat (SW) grain samples harvested in Umbria (central Italy). After visual observation of \textit{Fusarium} damaged kernels, samples were subjected to fungal isolation on PDA, DNA extraction for \textit{Fusarium} detection and identification by PCR, mycotoxin extraction for deoxynivalenol (DON) and T-2 toxin determination. DW and SW samples had 34% and 19% \textit{Fusarium}-damaged kernels while average the incidence of \textit{Fusarium} infections was 40% and 33%, respectively, in the two wheat species. PCR assays showed that the most frequent species were \textit{F. graminicola}, \textit{F. avenaceum} and \textit{F. culmorum}. \textit{F. equiseti} was also detected by PCR but was not isolated on PDA while \textit{F. poae} was mainly detected by traditional isolation rather than by PCR. DON levels exceeded the EU legal limits in 9% of DW and 8% of SW samples, reaching contamination levels of 53 mg kg\textsuperscript{-1} in DW (average 2 mg kg\textsuperscript{-1}) and 10 mg kg\textsuperscript{-1} in SW (average 0.7 mg kg\textsuperscript{-1}). T-2 toxin was constantly detected, with peaks of 187 µg kg\textsuperscript{-1} in DW (average 62 µg kg\textsuperscript{-1}) and 78 µg kg\textsuperscript{-1} in SW (average 40 µg kg\textsuperscript{-1}). Our results indicate that, even if previous studies carried out in Umbria showed a low mycotoxins risk, in years like 2008 that was characterized by climatic conditions extremely favourable to \textit{Fusarium} head blight, wheat contamination may represent an important problem also in the examined area.
DETECTION OF Fusarium spp. IN ASPARAGUS TISSUES AND SOIL BY POLYMERASE CHAIN REACTION.
L. Cozzolino, G. Popolo, A. Zoina. Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università degli Studi di Napoli “Federico II”, Via Università 100, 80055 Portici (NA), Italy. E-mail: luca.cozzolino@unina.it

Fusarium oxysporum f. sp. asparagi and Fusarium proliferatum are very dangerous pathogens of asparagus (Asparagus officinalis) associated with crown and root rot. Both fungal species can produce mycotoxins, thus their early detection is of great importance for prevention of pathogenic and toxicogenic risks arising from infections. One of the most affected places of Campania (southern Italy) is the asparagus cropping area around Avella, where in 2005 and 2006 repeated surveys were carried out in different farms where many samples consisting of roots, rhizomes and soil were collected. A molecular diagnostic method was developed using a PCR assay based on partial calmodulin gene sequence using primers CLOX1/CLOX2, specific for F. oxysporum f. sp. asparagi and primers CLPRO1/CLPRO2, specific for F. proliferatum. PCR assays were performed on DNA extracted from plant material and infested soil and confirmed the occurrence of F. oxysporum f. sp. asparagi only. The sensitivity threshold of the detection protocol from soil was about 10⁴ propagules/g of soil. Only F. oxysporum f. sp. asparagi was found as the agent of root rotting. Since this pathogen is specific to asparagus, and is unable to attack other crops, cultural practices such as the use of resistant cultivars and crop rotation can be very helpful in controlling the disease.

TURNIP MOSAIC VIRUS INFECTING Eruca sativa IN SICILY. S. Davino¹, M. Davino², L. Cavichi³, M.G. Bellardi².
¹Dipartimento di Scienze Entomologiche, Fitopatologiche, Microbiologiche, Agrarie e Zootecniche, Sezione di Patologia Vegetale e Microbiologia Agraria, Università degli Studi, Viale delle Scienze, 90128 Palermo, Italy. ²Dipartimento di Scienze e Tecnologie Fitosanitarie, Sezione di Patologia Vegetale e Microbiologia Agraria, Università degli Studi, Viale delle Scienze, 90128 Palermo, Italy. ³Plesso Didattico G. Scarabelli, Università degli Studi, Viale G. Ascarì 15, 40026 Imola (BO), Italy.

In the Spring 2010, plants of Eruca sativa (family Brassicaceae), also known as rocket, grown in a private garden of Sicily (southern Italy) were observed, showing a severe virus-like disease consisting of mosaic, interveinal yellowing and/or dark greening areas on crinkled leaves and stunting. Preliminary electron microscopy observations of leaf-dips revealed the presence of filamentous particles 700-730 nm long. Considering that in 1959, this species has been reported in Italy as a natural host of Turnip mosaic virus (TuMV), symptomatic leaf samples were tested serologically to verify the presence of this potyvirus. Both ISEM and PAS-ELISA analyses were positive for TuMV. Mechanical inoculations carried out using rocket leaf sap allowed transmission of the virus to Cucumis sativus murale (local symptoms) and C. quinoa. (necrotic spots and systemic veinal flecks). None of the other species belonging to family Brassicaceae were infected. To further characterize the virus at the molecular level, RT-PCR of CP gene of the isolate under study was amplified with the primers TuMV 3pr: 5’-CTAGGATAACATCTTTGATAAC-3’ and TuMV 5pr: 5’-TGGTGTTATCCAGCCGACAG-3’. The amplified product was cloned and sequenced and the sequence obtained was compared with that of other isolates retrieved from GenBank. Results indicated that the homology level of CP genes is probably related to the major differences in host specificity, rather than to geographic distribution. Notwithstanding the widespread presence of TuMV Italy, this virus does not appear to be associated with serious disease outbreaks.

CITRUS TRISTEZA VIRUS: TEN YEARS OF INVESTIGATION IN SICILY. S. Davino¹, M. Guardo², A. Caruso², M. Davino¹. ¹Dipartimento di Scienze Entomologiche, Fitopatologiche, Microbiologiche, Agrarie e Zootecniche, Sezione di Patologia Vegetale e Microbiologia Agraria, Università degli Studi, Viale delle Scienze, 90128 Palermo, Italy. ² CRA, Istituto Sperimentale per l’Agronomia, Corso Savoia 190, 93024 Acireale (CT), Italy.

Citrus tristeza virus (CTV) is the causal agent of a severe citrus disease. In the past, citrus budsticks imported illegally from abroad were infected by CTV. In 2000, in the course of a survey for selecting superior old citrus lines in the area of Cassibile (Syracuse) trees of Fortune, Nova, Satsuma mandarins and Marsh grapefruit grafted on sour orange rootstock showed stunting, decline, dieback small-size fruits and pin-holing on the bark below the bud union line. The samples collected and analyzed by DAS-ELISA and DTBIA for CTV infection were positive. Total RNA was extracted from 50 plants and tested by RT-PCR using primers specific for the genes encoding protein p20 and p23. In all cases, DNA fragments of the expected size were amplified. About two years later, thousands of CTV infections were ascertained in old cv. Tarocco sweet orange grafted on sour orange in the area of Catania. To study the genetic variation of CTV populations, 150 samples from each sampled area (Catania-Baé, Cassibile and Catania-Motta S. Anastasia) were examined by SSCP and nucleotide sequence analysis of protein p20. All isolates from the same area showed the same SSCP pattern, whereas for each area a different SSCP pattern was obtained. The isolates from Cassibile and Motta S. Anastasia had a nucleotide identity higher than 99% with a mild CTV isolate from Spain (T385) and about 92% with the severe isolate from Israel (CTV-VT), whereas the isolate from Catania-Baé was similar (> 99%) to severe isolates from California (SY68) and Japan (NUA9A). From 2000 to 2009 over 60,000 samples were collected in Sicily where propagating material was introduced or declining trees were present. The results showed that different varieties are affected by CTV.

SPREAD OF PEPINO MOSAIC VIRUS TO DIFFERENT TOMATO GENOTYPE CROPS BY BUMBLE BEES. S. Davino¹, G. Iacono², M. Davino². ¹Dipartimento di Scienze Entomologiche, Fitopatologiche, Microbiologiche, Agrarie e Zootecniche, Sezione di Patologia Vegetale e Microbiologia Agraria, Università degli Studi, Viale delle Scienze, 90128 Palermo, Italy. ²Dipartimento di Scienze e Tecnologie Fitosanitarie, Sezione di Patologia Vegetale, Università degli Studi, Via Santa Sofia 100, 95123 Catania, Italy. E-mail: davino@unipa.it

Pepino mosaic virus (PepMV, genus Potexvirus family Flexiviridae), first reported from pepino (Solanum muricatum Ait.) in Peru, was shortly after discovered in tomato (Solanum lycopersicum L.) in Israel. In 1999, PepMV was widespread in tomato throughout Europe including Italy (Sardinia and Sicily). PepMV is highly infectious, being readily transmitted to healthy plants during routine cultivation procedures and by plant-to-plant contact. PepMV is also transmitted via contaminated seeds, which could serve as the
primary inoculum for transmission mechanical means or by Bumble bees. How PepMV infection initiates in new areas is now understood, but tomato seed has been suspected as one of the key components of worldwide epidemics. The objective of this study was to investigate the role of PepMV transmission by Bumble bees. Observations were carried out in different farms in Ragusa province. The tomato genotypes were Genio and Shereen (severe symptoms on the fruits), Piccadilly (mild symptoms) and Belize (symptomless). In some farms 70 Bumble bees were used per 1,000 mq (thesis A), while in others 140 or more (thesis B). At the beginning of the observations 10% of plants were infected. By the end of March, 30% of plants were infected in the farms A, while in B the infection rate was between 60 and 90%. The cultivation procedures and transplant dates were practically identical in all farms. On the basis of our observations we can affirm that a high number of Bumble bees significantly contributed to the spread of PepMV.

INHIBITORY EFFECT OF FURFURALS CONTAINED IN THE STEAM-EXPLODED BIOMASS OF MISCANTHUS SINENSIS AGAINST SOIL-BORNE PLANT PATHOGENS. U. De Corato1, N. Sharma2, O. Maccioni2, F. Zimbardi3. Agenzia Nazionale per le Nuove Tecnologie, l’Energia e lo Sviluppo Economico Sostenibile (ENEA), Unità Tecnica Efficienza Energetica, Servizio Agricoltura, Centro di Consulenza Energetica Integrata di Bari, Via Roberto Da Bari 119, 70122 Bari, Italy. 2Laboratorio di Biotecnologie dell’ENEA, Centro Ricerche Trisaia, S.S. 106 Jonica Km. 419.300, 75026 Rotonella (MT), Italy. 3Laboratorio di Bioeconomia Sostenibile, Centro Ricerche Energetica di Bari, Via Roberto Da Bari 119, 70122 Bari, Italy. E-mail: ugo.decorato@enea.it

The Steam-Exploded Biomass (SEB) of Miscanthus sinensis, a herbaceous perennial species growing up to 3–4 m in height, with an annual yield of 20–25 tons/ha, is a good renewable energy source. SEB could also be useful in crop protection, as an alternative to the use of compost in the greenhouse against soil-borne plant pathogens. Detailed studies on disease suppressiveness of SEB yielded positive results against three pathosystems. In this plant pathogens. Detailed studies on disease suppressiveness of SEB could be related to its content of furfurals, organic acids and lignosulfonates produced during processing of fresh biomass in a pilot plant of Steam-Explosion.

THE INFLUENCE OF YEAST ORIGIN AND IDENTITY ON MODES OF BIODEGRADATION OF PATULIN BY BASIDIOMYCETOUS PINK YEASTS. F. De Curtis1, R. Quici2, G. Ianiri2, G. Palmgren3, V. De Cicco2, R. Castoria2, S.A.I. Wright2, Dipartimento di Scienze Animali, Vegetali e del-CIDES IN CHARACTERIZATION OF RESISTANCE TO QOI FUNGI-CIDES IN BOTRYOTINIA FUCKELIANA (BOTRYTIS CINEREA). R.M. De Miccolis Angelini, C. Rotolo, S. Pollastro, A. Santomauro, M. Masiello, F. Faretta. Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi “Aldo Moro”, Via Amendola 165/A, 70126 Bari, Italy. E-mail: faretta@ugr.uniba.it

Botryotinia fuckeliana (Botrytis cinerea), the grey mould fungus, is well known for its adaptability to adverse conditions that frequently lead to acquired resistance to fungicides. QoIs include fungicides effective against a broad spectrum of fungi. They act as mitochondrial respiration inhibitors at the cytochrome bc1-complex level and are considered at high risk of acquired resistance. The response of B. fuckeliana field isolates, collected prevalently from grapevine in Italy, France, Germany and Japan, to several QoI fungicides was evaluated by colony-growth and conidial germination assays. Media were amended with various concentrations of QoIs along with salicylhydroxamic acid, a specific inhibitor of alternative oxidase. Tested fungicides showed different effectiveness against B. fuckeliana. Positive cross resistance between QoI fungicides belonging to different chemical groups was observed. One-hundred-thirty-four isolates were characterized, 35 of which showed a high level of resistance (RF ≥ 200). All isolates carried the G143A mutation in the cytochrome b (cytb) gene, as shown by analysis of morphological traits, RFLP (restriction fragment length polymorphism) and sequencing of the ITS (internal transcribed spacer) regions. We have evidence suggesting that members of specific phylogenetic groups are capable of degrading patulin. No isolates from wild plants were able to biodegrade patulin.
In late spring-early summer 2010, many cases of severely damaged olive trees were reported to occur in Valdinievole, an extensive area in the south-western part of the province of Pistoia (Tuscany). Samples collected by local extension services and olive growers showed symptoms consistent with those induced by olive knot. In fact, trees were stunted and had desiccated leaves, twigs and terminal branches. Galls were present on the entire canopy including twigs, young branches, trunk and leaves. Most severe symptomatic organs and PCR was performed. As expected, the presence of *Pseudomonas savastanoi pv. savastanoi* DNA was extracted from symptomatic organs and PCR was performed. As expected, the presence of the bacterium was ascertained in all tested samples. To understand the cause of such outbreak, data recorded from different meteorological stations in Pistoia were collected and analysed. It was found that that numerous cold waves occurred during winter 2010 and unusual low temperatures were recorded. In addition, the following spring was characterized by frequent and intense rainfalls. Thus, we conclude that the wide distribution and severity of the disease observed in 2010 can be credited in part to lesions induced by agricultural practices and to exceptional environmental conditions which favoured the infection by *P. savastanoi*.

**LABORATORY ASSAYS ON NON-ENZYMATIC PROCESSES ASSOCIATED WITH IRON AND ESCA TRACHEOMYCOTIC FUNGI.** S. Di Marco and F. Osti. Istituto di Biometeo- 

rologia del CNR, Via P. Gobetti 101, 40129 Bologna, Italy. E-mail: s.dimarco@biomet.cn.it

The tracheomycotic fungi *Phaeomoniella chlamydospora* and *Pseudoacremonium aleophilum* are considered the main pathogens causing esca of grapevine, a widespread wood disease that affects vine yield and longevity. These pathogens can produce toxins and extracellular enzymes in a complex infection process not yet fully understood. The possibility that fungi employ also non-enzymatic means in such process was investigated. Biological tests were carried out in the laboratory to examine the role of iron in promoting non-enzymatic processes with the production of radicals. *P. chlamydospora* and *P. aleophilum* produced siderophores, and reduced ferric iron. Although both fungi lack specific enzymatic activity, they were able to degrade crystalline cellulose, but only in the presence of iron. It was therefore hypothesized that the activation of iron-dependent cellulose-degrading mechanisms that supplement the degrading activity of the enzymes, may have taken place with the production of hydroxyl radicals. This hypothesis was confirmed by a specific spectrophotometric test as capacity of the radicals to break down 2-deoxy-D-ribose. The present study has shown that *P. chlamydospora* and *P. aleophilum* can activate iron-dependent non-enzymatic mechanisms, amplifying their pathogenic capacity. These findings, if confirmed by further study, may provide a new way to explain the nature of grapevine/pathogen relationship in term of wood degradation and/or foliar symptom formation, leading to a better understanding of the esca complex infection process.

**SEVERE ATTACK OF OLIVE KNOT IN THE PROVINCE OF PISTOIA.** S. Di Napoli1, D. Rizzo2, A. Voiglar1. 1Istituto Tecnico Agrario Statale “D. Arizziotti”, Viale Ricciano 5, 51017 Pescia (PT), Italy. 2Agenzia Regionale per lo Sviluppo e l’Innovazione Tecnico Agrario Statale “D. Anzilotti”, Viale Ricciano 5, 51017 Pescia (PT), Italy. E-mail: domenico.rizzo@arsia.toscana.it

In late spring-early summer 2010, many cases of severely damaged olive trees were reported to occur in Valdinievole, an extended area in the south-western part of the province of Pistoia (Tuscany). Samples collected by local extension services and olive growers showed symptoms consistent with those induced by olive knot. In fact, trees were stunted and had desiccated leaves, twigs and terminal branches. Galls were present on the entire canopy including twigs, young branches, trunk and leaves. Most severe symptomatic organs were observed in cv. Frantoio, known to be particularly susceptible to olive knot. To verify the occurrence of *Pseudomonas savastanoi pv. savastanoi*, DNA was extracted from symptomatic organs and PCR was performed. As expected, the presence of the bacterium was ascertained in all tested samples. To understand the cause of such outbreak, data recorded from different meteorological stations in Pistoia were collected and analysed. It was found that that numerous cold waves occurred during winter 2010 and unusual low temperatures were recorded. In addition, the following spring was characterized by frequent and intense rainfalls. Thus, we conclude that the wide distribution and severity of the disease observed in 2010 can be credited in part to lesions induced by agricultural practices and to exceptional environmental conditions which favoured the infection by *P. savastanoi*.

**CANKER AND DIEBACK OF SYCAMORE MAPLE CAUSED BY EUTYPA FLAVOVIRENS IN ITALY.** R. Faedd1, A. Pane1, A. Sidoti2, G. Granata1. 1Dipartimento di Scienze e Tecnologie Fitosanitarie, Università degli Studi, Via S. Sofia 100, 95123 Catania, Italy. 2Regione Siciliana, Azienda Regionale Foresti Demaniali, UOB n 3, Via della Libertà 97, 90143 Palermo, Italy. E-mail: granata@unict.it

Sycamore maple (*Acer pseudoplatanus*, family Sapindaceae) is a deciduous tree native to Europe and southwestern Asia. In Italy it grows from the Alps to Sicily. Since spring 2007, cankers and dieback of entire branches of sycamore trees was observed in eastern Sicily. Cankers girdled entire branches and trunks. Yellowing and death of foliage above the cankers was also observed. Numerous dark pycnidia occurred on the surface of the necrotic bark of cankers. Colonies of *Cytosporina flavovirens* (Sacc.) Groves [anamorph of *Eutypa flavovirens* (Pers.) Tul. & C. Tul (syn. *Diatrype flavovirens*)] were consistently obtained from symptomatic tissues on oatmeal agar (OA), producing spindly and asceptate conidia (17-23 x 1.5-1.8 μm). The fungus was identified on the basis of morphological and cultural characteristics in comparison with reference isolates supplied by the Centraalbureau voor Schimmelcultures (Utrecht, The Netherlands). Ribosomal DNA (rDNA) sequences of the internal transcribed spacer region (ITS1-5.8S-ITS2) from sycamore isolates were amplified and sequenced. Consensus sequences showed 98% similarity with *E. flavovirens* isolates deposited in GenBank (accession Nos. AJ302457, AJ302430 and AJ302429). Pathogenicity tests were performed on ten 3-year-old seedlings of sycamore maple. A patch of bark was removed from the stems and replaced with a 5-mm-diameter OA plugs colonized by the fungus. Ten control plants were inoculated with sterile OA plugs. All trees were grown outdoors. After ten months, cankers and canopy wilting were observed on inoculated sycamores, whereas control plants remained symptomless. *E. flavovirens* was reisolated from symptomatic tissues. Several species of *Eutypa*, *Diatrype*, *Eutypella* and *Vala* are known to occur on *A. pseudoplatanus*. To our knowledge, this is the first report of *E. flavovirens* on sycamore maple in Italy.

**PROTEOMIC STUDY OF THE FOUR-WAY INTERACTION BETWEEN TRICHODERMA ATrovIRIDE, PSEUDOMONAS FLUORESCENS, RHIZOCTONIA SOLANI AND TOMATO.** M. Faraji1, M. Ruocco2, S.L. Woo3, M. Ahmazadeh3, K. Behbodi1, A.M. El-Tabey Eid3, M. Lorito1. 1Department of Plant Protection, Section of Agricultural and Natural Resources, University of Tehran, Karaj, Iran. 2Istituto per la Protezione delle Piante e delle Scienze e Tecnologie Fitosanitarie, Università degli Studi di Napoli “Federico II”, Via Università 100, 80055 Portici (NA), Italy. 3Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università degli Studi di Napoli “Federico II”, Via Università 100, 80055 Portici (NA), Italy. E-mail: matteo.lorito@unina.it

The use of biocontrol agents (BCAs) in synergistic combinations is a promising approach for the control of plant diseases and should be a priority in agricultural research. The efficacy, in terms of plant growth promotion and pathogen protection, provided by some elite bacterial (*Pseudomonas fluorescens*) and fungal (*Trichoderma* spp.) BCA strains has been widely demonstrated. The three-way interaction between plant, pathogen and antago-
nist, e.g. Trichoderma or P. fluorescens, has been previously investigated by using functional genomics and proteomic techniques. In this study we propose testing the contemporary presence of both BCAs in a very complex four-player system. The main strategy of this research was to use a proteomic approach in order to determine which molecular factors are activated in the interactions, identify those compounds that have an important affect on the activity of either or both BCAs during their concurrent interaction with tomato roots and/or pathogen, and the relative plant molecular responses to the different microorganisms. A method has been developed and optimized to obtain 2-D gel maps of separately collected proteomes from each partner, as well as all the possible combinations of the components. Several plant and microbial differentially accumulated proteins, selected from the four interacting proteomes, have been isolated and are being identified and characterized.

FIRST OBSERVATIONS ON THE FUNGAL ENDOPHYTIC COMMUNITY OF OLEA EUROPAEA IN SICILY. V. Ferraro, S. Lo Piccolo, G. Coniglario, V. Mondello, L. Torta, S. Burruano. Dipartimento di Scienze Entomologiche, Fitopatologiche, Microbiologiche, Agrarie e Zootecnici, Università degli Studi, Viale delle Scienze 2, 90128 Palermo, Italy. E-mail: santella@unipar.it

During an etiological and epidemiological study on a new decline (foliar chlorosis and withering twigs) of Olea europaea in Sicily, the composition of the endophytic community of symptomless and asymptomatic olive tree organs was investigated. Two Sicilian olive-groves, located in a hill and in a plain, respectively, comparable for plant age, cultivar and agricultural management, were taken into consideration from spring 2007 to autumn 2008. Samples from healthy and diseased leaves and twigs were collected to isolate fungal endophytes. The fungal endophytic community varied quantitatively and qualitatively in relation to (i) type and health condition of the sampled organ; (ii) site of collection and (iii) survey period. Fungi belonging to the genera Alternaria, Aureobasidium, Camarosporium, Cladosporium, Diplodia, Phoma, Pleospora Septoria, Stemphylium, and several Mycelia sterilis were detected in almost all samples. The overall colonization rates (CR) were always higher in leaves than twigs, in both localities. Moreover, CR showed variability also in relation to the health condition of the organs. In fact, both in the hill and the plain, the endophytic colonization was higher in symptomatic organs than in the healthy ones. Although many taxa were detected, the results of this investigation suggest that the relatively stable endophytic fungal community residing in O. europaea is characterized by a constant composition of common taxa. Studies to achieve fungal identification and to evaluate the role of fungal endophytes in O. europaea are in progress.

CHARACTERIZATION OF PSEUDOMONAS spp. STRAINS, CAUSAL AGENTS OF CANKERS AND WILT OF STONE FRUITS IN SARDINIA. M. Fiori, V. Ligios, A. Schiaffino. Dipartimento di Protezione delle Piante, Università degli Studi, Via E. De Nicola, 07100 Sassari, Italy. E-mail: fiorim@uniss.it

A collection of 94 bacterial strains was obtained from apricot, nectarine, peach and plum trees showing cankers on twigs, stems and branches, gum exudates, bud necrosis, wilting of shoots, and spots on the leaves. Thirteen strains, selected on the basis of morphology on NSA and hypersensitivity reaction on tobacco leaves, were able to infect cherry plum GF31, watermelon and vegetable marrow plantlets; two strains infected pepper and tomato and five lilac and lemon fruits. On the basis of the results obtained by Gram reaction, catalase, poly-hydroxybutyrate, fluorescence on KB and LOPAT tests, five strains were identified as Pseudomonas syringae (Ps), three as P. viridiflava (Pv), four as P. fluorescens. One isolate was not identified. Biolog and serological agglutination tests partially confirmed the above reported results, allowing the assignment of three strains of Pseudomonas syringae to the pathovar syringae (Pss). Ater fatty acid analysis on five isolates, one was ascribed to Ps (S.I., 0.967), one to Po (S.I., 0.979), one to P. putida (S.I., 0.847), while two were not identified. The analysis of the thirteen strains with BOX-PCR produced 5 reaction profiles. One of them was characteristic of the type strain Ps CFBP 700. SyR gene amplification of the three Pss strains produced the specific band of 752 bp like the type strain Ps CFBP 700, confirming their identification. In conclusion, while Ps is already present in Italy in all plant species analyzed, Pe was not yet reported in Europe on apricots and nectarine.

EFFECT OF WATER ACTIVITY AND FUNGICIDES ON COMPETING ABILITIES OF COMMON MAIZE FUNGI, S. Forementi1, N. Magan2, A. Pietri2, P. Battilani1, 1Institute of Entomology and Plant Pathology, Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, 29122 Piacenza, Italy. 2Cranfield Health, Vincent Building, Cranfield University, Bedford MK43 0AL, UK. 3Institute of Science of Food and Nutrition, Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, 29122 Piacenza, Italy. E-mail: paola.battilani@unicatt.it

Maize is colonized by a mixture of spoilage fungi in pre- and post-harvest. The occurrence of certain dominant fungal species depends on several abiotic and biotic factors that determine their prevalence in the maize grain ecosystem. In order to understand when Fusarium spp. are able to dominate the maize ecosystem, it is necessary to clarify the complex interactions which occur between abiotic and biotic factors and their impact on the growth and between fungal interactions. The aims of this study were: (i) to determine the competitiveness of F. verticillioides against a range of fungi commonly growing on maize (F. proliferatum, A. flavus, A. ochraceus, A. niger and P. verrucosum), on artificial media under different a_w levels (0.99, 0.98, 0.98); (ii) to establish how the presence of sub-optimal concentrations (50% effective dose) of commercial fungicides (tebuconazole, prothioconazole and prochloraz) affects interspecific interactions. The results showed how F. verticillioides was able to compete effectively in dual culture with other fungal species commonly isolated from maize, although the dominance against some species was modified by a_w and the presence of fungicides. The dominance of F. verticillioides without the presence of sub-optimal active ingredients could be mainly due to its ability to grow rapidly and invasively. A. flavus was the most competitive species at sub-optimal levels of active ingredients tested, which is not surprising because the tested products are considered as effective mainly towards Fusarium. However, no important variation in the inter and intra-specific interaction of common fungi in maize was noticed by the addition of fungicides.

ABILITY OF SOME SOIL FUNGI TO DECREASE THE ACTIVE PRINCIPLE CONCENTRATION OF BIOFUMIGATION BY BRASSICA CARINATA SEED MEAL. S. Galliati3, L. Ugolini1, P.L. Burzi1, S. Gianchetta1, R. Roberti2, C. Cerato1. 1CRA, Centro di Ricerca per le Coltture Industriali, Via di Corticella 133, 40128 Bologna, Italy. 2Dipartimento di Protezione e Valo-
Biofumigation is a low-impact method alternative to chemical disinfection of soil, based on the glucosinolate-myrosinase defensive system of Brassicaceae plants, ploughed into the soil as green manure or seed meal. The enzymatic degradation of glucosinolates by myrosinase occurring in the soil delivers volatile isothiocyanates, which are very effective against several soil pathogens. The combination of this technique with a biological control agent like the filamentous fungus Trichoderma, selected for tolerance to allyl-isothiocyanates in previous trials, gave interesting synergic effects in controlling sugar beet damping-off by Pythium ultimum. On the other hand, it was found that a non-pathogenic isolate of Aspergillus flavus was able to actively reduce the concentration of allyl-isothiocyanate in soil during biofumigation, thus hampering its effectiveness. A screening was carried out in vitro among different non-pathogenic fungal isolates in order to investigate their interaction with allyl-isothiocyanate. The results showed differential responses: in particular some isolates of Trichoderma were able to decrease allyl-isothiocyanate concentration similarly to A. flavus, while the same Trichoderma isolate used in the biofumigation experiment against P. ultimum, was not. These findings suggest that the soil fungal community could be one of the causes of the reduced isothiocyanate concentration often found in biofumigated soil with respect to expectancies. Therefore, the use of Trichoderma isolates, selected for their antagonistic behaviour against the pathogens, if employed in combination with biofumigation, should be carefully evaluated not only for their tolerance to isothiocyanates, but also for their ability to interact with these molecules.

**COMPARATIVE PROFILING OF SMALL RNA POPULATIONS IN VIRUS-INFECTED AND HEALTHY GRAPEVINE PLANTS.** A. Giampetruzzii, A. Gisell, P. La Notte, C. Pirolo, P. Saldarelli. Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi “Aldo Moro”, Via Amendola 165/A, 70126 Bari, Italy. 2Istituto di Virologia Vegetale del CNR, UO Bari, Via Amendola 165/A, 70126 Bari, Italy. 3Istituto di Tecnologie Biomediche del CNR, Via Amendola 122/O, 70126 Bari, Italy. E-mail: p.saldarelli@ba.inven.it

RNA silencing in plants is a process operating through a multitude of small RNAs (sRNAs) 18-24 nt in length, that maintain genome integrity, control plant development, and respond to abiotic and biotic stresses. The large majority of sRNAs derive from the host genome and mediate gene regulation through target-gene cleavage, translational inhibition or DNA methylation. However, in virus-infected plants some sRNAs originate from replicating viral genomes in response to infections. Grapevine fanleaf virus (GFLV), one of the most important grapevine viruses, causes fanleaf degeneration and is responsible for severe yield losses. The sRNAs fractions from leaves of a healthy (S5) and a fanleaf-infected vine (S3) with the same genotype, were sequenced by Illumina Genome Analyzer II. The 21-24 nucleotide size classes dominated both libraries, but while healthy S5 tissues showed a prevalent 24 nt over the 21 nt population, the opposite occurred in infected S3 tissues. BLASTN search, together with a de novo assembly approach, confirmed the presence of actively replicating GFLV in the S3 vine, and a small number of reads also from Grapevine rupewiei stem pitting-associated virus (GRSPaV), Hop stunt viroid (HSVd) and Grapevine yellow speckle viroid 1 (GYSVd1). The same analysis on the healthy vine (S5), confirmed its good sanitary status, as it only showed contig homologies to grapevine genes. Characterization of known grapevine microRNAs (miRNAs) done on both libraries, showed clearly different profiles. In particular, miRNA166, which is involved in morphogenesis in A. thaliana and aspen, is among the overexpressed miRNAs in the infected vine.

**PRESENCE AND ABUNDANCE OF ROOT ROT, BUTT ROT AND STEM ROT FUNGI IN PROTECTION FORESTS OF THE WESTERN ALPS.** L. Giordano, G. Nicolotti, P. Gontier. Dipartimento di Valorizzazione e Protezione delle Risorse Agroforestali, Università degli Studi di Torino, Via Leonardo da Vinci 44, 10093 Grugliasco (TO), Italy. E-mail: paolo.gontier@unito.it

Protection forests have an important role in reducing the effects of natural hazards, such as avalanches, rock falls and debris flow. An adequate understanding of the disturbances that these forests may undergo is a prerequisite for the successful maintenance of the protection function. Wood decay fungi may affect the stability of trees and thus they may behave as natural disturbances. In this work we investigated the presence of root rot, butt rot and stem rot fungi in some protection forests of Piedmont and Aosta Valley (western Italian Alps). Tree species composition was variable depending on sites, and included both conifers and broadleaves. Fungi were identified through traditional techniques or DNA sequencing. The most frequent fungi were Fomitopsis pinicola, Heterobasidion annosum sensu lato and Stereum sanguinolentum. In a Norway spruce-dominated subalpine stand, approximately 150 trees were sampled at the root collar by drilling them with a 4 mm diameter, 43 cm long bit. Fungal DNA was extracted from wood and analyzed by using universal and taxon-specific primers. A high percentage (56%) of the trees was infected by root and butt rot fungi. H. annosum sensu lato accounted for 70% of infected trees, while Armillaria mellea sensu lato for 8%. Remaining trees were infected either by both fungi or by unknown fungi. These results suggest that H. annosum sensu lato is by far the most frequent root and butt rot agent in the site.

**ULTRASTRUCTURE OF THE ALDER RUST MELAMPORSIDIUM HIRATSKANUM ON GREY ALDER (ALNUS INCANA).** B. Ginetti, G. Maresi, S. Moricca. Dipartimento di Biotecnologie Agrarie, Sezione di Protezione delle Piante, Piazzale delle Cascine 28, 50144 Firenze, Italy. 2FEM-IASMA-Centro Trasferimento Tecnologico, Via E. Mach, 38010 San Michele all’Adige (TN), Italy. E-mail: beatrice.ginetti@unifi.it

The alder rust pathogen Melamporsidiun hiratsukanum S. Ito ex Hirats. is currently spreading epidemiologically across several European countries. Outbreaks were reported in the past 15 years from the northernmost countries (Finland, Norway, Latvia) down to Hungary, the Balkan Peninsula and Turkey. It has been conjectured that this heteroecious rust, considered to be native to eastern Asia, may have been introduced into Baltic states with contaminated commercial material (seedlings) transported through the Baltic sea. The fungus is closely related to Melamporsidiun betulinum and M. alni, two rusts infecting also hosts in the family Betulaceae (Alnus spp. and Betula spp.). However, based on host specificity (M. hiratsukanum does not infect Betula sp.) and micro-morphometric data (spore dimensions, spore ornamentation and length of ostiolar cells) M. hiratsukanum was kept separate from the above congeneric rusts. In spite of the many reports on the distinguishing features of M. hiratsukanum, a clear identification of this rust at the ultrastructural level was never achieved. In the present work, the ultrastructure of M. hiratsukanum on grey alder
PRESENCE AND ABUNDANCE OF ROOT ROT, BUTT ROT AND STEM ROT FUNGI IN PROTECTION FORESTS OF THE WESTERN ALPS. L. Giordano, G. Nicolotti, P. Gonthier. Dipartimento di Valorizzazione e Protezione delle Risorse Agroforestali, Università degli Studi, Via Leonardo da Vinci 44, 10095 Grugliasco (TO), Italy. E-mail: paolo.gonthier@unito.it

Protection forests have an important role in reducing the effects of natural hazards, such as avalanches, rock falls and debris flow. An adequate understanding of the disturbances that these forests may undergo is a prerequisite for the successful maintenance of the protection function. Wood decay fungi may affect the stability of trees and thus they may behave as natural disturbances. In this work we investigated the presence of root rot, butt rot and stem rot fungi in some protection forests of Piedmont and Aosta Valley (western Italian Alps). Tree species composition was variable depending on sites, and included both conifers and broadleaves. Fungi were identified through traditional techniques or DNA sequencing. The most frequent fungi were *Fomitopsis pinicola*, *Heterobasidion annosum sensu lato* and *Stereum sanguinolentum*. In a Norway spruce-dominated subalpine stand, approximately 150 trees were sampled at the root collar by drilling them with a 4 mm diameter, 43 cm long bit. Fungal DNA was extracted from wood and analyzed by using universal and taxon-specific primers. The majority of the trees (56%) was infected by root and butt rot fungi. *H. annosum sensu lato* accounted for the 70% of the infected trees, while *Armillaria mellea sensu lato* for 8%. Remaining trees were infected either by both fungi or by unknown fungi. These results suggest that *H. annosum sensu lato* is by far the most frequent root and butt rot agent in the site.

ALTERNATIVE HOSTS OF CASSAVA MOSAIC BEGOMOVIRUSES IN TANZANIA. D. Guastella1, C. Busungu2, J. P. Legg2 and M. Tessitori1. 1Dipartimento di Scienze Fitosanitarie, Sezione Patologia Vegetale, Università di Catania, Via S. Sofia 100, 95123 Catania, Italy. 2IITA-Tanzania, P. O. Box 34441, Dar es Salaam, Tanzania. E-mail: mtessitori@unict.it

Cassava mosaic disease (CMD), the most important disease of this staple food crop in Africa, is caused by eight cassava mosaic begomoviruses (CMBs), seven of which occur in Africa. All viruses involved in the disease are efficiently transmitted in the field by *Bemisia tabaci*. Cassava was introduced to Africa in the 16th century, so it is recognized that this crop must have become infected by CMBs originating from wild African plant species. To date, however, little is known about the wild hosts of CMBs. This is an important shortcoming, since wild hosts may potentially play a significant role in the epidemiology of CMD. During a May 2010 survey that covered about 30,000 km² of north-western Tanzania, more than 30 samples were collected from different species of weeds or cultivated plants showing mosaic symptoms. PCR using universal primers (UniF and UniR) confirmed the association of wild cassava (*Manihot glaziovii*) with CMBs in Tanzania. Moreover, new hosts of these viruses were identified, i.e. *Combretum confertum*, *Arachis hypogaea*, *Hibiscus cannabinus*, *Leucaena leucocephala* and *Coryza sumatrensis*. Subsequent PCR by specific primers for both African cassava mosaic virus (ACMV) and East African cassava mosaic virus (EACMV) allowed the identification of specific virus infections. *L. leucocephala* and *C. sumatrensis* were infected by both ACMV and EACMV. Sequencing studies are currently underway to further characterize these novel CMB infections of non-cassava hosts.

BOTRYOSPHAERIA SPECIES ASSOCIATED WITH ESCA-DISEASED GRAPEVINE IN SOUTHERN ITALY AND LEBANON. W. Habib, S. Pollastro, F. Faretra. Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi “Aldo Moro”, Via Amendola 165/A, 70126 Bari, Italy. E-mail: faretra@agr.uniba.it.

Botryosphaeriaceae are fungi frequently isolated from vines showing decline or dieback symptoms in several countries. Wood symptoms such as cankers, sectorial V-shaped necroses in wood, bud mortality, shoot dieback and graft union failure have been associated with different species of Botryosphaeriaceae, and different disease names have been given to certain types of symptoms. In particular, in Mediterranean countries, black dead arm (BDA) was retained as an important vine-decline disease in addition to Eutypa dieback and esca. In the present work, morpho-taxonomic characterization and sequence analysis of rDNA internal transcribed spacer regions (ITS) were used to identify Botryosphaeriaceae isolates obtained from esca-affected vines in Apulia (southern Italy) and Lebanon. According to the morpho-taxonomic approach, three main groups of isolates were recognized: (i) characterized by pigmented, thick-walled conidia, including the genera *Diplodia*, *Lasiodiplodia*, and *Dothiorella*; (ii) characterized by hyaline, thin-walled conidia, comprising the genera *Fusicoccum* and *Neofusicoccum*; (iii) isolates which did not differentiate conidia in culture. Molecular identification of Botryosphaeriaceae species was based on the comparison of ITS sequences with those available in GenBank. In agreement with the results obtained in other countries, 55 tested isolates were identified as *Diplodia seriata* (32.7%), *Neofusicoccum parvum* (30.9%), *Fusicoccum aesculi* (12.7%), *Neofusicoccum australe* (9.1%), *Neofusicoccum luteum* (5.5%), and *Neofusicoccum vitisforme* (3.6%). The species *Dothiorella viticola*, *Diplodia mutila*, and *Lasiodiplodia theobromae* were represented by one isolate each. Further studies on the distribution and pathogenicity of Botryosphaeriaceae fungi are therefore essential to clarify their possible role in grapevine decline.

RAPID ANALYSIS IN BOTRYOSPHAERIAEAE FUNGI. W. Habib, S. Pollastro, F. Faretra. Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi “Aldo Moro”, Via Amendola 165/A, 70126 Bari, Italy. E-mail: faretra@agr.uniba.it.

For a long time, taxonomic studies on Botryosphaeriaceae fungi were based on morphological characters which exhibit high variability. Host specificity cannot be of help as a taxonomic criterion since several host plants can be colonized by a single fungal species and different species can occur on the same host plant. In the present study, 30 isolates of Botryosphaeriaceae fungi (*Diplodia mutila*, *Lasiodiplodia seriata*, *Dothiorella viticola*, *Fusicoccum aesculi*, *Lasiodiplodia theobromae*, *Neofusicoccum australe*, *Neofusicoccum luteum*, *Neofusicoccum parvum*, and *Neofusicoccum vitisforme*) were collected from standing vines showing symptoms of cankers, dieback and esca in commercial vineyards and from rootstocks in nurseries of southern Italy and Lebanon.
These isolates were identified by morphological and molecular methods. Fungal DNA was subjected to RAPD (Random Amplified Polyorphic DNA) analysis using 20 decamer primers. A total of 248 markers (from 209 to 1,800 bp in size) were obtained. The number of RAPD markers generated per primer varied between 15 and 28. Similarity matrix using Dice coefficient for pairwise comparisons gave a dendrogram that grouped the isolates in clusters corresponding to the anamorph. A broad inter-specific variability resulted, each species being well distinguished from the most closely related ones. RAPD analysis confirmed to be a reliable technique for investigating species differentiation and genetic diversity. The technique can be of help in the identification of some Botryosphaeriaceae species, being cheaper and faster than the commonly used methods based on sequencing of ITS region, often integrated with partial sequencing of EF-1α or β-tubulin genes.

DEVELOPMENT OF A MACROARRAY SYSTEM FOR THE DIAGNOSIS OF ROOT ROT AND TRACHEOMYCOSES OF ORNAMENTAL PLANTS. A. Haegi 1,2, M. Paoli 3, D. Rizzo 1, L. Riccioni 2, A. Grassotti 1. 1CRA, Unità di Ricerca per il Vivasiano e la Gestione del Verde Ambientale ed Ornementale (CRA-VIV), Via dei Fiori 8, 51012 Pescia (PT), Italy. 2CRA, Centro di Ricerca per la Patologia Vegetale (CRA-PAV), Via C.G. Bertengo 22, 00156 Roma, Italy. E-mail: anita.haegi@entecra.it

The industry of ornamental plants is characterized by continuous introduction of new crops and new production technologies that create new opportunities for pathogens to exploit. In this framework an early and precise identification of pathogens is critical for determining defence strategy. In this work we addressed root rot and trachecomyces diseases of perennial ornamental plants. Since phytosanitary surveys show an epidemiological complex situation, a macroarray device was chosen because it can detect multiple pathogens in a single assay, is sensitive, rapid and does not require a sophisticated equipment. Surveys were conducted in some Tuscan farms growing ornamentals to assess the main phytosanitary problems. Samples were collected and the fungi isolated from infected tissues were identified by morphological and molecular tools. On the same samples, a DNA extraction protocol from infected plant material have been set up, and the resulting DNA samples were amplified with ITS5/ITS4 primers for fungi and ITS6/ITS4 primers for oomycetes. The technique for macroarray procedure was set up using direct labelling (Gene Images AlkPhos, Amersham). Briefly, oligonucleotide detectors were immobilized on a nylon membrane and hybridized with the sample DNA, previously amplified and labelled. For each pathogen to be tested oligonucleotide detectors were looked for in the literature and validated or designed ex novo. Oligonucleotide detectors for Fusarium oxysporum, Phytophthora spp. and Pythium spp. were found in the literature and are now under validation. Four oligo detectors for Phytophthora cactorum have also been designed.

IN VITRO STUDY OF FUM GENES EXPRESSION AND FUMONISINS PRODUCTION IN Fusarium verticillioides UNDER DIFFERENT ECOLOGICAL CONDITIONS. I. Lazzaro 1, A. Susca 2, G. Mule 2, A. Ritiemi 3, P. Battilani 1. 1Istituto di Entomologia e Patologia Vegetale, Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, 29100 Piacenza, Italy. 2Istituto di Scienze delle Produzione Alimentari del CNR, Via Amendola 122/O, 70126 Bari, Italy. 3Istituto di Scienze degli Alimenti, Università degli Studi “Federico II”, 80055 Napoli, Italy. E-mail: paola.battilani@unicatt.it

F. verticillioides, a fungus associated with maize ears all over the world, induces Fusarium pink ear rot and produces fumonisins (FBs), mycotoxins found in maize kernels and their derivatives. FBs are toxic to humans, FB, in particular, which is classified as possibly carcinogenic. Fumonisins biosynthetic pathway is regulated by several genes belonging to the FUM cluster whose behaviour in different ecological conditions is poorly studied. The aim of this work was to investigate the behaviour of two F. verticillioides strains grown in vitro both on FB-inducing (Malt extract agar, MEA) and FB-inhibiting (Czapek yeast agar, CYA) media. Fungal growth, FBs production and gene expression were studied at different temperature (T; 20-30) and water activity (a_w) (0.90-0.99) regimes, in liquid cultures incubated for 7-21 days. The expression of two genes, FUM21 and FUM2, was considered; the relative quantification of gene expression was performed by Real time PCR. Results showed that, with a_w fixed at 0.99, maximum FUM21 and FUM2 expression was at 30°C after 14 days of incubation, while FBs production increased from 7 to 21 days. At fixed T (25°C), fungal growth at 0.90 a_w was not observed after 21 days of incubation. Gene expression increased with incubation time both at 0.95 and 0.99 a_w; FB production increased till 21 and 14 days respectively at 0.95 and 0.99 a_w. In all conditions studied the expression of the two genes considered followed a very similar trend, but FUM2 was much more expressed than FUM21 and more influenced by a_w conditions.

TRICHERODERMA VIRIDE AND PHOMOPSIS sp. ASSOCIATED WITH A DECLINE OF PINUS NIGRA PLANTLETS IN A REFORESTATION AREA OF CENTRAL ITALY. M.G. Li Destri Nicosia, S. Mosca, G.E. Agosteo, R. Mercurio, L. Schena. Dipartimento di Gestione dei Sistemi Agrari e Forestali, Università degli Studi Mediterranea, Località Feo di Vito, 89122 Reggio Calabria, Italy. E-mail: lschena@unirc.it

In winter 2009, a decline was observed on 4-year-old plantlets of Pinus nigra in a reforestation area of Abruzzo National Park (central Italy). More than 70% of the plantlets died during the first year after transplanting while survivors were stunted and showed leaf chlorosis as well as root and crown rot. Four fungal morphotypes, grouped according to the morphology of colonies and reproductive structures, were isolated from the necrotic subcortical tissues of the basal stem of symptomatic plantlets. ITS regions of representative isolates of each morphotype were examined by BLAST analysis and compared with available sequences in GenBank. Three isolates were identified as Phomopsis sp., Trichoderma viride and T. harzianum, respectively, based on a 99-100% identity with deposited sequences. Conversely, a morphotype that did not produce conidia was not identified since, surprisingly, its ITS sequence matched sequences of multiple taxonomic groups. Pathogenicity tests were conducted on both cuttings and potted 4-year-old plantlets of P nigra by inserting a plug of PDA with actively growing mycelium under the bark. T. viride and Phomopsis sp. caused necrosis of subcortical tissues around the inoculation site. Lesions caused by T. viride were significantly more extended than those caused by Phomopsis sp. Both fungi were reisolated from symptomatic tissues. No symptoms were observed on cuttings and plantlets inoculated with sterile agar, T. harzianum or the unidentified fungus. Both Phomopsis sp. and T. viride were reported previously as tree pathogens. However their actual role in the decline of P nigra should be further investigated.
EMERGING BOTRYOSPHAERIACEAE IN FOREST ECOSYSTEMS IN SARDINIA. B.T. Linaldeddu, B. Scanu, A. Schiaffino, A. Franceschini.

Fungal species belonging to the family Botryosphaeriaceae are well known as endophytes and pathogens of woody plants worldwide. Beginning in 2008, a field survey was conducted to study Botryosphaeriaceae that occur on declining trees and shrubs of Mediterranean maquis, in various Sardinian ecosystems. Fungal isolates from symptomatic plants were identified on the basis of morphological features, analysis of nucleotide sequences of the internal transcribed spacer region (ITS1-5.8S-ITS2) of rDNA and partial sequence of the translation elongation factor 1-α gene.

Seven species were constantly isolated from diseased plants: Diplodia corticola (from holm oak and kermes oak), Diplodia seriata (field elm), Diplodia sp. (narrow-leaved ash), Diplodia scrobiculata (strawberry tree), Neofusicoccum vitisvini forme (narrow-leaved ash) and two new species belonging to the genus Doliiturella (common hazel). Pathogenicity of all fungal species was verified by stem inoculation of seedlings of the same host from which they were isolated, under controlled laboratory conditions.

All fungal species, except for a Doliiturella taxon, proved to be pathogenic to their host. The results obtained emphasize that many Botryosphaeriaceae may represent a serious threat for Mediterranean trees and shrubs. Our findings have contributed to improve the knowledge of N. vitisvini forme and D. scrobiculata by expanding their host range that includes now narrow-leaved ash and strawberry tree, respectively.

CERATO-PLATANIN AND CERATO-POPLININ INDUCE DIFFERENTIAL RESISTANCE RESPONSES IN PLANE LEAVES. L. Lombardi1, I. Baccelli3, R. Bernardi2, G. Cappugi4, L. Pazzaglì1, P. Piccarielli2, A. Scala1.

Cerato-platanin (CP) and cerato-populin (Pop1) are small proteins produced by the phytopathogenic fungi Ceratocystis platani and C. populicola, respectively. CP and Pop1 behave as PAMPs, since they elicit typical defense responses in various host and non-host plants. CP and Pop1 are well-structured α/β proteins with an identity of about 65% and the conservative substitution of approximately 12% of the amino acids. Moreover, the analysis by circular dichroism shows differences in the secondary structure between the two proteins.

The present work aimed at ascertaining whether these structural differences are reflected in differences in their eliciting activity in plane leaves, which represents a study model on which we have been working for a long time and know deeply. We examined the ability of CP and Pop1 to induce in plane leaves: (i) production of hydrogen peroxide and nitric oxide by using the specific probe 2′-7′-dichlorodihydrofluorescein diacetate and the fluorescent dye 4,5 diaminofluorescein diacetate, respectively; (ii) programmed cell death by using the specific probe 2′-7′-dichlorodihydrofluorescein diacetate and the fluorescent dye 4,5 diaminofluorescein diacetate, respectively; (iii) PR5 (thraumatin), LTP (lipid transfer protein) and APX (ascorbate peroxidase). The inhibition of fungal growth on plane leaves treated with CP and Pop1 was also assessed. Results showed marked differences in the eliciting capacity of the two proteins. In particular the plane resistance responses are activated earlier by CP than by Pop1. These results are the basis for identifying the protein region(s) involved in the PAMP activity.

MOLECULAR DETECTION OF BISCOGNIAUXIA NUMMULARIA IN SYMPTOMLESS BEECH TREES IN THE APENNINE MOUNTAINS. N. Luchi1, B. Ceccarelli2, A.M. Vettriano3, A. Vannini2, P. Capretti1.

Spreading of weak fungal parasites on forest trees growing at the border of their natural range area is one of the expected consequences of global climate change. Studies on Fagus sylvatica show that the spread of populations towards the southern limit of the species distribution are limited strongly by drought. Biscogniauxia nummularia is an endophyte fungus of European beech, generally living in symptomless trees, but also able to cause strip-cankers and wood decay on individuals stressed by drought. In the present work the latent phase of the fungus in symptomless host tissues was studied taking samples from apparently healthy beech trees growing in two different Fagus type associations: (i) Luzulo-Fagion Wood, sub continental climate (Gavina, Pistoia); (ii) AceroDictyon-Fagion wood, Mediterranean climate with dry summers (Monti Cimini, Viterbo). Samples consisting of 1-2 year-old twigs were collected in different seasons and used for isolation of the fungus and DNA extractions following the referenced method described by the authors (Lett. Appl. Microbiol. 43: 33-38, 2006).

Real time PCR afforded higher fungal detection rates than isolation methods. The occurrence of B. nummularia as endophyte showed seasonal fluctuation in area (i) (Gavina) and was scarce in autumn and winter (3-20%) with respect to summer (40-100%), whereas it was constant in area (ii) (Monti Cimini), where detection of fungal DNA ranged from 80-100% of the samples throughout the year. Higher level of B. nummularia detection in area (ii), much more prone to drought stress, confirms the role of this fungus as bioindicator of water deficit.

HETEROBASIDION ABIETINUM POPULATION GENETIC STRUCTURE IN RESPECT TO THE EUROPEAN ABIES POPULATION. N. Luchi1, D. Paffetti3, K. Korhonen2, J. Hantula2, M.E. Sanchez2, T.D. Lehtijärvi2, A. Lehtijärvi2, P. Capretti1.

Heterobasidion abietinum is one of fungal pathogens colonizing almost all Abies species occurring in Europe. However consequences of its attacks may be different according to environmental characters. During the last glacial age in Europe both fungal and host populations, including Abies species survived in different refuges in mountain areas or in the southern Mediterranean...
peninsulas. Later, northbound migrations enabled those populations to recolonize their present natural habitats. To investigate the genetic divergence of *H. abietinum* populations, a collection of isolates from central to southern Europe (Spain, Italy, Greece and Turkey), was analysed using minisatellites (DAMD-M13) and microsatellites (RAMS). Genetic variation within and among groups of populations was compared and a dendrogram was constructed with the Neighbor-Joining method. Clusters generally showed that isolates grouped according to their geographical origin. However those from southern Spain (from *Abies pinsapo*) were clearly differentiated from the others. More than 20 main haplotypes of *H. abietinum* were observed. Their number was higher in the central part of the area considered in this study and lower in the peripheral regions. In the western part of the distribution area (Andalucía, Spain) their number was very scarce. This study confirms the hypothesized relationship between variability of *H. abietinum* population and migration history of *Abies* species in Europe. The occurrence of several haplotypes in the eastern part of the distribution area could be a consequence of historical trade routes; in this case man activities may have helped fungal spreading.

**INFLUENCES OF MULCHES ON SOLARIZED SOIL-BORNE MICROBIAL COMMUNITY.** A. Luvisi¹, A. Panattoni¹, A. Colosimo¹, F. Filippi², G. Magnani², E. Triolo³,¹ Dipartimento di Coltivazione e Difesa delle Specie Legnose "G. Scaramuzza", Università degli Studi, Via del Borghetto 80, 56124 Pisa, Italy. ²Dipartimento di Biologia delle Piane Agrarie, Università degli Studi, Viale delle Piagge 23, 56124 Pisa, Italy. E-mail: aluvisi@agr.unipi.it

To investigate the effectiveness of plastic mulches for soil solarization, transparent polyethylene (PE), EVALux (EL) and HT Supersol (HT) (Agriplast, RG, Italy) were used. The experiment was carried out in sandy soil in 2008 and 2009, from 20 July to 20 August, near Pisa (Italy). Artificial soil infestation with *Sclerotinia minor* Jagger was carried out and untreated plots were used as control. Temperatures in the soil profile were monitored at 3-5 cm depth. Seedlings of *Lactuca sativa* cv. Justine were planted after solarization. Sclerotinia-induced lettuce drop was evaluated by counting the number of healthy and diseased plants in the plots. Soil samples were collected after solarization at a depth of 3-5 cm to evaluate total fungi (F), *Trichoderma* spp. (T) and actinomycetes (A) as CFU/g of soil, using PDA, P190 and water-agar medium, respectively. Microbial community analysis using Biolog EcoPlates was performed to estimate total activity, Shannon-Weaver index and Evenness. Different temperature profiles were registered and PE, EL and HT increased maximum soil temperature by 4.3, 6.7 and 7.2°C respectively. Lettuce drop was reduced by more than 90% compared to control, with no significant differences between treatments. In HT-treated soil, levels of F, T and A were more than 31.2, 2.5 and 39.0% higher than in PE- or EL-treated soils. Biolog parameters confirmed the milder effects of HT film on non pathogenic microbial population. Improved plastic mulches can control soil-borne pathogens efficiently, limiting shifts in the soil microflora.

**SANITARY SELECTION OF GRAPES VINE SUPPORTED BY RFID SYSTEM.** A. Luvisi¹, A. Panattoni¹, A. Colosimo¹, M. Pagano², R. Bandinelli³, E. Rinaldelli², E. Triolo¹,¹ Dipartimento di Coltivazione e Difesa delle Specie Legnose "G. Scaramuzza", Università degli Studi, Via del Borghetto 80, 56124 Pisa, Italy. ²Dipartimento di Scienze delle Produzioni Vegetali, del Suolo dell’Ambiente Agroforestale, Università degli Studi di Firenze, Viale delle Iride 30, 50019 Sesto Fiorentino (FI), Italy. ³Associazione Toscana Costitutori Viticoli TOS.CO.VIT., Via Vecchia di Marina 6, 56010 San Piero a Grado (PI), Italy. E-mail: aluvisi@agr.unipi.it

Radio-frequency identification (RFID) technology was suggested for tracking of numerous and diverse materials in plant pathology trials. Keeping track of the sample identification number and of the precise location where samples were collected represents an essential step. Phenotypic, sanitary and genomic data can also be added to RFID tags associated to the assayed plants. Grapevine selection is based on sub-sequential steps in which many grapevines are monitored in vineyard(s) for years, as well as their relative propagated grapevines used for indexing, compares studies or conservation in screenhouses. These steps can be supported by RFID technology. Traditional labels may undergo discoloration, degradation, loss or removal: these issues represent critical points in plant selection, considering the long periods in which plants have to be monitored. The association of RFID tag to plant reduce the occurrence of errors or losses, in particular if microchips are inserted in the grapevine, making impossible the removal or errors in data-to-plant association. The tagging trial, started in 2009, regarded accesses of *Vitis vinifera* cv San giovese selected in Montalcino (Siena, Italy) for clonal selection purpose, propagated materials stored in screenhouse, grafted cuttings used for indexing with biological indicators. RFID wristband was used for tagging non-grafted plants, whereas microchips were implanted in the grafted ones. A database was created to monitor the tagged plants during the whole sanitary selection procedure, as well as for storing and updating data pertaining to each plant and relative propagated material.

**DETECTION OF PHYTOPHTHORA CAMBIVORA IN SOIL PARTICLES BY REAL TIME QUANTITATIVE PCR ASSAY.** V. Mancini, N. Luchi, P. Capretti. Dipartimento di Biotecnologie Agrarie, Sezione di Protezione delle Piante, Università degli Studi, Piazzale delle Cascine 28, 50144 Firenze, Italy. E-mail: vale.mancini@yahoo.it

*Phytophthora cambivora* is one of the most harmful pathogens causing root rot, collar rot and or stem canker of *Castanea sativa* and a well known agent of “ink disease”. According to the data collected since 2002 by the regional monitoring service (META http://meta.arisia.toscana.it/) disease centres are increasing in Tuscany (central Italy), where a number of new foci are detected annually in coppice forests and orchards. As disease centres are mainly found near streams and along roads and trails it could be useful to setup a protocol to detect the source of infection. Aim of this work was the optimization of a real time PCR assay, by using SYBR Green chemistry, to detect and quantify the occurrence of *P. cambivora* from potential sources of infection as plant tissues and soil particles. To this purpose DNA was extracted from chestnut tissue according to the method described by the authors (Lett. Appl. Microbiol. 41: 61-68, 2005) Later on, soil samples were artificially infected with *P. cambivora* mycelium. Real time PCR was developed using genus- and specie-specific primers. Notwithstanding some failures, probably due to PCR inhibitors, real-time PCR proved to be an efficient method for detecting and quantifying *P. cambivora* from both host tissues and soil. The presence of *P. cambivora* DNA in soil samples, confirmed the hypothesis that the soil can be a source of infection.
SHOOT AND TIP BLIGHT BY DIPLODIA PINEA AND D. SCROBICULATA DETECTED BY MOLECULAR METHODS IN PSEUDOTSUGA MENZIESII IN CENTRAL ITALY. Y. Mancini, N. Luchi, P. Capretti. Dipartimento di Biotecnologie Agrarie, Sezione di Protezione delle Piante, Università degli Studi, Piazzale delle Cascine 28, 50144 Firenze, Italy. E-mail: vale.mancini@yahoo.it

The occurrence of Diplodia pinea as the causal agent of tip and shoot blight has often been reported from Pinus spp. and other conifers. In Italy, D. pinea is quite frequent on pines but also D. scrobiculata has occasionally been found on different hosts species in Sardinia and other localities of southern Italy. Recently in Tuscany (central Italy) during the annual regional monitoring survey (META http://meta.aris.arsnova.it/) symptoms of crown disease were observed in Douglas fir (Pseudotsuga menziesii) stands. The disease was especially frequent in the lower part of the crowns of 20- to 40-year-old trees. Terminal shoots and small branches were completely defoliated, the bark was necrotic and showed sporadic black picnidia. In the last few years a fungus was isolated from branches and shoots. Single spore colonies were obtained, grown in pure culture and DNA was extracted. Diplodia was identified on the basis of both morphological (fungal structures and colonies) and molecular methods. DNA amplification assays using specific primers available in the literature confirmed the identification of the fungal species as D. pinea in most of the samples but revealed also the occurrence of D. scrobiculata in some of the Douglas fir trees.

DIPLODIA PINEA DETECTION ON LEPTOGLOSSUS OCCIDENTALIS (INSECT VECTOR) BY REAL TIME-PCR. V. Mancini, N. Luchi, M. Feducci, P. Capretti. Dipartimento di Biotecnologie Agrarie, Sezione di Protezione delle Piante, Università degli Studi, Piazzale delle Cascine 28, 50144 Firenze, Italy. E-mail: vale.mancini@yahoo.it

Leptoglossus occidentalis (Hemiptera: Coreidae), an insect native to North America, is present in Italy since 1999. It now occurs mainly in Pinus pinea stands where it is thought to cause extensive damage to seed production. Considering that this insect has the same habitat (cones of conifers) of Diplodia pinea, a fungus responsible for damaging pine cones, it is possible that it may be involved in spreading fungal conidia. Real time PCR was used for detecting and quantifying fungal DNA on both cones and insects, This is taken as an indication that L. occidentalis may have a role as possible vector of D. pinea.

INTERACTION OF CERATO-PLATANIN AND CERATO-POPULIN WITH INANIMATE AND PLANE LEAF SURFACES: A STRUCTURAL STUDY. F. Martellini1, L. Pazzaglì1, L. Carresì2, F. Scbrana1, B. Tiribili1, B. Pantera1, G. Cappugi1, F. Faoro1, A. Scala2. 1Dipartimento di Scienze Biochimiche, Università degli Studi, Viale Morgagni 30, 50134 Firenze, Italy. 2Dipartimento di Biotecnologie Agrarie, Sezione di Protezione delle Piante, Laboratorio di Patologia Vegetale Molecolare, Università degli Studi, Viale della Lastruccia 10, 50019 Sesto Fiorentino (FI), Italy. 

INTRODUCTION OF CERATO-PLATANIN (CP) and cerato-populin (Pop1) are proteins abundantly secreted by and localized in the cell wall of Ceratocystis platani and C. populicola, respectively. Both are assumed to play a role in plant interaction, since they induce accumulation of H₂O₂ and NO₃, programmed plant cell death, overexpression of defence genes, phytoalexin synthesis and restriction of conidia growth. Thus, CP and Pop1 appear to act as PAMPs able to activate effective primary defence systems. CP and Pop1 are members of the “cerato-platani family” containing proteins involved in many microbe-host interactions acting as phytoxins, elicitors of defence responses or human allergens. Cellular localization in fungi suggests a role of these proteins in interaction with host surfaces. To investigate the mechanism of interaction, in vitro and in vivo experiments have been performed. CP and Pop1 strongly interacted with Tellon, a colloidal suspension used to mimic hydrophobic surfaces. During reaction, these proteins lost their native structure and adopted an unfolding conformation with a small percentage of α-helix. Moreover, CP and Pop1 were adsorbed on hydrophobic surfaces (silanized mica, gold sheets and graphite) and appeared as supra-molecular aggregates, which resemble the ordered assemblages that the proteins form in vitro, and are able to enhance host-defences. In vivo, the proteins seemed to interact with the hydrophobic cuticle of the plane leaves and did not penetrate the cell wall and the membrane. The results suggest that CP and Pop1 interact with hydrophobic components of the host before inducing defence events.

ANTIFungal ACTIVITY OF TERPENES IDENTIFIED IN THE LEAVES OF ROSMARinus OFFICINALIS. Y. Marrini1, C. Comaggi1, P. Capretti2, M. Michelozzi1, A. Scala2. 1Dipartimento di Biotecnologie Agrarie, Sezione di Protezione delle Piante, Università degli Studi, Piazzale delle Cascine 28, 50019 Sesto Fiorentino (FI), Italy. 2Istituto di Genetica Vegetale del CNR, Via Madonna del Piano 10, 50019 Sesto Fiorentino (FI), Italy. E-mail: martini@imgf.fci.cnri.it

Plants of Rosmarinus officinalis are widely distributed in Europe, Asia and Africa. Mediterranean is the area where spontaneous plants are commonly found. This plant is known for its use in cookery, and the increasing interest for its pharmaceutical properties. Two groups of compounds are mainly responsible of the biological activities of rosemary: the volatile fraction and the phenolic constituents. Alternaria leaf spot of rosemary has been reported in various Italian regions, as the cause of black spots on leaves and stems followed by defoliation. The aim of the present work was to investigate the antifungal activity of different rosemary monoterpens against a strain of Alternaria alternata (Fr.) Keissl. recently isolated from an Alternaria-diseased rosemary plant. Their antifungal activity was evaluated as inhibition of the mycelial growth using “special vials” containing potato dextrose broth and increasing concentrations (0.025, 0.1, 0.4, 1.6, 6.4, 10.0 and 100 mM) of 1,8-cineole, (+)-alpha-pinene, (-)-alpha-pinene, (+)-limonene, (-)-limonene, (-)-beta-pinene, (+)-beta-pinene, myrcene and linalool. The minimum inhibitory concentration...
THE ROLE OF VOLATILE COMPOUNDS IN THE CHEMICAL DEFENSE SYSTEM OF PICEA STICHTHENSIS AGAINST HETEROBASIDION ANNOSUM ATTACK. V. Martin1, S. Woodward2, G. Dellorio1, P. Capretti3, M. Michelozzi3, E. Materazzi1, D. Rizzo1, H. Bouyahia1, P. Braccini1, M. della Bartola1, 1 Dipartimento di Coltivazione e Difesa delle Piante, Università degli Studi “Aldo Moro”, Via Amendola 165/A, 70126 Bari, Italy. 2PTU-K Palestinian Technical University Kadoori, P.O. Box 7, Tulkarm, Palestine. E-mail: m.miazzi@agr.uniba.it

Picea sitchensis (Bong.) is very susceptible to Heterobasidion annosum (Fr.) Bref., the agent of root and butt-rot of conifers that usually enters the host through wounds or stumps, causing wood decay, with significant economic losses when monoculture plantations are attacked. In some cases, host tree populations may remain free of infection but for the basis for this apparent resistance to the pathogen is unknown. Plants produce a vast array of secondary metabolites such as terpenoids and phenolics, to defend themselves against their natural enemies. The aim of this study was to examine the variation in the chemical responses to H. annosum attack in P. sitchensis. Terpene composition was analyzed in cortical tissue samples of four Sitka spruce clones growing at the Scootmore site (Ref: NJ172392; Moray, Scotland, UK). The Sitka spruce clones included the two from among 30 tested in a previous screening trial forming the shortest stem bark lesions (Clones 20198 and 20206) and the two forming the longest stem bark lesions (Clones 20179 and 20204) following inoculation with H. annosum. The results showed significant variation in the constitutive terpene profiles between the different clones. Proportions of some volatile terpenes varied in the secondary resin produced in bark tissues surrounding the lesions. Total absolute quantities of monoterpenes were significantly higher in the secondary resin than in the primary resin, in both resistant and susceptible clones. This effect was more evident in tissues following inoculation, compared with the pseudo-inoculated samples. These findings are discussed in the context of ecological interactions and show that terpenoid metabolism may provide useful biochemical markers for resistance to H. annosum in selection and breeding programmes.

FURTHER DATA ON THE DISTRIBUTION OF MAIN GRAPEVINE VIRUSES IN TUSCANY. A. Materazzi1, D. Rizzo2, H. Bouyahia1, P. Braccini1, M. della Bartola1. 1 Dipartimento di Coltivazione e Difesa delle Specie Legnose “G. Scaramuzzi”, Sezione di Fitopatologia, Via dei Fiori 8, 51012 Pisa (PT), Italy. E-mail: amatazzi@agr.unipi.it

From 2005 to 2009, 451 accessions of V. vinifera belonging to major and minor varieties, were collected from the most important and the emerging grape-growing areas of Tuscany and subjected to virological tests. The sanitary status was evaluated by symptoms observation in the field and subsequently by laboratory analysis (ELISA and/or RT-PCR). The presence of the following viruses was investigated: Arabis mosaic virus (ArMV), Grapevine fanleaf virus (GFLV), Grapevine leafroll-associated virus 1 (GLRaV-1), 2 (GLRaV-2), and 3 (GLRaV-3), Grapevine virus A (GVA) and B (GVB), and Grapevine fleck virus (GFkV). Results showed that 308 (68.3%) vines were infected with at least one virus. In detail, 166 plants had single infections and 142 had mixed infections. The most widely spread virus was GLRaV-3, detected in 164 of 308 vines (53.2%). GFkV infections concerned 128 (41.6%) vines, while GLRaV-1 and GVA were found in 88 (28.6%) vines. GFLV, singly or in association with other viruses, was detected in 46 accessions (14.9%). Analysis confirmed the limited distribution in Tuscan vineyards of GLRaV-2 and GVB, that were found in 6 and 4 vines, respectively. Except for the viticultural areas of Garfagnana and Media Valle del Serchio, where an unusually high incidence of ArMV was recently reported, the occurrence of this virus was confirmed to be very low. In fact, ArMV was detected in only 2 accessions, always in association with GFkV.

ARE POINT MUTATIONS IN THE CYP51 GENE ASSOCIATED WITH RESISTANCE TO DMI FUNGICIDES IN ERYSPHE NECATOR? M. Miazzi1, H. Hajjeh2, F. Faretra1, Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi “Aldo Moro”, Via Amendola 165/A, 70126 Bari, Italy. 2PTU-K Palestinian Technical University Kadoori, P.O. Box 7, Tulkarm, Palestine. E-mail: m.miazzi@agr.uniba.it

Sterol 14α-demethylation inhibitors (DMIs) fungicides are still important in grapevine protection against powdery mildew, although acquired resistance has been reported since 1989. Previous studies showed that the point mutation A495T in the CYP51 gene, coding for a cytochrome P450, is responsible for a high level of resistance in E. necator. An allele-specific PCR assay with primers MUT1 (5’-AATTTGGACAATCAA-3’) and U14DM (5’ATGTACATTGCTGACATTTTGTCGG-3’) was performed on 50 fungal isolates sampled in 7 vineyards. Only four isolates (X109, X112, X113 and X115) carried the point mutation yielding a DNA band of the expected size. The response of E. necator isolates to biocides was evaluated through an in vitro bioassay on grapevine leaf disks. Wild-type sensitive isolates showed EC50 ≤0.1-3 µg ml-1 and MIC = 1-6 µg ml-1, while mutants showed EC50 = 6-10 µg ml-1 and MIC = 10 µg ml-1. Isolate X109, although carrying the point mutation, was normally sensitive (EC50 = 0.3 µg ml-1 and MIC = 3 µg ml-1). In these four isolates, the whole CYP51 gene was amplified with the primer pairs C14 (5’-TAAGG-TAGTATTGAGGCGGG-3’) and C14R (5’-TTCTAACCC-TAACACCTGCC-3’) and sequenced. The alignment with the nucleotide sequence available in GenBank (accession N. U83840) confirmed the presence of the point mutation A495T, but additional point mutations were detected. Results suggest that the A495T mutation is not strictly associated with resistance to DMIs in E. necator. Different mutations in the same gene or in other genes may be significant, but remain to be clarified.

RESISTANCE OF PODOSPHERA XANTHI TO QoI FUNGICIDES IN APULIA. M. Miazzi, C. La Guardia, F. Faretra. Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi “Aldo Moro”, Via Amendola 165/A, 70126 Bari, Italy. E-mail: faretra@agr.uniba.it
Powdery mildew, caused mostly by *Podosphaera xanthii*, is an important disease of cucurbits in the Mediterranean basin. The fungicides known as QoI are largely used to control the disease, but resistance has been observed in many phytopathogenic fungi, including *P. xanthii*. Here we present the results of a monitoring of resistance to trifloxystrobin in *P. xanthii* populations in Apulia. Sixty-four isolates of *P. xanthii* were sampled from 32 cucurbit fields in 2002-2007, and assayed at seven trifloxystrobin (Flint®) concentrations. Alternative respiration was inhibited by adding 1 mg ml\(^{-1}\) of salicylhydroxamic acid to fungicide suspensions. Portions of zucchini cotyledons (cv. Diamant 1) were dipped for 1 min in the fungicide suspension, placed on Blaich medium in Petri dishes, inoculated at a single point with about 20 conidia, and maintained at 25°C under a 12 h photoperiod. After 10 days, the percentage of infected surface was estimated using an empirical scale based on six infection classes, and EC\(_{50}\) and MIC were assessed. Results pointed out a high variability in the response to trifloxystrobin, with EC\(_{50}\) ranging from <0.1 to >375 µg ml\(^{-1}\), and MIC from 10 to >375 µg ml\(^{-1}\). About half of isolates had EC\(_{50}\) >375 µg ml\(^{-1}\), a concentration three times greater than the normal field rate (125 µg ml\(^{-1}\)), with no differences attributable to the host plants or the geographical origin. In conclusion, resistance to QoIs is very common in Apulia, and this should induce growers to implement more stringent anti-resistance strategies.

**FIRST REPORT OF LEAF SPOT CAUSED BY STEMPHYLIUM HERBARUM ON BORAGO OFICINALLIS.** L.C. Moretti, M. Quaglia, M. Orfei, C. Cappelli. Dipartimento di Scienze Agrarie e Ambientali, Università degli Studi, Borgo XX Giugno 74, 06121 Perugia, Italy. E-mail: chiara.luce@uni.pg.it

Borage (*Borago officinalis* L.) is an ornamental plant, widely present in nature in different areas of Italy. During spring 2009, in private gardens and on the border of Trasimeno lake (Central Italy), leaf spots on cultivated and wild borage plants were observed. Initial symptoms were recorded on basal leaves as small brown circular spots (2 mm diameter), greyish-white in the middle and surrounded by a reddish halo. The symptoms developed on the upper leaves and later severe yellowing and necrosis of the leaf and surrounding a reddish halo. The symptoms developed after 7-8 days, the plants inoculated with both fungal isolates showed small spots, identical to those observed under natural conditions, whereas no symptoms were observed on control plants sprayed with water. *S. herbarum* was consistently reisolated from inoculated borage leaves. The fungus is known to cause leaf spots on several herbaceous hosts, including lettuce and onion. However, to our knowledge, this is the first report of a leaf spot disease caused by *S. herbarum*.

**MULTIPLE BOTRYOSPHAERIACEAE INFECTION IN FOREST TREES: SYNERGISTIC OR ANTAGONISTIC INTERACTION?** S. Moricca\(^1\), A. Uccello\(^1\), E. Turco\(^2\), B. Ginetti\(^3\), A. Ragazzi\(^2\). \(^1\)Dipartimento di Biotecnologie Agrarie, Sezione di Protezione delle Pianta, Piazzale delle Cascine 28, 50144 Firenze, Italy. \(^2\)Istituto per la Protezione delle Piante del CNR, Via Madonna del Piano, 50019 Sesto Fiorentino (FI), Italy. E-mail: andrea.uccello@unifi.it

Botryosphaeriaceae as Ascomycetes family well known as endophytes of woody hosts under temperate and subtropical climates. It includes several species that may turn to a pathogenic *habitus* when hosts undergo physiological stress. Previous pathogenicity studies have focused their attention on the interactions between a single fungal species and their hosts. However, the type of interaction established when two or more botryosphaeriaceous fungi colonize the same host tissues is till unknown. Isolates of *Botryosphaeria dothidea*, Diplospora seriata and Neofusicoccum parvum were tested on two-year-old seedlings of *Acer campestre*, *Carpinus betulus*, *Fraxinus excelsa* and *Quercus cerris* to verify disease severity and to evaluate the effect of single and combined infections. *N. parvum* proved the most virulent of the three species, causing lesions significantly larger than those caused by the other species. Seedlings inoculated with the mixture of isolates showed smaller lesions than those caused by *N. parvum* alone. These preliminary results allow us to hypothesize that a sort of antagonistic interaction may occur between Botryosphaeriaceae in host tissues.

**STUDIES ON THE AETIOLOGY OF THE DISEASE KNOWN AS OCHRACEOUS LEAF SPOTS OF APPLE IN FRIULI VENEZIA GIULIA.** S. Moruzzi, M. Martini, R. Musetti, P. Ermacora, S. Borselli and R. Osler. Dipartimento di Biologia e Protezione delle Piante, Università degli Studi, Via delle Scienze 208, 33100 Udine, Italy. E-mail: serena.moruzzi@uniud.it

An apple disease known as ochraceous leaf spots is known since 1950, but its aetiology is still unclear. The disease is common, especially in orchards where apples are grown under organic regime. In Friuli Venezia Giulia, this disease is present in different areas, in orchards of commercial and autochthonous apple varieties. Symptomatic leaves were sampled in three farms to study disease aetiology. In total 825 fungal colonies were obtained, grouped according to morphological features, and characterized by molecular tools, by analysis of rDNA ITS region. Isolates yielded fungi belonging to *Phoma* spp. (about 37% subdivided in: *P. macrostoma*, 16.9%; *P. glomerata*, 4%; *P. epicea*, 15%; *P. exigua*, 2.5%), *Alternaria* spp. (about 35%), and other minor genera. In pathogenicity tests, leaves inoculated with all four *Phoma* species developed classical necrotic lesions, while *Alternaria* spp. developed some atypical necrosis, suggesting *Phoma* spp. as the most probable causal agent of leaf spots. Portions of symptomless leaves were also investigated: *Phoma* spp. accounted for 23% of the obtained colonies and *Alternaria* spp. for 58%, indicating that the latter fungal genus might behave mainly as a plant endophyte. In plants with leaf symptoms, part of the xylem appeared to be damaged under the electron microscope. Therefore, further investigations were conducted using different techniques, showing that *P. macrostoma* colonized also young branches. *P. macrostoma* isolates from leaves and young branches were characterized molecularly based on the ITS and 18S rDNA gene sequences. No molecular differences were found in these genomic regions among fungal isolates.

**FUNGAL ENDOPHYTES IN NEEDLES AND BRANCHES OF PICEA ABIES FROM THE PANEVEZZIO FOREST.** R. Musetti\(^1\), R. Polizzotto\(^1\), F. De Luca\(^2\), S. Grisan\(^3\), R. Osler\(^1\). Dipartimento di Biologia e Protezione delle Piante, Università degli
Boron (B) toxicity is an important disorder that causes negative physiological effects such as decrease in leaf chlorophyll content, inhibition of photosynthesis and increased membrane leakage. To gain an insight into the role of photosynthetic mechanisms in response to B toxicity, physiological parameters were analyzed in Eucalyptus globulus plants treated with 0.1 (control), 1 and 10 mg l⁻¹ (excess) H₃BO₃ in nutrient solution for 12 weeks. After 42 days, plants grown with excess B developed leaf symptoms in the form of marginal necrosis. At the end of treatment, CO₂ assimilation and stomatal conductance decreased (-71% and -30%, respectively, compared to control) when plants were supplied with 10 mg l⁻¹ H₃BO₃. Growth reduction (-30%) and increase of B concentration in the roots as a consequence of the treatment were also observed. Results indicate that B excess leads to: (i) visible injury in mature leaves; (ii) reduced root growth; (iii) increase in B concentration in all parts of the plant in leaves > stems > roots in the order; (iv) strong decrease in photosynthetic activity, because of structural damage of membranes. Under these circumstances, E. globulus should be regarded as sensitive to B toxicity.

**PHOTOSYNTHETIC RESPONSE OF EUCALIPTUS TO BORON TOXICITY.** C. Nali¹, A. Francini¹, E. Pellegrini¹, S. Loppi², G. Lorenzini¹.¹Dipartimento di Coltivazione e Difesa delle Specie Leggose “G. Sgarlato”, Università degli Studi, Via del Borghetto 80, 56124 Pisa, Italy. ²Dipartimento di Scienze Ambientali “G. Sartetti”, Università degli Studi, Via P.A. Matteoli 4, 53100 Siena, Italy. E-mail: cristina.nali@agr.unipi.it

To determine the accumulation of patulin in different fruits, four isolates of Penicillium expansum were cultured on fruit puree agar media (PAMs) and inoculated in wounded fruits of common (pear and apple) and less common (apricot, peach and kiwifruit) hosts. The concentration of patulin accumulated in mycelia grown on fruit PAMs was higher than that detected in infected fruit tissues. Three P. expansum isolates accumulated patulin when grown on all fruit PAMs, while one isolate produced patulin only on apricot PAM. Apple PAM substrates were the most suitable for in vitro patulin accumulation (maximum concentration 173.1 and 74.1 µg/ml in ‘Pink Lady’ and ‘Golden Delicious’ PAMs, respectively). However, infected tissue of cv. Golden Delicious showed lower average accumulation of patulin (1.7 µg/ml) compared with cv. Pink Lady (19.1 µg/ml), and no significant differences in patulin concentrations were found among ‘Golden Delicious’ apples and tested cultivars of pear, kiwifruit and strawberry. Peaches were highly susceptible to patulin accumulation, showing average concentrations of 27.4 and 18.6 µg/ml in vitro and in vivo, respectively. Apricots were also consistently positive to patulin accumulation, both in vitro and in vivo. Our study showed the potential of some uncommon hosts of P. expansum to support patulin production, indicating that a steady monitoring of patulin contamination should be carried out in fruit substrates other than apples and pears.

**PATULIN ACCUMULATION BY PENICILLIUM EXPANSUM ISOLATES IN DIFFERENT FRUITS.** F. Neri, I. Donati, F. Veronesi, D. Mazzoni and M. Mari. Dipartimento di Protezione e Valorizzazione Agroalimentare, Centro per la Protezione e Conservazione dei Prodotti Otofrutticoli “G. C. Pratella”, Università degli Studi, Via Gandolfi 19, 40057 Cadorino (BO), Italy. E-mail: fiorella.neri@unibo.it

**EFFECT OF DEOXYNIVALENOL-PRODUCING FUSARIUM GRAMINEARUM STRAINS ON SEEDS OF TOLERANT AND SUSCEPTIBLE TRITICUM AESTIVUM VARIETIES.** C. Nobsli¹, A. Ricelli², M. Reverberi², V. Scala², G. Au-
To shorten the screening of wheat varieties tolerant to *Fusarium* head blight (FHB) and to deoxynivalenol (DON) synthesis the development of rapid and reliable assays is required. In this work, active but ungerminated seeds of two *Triticum aestivum* varieties, Blasco and Sagittario, respectively tolerant and susceptible to FHB, were inoculated with two *F. graminearum* strains (Fg126 and Fg8308), having a different toxigenic profile. Wheat seeds reacted to *F. graminearum* infection by early production of reactive oxygen species (ROS) and activating antioxidant enzymes. Whilst cv. Blasco showed an important antioxidant reaction which apparently lead to a marked decrease in ROS content, cv. Sagittario partly missed this counteraction. Compared to cv. Blasco, cv. Sagittario also produced more 9-hydroxyoctadecenoic acid (9-HODE), considered a susceptibility factor toward mycotoxicogenic fungi. Moreover, some genes related to fungal aggressiveness were up-regulated in Fg126 when grown on susceptible wheat seeds and some plant defence genes advanced their expression into cv. Blasco as compared with cv. Sagittario. Finally, it turned out that DON production may trigger apoptosis, occurring very quickly after fungal inoculation and with the typical formation of pre-apoptotic vesicles into aleuronic cells. As a matter of fact, wheat seeds, irrespective of the variety, are able to partly (5-10%) convert DON in its less toxic glucosylated form (3-Glu-DON). Each of these parameters might be considered as a wheat tolerance marker and used for diagnostic purposes or, through a forward genetic approach, for selecting wheat varieties hampering DON biosynthesis.

ALARMING SPREAD OF PLUM POX VIRUS STRAIN M IN SOME AREAS OF SOUTHERN ITALY. F. Palmisano1, M. Calderaro2, D. Boscia1. 1Istituto di Virologia Vegetale del CNR, UCB Barri, Via Amendola 165/A, 70126 Bari, Italy. 2Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi “Aldo Moro”, Via Amendola 165/A, 70126 Bari, Italy. E-mail: d.boscia@ba.ivv.cnr.it

*Plum pox virus* (PPV), the causal agent of Sharka, the most harmful virus disease of stone fruits, is characterised by a great variability represented by seven types or strains, among which “Marcus” (PPV-M) is considered the most dangerous, particularly for the peach industry. Sharka appeared in the south-eastern part of Italy (Basilicata and Apulia) in 1987 with few outbreaks of strain “Dideron” (PPV-D), and was maintained under control for long time, thanks to the timely eradication of all infection foci. The situation turned to worse in 2007, when PPV-M appeared for the first time in Basilicata, followed two years later by a couple of outbreaks of the same strain in northern Apulia. Although eradication actions were intensified by the local Plant Protection Services, during spring 2010 new outbreaks popped up in both regions. Object of this study was the characterization of PPV samples collected in different places, for identifying the viral strains involved and studying the level of variability among them. Isolates were first analysed by ELISA with strain-specific monoclonal antibodies. Serological characterization was followed by molecular characterization based on: (i) RT-PCR; (ii) sequencing of amplicons; (iii) multiple sequence alignments; (iv) phylogenetic analysis. Results showed that the majority of the outbreaks were caused by PPV-M, thus confirming the high rate of natural transmission of this strain and the need for enforcing eradication and, even more, for preventing the introduction in the region of infected nursery productions from elsewhere.

RESPONSE OF MICROBIAL COMMUNITIES TO COMPOST AMENDMENT OF SOIL AND EFFECT ON DISEASE SUPPRESSIVENESS. C. Pane, D. Villecco, M. Zacardelli. CRA, Centro di Ricerca per l’Orticoltura di Pontecagnano, S.S. 18 n. 204, 84091 Battipaglia (SA), Italy. E-mail: catello.pane@enteira.it

Compost can be used to improve organic matter in cultivated soils, so as to reduce the use of mineral fertilizers, stimulate soil microbial activities, and improve suppressiveness of soil-borne pathogens. In this study, the response of the soil-borne microflora to soil waste compost amendment was evaluated at functional biodiversity (Biolog™ CLPPs) level and at global (CO₂ release, beta-glucosidase, FDA hydrolysis) level, in a short post-amendment period. *Rhizoctonia* damping-off suppressiveness was also measured in a laboratory experiments on the host *Lepidium sativum*. Soil chemical parameters such as electrical conductivity (EC) and pH were monitored at the same time. Other than compost-amended (CA) soils, mineral fertilized (MF) and non-treated (NT) soils were used as control. The addition of compost in-
increased all microbial activities, suppressivity levels and EC for the whole experimental period, whereas pH did not changed. Conversely, Biolog® CLPPs were enhanced in compost-amended soil at the beginning, but decreased with the time and disappeared at the end of the incubation period. FDA hydrolysis rate was strongly positively correlated to EC, soil beta-glucosidase activity and Biolog CLPPs. Results indicate that compost amendment affected microbial activities, both at the global and functional level, as a consequence of supplied carbon source. CO₂ release and soil beta-glucosidase activity were strongly auto-correlated and negatively related to the percentage of Rhizoctonia diseased plants, suggesting a mechanism of general suppression, where antagonistic microbial populations are stimulated by compost addition.

A PROTEOMIC APPROACH TO THE STUDY OF THE ROLE OF CERATO-PLATANIN IN CERATOCYSTIS PLATANI AND IN THE CANKER STAIN DISEASE. B. Pantera1, L. Pazzaglì1, L. Carrese2, C. Comparini2, F. Martellini2, M. Capuana3, G. Cappugi4, I. Baccelli5, R. Bernardi6, A. Scala7. 1Dipartimento di Scienze Biossonomiche, Università degli Studi, Viale Moregiani 30, 50134 Firenze, Italy. 2Dipartimento di Biotecnologie Agrarie, Sezione di Protezione delle Piante, Laboratorio di Patologia Vegetale Molecolare, Università degli Studi, Via della Lastruccia 10, 50019 Sesto Fiorentino (FI), Italy. 3Istituto di Genetica Vegetale Molecolare, Università degli Studi, Via della Piagge 23, 56124 Pisa, Italy. E-mail: barbarapanterac@unifi.it

Cerato-platanin (CP) is a moderately hydrophobic protein abundantly secreted and localized in the cell wall of Ceratocystis platani. CP is assumed to play a role in host-plant interaction, since it induces production of H₂O₂ and NO, programmed plant cell death, overexpression of defence genes, phytoalexin synthesis and restriction of conidial growth. Therefore, CP appears to act as PAMP able to activate effective primary defence mechanisms. Moreover, CP is the founder of the “cerato-platanin family”, whose members are secreted proteins involved in the microbe-host interaction acting as phytoxins, elicitors of plant defence responses or human allergens. The proteomic project that we are setting up has the aim to investigate the role of CP in the physiology of the fungus and in the physiopathology of the disease. Conidia of C. platani were harvested from fruiting cultures and suspended in PDB (2x10⁴ conidia ml⁻¹). Aliquots of 100 μl droplets were then applied to plane leaves and in empty Petri dishes as controls. All samples were maintained in a moist chamber at room temperature. After 48 h the mycelium was removed and lyophilized, and leaves were put at -80°C for further analysis. An aliquot of 0.05 g of dry mycelium was re-suspended in an acidic extraction buffer containing dodecyl-maltoside as a detergent. Results from 2D-gels have highlighted: (i) proteins expressed in the mycelium grown in PDB vs that grown on plant leaves, and (ii) proteins extracted from the treated leaves vs proteins from control leaves.

EPIDEMIOLOGICAL AND MOLECULAR ASPECTS OF ALFALFA MOSAIC VIRUS OCCURRENCE IN LAVANDULA VERA CROPS OF LIGURIA. G. Parrella1, C. Cavicchi2, G. Zamara3, M.G. Bellardi3. 1Istituto per la Protezione delle Piante del CNR, Via Università 133, 80055 Portici (NA), Italy. 2Plesso Didattico G. Scarabelli, Università degli Studi di Bologna, Viale G. Ascoli 15, 40026 Imola (BO), Italy. 3Dipartimento di Scienze e Tecnologie Agroambientali, Sezione di Patologia Vegetale, Università degli Studi, Viale F. Tanini 42, 40127 Bologna, Italy. E-mail: giuseppe.parrella@ipp.cnr.it

From 2008 to 2010, a study was carried out on the occurrence of Alfalfa mosaic virus (AMV) in some Lavandula vera crops of Albenga (Liguria, northern Italy). This non-persistent aphid-borne virus represents one of the most dangerous and economically important pathogens of lavender affecting both plant growth and appearance. In 2008 and 2009 numerous inspections were made at several lavender producers with accurate examination of pot-grown plants before blooming. AMV was detected by DAS-ELISA and PCR in association with stunting and yellow mosaic symptoms. Considering that L. vera is propagated by shoots and that mother-plants (10-12-year-old) are grown at the borders of pot-plant crops, in 2010 Ligurian propagation materials were checked serologically. Some L. vera mother plants showing leaf mosaic proved to be infected by AMV. One AMV isolate from symptomatic mother plants was characterized molecularly and compared with previously characterized Italian lavender isolates of the same virus, including an isolate recovered in 2010 from L. vera cv. None Blue in Emilia-Romagna. Results showed that AMV isolates from Liguria and Emilia-Romagna belong to subgroup I, according with restriction profiles of the coat protein gene and phylogenetic relationships with other AMV isolates of subgroups I and II. As prevention measure, mother plants could be selected by periodic visual inspections and serologically tested for AMV presence. Virus-free shoots used as propagation material and crop rotation are the most effective type of control.

ATTEMPTS TO CONTROL PSEUDOMONAS VIRIDIFLAVA IN RANUNCULUS ASIATICUS. C. Pasini and G. Boeri. CRA, Unità di Ricerca per la Floricoltura e le Specie Ornamentali, Corso degli Inglesi 508, 18038 Sanremo (IM), Italy. E-mail: c.pasin6@istflori.it

Pseudomonas viridiflava is the prevailing bacterial pathogen in ranunculus-growing areas of the Italian Riviera, where it can cause serious damages to commercial flower production. Infections appear during autumn and winter, and consist of necrotic spots on leaves and stems. To develop a defence strategy, a series of trials were undertaken to evaluate the effects against P. viridiflava of: (i) chemical dressing by immersion of rhizomes in a suspension of various active ingredients; (ii) dry heat thermotherapy of dry rhizomes at 60 and 65°C, for different times; (iii) fungicide sprays with acibenazerl-S-methyl, Bacillus subtilis, dithianon, harpin protein, phosphetyl-Al copper oxychloride and several copper salts to ranunculus potted plants grown in a climatic cell at 15°C, and on cut flowers stored at 6-8°C. Results showed that thermotherapy inhibited rhizome germination. Dressing with phosphetyl-Al, monopotasial phosphate and copper hydroxide provided a moderate activity, while several concentrations of ammonium salts and commercial bleach solutions were phytotoxic. Sprays with acibenazerl-S-methyl, different copper salts and phosphetyl-Al⁺ copper oxychloride reduced the infection both on emerged plants or on cut flowers. By applying the biocontrol agent B. subtilis a partial and inconsistent protective action was observed.

INTERACTIONS BETWEEN ENDOPHYTIC STRAINS OF ALTERNARIA SP. AND VITIS VINIFERA PLANTS. R. Polizotto, S. Grisan, R. Osler, R. Musetti. Dipartimento di Biologia e Protezione delle Piante, Università degli Studi, Via delle Scienze
The genus *Alternaria* is characterized by a large spectrum of plant-host interactions. It is therefore important to recognize and discriminate the pathogenic forms from the saprophytic or endophytic ones. It was reported that fungal endophytes recovered from symptomless natural hosts could cause severe symptoms when inoculated to other plant species. Since *Alternaria* endophytes from grapevine were effective in the biocontrol of *Plasmopara viticola*, both in pre- and post-infection treatments, the scope of this study was to investigate the behaviour of 12 *Alternaria* grapevine endophytic strains when artificially inoculated in different tissues of *Vitis vinifera*. Pathogenic assays were carried out using tomato (*Solanum lycopersicum*) as a non-host test plant. A conidial suspension of each *Alternaria* obtained from single-spore cultures was sprinkled over six wounded leaves of grapevine and tomato seedlings. Grapevine and tomato berries were artificially inoculated by immersion (grapevine) or by wounding (tomato), using the *Alternaria* conidial suspension as above. Symptoms were evaluated 4 weeks after infection on leaves and one week on fruits. Re-isolation rate from inoculated tissues was estimated. *Alternaria* did not induce symptoms in inoculated leaves of both plant species, but was pathogenic (dark brown or black circular necrotic lesions) to tomato berries. Re-isolation frequency was 7% in grapevine and 41% in tomato. Results showed that *Alternaria* strains behave differently on the different plant tissues inoculated, as they are true endophytes of grapevine whereas they can cause severe damage to tomato berries.

**INFLUENCES OF CULTURAL PRACTICES ON BUNCH ROTS AND OCHRATOXIN A CONTAMINATION IN WINE.** S. Pollastro¹, C. Dongiovanni², R.M. De Miccolis Angelini¹, P. Natali², D. Perrelli², F. Faretra¹, ¹Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi “Aldo Moro”, Via Amendola 165/A, 70126 Bari, Italy. ²CRA, Centro delle Piante e Microbiologia Applicata, Università degli Studi “Aldo Moro”, Via Amendola 281, Locorotondo (BA), Italy. E-mail: stefania.pollastro@agr.uniba.it.

Grapevine bunch rots are a complex of diseases and alterations caused by several microorganisms affecting the quality and safety of wine, as in the case of *Aspergillus carbonarius*. This fungus is the main responsible for ochratoxin A (OTA) contamination of grape and wine in the Mediterranean area. OTA is a mycotoxin frequently found in foods and beverages. The Reg. (EC) N. 123/2005 of 26.1.2005 established in 2.0 mg kg⁻¹ the maximum tolerable limit of OTA in wine and other grape-juice derivatives. To improve the efficacy of IPM strategies in limiting bunch rots and OTA contamination, the role of some cultural practices, especially grey mould (100%), followed by *A. carbonarius* (70%) and OTA contamination (50%). Opposite results were obtained when canopy was abundant and almost free to grow. In this case, bunch rots, especially grey mould (+30%), *A. carbonarius* (+33%) and OTA contamination (+47%) were higher. The severity of bunch rots was high when grass-cut soil was in between vine rows. *A. carbonarius* (+33%) and OTA contamination (+30%) also increased when ploughing between rows was carried out 20 days before vintage. Such findings suggest that a careful integrated management of both cultural practices and crop protection in vineyards is essential for reducing the risk of OTA contamination of wine.

**OBTAINMENT, CHARACTERIZATION AND FIRST APPLICATIONS OF A PHAEOMONIELLA CHLAMYDOSPORA BENZIMIDAZOLE-RESISTANT MUTANT IN EPIDEMIOLOGICAL STUDIES.** S. Pollastro, W. Habib, F. Faretra. Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi “Aldo Moro”, Via Amendola 165/A, 70126 Bari, Italy. E-mail: faretra@agr.uniba.it.

Studies on the fungal mycflora of grapevine propagation materials showed a very high frequency of *Phaeomoniella chlamydospora*, which was also detected at different stages of the nursery propagation process. The availability of *P. chlamydospora* isolates carrying genetic markers easily detectable on semi-selective media can be helpful for clarifying: (i) the possible role of vine propagation steps in the contamination of vine materials; (ii) the transmission of Esca disease to new vineyards; (iii) the effectiveness of control measures. Preliminary, the response to benzimidazole fungicides (benomyl, carbendazim and thiophanate methyl) of 10 wild-type isolates of *P. chlamydospora* was evaluated in colony-growth and conidial-germination assays. Colony-growth of all isolates was inhibited by 0.3-1 µg ml⁻¹ of fungicides whereas conidial germination was poorly affected, although germ tubes appeared deformed and bent from 0.3 µg ml⁻¹ a.i. Resistant mutants were selected on a medium added with 1 µg ml⁻¹ benomyl or carbendazim (mutation frequency: 2.1-2.5×10⁻⁸ and 1.4-2×10⁻⁸, respectively). Generally, mutants showed high resistance. The mutant CI.A43.2 (EC₅₀>100 µg ml⁻¹) was selected and used in preliminary experiments. Grapevine propagation material was artificially inoculated at different stages of the nursery process with conidia of the mutants and its ability to colonize wood tissues was verified 1-3 months after inoculation. The mutant was always reisolated from inoculated rootstocks and scions, even far away from the inoculation point, confirming the capability of *P. chlamydospora* to move in xylem vessels. The traceable mutant is being now applied in large-scale epidemiological studies on Esca disease.

**PRODUCTION OF AN ANTISERUM SPECIFIC TO CITRUS LEAF BLIGHT VIRUS.** O. Potere¹, M. Guardo², A. De Stradis³, A. Caruso¹, D. Bosia¹. ¹Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi “Aldo Moro”, Via Amendola 165/A, 70126 Bari, Italy. ²CRA, Centro di Ricerca per l’Agrumicoltura e le Colture Mediterranee, Corso Savoia 190, 95024 Acireale (CT) Italy. ³Istituto di Virologia Vegetale del CNR, UOS Bari, Via Amendola, 165/A, 70126 Bari, Italy. E-mail: o.potere@agr.uniba.it.

Different *Citrus* species used as ornamentals and some trifoliate rootstocks were recently found infected by *Citrus leaf blotch virus* (CLBV), the type species of the genus *Citrivirus*, family *Betacryptoviridae*. This virus is currently detected by RT-PCR following nucleic acid extraction. For a faster virus detection from crude plant extracts, the feasibility of raising a CLBV-specific antiserum to be utilized for serological testing was investigated. To this aim, virus purification protocols were compared using several kinds of tissue (leaves, petioles and cortical scrapings) collected from Nagami kumquat and citron ‘Etrò’ in different seasons. Virus concentration was highest in spring, decreasing progressively as temperature arose. The successful mechanical transmis- sions of CLBV to some hosts enabled the use of *N. benthamiana* as host for virus propagation. Concentrated preparations of CLBV were evaluated by electron microscope before injecting them into a rabbit for immunization. The antiserum obtained decorated isolated ISA-10-CT-I particles used as inject antigen at a dilution of 1:20. Immunoglobulins (IgGs), purified from crude
antiserum preabsorbed with healthy plant extracts and conjugated with alkaline phosphatase, were comparatively used in Western blotting (WB) and DAS-ELISA. Specific detection of coat protein bands was obtained only in WB of infected Citrus and Nicotiana extracts. The test was successfully extended to ornamental Citrus trees, such as Calamondin (Citrus microcarpa) and Fortunella spp., from nurseries and commercial groves.

**PRESENCE OF DEOXYNIVALENOL AND NIVALENOL CHEMOTYPES OF FUSARIA MULCUM ISOLATED FROM DURUM WHEAT IN SOME ITALIAN REGIONS. A. Prodi, D. Salomoni, D. Alkadi, S. Tonelli, P. Nipoti, A. Pisit, D. Pancaldi.** Dipartimento di Scienze e Tecnologie Agro-Ambientali, Università degli Studi, Viale G. Fannin 40, 40127 Bologna, Italy. Dipartimento di Protezione e Valorizzazione Agro-Alimentare, Università degli Studi, Viale G. Fannin 40, 40127 Bologna, Italy. Ente Nazionale Sementi Elette, Via Cà Nova Zampieri 37, 37057 San Giovanni Lupatoto (VR), Italy. E-mail: antonio.prodi@unibo.it

Durum wheat production in Italy is of great economical importance. Fusarium Head Blight (FHB) is an important and widespread disease of wheat that, in Italy, causes serious damages in terms of yields and quality of grains. Fusarium culmorum, one of the main causal agents of FHB, is spread in all the Italian regions, but especially in the center-northern areas. It produces mycotoxins, such as deoxynivalenol (DON), nivalenol (NIV) and zearalenone (ZEA), representing potential health hazards for both humans and animals. This work investigated a population of F. culmorum strains isolated from durum wheat grains collected in different regions, but especially in the center-northern areas. To our knowledge, these data represent the first case where a mutation in gacA did not totally abolish the production of AHL and PHZ in a Pca member.

**COMPARISON OF BIOCONTROL FEATURES OF THREE BACTERIAL ANTAGONISTS BELONGING TO PSEUDOMONAS CHLORORAPHIS subsp. AUREOFACIENS. G. Puopolo, V. Battaglia, A. Russo, L. Cozzolino, A. Zoina.** Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università degli Studi di Napoli “Federico II”, Via Università 100, 80055 Portici (NA), Italy. E-mail: puopolo@unina.it

Members of the subspecies Pseudomonas chlororaphis aureofaciens (Pca) share several physiological features exploitable in the biological control of phytopathogenic fungi. The production of phenazines (PHZ), antibiotic molecules with a broad host range activity, is the main mechanism through which these bacteria control important fungal pathogens. PHZ production relies on the synthesis of N-acyl homoserine lactones (AHL) mediated by synthase enzymes belonging to the LuxR family. Once signal molecules reach a threshold density, they bind transcriptional factors of the LuxR family, responsible for the transcription of the operon involved in PHZ production. This molecular mechanism has been termed Quorum Sensing. In this study, three Pca strains were analysed for their biocontrol aptitudes, paying particular attention to the production of PHZ and AHL. These strains were also evaluated for their ability to control Fusarium oxysporum f. sp. radicis-lycopersici (Forl') attacks on young tomato plantlets and for their ability to persist on tomato roots. Pca strains 30-84, AZ10C2 and M71 were able to produce proteases, lipases, siderophores and to synthesize PHZ and AHL in complex growth media. Interestingly, M71 was the only strain capable of producing PHZ and AHL in minimal growth media. Pca strains were effective in the protection of tomato plantlets against Forl reducing up to 50% the incidence of the disease. Furthermore, these bacterial strains showed a good aptitude to colonization of tomato roots, but Pca M71 was the only strain that persisted on tomato roots up to three months in field trials.

**OCCURRENCE OF MUTANTS DEFECTIVE IN BIOCONTROL ACTIVITY IN PSEUDOMONAS CHLORORAPHIS subsp. AUREOFACIENS STRAIN M71. G. Puopolo, A. Russo, V. Battaglia, A. Zoina.** Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università degli Studi di Napoli “Federico II”, Via Università 100, 80055 Portici (NA), Italy. E-mail: puopolo@unina.it

The production of phenazines (PHZ) plays a key role in the biocontrol activity of several members of Pseudomonas chlororaphis subsp. aureofaciens (Pca). The biosynthesis of these molecules is regulated by a Quorum Sensing (QS) mechanism, that relies on the production of N-acyl homoserine lactones (AHL). In fluorescent pseudomonads, the GacS/GacA two-component system allows to perceive and respond to environmental stimuli by activating QS. Mutations in the genes gacS and/or gacA determine the loss of AHL and PHZ production in Pca members. Occurrence of mutants defective in the production of phenazines has been shown in Pca strain M71. These mutants, named M71a and M71b, had a siderophore production higher than that of the wild type strain. Strain M71b did not produce PHZ and AHL, while strain M71a was able to produce these metabolites only when grown in complex media. Furthermore, strain M71a and M71b were impaired in the colonization and persistence on tomato roots and in the control of Fusarium oxysporum f. sp. radicis-lycopersici. The cosmids pEMH97 (carrying gacS) and PME3066 (carrying gacA) were moved by triparental mating in the mutants in order to assess the loss of a functional GacS/GacA system. Restoration of wild type properties in M71b was achieved by introducing a functional copy of gacS, while the introduction of PME3066 (gacA) in M71a restored the wild type phenotype.
which is the primary phytotoxic compound of the fungus but can also have a limiting effect on its growth. Botrytial biosynthetic intermediates appear to be cyclization products of caryophyllene. Terpenes are already present in the food chain and do not display significant human toxicity, thus their use for the postharvest control of fruit and vegetable diseases could be interesting. However, only few studies deal with the effect of pure terpenes against phytopathogenic fungi. For this reason, we investigated the effectiveness of caryophyllene, linalool, nerolidol and 3-pinene against B. cinerea. Nerolidol and linalool reduced significantly fungal growth in vitro when used at concentrations ranging from 1000 to 2500 µl/l and from 1500 to 2500 µl/l, respectively. In particular, linalool completely inhibited the fungal growth at the highest concentration. In vivo, linalool (2500 µl/l) reduced significantly infection percentage when applied by dipping the berries in a solution, while it was not effective when applied by evaporation. Nerolidol (2500 µl/l) was not effective in both cases. Unfortunately, the two compounds exerted a strong phytotoxic activity when applied by dipping, causing browning of the berries. In further investigations, other terpenes and other strategies of application will be tested.

HOST PECTIN METHYLESTERASE PLAYS A ROLE IN THE SUSCEPTIBILITY TO NECROTOPHIC PATHOGENS. A. Raiola1, V. Lionetti2, I. Elmaghraby1, F. Cerbone3, D. Bellincampi2,3. 1Dipartimento Territorio e Sistemi AgroForestali, Università degli Studi di Padova, Viale dell’Università 16, 35020 Legnaro (PD), Italy. 2Dipartimento di Biologia Vegetale, Università degli Studi “La Sapienza”, Piazzale Aldo Moro 5, 00185 Roma, Italy. 3Dipartimento di Chimica, Università degli Studi “La Sapienza”, Piazzale Aldo Moro 5, 00185 Roma, Italy. E-mail: alessandro.raiola@unipd.it

The host cell wall is a primary target during growth of necrotrophic pathogens. During the first stages of infection, pectin, one of the main components of the plant cell wall, is degraded by pectinolytic enzymes produced by the majority of fungal and bacterial pathogens. Some evidence indicates that variation of the pectin structure and composition may cause an altered disease response upon infection with pathogens. Pectin is synthesized and secreted into the cell wall in a highly methylesterified form and, soon thereafter, deesterified in vivo by pectin methylesterases (PMEs). The action of PME makes pectin susceptible to degradation by enzymes such as endo-polygalacturonases (PGs) and pectate lyases (PEls). Endogenous PME activity is controlled through the interaction with the pectin methylesterase inhibitor (PMEI). PMEI over-expression and PME knockout have been used to stably increase pectin methylesterification in Arabidopsis plants. We have shown that the increase of pectin methylesterification and the lack of a specific PME activity correlate to a decreased susceptibility of Arabidopsis to the necrotrophic pathogens Pectobacterium carotovorum and Botrytis cinerea. The reduced symptoms of transformed plants have been related to the inability of the pathogens to take advantage of host PMEs and to their impaired ability to grow on methylesterified pectins.

FIRST REPORT OF ANTHRACNOSE OF HOYA CARNOSA IN ITALY. G.L. Rana1, I. Camele1, F. Marziano2. 1Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali, Università degli Studi della Basilicata, Via Ateneo Lucano 10, 85100 Potenza, Italy. 2Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università degli Studi di Napoli “Federico II”, Via Università 100, 80055 Portici (NA), Italy. E-mail: gianluigi.rana@unibas.it

During spring 2009 some plants of Hoya carnosa (wax plant, Asclepiadaceae) with leaves showing sunken dry lesions 1-2 cm in diameter, bordered by a slightly raised rim, were observed in Molfetta territory (Bari province, southern Italy). The above lesions, as it was shown by stereomicroscopic and microscopic observations of entire and dissected symptomatic leaves, contained numerous, unitarily arranged acellular conidiomata. The microcyste present in infected organs, tentatively considered as the aetiological agent of the leaf alterations, was isolated in pure culture in Petri dishes on PDA then used for: (i) studying colony, conidioma and conidium development at 24-26°C on PDA, OMA and SNA; (ii) DNA extraction, amplification with primers ITS4/ITS5 and sequence analysis and (iii) artificially inoculating leaves of healthy wax plants. Results showed that the microfungus under study was Colletotrichum gloeosporioides (Teleomorph: Glomerella cingulata, complex species). Its DNA sequence (547 bp) was compared with those of the same fungus present in GeneBank with accession No. AJ310917 and EU326191 and showed 100% and 99% similarity, respectively. One of the sequences obtained was deposited at the European Molecular Biology Laboratory (EMBL) with accession code FN68840. In pathogenicity tests, inoculated leaves of H. carnosa showed symptoms very like those observed on naturally infected plants. Acellular conidiomata were also formed subepidermally inside necrotic foliar tissues. C. gloeosporioides, previously unreported on H. carnosa in Italy, was found infecting wax plant only in USA in 1984.

A NEW PROTOCOL FOR THE RAPID DETECTION AND CHARACTERIZATION OF CITRUS TRISTEZA VIRUS ISOLATES BY SEQUENTIAL ELISA-CE-SSCP. D. Raspagliesi1,2, G. Liciardiello2, A. Lombardo2, S. Rizza1, M. Bar-Joseph3, A. Catarà2, 1Dipartimento di Scienze e Tecnologie Fitosanitarie, Università degli Studi, Via S. Sofia 100, 95123 Catania, Italy. 2Laboratorio di Diagnosi e Biotecnologie Fitosanitarie, Parco Scientifico e Tecnologico della Sicilia, Blocco Palma I, Z.I., 95121 Catania, Italy. 3The S. Tolkoasby Laboratory, ARO, The Volcani Center, Bet Dagan 50230, Israel. E-mail: gliciardielle@psicilicia.org

Management and control of emerging Citrus tristeza virus (CTV) epidemics is based on the ability to rapidly detect infected trees and to differentiate between prevailing isolates. Previously, ELISA and CE-SSCP were used separately for CTV detection and to isolate differentiation. Here we combined these two diagnostic methods into a continuous process allowing the detection of infected trees among unknown samples and the rapid analysis of the genetic structure of samples positive to ELISA, thus saving both time and resources. Crude extracts from different samples were tested by DAS-ELISA, positive wells were washed, RNA eluted and used as template for cDNA synthesis and subsequent CE-SSCP analysis. Different susceptible citrus species, as sour orange, Etrog citron and Mexican lime, were used for preliminary tests using the p18 gene. A total of 41 plants inoculated with five CTV isolates (Tapi, TDV, RPC3, SG29, M1), or naturally infected, were analysed and successfully compared with stored profiles. Size, conformation, data point and migration of the two strands, showing sequences obtained was deposed at the European Molecular Biology Laboratory (EMBL) with accession No. AJ310917 and EU326191 and showed 100% and 99% similarity, respectively. One of the sequences obtained was deposited at the European Molecular Biology Laboratory (EMBL) with accession code FN68840. In pathogenicity tests, inoculated leaves of H. carnosa showed symptoms very like those observed on naturally infected plants. Acellular conidiomata were also formed subepidermally inside necrotic foliar tissues. C. gloeosporioides, previously unreported on H. carnosa in Italy, was found infecting wax plant only in USA in 1984.
DETECTION AND CHARACTERIZATION OF CITRUS TRISTEZA VIRUS IN THE NATIONAL GERMPLASM COLLECTION OF AFGHANISTAN. S. Rehman1, J. Ahmad1, C. Lanzoni2, C. Rubies Autonell3, C. Ratti3. 1Agra Khan Foundation-Afghanistan, Wazir Akbar Khan, Road 13, H43, Main road - Kabul, Afghanistan. 2Dipartimento di Scienze e Tecnologie Agroambientali, Sezione di Patologia Vegetale, Università degli Studi, Viale Fanin 40, 40127 Bologna, Italy. E-mail: shams-u-rahman.shams@akdn.org

Reviving horticultural industry is a government priority in Afghanistan. To the same purpose, EC supported programmes specifically focus on increasing the access to improved and appropriate planting material, in order to increase the quantity and quality of more competitive horticultural products. The availability of a biotechnology laboratory and of its services for quality control and management along the horticulture supply chains were considered an essential support to horticulture development. This laboratory started screening the health state of the Afghan Germplasm National Collection not only to ensure multiplication of the best selected varieties or ecotypes, but also to avoid production and distribution of virus-infected trees. During surveys of citrus at the National collection experimental farm in Jalalabad, plants from accessions showing vein flecking, yellowing and decline symptoms were sampled and analyzed by ELISA. Four accessions, kamquat cv. Margarita, orange cv. Mahali, mandarin cv. Fruter and rough lemon cv. Mahali, proved to be infected by Citrus tristeza virus (CTV). Known to be transmitted by aphids, CTV causes a serious disease that can rapidly spread and destroy orchards. In order to preserve the citrus national collection from CTV infection, a study for the characterization of virul isolates was undertaken. A 659 nt long fragment, corresponding to the major coat protein gene, was amplified by RT-PCR from all ELISA-positive samples. Preliminary analysis revealed sequence identity ranging from 91 to 100% within Afghan CTV isolates and high similarity with GenBank isolates from Angola, India and USA.

SERIOUS DAMAGES BY IMPATIENS NECROTIC SPOT VIRUS IN ZANTEDESCHIA AETHIOPICA. D. Rizzo1, S. Lazzereschi2, B. Nest2, A. Grassotti1. 1Agenzia Regionale per lo Sviluppo e l’Innovazione nel Settore Agricolo-Forestale (ARSIA), Laboratorio di Diagnostica Fitopatologica, Via dei Fiori 8, 51012 Pescia (PT), Italy. 2CRA, Unità di Ricerca per il Vivaismo e la Sviluppo e l’Innovazione nel Settore Agricolo-Forestale (ARSIA), Studi di Bologna, Via Gandolfi 19, 40057 Cadriano (BO), Italy. E-mail: sara.lazzereschi@entecra.it

Calla lily (Zantedeschia aethiopica, family Araceae) has become one of the most popular cut flowers worldwide because of its ornamental characteristics, such as spathé beauty and post-harvest shelf-life. Calla lily is mainly used for cut flower, but also for potted plants production. Tospoviruses (family Bunyaviridae) are agriculturally important as they cause severe economic damage to various crops and flowers. Tospovirus diseases, induced by Impatiens necrotic spot virus (INSV) mainly affect a lot of ornamental plants, i.e. cineraria, cyclamen, prairie gentian and many others, including perennials. INSV causes yellow or brown ring spots, round brown black or white spots, brown or black stem sections, black or brown necrosis at the leaf base, stunting, etc. Symptoms vary with the plant species, cultivar and age. In calla lily, the first symptoms of infection are chlorotic or yellow spots radiating from the midrib towards the edge on the leaf. ELISA is a routine method for diagnosis of plant viruses including tospoviruses and RT-PCR has been widely used as a highly sensitive and specific detection method. In the present work the collection of calla lily plants of the CRA-VTV was analysed in collaboration with the phytopathological laboratory of ARSIA. INSV was detected by the immunocromatographic ‘Lateral Flow’ technique and a protocol for double step RT-PCR was performed, using different amplification profiles.

ANTIFUNGAL ACTIVITY OF VOLATILE COMPOUNDS PRODUCED BY PENICILLIUM sp. ISOLATE R82 AGAINST POSTHARVEST FUNGAL PATHOGENS. W. Rouissi, P. Bertolini, M. Mari. Centro per la Protezione e Conservazione dei Prodotti Ornifruttioli “G.C. Pratella”, Università degli Studi di Bologna, Via Gandolfi 19, 40057 Cadriano (BO), Italy. E-mail: wafa.rouissi@studio.unibo.it

The thiabendazole (TBZ) sensitive Penicillium R82 isolate, was grown in PDB for 10 days at 20°C. The liquid culture (LC) was lyophilized, resuspended in distilled water (1:10, 1:100, 1:1000 v/v) and sterilized. Its influence on in vitro growth of Penicillium expansum, Monilinia laxa, Botrytis cinerea and Colletotrichum acutatum was evaluated by measuring the decrease of mycelia dry weight (DWM) and conidia germination. The LC of R82 reduced significantly the DWM of all pathogens tested, while it increased the length of the germ tubes compared to the control, however an abnormality in mycelium growth was observed. In vivo assays were performed on apples and pears. Wounded fruits were treated with a conidial suspension of R82 (105 conidia/ml), inoculated with two isolates of TBZ-resistant P. expansum and stored for 10 days at 20°C. All fruit were rotted.
However lesions were produced only by isolate R82 since no fungal growth was observed on malt agar plates amended with TBZ (400 mg/g) and inoculated with small pieces of rotted tissues. To explain the mode of action of isolate R82, a double Petri-dish assay was performed. Antifungal activity was probably due to the production of volatile substances whose identification through Gas Mass technique is in progress. In conclusion, fusible substances generated by Penicillium R82 isolate and present in culture filtrate showed activity against the above listed postharvest pathogens. A possible application of these natural substances could be the postharvest biofumigation of fruit in storage room, to prevent losses through the distribution chain.

THE ROLE OF PLANT GENOTYPE IN THE BENEFICIAL INTERACTION BETWEEN TOMATO AND THE BCAS TRICHODERMA spp. M. Ruocco1, M. Tucci2, L. De Masi2, M. de Palma2, S. L. Woo3, F. Vinale3, S. Lanuzise3, M. Nigro, A.M. El-Tabey Eid3, M. Lorito4,5. Istituto di Protezione delle Piante del CNR, Via Università 133, 80055 Portici (NA), Italy. 1Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università degli Studi di Napoli “Federico II”, Via Università 100, 80055 Portici (NA), Italy. E-mail: ruocco@ipp.cnr.it

Selected fungi of the genus Trichoderma have the ability to interact simultaneously with plants and pathogens. These antagonists can trigger systemic and localised resistance to diseases and promote plant growth and development. Additional effects include the suppression of deleterious soil microflora/fauna, degration of toxic compounds, direct stimulation of root development, by producing hormone-like compounds or affecting plant synthetic pathways, and/or promotion of water and nutrient uptake. However, the occurrence of the above benefits depends on the specific plant genotype, although this concept has been poorly investigated. In this work we have studied the effects of two Trichoderma species (T. harzianum T22 and T. atroviride P1) on several cultivated and wild tomato genotypes (Solanum lycopersicum and S. habrochaites) in terms of promotion of seed germination and plant development, protection against pathogens and transcriptional modifications of response genes. Strong differences in the response to the symbiotic interaction with the two Trichoderma strains were found among different tomato varieties. In fact, the effect of T. harzianum T22 and T. atroviride P1 on the growth and systemic resistance against B. cinerea depended strongly on the genotype tested. Our data on the induction of defense response pathways may help the selection or breeding of tomato lines with enhanced ability to benefit from the interaction with Trichoderma, which may support a correct application of Trichoderma-based bio-pesticides and bio-fertilizers in agriculture.

ACTIVITY OF ELECTROLYZED OXIDIZING WATER GENERATED BY DIAMOND THIN FILM ELECTRODES IN REDUCING PATHOGENIC MICROBIAL POPULATION IN PACKINGHOUSE WASH WATER. S.M. Sanzani1,2, F. Fallanaj, C. Zavanella1, A. Ippolito1. 1Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi “Aldo Moro”, Via Amendola 163/A, 70126 Bari, Italy. 2Adamt Technologies SA Eplatures-Girte 17, 2300 La Chaux-de-Fonds, Switzerland. E-mail: simona.sanzani@agz.uniba.it

Electrolyzed Oxidizing Water (EOW), generated by electrolysis of a salt solution, recently gained attention for its possible use as eco-friendly alternative method for the inactivation of the pathogenic microflora of fresh fruit and vegetables. Aim of the present investigation was to determine if EOW generated by Diamond Thin Film Electrodes has potential for use in commercial packinghouses to control the main pathogens of harvested fruits. EOW was generated with Diacell, an electrolytic cell based on boron-doped diamond electrodes (Adamt Technologies SA, CH) that are known for their outstanding electrochemical properties, including the production of a wide range of oxidising species. Spores of Penicillium expansum were suspended in EOW produced either in the presence or absence of 1 g/l of NaCl to increase conductivity. Furthermore, the suspension itself was subjected to direct electrolysis. Fungal growth was assessed after 24-48 h incubation at room temperature. When NaCl was used, EOW exhibited nearly 100% inhibition of P. expansum spore germination, whereas in the absence of NaCl a significant although lower efficacy (around 60% reduction) was recorded. The efficacy of disinfection increased to 98% after 75 min of direct electrolysis. On the basis of the results obtained a further trial was conducted on the wash water from a local packinghouse, which was subjected to electrolysis in presence of NaCl for 3 h. A visible improvement of water clarity and a significant reduction of total microbial population was obtained after only 30 min treatment. Trials on sweet cherries treated with EOW in commercial packinghouses are in progress.

PROFILING OF SMALL RNAs POPULATIONS DERIVED FROM SOUR ORANGE SEEDLINGS SHOWING CROSS-PROTECTION AGAINST SEEDLING YELLOWS STRAINS OF CITRUS TRISTEZA VIRUS. M. Saponari1,4, H. Doddapaneni2, G. Loconsole3, A. Giampetruzzi3, P. Saldarelli1, R.K. Yokomi4. 1Istituto di Virologia Vegetale del CNR, Via Amendola 165/A, 70126 Bari, Italy. 2Carver Center for Genomics, Department of Biology, University of Iowa, 52242 Iowa City, IA, USA. 3Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi “Aldo Moro”, Via Amendola 165/A, 70126 Bari, Italy. 4USDA, Agricultural Research Service, 93648 Parlier, CA, USA. E-mail: m.saponari@ba.ivic.cnr.it

Cross protection against the stem pitting strain of Citrus tristeza virus (CTV) has been used to reduce losses to grapefruit and sweet orange. Recent studies have shown that cross-protection occurs between isolates of the same genotype, but its mechanism is unknown. Recently, endogenous small (s) RNAs were shown to play a role in plant stress response. During viral infections they originate either from the viral or plant genomes. High-throughput sequencing to assess sRNA profile is a powerful new technology for discovery of new approaches for disease control by understanding plant/virus interactions at the sRNA level. This technology was used to examine seedling yellows cross protection of CTV. Small RNAs fractions from a symptomless sour orange seedling (SO) infected with a mixture of T3, VT and non-standard CTV genotypes (S2) vs. SO infected with the VT component which resulted in severe seedling yellows (S1) were sequenced by Illumina Genome Analyzer II. The 21-24 nucleotide size classes dominated both libraries. Among the virus-derived sRNAs those from the 3’end genomic region, which includes the three gene silencing suppressors were predominant in both libraries, but with significant differences in the accumulation of one or the other suppressor. The largest class of the host-derived sRNAs localized in the CTV resistance gene locus; specifically they derived from the Gipsy-like retrotransposone C. More comparative bioinformatics analyses are underway to correlate sRNA profiles with the phenotypes for a better understanding of the CTV and host genes involvement and regulation.
INTRASPECIFIC VARIABILITY OF Armillaria mellea IN LOMBARDY. M. Saracchi, F. Rocchi, P. Sardi. Dipartimento di Protezione dei Sistemi Agroalimentare e Urbano e Valorizzazione delle Biodiversità, Università degli Studi, Via Celoria 2, 20133 Milano, Italy. E-mail: marco.saracchi@unimi.it

Within the Ticino River Regional Park root rots are among the factors that significantly contribute to the death of plants involved in oak decline. In most cases they are due to the action of basidiomycetes referable to the genus Armillaria. In this preliminary study 12 groups of carpophores, collected in the central part of the park and 23 fungal isolates obtained in vitro were involved in oak decline. In most cases they are due to the action of those same isolates. In most cases they are due to the action of those same isolates. The two microsatellites gave interesting results, generating respectively 9 and 12 polymorphic bands, well defined by agarose gel electrophoresis. Primer M13 produced a larger number of bands but some, especially those less than 1000 bp in size, could not be easily defined. Cluster analysis of all profiles revealed a wide genetic variability between isolates, even when coming from woods not too far from each other.

DETECTION OF OCHRATOXICENIC ASPERGILLI ON GRAPE BERRIES DURING DRYING FOR PRODUCTION OF “VIN SANTO”. A. Scala1, N. Parissi1, C. Comparini1, F. Abissi1, C. Fanelli2, M. Reverberi2, A. Ricelli3. 1Dipartimento di Biotecnologie Agrarie, Università degli Studi, Piazzale delle Cascine, 50144 Firenze, Italy. 2Dipartimento di Biologia Vegetale, Università degli Studi “La Sapienza”, Largo Cristina di Svezia 24, 00165 Roma, Italy. 3Istituto di Chimica Biomolecolare del CNR, Piazzale Aldo Moro 5, 00185 Roma, Italy. E-mail: aniele.scalo@unifi.it

Aspergillus carbonarius, A. niger and A. tubingensis are known to produce ochratoxin A (OTA), a secondary metabolite with very dangerous effects to animals and humans. The International Agency for Research on Cancer has classified OTA as a possible carcinogen to humans (group 2B). As a consequence, the European Commission has imposed regulatory limits for the maximum tolerable presence of this toxin in different foodstuffs. After cereals, grape products are accounted as a considerable source of OTA in the diet. Based on the fungal requirements of environmental temperature and relative humidity, it is easy to suppose that wines, as the Tuscan “Vin Santo”, obtained from grapes partially dried for several months to concentrate sugar content to at least 30% (w/v), are potentially at risk more than table wines. The contamination of food commodities by mycotoxins, health hazardous and carcinogenic secondary metabolites produced by different fungi, has to be closely controlled to safeguard human and animal health. The strategies applied to achieve this aim often induce environmental pollution and are toxic to the end users. Trametes versicolor is a basidiomycete known for its therapeutic effects, mainly based on the production of several anti-oxidative effects. In the culture filtrate of this fungus two different components were identified, one polysaccharidic and one proteic. The exo-polysaccharidic component was analysed by chromatographic separation techniques (Sephacyrl S-300) and 1H-NMR analysis showing the presence of highly complexed glucans (alpha- and beta-), with a MW of ~20 kDa. It can be hypothesised that these fungal polysaccharides can act as non-self signals able to modulate secondary metabolism in mycotoxigenic fungi. The proteic component has a clear antimicrobial effect which slows or completely blocks conidia germination at high

PHYTOPHTHORA PSEUDOSYRINGAE ON SWEET CHESTNUT TREES IN SARDINIA. B. Scanu, B.T. Linaldeddu, A. Franceschini. Dipartimento di Protezione delle Piante, Sezione di Patologia Vegetale, Università degli Studi, Via E. De Nicola 9, 07100 Sassari, Italy. E-mail: bscanu@uniss.it

Phytophthora pseudosyringae is a well known soil-borne root pathogen of deciduous tree species in several European countries. We now report a new finding of P. pseudosyringae on mature trees of Castanea sativa in Sardinia. Infected trees showed classic symptoms of ink disease, such as microphylly and yellowish foliage as well as necrosis of collar and root flares. Isolations were made from infected roots and soil using green apples as bait. Small pieces of tissue were cut from the lesions that developed in the apples, plated on Phytophthora selective medium (SMA) and then subcultured onto carrot agar. Cultural and morphological features were in close agreement with those described for P. pseudosyringae. Identity was confirmed by analysis of the internal transcribed spacer region (ITS1-5.8S-ITS2) of rDNA. BLAST searches in GenBank showed 100% identity with reference sequences of P. pseudosyringae. Pathogenicity was demonstrated by both stem and root inoculations of 5-month-old chestnut seedlings grown in pots. Aggressiveness was also confirmed by inoculating living chestnut logs (1 m long x 10 cm diameter). This finding represents the first record of P. pseudosyringae on C. sativa in a woodland area. Previously, this pathogen had been reported as causal agent of stem necroses only on chestnut seedlings in a nursery in Spain.

NATURAL COMPOUNDS FROM TRAMETES VERSICOLOR INHIBIT GROWTH AND MYCOTOXIN BIOSYNTHESIS IN DIFFERENT FUNGI. M. Scarpari1, A.A. Fabbri1, C. Fanelli1, P. Cescutti2, R. Rizzo2, Y. Herasimenka3, M. Punelli3, S. Zaliec1, A. Ricelli1, M. Reverberi1. 1Dipartimento di Biologia Vegetale, Università degli Studi “La Sapienza”, Largo Cristina di Svezia 24, 00165 Roma, Italy. 2Dipartimento di Scienze della Vita, Università degli Studi, Via L. Giorgieri 1, 34127 Trieste, Italy. 3Istituto di Chimica Biomolecolare del CNR, Università degli Studi “La Sapienza”, Piazzale Aldo Moro 5, 00185 Roma, Italy. E-mail: marzia.scarpari@uniroma1.it

The contamination of food commodities by mycotoxins, health hazardous and carcinogenic secondary metabolites produced by different fungi, has to be closely controlled to safeguard human and animal health. The strategies applied to achieve this aim often induce environmental pollution and are toxic to the end users. Trametes versicolor is a basidiomycete known for its therapeutic effects, mainly based on the production of several glycoproteins and exoglucans displaying anti-tumoral action and anti-oxidative effects. In the culture filtrate of this fungus two different components were identified, one polysaccharidic and one proteic. The exo-polysaccharidic component was analysed by chromatographic separation techniques (Sephacryl S-300) and 1H-NMR analysis showing the presence of highly complexed glucans (alpha- and beta-), with a MW of ~20 kDa. It can be hypothesised that these fungal polysaccharides can act as non-self signals able to modulate secondary metabolism in mycotoxigenic fungi. The proteic component has a clear antimicrobial effect which slows or completely blocks conidia germination at high
concentrations (2% w/v). Proteome maps of extracellular proteins of T. versicolor grown in two conditions, supporting or not the production of bioactive proteins, were analyzed by 2D gel electrophoresis identifying spots with qualitatively and quantitatively different profiles between the two growth conditions and subsequently characterized by MALDI-TOF. These results might be promising in view of the application of a more environmentally friendly strategy aimed at achieving an improved control of the different toxins which are often present in foods and feeds.

Intraspecific Variability of Erwinia amylovora Strains from Morocco. G. Scuderi1, E. Valentini2, C. Platania3, A.M. D’Onghia4, M. Fatmi2, G. Cirvilleri2. 1Dipartimento di Scienze e Tecnologie Fitosanitarie, Università degli Studi, Via S. Sofia 100, 95123 Catania, Italy. 2Centre International de Hautes Etudes Agronomiques Méditerranéennes, Mediterranean Agronomic Institute of Bari, Via Ceglie 9, 70010 Valenzano (BA), Italy. 3Dipartimento Protection des Plantes, Institut Agronomique et Vétérinaire Hassan II, Complexe Horticole d’Agadir, Morocco. E-mail: gcirvill@uniss.it

Fire blight disease, caused by Erwinia amylovora, detected for the first time in Morocco in 2006, has spread rapidly throughout the main orchards areas. Surveys were carried out in these regions to evaluate the current situation of the disease in the country. In 2009 the disease was observed in 71 farms of different regions, i.e. Melkes, El Hajeb, Sefrou, Ifrane, Taounate and Khenifra. In 2009 the disease was observed in 71 farms of different regions, i.e. Melkes, El Hajeb, Sefrou, Ifrane, Taounate and Khenifra. In 2009 the disease was observed in 71 farms of different regions, i.e. Melkes, El Hajeb, Sefrou, Ifrane, Taounate and Khenifra. In 2009 the disease was observed in 71 farms of different regions, i.e. Melkes, El Hajeb, Sefrou, Ifrane, Taounate and Khenifra.

Diagnostic Analyses on Fourth Range Production in the Province of Salerno. L. Sigillo, V. Senape, G. Serratore, V. Spina, R. Bravi. Ente Nazionale Sementi Elette, SS 18 km 77.700, 84091 Baitapaglia (SA), Italy. E-mail: lsigillo@ense.it

The production of species for fourth range market has strongly increased in the last years. Important economical losses are reported due to the widespread presence of bacterial and fungal diseases. In our laboratory, 73 samples of rocket (Eruca sativa), 67 of lettuce (Lactuca sativa), 19 of corn salad (Valerianella locusta), 9 of spinach (Spinacia oleracea) and many other minor species were analysed from 2005 to 2010. Among these, 108 and 68 seed and symptomatic plant samples were analysed for detection of the most important pathogens. Rocket seed samples were contaminated by Xanthomonas campestris (15%) and pathogenic strains of Fusarium oxysporum (8%). Instead, 50% and 20% of rocket plants were infected by F. oxysporum and X. campestris, respectively. F. oxysporum, Verticillium sp. and Rhizoctonia sp. occurred rarely on lettuce seeds or plant tissues (about 3%). Generally, corn salad, beet and spinach seeds were healthy. X. campestris was detected only in a sample of corn salad. In conclusion, an increase of F. oxysporum and X. campestris incidence on rocket has been observed in the last years. This increase is probably due to the diffusion of monoculture system and the use of contaminated seeds in the fourth range production.

Identification and Pathogenicity of Botryosphaeria spp. Associated with Grapevine Trunk Diseases in Sardinia. S. Serra, B. Scanu, A. Schiaffino, A. Deidda, B.T. Linaldeddu. Dipartimento di Protezione delle Piante, Sezione di Patologia Vegetale, Università degli Studi, Via E. De Nicola 9, 07100 Sassari, Italy. E-mail: salvase@uniss.it

Grapevine spur, cordon, and trunk dieback constitute a serious economic problem in Sardinian vineyards. Recent studies have indicated that several grapevine trunk pathogens belong to the genus Botryosphaeria. Thus, during 2009 a field survey was carried out to study Botryosphaeria spp. involved in the aetiology of grapevine trunk diseases in several vineyards located in north Sardinia (Italy). Botryosphaeria spp. were the most common fungal species isolated from symptomatic tissues. Four species (Botryosphaeria australis, B. obtusa, B. parva and Lasiodiplodia theobromae) were identified on the basis of morphological and cultural features. The identity of B. australis was also confirmed by analysis of nucleotide sequences of the internal transcribed spacer region (ITS1-5.8S-ITS2) of 16S rDNA. BLAST searches in GenBank showed 100% similarity with reference sequences of B. australis and confirmed differences in three nucleotide positions from sequences of Neofusarium luteum, a closely related Botryosphaeria species. Pathogenicity of all species was assessed by inoculation of excised green grapevine shoots of cvs Vermentino and Cannonau under controlled laboratory conditions. B. australis and L. theobromae were more virulent than the other species. Inoculated fungi were successfully reisolated from all infected tissues, thus fulfilling Koch’s postulates. These findings confirm the active role of Botryosphaeria spp. in the aetiology of grapevine trunk diseases. For each fungal species, except for B. obtusa, this represents the first record on grapevine in Sardinia.

Distribution of Three Different Isolates of Citrus tristeza Virus in Southern Italy. G. Sorrentino1, S. Davino2, M. Davino3. 1CRA, Istituto Sperimentale per l’Agronomia di Cultura, Corso Savoia 190, 95024 Acireale (CT), Italy. 2Dipartimento di Scienze Entomologiche, Fitopatologiche, Microbiologiche, Agrarie e Zootecniche, Sezione di Patologia Vegetale e Microbiologia Agraria, Università degli Studi, Viale delle Scienze, 90128 Palermo, Italy. 3Dipartimento di Scienze e Tecnologie Fitosanitarie, Sezione di Patologia Vegetale, Università degli Studi, Via Santa Sofia 100, 95123 Catania, Italy. E-mail: davino@unipa.it

The discovery of large concentrations of different citrus species affected by the Citrus tristeza virus (CTV) in southern Italy has provided the opportunity to investigate the distribution of three main viral isolates. The survey was carried out in different farms mainly in the Baś area (Catania province). All trees in each area were sampled yearly from 2001 to 2008 in May and September. Four young apical shoots were collected and analyzed by DAS-ELISA or DTBIA. Molecular tests (SSCP and p20 and
MOLECULAR CHARACTERIZATION BY IGS SEQUENCING OF FORMAE SPECIALES OF FUSARIUM OXYSPO-RUM PATHOGENIC TO LAMB’S LETTUCE AND ROCK-ET. D. Spadaro1,2, K. Srinivasan1, G. Giraldi3, M.L. Gullino1, A. Garibaldi1. 1Centro di Competenza per l’Innovazione in Campo Agro-Ambientale (AGROINNOVA), Università degli Studi di Torino, 10095 Grugliasco (TO), Italy. 2Dipartimento di Valorizzazione e Protezione delle Risorse Agroforestali, Università degli Studi di Torino, Via Leonardo da Vinci 44, 10095 Grugliasco (TO), Italy. E-mail: davide.spadaro@unito.it.

Twenty-nine isolates of Fusarium oxysporum collected from wilted lamb’s lettuce plants (Valerianella olitoria) and 36 Fusarium oxysporum isolates collected from wilted rocket plants (Eruca vesicaria L., syn. E. sativa, cv. ‘Rucola coltivata’), including ATCC strains, were examined for differences in the nucleotide sequences of the ribosomal DNA (rDNA) intergenic spacer (IGS) region, approx. 2.5 kb long in the isolates analyzed. The isolates were tested for pathogenicity on lamb’s lettuce or rocket in glasshouse. Results showed that the isolates were slightly, moderately and highly pathogenic except for four non-pathogenic isolates from lamb’s lettuce. Most of the isolates from wilted rocket and lamb’s lettuce plants collected in Italy were very similar to F. oxysporum Esp. rapabij. In conclusion, the analysis of the IGS sequences revealed that the isolates studied had different origins and that phylogeny and pathogenicity were related. Non-pathogenic isolates differed genetically from those with low, moderate and high virulence. To our knowledge, this is the first report of differentiation of formae specialis of F. oxysporum on rocket and lamb’s lettuce by IGS sequence analysis.

TESTING THE EFFICACY OF A TRANPOSON-BASED DOUBLE COMPONENT SYSTEM TO TAG PATHOGENICITY GENES IN THE WHEAT PATHOGEN FUSAR-IUM CULMORUM. F. Spanu1, B. Scherm1, V. Balmas1, A. Marcello1, M. Dufresne2, M.J. Daboussi3, Q. Miglioli1. 1Dipartimento di Protezione delle Piante, Unità di Ricerca Istituto Nazionale Biostrutture e Biosistemi, Università degli Studi, Via E. De Nicola 9, 07100 Sassari, Italy. 2Institut de Biologie des Plantes, Bâtiment 630, Université Paris Sud 11, F-91405 Orsay Cedex, France. 3Institut de Génétique et Microbiologie, Bâtiment 400, Université Paris Sud 11, F-91405 Orsay Cedex, France. E-mail: qmiglioli@uniss.it.

As it has been already achieved for other economically relevant Fusarium species (i.e., Gibberella zeae, F. sporotrichioides and F. oxysporum), the genome of F. culmorum, incitant of crown and foot rot on wheat and type B trichothecene producer, is now being sequenced. Based on available data, the number of predicted genes present in this genome is estimated to exceed 10,000. For many genes the function is yet unknown and consequently there is a strong need for a high-throughput method for functional genomic analysis. We tested the efficacy of two double component systems based on defective transposable elements mobilised by a separate source of transposase. In both systems, the tagging element is inserted into the first intron of the A. nidulans niaD gene, and a phenotypic assay for excision allows recovery of excision events on minimal medium containing nitrate as the sole nitrogen source. The first system is based on a bop copy that has been engineered by insertion of the A. nidulans bph gene conferring resistance to hygromycin B. This defective element is mobilized by the bop transposase under the control of the constitutive A. nidulans gpdA promoter on a separate vector. The selectable marker facilitates the recovery of strains with a reinserted element on hygromycin B-containing medium. The second system is based on the ability of the impala transposase to transactivate nimm1, which shows features of MITEs. Preliminary experiments demonstrate that the excised bop element is unable to reinsert in the genome of F. culmorum, making this tool unsuitable for the pathogen.

USE OF MEDITERRANEAN PLANT ESSENTIAL OILS TO CONTROL POSTHARVEST ROts CAUSED BY BOTRYTIS CINEREA AND PEnICILLIUM EXPANSUM ON FOUR CULTIVARS OF APPLES. D. Spadaro1,2, J.G. Lopez-Reyes1, M.L. Gullino1, A. Garibaldi1. 1Centro di Competenza per l’Innovazione in Campo Agro-Ambientale (AGROINNOVA), Università degli Studi di Torino, 10095 Grugliasco (TO), Italy. 2Dipartimento di Valorizzazione e Protezione delle Risorse Agroforestali, Università degli Studi di Torino, Via Leonardo da Vinci 44, 10095 Grugliasco (TO), Italy. E-mail: davide.spadaro@unito.it.

The efficacy of essential oils of different Mediterranean plants was evaluated on apple cvs Golden Delicious, Granny Smith, Red Chief, and Royal Gala, to control Botrytis cinerea and Penicillium expansum in postharvest. The essential oils of basil (Ocimum basilicum), fennel (Foeniculum vulgare), lavender (Lavandula officinalis), marjoram (Origanum majorana), oregano (Origanum vulgare), peppermint (Mentha piperita), rosemary (Rosmarinus officinalis), sage (Salvia officinalis), savory (Satureja montana), thyme (Thymus vulgaris) and wild mint (Mentha arvensis) were tested at different concentrations. Fruits were artificially wounded and inoculated with a suspension at 1x10^5 conidia/ml of each pathogen. After 12 h, emulsions at 1% and 10% of each essential oil were introduced into each inoculated wound. Tebuconazole chemical control and an inoculated control were also included. All treated fruit were stored at 4°C. After 15 and 30 days, the diameter of rots was measured. Results showed that the efficacy of the essential oils tested was cultivar- and storage time-dependent. Treatments with essential oils from oregano, savory, and thyme showed significant efficacy in all apple cultivars tested. Treatments with essential oil emulsions at 10% were phytotoxic for all apple cultivars evaluated.

CHARACTERIZATION OF p20 AND p23 GENES IN COR- SICAN ISOLATES OF CITRUS TRISTEZA VIRUS. M. Tessitori1, J. P. Thermo2, M. Davino3, S. Davino3. 1Dipartimento di Scienze Fitosanitarie, Sezione di Patologia Vegetale, Università degli Studi, Via S. Sofia 100, 95123 Catania, Italy. 2Unité Gene-
SYNERGISTIC INTERACTION BETWEEN POTATO SPINDE TUBER VIROID AND CUCUMBER MOSAIC VIRUS IN TOMATO. E.M. Torcetti, B. Navarro, F. Cillo, F. Di Serio. Istituto di Virologia Vegetale del CNR, Via Amendola 165/A, 70126 Bari, Italy. E-mail: em.torcetti@ba.ivv.cnr.it

Potato spindle tuber viroid (PSTVd), a nuclear replicating viroid included in the EPPO A2 list of quarantine pests, causes severe diseases to potato and tomato. In the last few years, this viroid has been detected in several symptomless ornamental solanaceous species in many European countries, producing a general alert on the risk of PSTVd outbreaks in horticultural crops. The recent report of symptomatic tomato plants infected by PSTVd in Italy has increased such concerns. Since tomato is also a natural host of Cucumber mosaic virus (CMV), its response to mixed infections by this virus and PSTVd was investigated. Here, we report the synergistic interaction of contemporary infections by a mild strain of PSTVd and the Fny strain of CMV in tomato cvs. Micro-Tom and UC32. Plants infected by both pathogens showed severe stunting, delay of flowering, and frequent systemic necrotic lesions that completely impaired plant growth. These symptoms were never observed in PSTVd and CMV single-infected plants, which only showed slight stunting and leaf deformation, respectively. Data about the accumulation levels of each pathogen in single- and mix-infected hosts will be discussed in the context of a possible role of RNA silencing in the synergistic interaction. These findings highlight the need of implementing control measures to restrain PSTVd spread in Europe.

ALTERNARIA LEAF SPOT ON OKRA IN TANZANIA: PROSPECTS FOR BIOLOGICAL CONTROL IN SUSTAINABLE AGRICULTURE. E. Turco⁴, M. Galli⁴, D. Bocciolini⁴, S. Tofani⁴, A. Ragazzi⁴. ¹Istituto per la Protezione delle Piante (IPP) del CNR, Via Madonna del Piano 10, 50019 Sesto Fiorentino (FI), Italy. ²Dipartimento di Bioprotezione Vegetale, Sezione di Protezione delle Piante, Università degli Studi, Piazzale delle Cascine, 28, 50144 Firenze, Italy. ³Cooperativa Agricola di Legnago, Via di Sollicciano 13a, 50142 Firenze. E-mail: e.turco@ipp.cnr.it

The “Progetto Tanzania” was funded in 2006 by the Cooperativa Agricola di Legnago, the oldest and largest agricultural cooperative in Tuscany. In 2007, the Faculty of Agriculture of the University of Florence joined the project. Besides the humanitarian and economical assistance to “Villaggio della Speranza” in Dodoma and San Gaspare hospital in Itigi, the project is involved in improving and upgrade livestock and crop plants (mainly vegetables) of local communities. Okra (Abelmoschus esculentus L.), a traditional food plant in Africa with a high nutritive value, is essential to the daily diet of local population, consisting almost exclusively of carbohydrates. Eco-compatible measures to control and eradicate fungal diseases are therefore required to manage sustainable agriculture and rural development. Periodical surveys of the health status of okra plantations in the area of Itigi (Manyoni district, 4°2’S 34°29’E, 1300 m a.s.l.) revealed leaf spots and blight caused by Alternaria alternata. The biological control of the disease by the application of Trichoderma viride and Epigonium nigrum is the scope of this report. Field experiments were carried out during the rainy season of 2009. Leaf spots and blight, before and after different treatments with the fungal antagonists, were scored and DI (disease score index) using a three class scale was assessed over 8 weeks. DI above 2 was observed starting 6 weeks after pathogen inoculation. Leaf symptoms caused by A. alternata were not significantly reduced by the presence of either T. viride or E. nigrum. The efficacy of fungal antagonists and validity of the experimental protocol are discussed in relation to the environmental and climatic conditions.

PRELIMINARY RESULTS ON THE EFFECT OF GOSSEPOL ON FEW AUSTRALIAN FUSARIUM OXYSPORUM F. SP. VASINFECTUM ISOLATES. E. Turco¹, A. Ragazzi², B. Wang³, C.L. Brubaker³. ¹Istituto per la Protezione delle Piante (IPP) del CNR, Via Madonna del Piano 10, 50019 Sesto Fiorentino (FI), Italy. ²Dipartimento di Bioprotezione Vegetale, Sezione di Protezione delle Piante, Università degli Studi, Piazzale delle Cascine, 28, 50144 Firenze, Italy. ³Centre for Plant Biodiversity Research, CSIRO Plant Industry, Clunies Ross Street and Barry Drive, GPO Box 1600, Canberra ACT 2601, Australia. ⁴Bayer CropScience, Technologypark 38, B-9032, Gent, Belgium. E-mail: e.turco@ipp.cnr.it

The Australian cotton industry is an important component of the Australian economy. Researchers and farmers are working to build sustainable production based on new cotton cultivars with high fibre quality and yield coupled with improved pest and disease resistance. Fusarium wilt of cotton (caused by Fusarium oxysporum f. sp. vasinfectum or FOV) appeared unexpectedly in Australia in 1995 and represents a considerable threat to the sustainability of cotton production. Therefore, considerable efforts are directed toward developing and obtaining new cotton varieties with enhanced levels of FOV tolerance. In some breeding programs attention as turned to native Australian Gossypium species with variable concentrations of gossypol, a terpenoid aldehyde with a suspected potential to improve resistance to Fusarium wilt in cotton cultivars. Because the Australian FOV isolates are genetically distinct from FOV isolates found outside Australia and the probable co-evolution pathogen and wild cotton cultivars, the susceptibility of FOV conidia to varying levels of gossypol was deter-
crawled for fourteen isolates collected from different locations in the cotton growing regions of Australia. Four gossypol concentrations (0, 4, 10 and 20 mg/l) and five FOV isolates (two from each vegetative compatibility group, VCG 0111 and VCG 0112, and a fifth isolate not yet assigned to a VCG) were here considered. Different conidial germination rates were observed over 8 h among the isolates tested. The results are discussed according to the vegetative compatibility group to which the isolates belong and to their different virulence on cotton germlasm.

**Ophiostomatoidei fungi associated with Ips acuminatus (Coleoptera: Curculionidae) in the Italian Alps. C. Villari¹, A. Battisti¹, P. Capretti², M. Faccioli¹, ¹Dipartimento di Agronomia Ambientale e Produzioni Vegetali, Università degli Studi di Palermo, Agrigoli, Viale dell’Università 16, 35020 Legnaro (PD), Italy. ²Dipartimento di Biotecnologie Agrarie, Sezione di Protezione delle Piante, Università degli Studi, Piazzale delle Cascine 28, 50144 Firenze, Italy. Email: caterina.villari@unipd.it

Most bark beetles are associated with symbiotic fungi, assisting beetles in killing healthy trees. The Scots pine beetle Ips acuminatus is reported to be associated with Ophiostoma bruno-neo-ciliatum, O. ips and Ambrostella macrospora. The first two species are weak blue-stain pathogens thought to be involved in lowering the critical threshold of beetle attack density. A. macrospora is a non-pathogenic fungus used as a direct food source for the larvae. This study aims at explaining the role of the fungi associated with I. acuminatus in the decline of alpine Scots pine stands, exploring the possible quantitative and qualitative variations of fungal flora. Epidemic and endemic beetle populations were sampled in early spring 2009 from three sites at each of six locations selected across the Italian Alps. Adult beetles were obtained from logs incubated in rearing cages and a subset was washed in 1% Tween 80. The washing solution was then siphoned. A. macrospora was never isolated, probably because it is difficult to culture. The insect pathogenic fungus Ambrostella was isolated with high frequency. No differences were found between epidemic and endemic populations. Molecular markers specific for each fungal species were developed, in order to simplify and speed up the species identification. A. macrospora included, and to compare the fungal communities associated with I. acuminatus among populations.

**Harzianic acid, a siderophore from Trichoderma harzianum with antifungal and plant growth promoting activity. F. Vinale¹, G. Flematti², K. Sivasithamparam³, E.L. Ghisalberti⁴, R. Marra¹, M. Ruocco¹, S.L. Woo⁵, S. Lanzuise¹, M. Nigro¹, M. Lorito¹, ¹Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi “A. Moro”, Via Amendola 163/A, 70126 Bari, Italy. ²Dipartimento di Arboricoltura Botanica e Patologia Vegetale, Università degli Studi di Napoli ‘Federico II’, 80055 Portici (NA), Italy. ³Istituto di Protezione delle Piante del CNR, Via Università 133, 80055 Portici (NA), Italy. E-mail: frinale@unina.it

Many strains of the fungus Trichoderma are well known producers of secondary metabolites having antibiotic activity. Their production varies in relation to: (i) the specific compound; (ii) the species and strain; (iii) the presence/absence of other microbes and; (iv) the balance between elicited biosynthesis and biotransformation rates. The involvement of secondary metabolites in the ability of Trichoderma spp. to activate plant defence mechanisms and regulate plant growth has recently been investigated. A T. harzianum strain, isolated from composted hardwood bark in Western Australia, produces a metabolite that showed antifungal activity in vitro against Pythium irregularure, Sclerotinia sclerotiorum and Rhizoctonia solani. The structure and absolute configuration of the fungal metabolite, harzianic acid, was determined by X-ray diffraction studies. The effect of harzianic acid on plant growth promotion was evaluated by treating seeds of canola (Brassica napus) with different concentrations of the purified Trichoderma metabolite and measuring the stem length of developing seedlings. Applications of the compound at concentrations of 100, 10 and 1 ng per seed, stimulated plant growth as indicated by an increase of 42%, 44% and 52% of stem length, respectively, compared with the untreated control (water). However, this same harzianic acid compound also inhibited plant growth up to 45% and 33% (stem length) when augmented concentrations of 100 and 10 μg, respectively, were applied to the seed. Additionally, we have discovered that this tetrac acid is capable of binding essential metals such as Fe³⁺ with a good affinity, providing an important mechanism for iron solubilization, influencing nutrient availability in the soil environment to other microorganisms and to the plant.

**EVALUATION OF ANTIFUNGAL ACTIVITY OF “BIO-PROTEGE”, A MIXTURE OF SODIUM BICARBONATE AND SILICIC DIOXIDE, AGAINST MAJOR POSTHARVEST FUNGAL PATHOGENS OF FRUIT AND VEGETABLES. K. Youssef¹, P. Di Primo², M. Congilione³, A. Ippolito¹. ¹Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi “A. Moro”, Via Amendola 163/A, 70126 Bari, Italy. ²Decco Italia srl, Contrada Barriera, 95032 Belpasso (CT), Italy. E-mail: ippolito@agr.uniba.it

In recent years, public demands to reduce pesticide use, stimulated by increased awareness of environmental and health issues associated with fungicide residues, as well as the development of fungicide-resistant strains of pathogens, have created the need to find and develop safe alternative control means. The effectiveness of a formulation based on a mixture of sodium bicarbonate and silicic dioxide (Bioprotege, Decco Italia) as a possible alternative to synthetic fungicides for controlling postharvest pathogens was evaluated. Bioprotege at increasing concentrations (0, 0.1, 0.2, 0.3, 0.4, 0.5, 1, 2, 4, 6%, w/v) mixed with potato dextrose agar (PDA) medium was tested. The concentration causing 50% growth reduction (ED₅₀) SAS probit analysis) and the minimum inhibition concentration (MIC) values, were also evaluated. The ED₅₀ was 0.11, 0.10, 0.08, 0.08, 0.25, 0.53, and 1.3% (w/v) for Penicillium digitatum, P. italicum, P. ulasiense, Monilinia laxa, Phytophthora nicotianae, Geotrichum candidum, and Botrytis cinerea, respectively. Complete growth inhibition of the same pathogens, except for B. cinerea, was achieved at 0.3, 0.3, 0.2, 0.2, 2, and 2% (w/v), respectively. No MIC was found for B. cinerea since complete inhibition was not achieved till 6%. The effect of Bioprotege was primarily fungistatic because when fungal discs showing no growth were re-seeded onto fresh PDA, their growth resumed. Bioprotege confirmed its activity in vivo trials performed on fruits of different citrus species. Overall, the results show the potential benefits of Bioprotege for controlling postharvest pathogens attacking fruit and vegetables.
RESISTANCE OF MUSKMELON TO *Fusarium* WILT. A. Zechini D’Aulerio, R. Roberti, F. Piattoni, G. Servidio. Dipartimento di Protezione e Valorizzazione Agroalimentare, Università degli Studi, Viale Fanin 46, 40126 Bologna, Italy. E-mail: aldo.zechini@unibo.it

In Italy, *Fusarium oxysporum* f. *melonis* induces an important disease of muskmelon. Four races of this pathogen are currently known, i.e. 0, 1, 2 and 1-2. Varietal resistance is the only reliable approach for preventing wilt induced by race 0, 1 and 2, which were studied in this work. Investigations carried out between 2008 and 2010 aimed first at verifying the resistance of the cultivars certified as “resistant” by seed companies. To this aim, three “resistant” cultivars (Bingo, Giusto and Sweetness) were compared with three susceptible cultivars (Cantalupo, Harper and Retato degli Ortolani), by infecting plants with each of the three pathogenic races (spore suspension: $10^6$ conidia/ml). Among the “resistant” cultivars, one resulted totally resistant (Bingo), whereas Giusto and Sweetness showed a partial resistance (disease severity: 10%). Similar differences were observed among the susceptible cultivars for disease severity was close to 100% in Retato whereas Cantalupo and Harper it ranged between 80 and 85%. Differences in pathogenicity were also observed among the pathogenic races tested: race 0 and 2 caused the highest disease severity in Bingo and Retato. Attempts were made to investigate the mechanisms involved in plant resistance. We found that root exudates did not affect neither conidial germination rate nor mycelial growth. Currently, studies on the development of promycelium are being carried out. Further investigations will aim at highlighting possible differences between resistant and susceptible cultivars, in post-infection root morphological modifications, by means of fluorescence microscopy techniques.