

BIOLOGICAL AND INTEGRATED CONTROL OF *ASPERGILLUS CARBONARIUS*. P. Battilani¹, V. De Cicco², F. De Curtis², L. Macchia³, A. Pietri¹, A. Ritieni⁴ and A. Silva¹. ¹Istituto di Entomologia e Patologia Vegetale, Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, 29100 Piacenza, Italy. ²Dipartimento di Scienze Animali Vegetali e dell'Ambiente, Università degli Studi del Molise, Via De Sanctis, 86100 Campobasso, Italy. ³Cattedra di Allergologia ed Immunologia Clinica, Piazza Giulio Cesare, 70124 Bari, Italy. ⁴Chimica e Biotecnologia delle Fermentazioni, Dipartimento di Scienza degli Alimenti, Università degli Studi di Napoli "Federico II", Via Università 100, Parco Gussone ed. 84, 80055 Portici (NA), Italy. E-mail: paola.battilani@unicatt.it

The main purpose of this research was to minimize ochratoxin A (OTA) content in grapes. It followed a "farm to fork" approach and was focussed on protecting consumer's health. Biocontrol agents (BCAs) active against *A. carbonarius* were studied for their application in organic and integrated farming. The possible effect of BCAs on wine making, the identification of OTA degradation products and their toxicity in mammalian cell models, compared to OTA toxicity, were also studied. During the first year, a biocontrol agent (*Aureobasidium pullulans* LS30) was applied in two different vineyards, located in Molise and Puglia (southern Italy) both in integrated and organic farming. The BCA became well established in the vineyards, especially in integrated farming, and its population increased over time. No conclusions could be drawn on OTA control because of the very low level of contamination in 2006. The same BCA was used during grape drying, but it showed a limited resistance to available water reduction and little effect on *A. carbonarius* control. Some differences were observed in musts and wines produced from grapes subjected to different disease control approaches, but further results are necessary for ultimate conclusions. Specific studies were carried out, finalised to multi-fungicide residues detection. Analytic and spectrometric conditions using LC/MS/MS were optimised as well as conditions to conduct toxicity trials. Three cell lines were used to test OTA toxicity: RBL-1, HL-60 and HepG2. The latter was the most sensible to OTA, but difficult to grow, while HL-60 showed an opposite behaviour. RBL-1 was considered the best cell line, as it showed an acceptable susceptibility and was readily grown.

BIOCHEMICAL AND MOLECULAR CHARACTERIZATION OF SYSTEMIC INDUCED RESISTANCE IN PINE. P. Bonello. Department of Plant Pathology, The Ohio State University, 201 Kottman Hall, 2021 Coffey Road, Columbus, OH 43210, USA. E-mail: bonello.2@osu.edu

Coniferous trees are often dominant species in both boreal and temperate forests, wherein they play critical roles in ecosystem function. In natural environments, ecosystem stability appears to be the norm, notwithstanding the co-occurrence of microbial species inherently capable of killing their host trees. Adaptive plasticity of host trees involving inducible mechanisms of resistance against invading organisms is likely to play a crucial role in these interactions. We hypothesize that systemic induced resistance (SIR) represents a common and important phenomenon in coniferous trees, allowing for a balanced allocation of resources between growth and defense. Here we present metabolomics and proteomics-based data illustrating the role of secondary metabolism (including both phenolic and terpenoid networks) and defense proteins in the expression of SIR in the Austrian pine/*Diplodia pinea* model pathosystem. We found that: (i) SIR is bidirectional; (ii) phenolics, particularly phenolic glycosides, are likely to play a key role in SIR, while terpenoids do not;

(iii) defense proteins do not appear involved in resistance while heat shock proteins are upregulated in systemically induced trees. These results are discussed in the context of current understanding of plant defense mechanisms and plant defense theory.

MODELLING ASCOSPORIC INFECTIONS BY *UNCINULA NECATOR*. T. Caffi and V. Rossi. Istituto di Entomologia e Patologia Vegetale, Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, 29100 Piacenza, Italy. E-mail: tito.caffi@unicatt.it

Uncinula necator (Schw.) Burr. is the causal agent of powdery mildew, a major grapevine disease throughout the world. In many grape-growing areas, the source of primary inoculum is essentially provided by cleistothecia. These ascocarps are formed from late summer on the affected host tissue and are dispersed by rain splashes to the bark, where they overwinter. Ascospores are repeatedly released from cleistothecia in spring and cause (primary) infection on the leaves. Primary infections represent the starting point for the powdery mildew epidemics driven by the asexual infection cycles. Epidemiological models have been elaborated to simulate secondary infections, while no models are available for the ascosporic infections in spite of their key role in the pathogen's life cycle. A dynamic mechanistic model simulating *U. necator* ascosporic infections was elaborated according to principles of the systems analysis. The model uses data of air temperature, relative humidity, leaf wetness duration, rainfall, and vapour pressure deficit to calculate: (i) dynamics of ascospore maturation; (ii) ascospore dispersal events; (iii) infection efficiency of ascospores; (iv) probable onset of disease symptoms, and (v) latent periods between infection and production of asexual spores. Model validation is ongoing in environment-controlled conditions and vineyards in different grape-growing areas. Appearance of symptoms from ascosporic infections is carefully detected and compared with model simulations. Preliminary results are very promising.

IDENTIFICATION OF A LYTIC GRAM-NEGATIVE BACTERIUM SHOWING APPRECIABLE ANTAGONISTIC ACTIVITY TOWARDS A BROAD RANGE OF FUNGAL SPECIES. V. Cappio¹, G. Puopolo¹, V. Battaglia¹, A. Raio² and A. Zoina¹. ¹Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università degli Studi di Napoli "Federico II", Via Università 100, 80055 Portici, Italy. ²Istituto per la Protezione delle Piante del CNR, Via Università, 133, 80055 Portici, Italy. E-mail: puopolo@unina.it

The production of lytic enzymes such as chitinases, proteases and lipases is often associated with the ability of several biological control agents to inhibit the development of phytopathogenic fungi. A Gram-negative bacterial isolate named PG4, recovered from tobacco rhizosphere, showed high lipase, protease and chitinolytic activities *in vitro*. PG4 was identified by using Biolog Test and the analysis of its 16S rDNA sequence. On the basis of the latter test, bacterial isolate PG4 was identified as belonging to the *Lysobacter* genus, a group of α -proteobacteria related to the family *Xanthomonadaceae*. 16S rDNA sequence of strain PG4 clustered with the related sequences of *Lysobacter antibioticus* and *L. gummosus*. However, the Biolog Test failed to give a precise identification as no *Lysobacter* species has been included in Biolog Databases. *Lysobacter* sp. strain PG4 was evaluated *in vitro* against a broad range of fungal isolates including beneficial, saprophytic and phytopathogenic species. In these tests strain PG4 showed a high inhibitory activity that can be attributed to the production of diffusible antibiotic compounds. The attitude to control *Rhizoctonia solani*, *Fusarium oxysporum* f. sp. *lycopersici*

ci and *F. oxysporum* f. sp. *radicis lycopersici* on tomato plantlets was assessed by using a predictive *in vivo* approach. In these trials, strain PG4 reduced significantly disease severity in all the cases and had a stimulatory effect on root growth. These preliminary results showed that *Lysobacter* sp. strain PG4 represents a new potential biological control agent able to counteract phytopathogenic fungi responsible for important tomato diseases.

FUNGI INVOLVED IN DAMPING OFF OF CUCURBITACEOUS PLANTS IN APULIA. A. Carlucci¹, M. Mucci¹, F. Lops¹, M.L. Raimondo¹, S. Somma², A. Moretti² and S. Frisullo¹. ¹Dipartimento di Scienze Agronomiche, Chimica e Difesa Vegetale, Università degli Studi, Via Napoli 25, 71100 Foggia, Italy. ²Istituto di Scienze delle Produzioni Alimentari del CNR, Via Amendola, 122/O, 70126 Bari, Italy. E-mail: s.frisullo@unifg.it

Surveys of diseases caused to cucurbits by telluric pathogens were carried out during every summer in 2003-2005. *Plectosporium tabacinum*, *Acremonium cucurbitacearum*, *Macrophomina phaseolina*, *Verticillium dahliae*, *Rhizoctonia solani* and *Rhizopycnis vagum* were the pathogens most frequently recovered from the root system of wilted plants. Some severely pathogenic *Fusarium* species were also isolated. Because of their morphological similarity, *Fusarium* isolates were characterized at the species level by molecular tools. In this note, the first occurrence of *F. redolens* on melon and *F. hostae* on watermelon is reported. Finally, pathogenicity tests carried out under controlled conditions showed that *F. oxysporum*, *F. solani*, *F. redolens*, *F. proliferatum* and *F. avenaceum* caused severe damping off of melon seedlings and wilt symptoms on melon plants cv. Amarillo oro.

ENZYMATIC FUNCTIONS AND MYCOPARASITISM VERSUS SCLEROTIA IN TRICHODERMA. V. Catalano¹, S. Sarrocco¹, M. Vergara² and G. Vannacci¹. ¹Dipartimento di Coltivazione e Difesa delle Specie Legnose "G. Scaramuzzi", Sezione di Patologia Vegetale, Università degli Studi, Via del Borghetto 80, 56124 Pisa, Italy. ²Scuola Normale Superiore di Pisa, Piazza dei Cavalieri 7, 56126 Pisa, Italy. E-mail: vcatalano@agr.unipi.it

The mycoparasitic activity of *Trichoderma* spp. against sclerotial phytopathogenic fungi is a powerful tool for a biocontrol agent, since these highly resistant vegetative structures represent the pathogen primary survival form in soil. Parasitic interactions established by *Trichoderma* against sclerotial fungi have been investigated mainly by histological and biochemical analysis. Several enzymatic activities have been related to sclerotia mycoparasitism, such as enzymes degrading cell wall components (chitinase, cellulase) or phenolic compounds (laccase, lignin and melanin degrading enzymes), proteases and lipases. The availability of a *T. virens* isolate (I10) transformed with genes coding for fluorescent proteins, red (I10DsRed) and green (I10GFP), allowed to monitor *Trichoderma* as antagonist in natural environments (Mikkelsen *et al.*, *FEMS Microbiol. Let.* **223**: 135-139, 2003.) and to follow *in vitro* sclerotia colonisation by *Sclerotium rolfii* and *Sclerotinia sclerotiorum* (Sarrocco *et al.*, *Mycol. Res.* **110**: 179-187, 2006). The present work aims at identifying enzymatic activities related to the mycoparasitic ability by a genetic approach. Mutants of I10DsRed and I10GFP were selected for specific functions in order to evaluate their sclerotia colonisation ability. *S. sclerotiorum* and *Botrytis cinerea* were used as phytopathogenic sclerotia-producing fungi. Mutants were screened for cellulase, chitinase, lipase, protease and laccase activity. The correlation between defective traits and sclerotia colonisation is under investigation.

SEED DISINFESTATION BY OZONE TREATMENTS. F. Ciccarese¹, N. Sasanelli², T. Ziadi¹, M. Gallo¹ and I. Papajova³. ¹Dipartimento di Biologia e Patologia Vegetale, Università degli Studi, Via G. Amendola 165/A, 70126 Bari, Italy. ²Istituto per la Protezione delle Piante del CNR, Via G. Amendola 122/O, 70126 Bari, Italy. ³Parasitological Institute, SAV, Hlinkova 3, Košice, Slovak Republic. E-mail: fcicare@agr.uniba.it

Seed contamination by fungi may be dangerous both when seeds used for sowing or when they are stored for human or animal consumption. Several diseases of horticultural crops derive from infected seeds that act as carriers of infections. For each plant species at least a seed-transmitted disease is known. Superficial contamination during storage and marketing of seeds intended for human or animal consumption derives from infections contracted in the field primarily by saprophytic fungi as *Aspergillus* spp. and *Penicillium* spp. that produce dangerous metabolites for human or animal health (aflatoxin B1, B2, G1, G2, M1 and ocratoxin A). This work reports the results of two experimental trials using ozone treatments for seed disinfestation. A trial concerned seeds of a local pea variety obtained in Southern Italy from farmers, who produce their own seed. The second trial was carried out on 1-year-old stored wheat kernels cv. Cappelli, for industrial use. In both experiments ozone at 120 ppm concentration was applied as a mixture of ozone + air for 1, 1.5 and 3 min. Untreated seeds were used as control. Treated and untreated seeds were plated on PDA in Petri dishes for 4 days. The developed fungi were identified and counted. *Fusarium oxysporum*, *Alternaria alternata* and *Penicillium* spp. were frequently observed. The longest exposure time to ozone was effective in seed disinfestation without influencing germination.

TOLERANCE OF TOMATO AND WILD SOLANUM spp. ACCESSIONS TO CUCUMBER MOSAIC VIRUS AND SATELLITE RNA INFECTIONS. F. Cillo¹, M. Pasciuto¹, C. De Giovanni², M.M. Finetti-Sialer¹, L. Ricciardi² and D. Gallitelli¹. ¹Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi e Istituto di Virologia Vegetale del CNR, Sezione di Bari, Via Amendola 165/A, 70126 Bari, Italy. ²Dipartimento di Biologia e Chimica AgroForestale ed Ambientale, Sezione di Genetica e Miglioramento Genetico, Università degli Studi, Via Amendola 165/A, 70126 Bari, Italy. E-mail: f.cillo@ba.ivv.cnr.it

The differential response of twenty-nine genotypes of tomato and wild tomato relatives (*Solanum* section *Lycopersicon* spp.) to *Cucumber mosaic virus* strain Fny (CMV-Fny), alone or in combination with three different satellite RNA (satRNA) variants, allowed the identification of four disease phenotype patterns, each including plants that reacted with very severe symptoms (leaf malformations, apical stunting, lethal necrosis) and plants that remained symptomless. No resistance or tolerance to CMV-Fny was observed, while individual host genotypes displayed latent infection upon inoculation with one (CMV-Fny/Tfn-satRNA, phenotype pattern 1 and 4), two (CMV-Fny/Tfn-satRNA and CMV-Fny/TTS-satRNA, phenotype pattern 2) or all three (the former two plus CMV-Fny/77-satRNA, phenotype pattern 3) CMV/satRNA combinations. RNA gel blot analyses showed that latent infection generally correlated with a down-regulation of CMV RNA accumulation levels. Introgression lines deriving from a cross between *S. habrochaites* LA1777, that displayed a disease phenotype pattern 2, and *S. lycopersicum* were screened for tolerance to the stunting phenotype induced by CMV-Fny/TTS-satRNA, and only one line carrying an introgression on chromosome 6 was identified as partially tolerant. *S. chilense* LA1932 x *S. lycopersicum* backcross introgression lines were screened for toler-

ance to lethal necrosis induced by CMV-Fny/77-satRNA (phenotype pattern 3), and the tolerant phenotype was observed in 33% plants of the BC₁F₂ progeny, less than 1% plants of the BC₁F₃ progeny and 66% plants of one line of the BC₁F₄ progeny. Thus, potentially useful sources of tolerance to CMV/satRNA-induced diseases were identified, and the tolerant phenotype seems to be controlled by quantitative trait loci.

MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF THE NSs PROTEIN OF A NEW TOSPOVIRUS IN *POLYGONUM CONVOLVULUS*. M. Ciuffo, D. Pacifico and M. Turina. *Istituto di Virologia Vegetale del CNR, Strada delle Cacce 73, 10135 Torino, Italy. E-mail: m.ciuffo@ivv.cnr.it*

Post-transcriptional gene silencing is a natural defence mechanism of plants to protect themselves against viral infections. The non-structural small protein (NSs) of tospoviruses is known to be a gene silencing suppressor (Takeda *et al.*, 2002. *Fed. Eur. Biochem. Soc. Lett.* 352: 75-79). Furthermore, evidence of its role as avirulence determinant in the interaction TSWV-Tsw resistant pepper has been provided (Margaria *et al.*, 2007. *Mol. Plant-Microbe Interact.* 20: 547-558). In summer 2005, we found a new tospovirus in *P. convolvulus*, temporary called Polygonum ring spot virus. The virus was characterized biologically, purified and a specific antiserum was prepared to obtain a diagnostic kit for DAS ELISA and a lateral flow device for its rapid detection. Full-length small and medium segments were cloned and sequenced for comparison with other tospovirus sequences available in the database. The sequence of the NSs gene was amplified with specific primers containing the BamHI cutting site, cloned in the pRSET-A vector (Invitrogen, Carlsbad, CA, USA) and transformed to obtain the expression of the recombinant protein. This protein will be used as antigen to produce a specific antiserum suitable to detect it in the host plants or in the vector *Dicthiothrips betae* during infection. The NSs protein was also cloned in p-Bin61 vector for transient expression *in planta* through agroinfiltration. Plasmids will be co-infiltrated in *Nicotiana benthamiana* 16C leaves with plasmid containing GFP gene and green fluorescence will be checked to verify its activity as gene-silencing suppressor.

EFFECTIVENESS OF CALCIUM CYANAMIDE AND SOIL SOLARISATION ON TOMATO CORKY ROOT CONTROL. C. Colella, M. D'Amico, M. Amenduni, G. Bubici and M. Cirulli. *Dipartimento di Biologia e Patologia Vegetale, Università degli Studi, Via G. Amendola 165/A, 70126 Bari, Italy. E-mail: c.colella@agr.uniba.it*

The present study evaluated the effect of calcium cyanamide (granular and powdery formulations) and soil solarisation - either singly or in combination - on corky root caused by *Pyrenochaeta lycopersici*, and tomato yield in a three-year trials under greenhouse conditions. All treatments significantly reduced corky root severity over the three years of testing. Treatments using each chemical formulation singly reduced the disease by 20-30%, while soil solarisation used alone reduced the disease by 50-70%. The combination of calcium cyanamide with soil solarisation reduced disease severity by 60-70% when compared with the control treatment. The two calcium cyanamide formulations were statistically the same in reduction of corky root. Tomato yield was not significantly affected by calcium cyanamide or soil solarisation. Results obtained from this investigation provide a new effective integrated strategy for the control of tomato corky root under greenhouse conditions.

IN VITRO TRANSFORMATION OF *PSEUDOMONAS CORRUGATA* BY DNA OF TRANSGENIC TOMATO PLANTS. L. Cozzolino¹, S. Gaglione¹, A. Zoia¹ and A. Raio². ¹Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Sezione di Patologia Vegetale Università degli Studi di Napoli "Federico II", Via Università 100, 80055 Portici, Italy. ²Istituto per la Protezione delle Piante del CNR, Via Università 133, 80055 Portici, Italy. E-mail: lucia.cozzolino@unina.it

Occurrence of horizontal gene transfer from plants to soil bacteria has been shown by several studies, but very few studies have been carried out about gene transfer from GM plants to phytopathogenic bacteria. In the last years several analytical protocols have been developed in order to monitor transfer of DNA from transgenic plants to other microorganisms. These procedures are mainly based on the ability of bacteria to take up foreign DNA and integrate it into their genome by homologous recombination. Our preliminary experiments demonstrate that horizontal gene transfer occur, under optimized laboratory conditions, between GM tomato plants and the phytopathogenic bacterium *Pseudomonas corrugata*. *Pseudomonas corrugata* (pLaf Δ*npII*), carrying a plasmid with a deleted *npII* gene, was able to capture DNA extracted from transgenic tomato plants and integrate it by homologous recombination. Restoration of *npII* originated kanamycin-resistant transformants, that were detected when transformation was performed using plasmid DNA, plant DNA, and homogenates of tissues of GM plants carrying the functional gene *npII*. DNA transfer was not detected when *P. corrugata* wild type strain (without *npII* gene) was used as control. Greenhouse experiments are being planned to verify a possible transformation of *P. corrugata* by DNA of GM tomato plants in an *in vivo* interaction.

GENETIC VARIATIONS AMONG *XANTHOMONAS ARBORICOLA* pv. *PRUNI* POPULATIONS FROM PEACH ORCHARDS IN ROMAGNA. D. Dallai and E. Stefani. *Dipartimento di Scienze Agrarie e degli Alimenti, Università degli Studi di Modena e Reggio Emilia, Via J.F. Kennedy 17, 42100 Reggio Emilia, Italy. E-mail: emilio.stefani@unimore.it*

Xanthomonas arboricola pv. *pruni*, the causal agent of bacterial canker and leaf spots of stone fruits, elicited several outbreaks in peach orchards during the last years in Romagna. Studies on population variability during the early '90s based on phenotypic (SDS-PAGE) and genotypic (AFLP) fingerprints did not show the presence of any differential sub-population among the pathovar. In 2007, the use of bacterial antagonist(s) in commercial orchards was started, with the aim of implementing alternative, sustainable and environmentally friendly control strategies. Thus, for securing a better approach to possible and effective biocontrol strategies a study was initiated of the population structure and genetic variations of field isolates of the pathogen. A number of *X. arboricola* pv. *pruni* isolates from peach orchards were collected in 2006 and analysed to check variability of protein profiles (SDS-PAGE) and of genomic fingerprints (rep-PCR, using the REP, BOX and ERIC primers). Protein profiling was again not able to differentiate any sub-population among the pathogen and cluster analysis grouped all the isolates at a correlation coefficient *r* higher than 0.9. REP and BOX primers gave the same amplicon patterns for all isolates considered, whereas the ERIC primers were able to discriminate a few isolates showing an additional band in the amplicon pattern. Results suggest the presence of genetic variations within the *X. arboricola* pv. *pruni* population. Thus, biological control with microbial agent should take into consideration the presence of a second population of the pathogen, which might have a different behaviour towards the antagonist(s).

EVOLUTION OF TOMATO YELLOW LEAF CURL VIRUS AND TOMATO YELLOW LEAF CURL SARDINIA VIRUS IN ITALY: APPEARANCE OF INTERSPECIFIC HYBRIDS. S. Davino¹, M. Davino¹, M. Testa², M. Nannini², C. Napoli³ and G.P. Accotto³. ¹Dipartimento di Scienze e Tecnologie Fitosanitarie, Sezione di Patologia Vegetale, Università degli Studi, Via S. Sofia, 100, 95123 Catania, Italy. ²AGRI Sardegna, Viale Trieste 111, 09123 Cagliari, Italy. ³Istituto di Virologia Vegetale del CNR, Strada delle Cacce 73, 10135 Torino, Italy. E-mail: wdavino@unict.it

Tomato yellow leaf curl disease (TYLCD) has been known for more than 40 years, but only since the end of the 1980s it became widespread in all important tomato growing areas of the Mediterranean basin. Two virus species have been recorded in Italy, *Tomato yellow leaf curl virus* (TYLCV), and, *Tomato yellow leaf curl Sardinia virus* (TYLCSV), both in the genus *Begomovirus*, family *Geminiviridae*. TYLCSV is present in Sicily and Sardinia since 1989, while TYLCV was first reported in Sicily in 2002 and in Sardinia in 2004. Today the two viral species coexist in tomato crops of the most relevant Italian tomato growing areas. This natural coexistence in the field may favour possible genetic exchange: the DNAs of the two viruses share about 70% sequence identity, with some short regions nearly identical. This prompted us to search for interspecific hybrids in field samples collected in Sicily and Sardinia. PCR assays were designed for detecting possible recombination within the IR region, that is considered a putative recombination site in geminivirus genomes. In 27 of 67 plants infected by TYLCV and/or TYLCSV hybrid DNAs could be amplified. Sequencing of these amplified DNAs showed that the recombination site is located in the stem-loop region and that the left portion is always constituted by TYLCV, while on the right side sequences deriving from TYLCSV (either type strain or TYLCSV-Sic strain) can be found.

Research supported by the Ministry of Agricultural, Alimentary and Forestry Politics (MiPAAF) in the framework of the project PROM

EARLY DETECTION OF OCHRATOXIGENIC FUNGI ON GRAPE AND OCHRATOXIN A IN WINE. P. De Rossi¹, M. Reverberi², A. Ricelli³, D. Caputo⁴, G. De Cesare⁴, A. Nascetti⁴, R. Scipinotti⁴ and C. Fanelli². ¹Dipartimento di Biotecnologie, Agroindustria e Protezione della Salute, ENEA, Centro Ricerche Casaccia, Via Anguillarese 301, 00123 Roma, Italy. ²Dipartimento di Biologia Vegetale, Università "La Sapienza", Largo Cristina di Svezia 24, 00165 Roma, Italy. ³Istituto di Scienze delle Produzioni Alimentari del CNR, Via Amendola 122/O, 70126 Bari, Italy. ⁴Dipartimento di Ingegneria Elettronica, Università "La Sapienza", Via Eudossiana 18, 00165 Roma, Italy. E-mail: patrizia.derossi@casaccia.enea.it

Ochratoxin A (OTA), a mycotoxin with nephrotoxic, nephrocarcinogenic, teratogenic and immunosuppressive properties, has been detected in different kinds of foods and beverages, including grape juice and wine. Among the OTA-producing fungal species, *Aspergillus carbonarius* shows the highest ochratoxigenic potential in grapes. The objective of this research was to investigate the presence of *A. carbonarius* in grapes and of OTA in wine. To detect the presence of *A. carbonarius* in grapes, two PCR assays were developed using species-specific primers designed on sequences of: (i) Internal Transcribe Spacers of rDNA units (ITS), used to carry out phylogenetic studies; (ii) polyketide synthases (PKSs) genes responsible for the biosynthesis of ochratoxin A. Currently, most of the methods available for the determination of OTA in wine are based on an extraction step, a cleanup passage and assessment by High Performance Liquid Chromatography (HPLC) with fluorescence detection.

In this work we investigated the performance of a system based on hydrogenated amorphous silicon photosensors for the early detection of Ochratoxin A, based on the measurement of the photocurrent induced in a hydrogenated amorphous silicon (a-Si:H) photodiode by the fluorescence of the mycotoxin excited by UV radiation. The mycotoxin is deposited on a thin layer chromatographic plate and aligned with the sensor. The photocurrent value is proportional to the mycotoxin quantity deposited.

TEMPERATURE DOES NOT AFFECT RESISTANCE TO PLUM POX VIRUS CONFERRED BY RNA SILENCING OF PPV 5' UTR/P1 SEQUENCE. E. Di Nicola-Negri and V. Ilardi. CRA, Istituto Sperimentale per la Patologia Vegetale, Via C.G. Bertero 22, 00156 Roma, Italy. E-mail: v.ilardi@ispave.it

Plum pox virus (PPV) is the agent of Sharka disease of stone fruits. In order to obtain resistance to PPV infection we have developed PPV-derived gene constructs based on the hairpin RNAi technology (Di Nicola-Negri *et al.*, 2005. *Trans. Res.* 14: 489-494). The PPV 5' UTR/P1 construct was able to induce immunity in *Nicotiana benthamiana* plants to the transgene-homologous PPV isolate and to distantly related PPV strains (Di Nicola-Negri and Ilardi, *J. Plant Pathol.* 88: S19-S20, 2006). However, the evidence that low temperature can impact the effectiveness of this strategy poses the question of whether RNA silencing is suited to confer resistance to viruses that infect, like PPV, perennial plant species growing in temperate and continental climate. Thus, to evaluate the field applicability of self-complementary hairpin constructs to control Sharka we decided to analyze these transgenic plants for the ability to resist to PPV infection at different temperatures. Our data show that transgenic *Nicotiana benthamiana* plants expressing the PPV 5' UTR/P1 construct are resistant to PPV infection also at low temperature (15°C). The overall data suggest that the 5' UTR/P1 construct can be profitably used to confer resistance to Sharka in *Prunus* species.

IDENTIFICATION AND CHARACTERIZATION OF POTATO SPINDLE TUBER VIROID INFECTING ORNAMENTAL SOLANACEAE IN ITALY. F. Di Serio¹, M.R. Silletti², N. Trisciuzzi², A. Guarino³, A. Percoco³ and A. Lillo³. ¹Istituto di Virologia Vegetale del CNR, Sezione di Bari, Via Amendola 165/A, 70126 Bari, Italy. ²Centro di Ricerca e Sperimentazione in Agricoltura Basile Caramia (CRSA), Via Cisternino 281, 70010 Locorotondo, Italy. ³Osservatorio Fitosanitario Regionale, Lungomare N. Sauro 47, 70121 Bari, Italy. E-mail: f.diserio@ba.ivv.cnr.it

Potato spindle tuber viroid (PSTVd) is a quarantine pathogen included in the EPPO A2 list. Depending on the strain and the environmental temperature, PSTVd causes mild to severe diseases to potato and tomato crops. A preliminary survey in central and southern Italy allowed the identification of symptomless *Solanum jasminoides* and *S. rantonnetii* plants infected by PSTVd, thus showing for the first time that this quarantine pathogen is present in Italy and that *S. rantonnetii* is a previously unreported natural host of PSTVd. Molecular characterization of four *S. jasminoides* and one *S. rantonnetii* PSTVd isolates showed that viroid populations had a low sequence variability and shared the same master sequence variant (357 nt long), which was identical to the PSTVd variant previously found in *S. jasminoides* in the Netherlands. PSTVd variants from *S. jasminoides* were mechanically transmitted to tomato seedlings cv. Rutgers, as shown by the typical symptoms expressed by inoculated plants and by molecular hybridization assays. These results call for a prompt extension

of surveys for the presence of PSTVd in other Italian regions and other ornamental plant species, which could constitute concealed reservoirs of this pathogen. To this aim, a tissue-printing hybridization method for detecting PSTVd was developed and its potential for large scale surveys tested and validated. The epidemiological risk of this finding for cultivated potato and tomato crops will be discussed.

CROSS TALK AMONG SIGNAL MOLECULES IN THE REGULATION OF THE DIVINYL ETHER BIOSYNTHETIC PATHWAY IN TOBACCO. A. Fammartino¹, V. Aramini¹, B. Verdagner², M.-T. Esquerré-Tugayé², F. Cardinale¹. ¹Dipartimento di Valorizzazione e Protezione delle Risorse Agroforestali, Sezione di Patologia Vegetale, Università degli Studi di Torino, Via Leonardo da Vinci, 44, 10095 Grugliasco (TO), Italy. ²Université Paul Sabatier, Unité Mixte de Recherche 5546 CNRS-UPS, Pôle de Biotechnologies Végétales, 24 Chemin de Borde-Rouge, BP 17 Auzeville, 31326 Castanet-Tolosan, France. E-mail: francesca.cardinale@unito.it

In tobacco, fungitoxic 9-divinyl ethers (DVEs) produced by the lipoxygenase NtLOX1 and DVE synthase NtDES1 are important for full resistance to pathogens. In this work, the regulation of NtLOX1 and NtDES1 expression by signal molecules related to defense was investigated by RT-qPCR on both genes, and in transgenic plants where GUS expression was driven by the NtLOX1 putative promoter sequence. Methyl jasmonate, ACC and an elicitor molecule were shown to coordinately trigger the DVE pathway; induction was strongly attenuated in the presence of salicylic acid, which seems to act as a negative regulator of 9-DVE production. Dose-dependent activation patterns are currently being checked out by RT-qPCR. Our data suggest that, in tobacco, DVE biosynthesis is cross-regulated by another oxylipin branch (jasmonates), and by hormonal and signal molecules such as ethylene and SA.

GRAPE BERRY PROTEINS ACT AS SCAVENGERS OF GRAPE POLYPHENOLS AND PROTECT POLYGALACTURONASE ACTIVITY OF BOTRYTIS CINEREA FROM INHIBITION. F. Favaron, M. Garbin, M. Lucchetta, L. Sella and S. Odorizzi. Dipartimento Territorio e Sistemi Agro-Forestali, Università degli Studi di Padova, Viale dell'Università 16, 35020 Legnaro (PD), Italy. E-mail: francesco.favaron@unipd.it

At maturity grape berries contain a large amount of proteins and polyphenols with potential anti-fungal activity. The protein fraction of grape extract includes mostly thaumatin-like proteins and chitinase while flavonoids predominate among polyphenols. However, *Botrytis cinerea* grows well in the presence of proteins and polyphenols when these molecules are supplied to the culture in a ratio similar to that measured in the grape fruit extract. *In vitro* it was observed that a fraction of grape proteins interacts with polyphenols forming complexes with reduced solubility and that *B. cinerea* laccase enhances this process. Polyphenols are well known inhibitors of fungal polygalacturonases (PGs) activity, and *B. cinerea* PG activity is inhibited by grape polyphenols *in vitro*. However, the simultaneous administration of grape proteins diminishes the inhibitory activity of grape polyphenols, and laccase addition restores completely *B. cinerea* PG activity. Similar results are obtained when the stylbenic phytoalexin resveratrol is used in combination with grape proteins and *B. cinerea* laccase. The scavenging of polyphenols by grape proteins could favour the berry infection by *B. cinerea*.

HOST-PATHOGEN-VECTOR INTERACTIONS: THE CASE OF FRUIT TREE PHYTOPLASMAS. F. Ferrini, L. Carraro and N. Loi. Dipartimento di Biologia Applicata alla Difesa delle Piante, Università degli Studi, Viale delle Scienze 208, 33100, Udine, Italy. E-mail: ferrini@uniud.it

In nature, the behaviour of phytoplasmas is different, some having a wide host range, others infecting only one or a few hosts. Similarly, phytoplasmas are transmitted either by a single or by several insect vectors. '*Candidatus Phytoplasma mali*', '*Candidatus Phytoplasma pyri*' and '*Candidatus Phytoplasma prunorum*', causal agent respectively of apple proliferation, pear decline and European stone fruit yellows, are known for their high specificity regarding both host and vector. For example, '*Candidatus Phytoplasma prunorum*' infects *Prunus* spp. and is transmitted by *Cacopsylla pruni*. At the same time, the three agents have common characteristics as high 16SrDNA sequence similarity, hosts belonging to the family *Rosaceae*, vectors belonging to *Cacopsylla* spp. So, they are clearly different and highly specific agents with important similarities. With different experiments carried out in controlled conditions, it was investigated if the characteristics that differentiate the three phytoplasmas are mainly due to the interaction host-pathogen, host-vector or vector-pathogen. Results showed that '*C. Phytoplasma mali*', '*C. Phytoplasma pyri*' and '*C. Phytoplasma prunorum*' are both host-specific and vector-specific agents; besides, the vectors *Cacopsylla picta*, *Cacopsylla pyri* and *Cacopsylla pruni* proved to be host-specific insects. On the contrary, phytoplasmas belonging to the Elm yellows group (16SrV) resulted non host-specific, able to infect different plant species.

DOES TRICHODERMA HARZIANUM REALLY PRODUCE TRICHOHECENE MYCOTOXINS? A. Gallo¹, M. Marzano^{1,2} and C. Altomare¹. ¹Istituto di Scienze delle Produzioni Alimentari del CNR, Via Amendola 122/O, 70126 Bari, Italy. ²Dipartimento di Scienze Animali, Vegetali e dell'Ambiente, Università degli Studi del Molise, Via De Sanctis, 86100 Campobasso, Italy. E-mail: antonia.gallo@ispa.cnr.it

Trichoderma harzianum is an ubiquitous fungal species known for its effectiveness in the biological control of a variety of plant pathogens. Possible production of mycotoxins by *T. harzianum* is of great concern for the assessment of toxicological potential of strains used as biocontrol agents. The strain *T. harzianum* ATCC 90237 produces harzianum A, belonging to the group of trichothecene mycotoxins. The putative gene of trichodiene synthase (*tri5*), the key enzyme required for trichothecene biosynthesis, was identified in this strain by Gallo *et al.* (*Physiol. Mol. Plant Pathol.*, **65**: 11-20, 2004). Here we report the molecular characterization of ATCC 90237 through the analysis of ITS1 and ITS2 regions of ribosomal DNA and of *tef1*, translation elongation factor 1-alpha gene. The sequence comparison analysis showed a 100% similarity with *T. brevicompactum* sequences. This result suggests that production of harzianum A was erroneously attributed to *T. harzianum* and supports the findings of Nielsen *et al.* (*J. Agric. Food Chem.*, **53**: 8190-8196, 2005) who reported that among different *Trichoderma* species, only the *T. brevicompactum* strains were trichothecene producers. Twenty *T. harzianum* isolates, identified by ITS and *tef1* sequences, were examined for the presence of *tri5*. The screening was approached using two pairs of specific primers designed on the ATCC 90237 gene sequence. The first pair of primers allowed the amplification of an internal 500 bp fragment in most isolates. Amplicons showed similarity from 70% to 100% with the corresponding segment of the ATCC 90237 *tri5*. The attempt to amplify the whole gene using two specific primers positioned at the ends of *tri5* sequence was unsuccessful, suggesting a high sequence variability in those regions.

JEKYLL OR HYDE? THE STRANGE CASE OF A BIOFILM-FORMING STRAIN OF *PICCHIA FERMENTANS*, WHICH CONTROLS *MONILINIA* BROWN ROT ON APPLE BUT IS PATHOGENIC ON PEACH FRUIT. S. Giobbe¹, B. Scherm¹, G. Zara², M. Budroni² and Q. Migheli¹. ¹Dipartimento di Protezione delle Piante, Centro interdisciplinare di eccellenza per lo sviluppo della ricerca biotecnologica e per lo studio della biodiversità della Sardegna e dell'area mediterranea, Università degli Studi, Via E. De Nicola 9, 07100 Sassari, Italy. ²Dipartimento di Scienze Ambientali e Biotecnologie Agroalimentari, Università degli Studi, Via E. De Nicola 9, 07100 Sassari, Italy. E-mail: saragio80@yahoo.it

A biofilm-forming strain of *Pichia fermentans* proved most effective in controlling brown rot on apple fruits when co-inoculated into artificial wounds with a phytopathogenic isolate of *Monilinia fructicola*. Culture filtrates and autoclaved cells had no significant influence on the disease. When sprayed onto the apple fruit surface, this yeast formed a thin biofilm but failed to colonise the underlying tissues. When inoculated into wounds artificially made to peach fruits or when sprayed onto peach fruit surface, the same strain showed an unexpected pathogenic behaviour, causing rapid decay of tissues even in the absence of *M. fructicola*. Both optical and scanning electron microscopy were used to evaluate the pattern of fruit tissue colonisation by *P. fermentans*. While on apple surface and within apple wound the antagonist retained its yeast-like shape, colonisation of peach fruit tissue was always characterised by a transition from vegetative growth to pseudohyphal growth. These results suggest that pseudohyphal growth plays a major role in governing the potential pathogenicity of *P. fermentans*, further emphasizing the importance of a thorough risk assessment for the safe use of any novel biocontrol agent.

Work carried out within the frame of the Research Programme MiPAAF-CIPE "FRU.MED." - Project "DAFME".

PHYSIOLOGICAL CHARACTERIZATION OF *MONOSPORASCUS CANNONBALLUS* STRAINS ISOLATED FROM CURCUBIT ROOTS. R. Gregori¹, M. Mari¹, A. Veronesi¹, M.P. Aleandri², P. Magro², G. Chilosi², R. Roberti¹. ¹Dipartimento di Protezione e Valorizzazione Agroalimentare, "Alma Mater Studiorum" Università degli Studi, Via Fanin 46, 40127, Bologna, Italy. ²Dipartimento di Protezione delle Piante, Università degli Studi della Tuscia, Via S. Camillo del Lellis, 01100 Viterbo, Italy. E-mail: rgregori@agrsci.unibo.it

M. cannonballus is the causal agent of root rot and decline of cucurbits worldwide and causes severe losses to cucurbit crops. The objective of this research was to investigate the possible relationship among pathogen growth and enzyme activities involved in pathogenesis. Nine strains (MA1, 234, 235, 236, 237, 350, 351, 354 and 357) of this pathogen, were isolated from roots of melons grown in Italy and their polygalacturonase (PG), pectin lyase, cellulase and protease activity were evaluated. The mycelium dry weight of the strains was measured in mycelial mats obtained by filtration of 9-day liquid cultures at 26°C. The enzymatic assays were carried out in cultural extracts by agarose diffusion assay (ADA), spectrophotometric assays and isoelectric focusing analysis. The ADA screening revealed the presence of PG activity in all strains. The spectrophotometric assays showed the highest PG activity of MA1, 235, 236, 237 and 357 and the highest mycelial growth for MA1, 235, 236 and 357. The mycelial growth of MA1, 235, 236 and 357 was positively correlated with PG activity ($r^2=0.99$).

FIRST DETECTION OF *CITRUS LEAF BLOTCH VIRUS* IN ITALY. M. Guardo^{1,2}, M. Castellano², V. Savino² and A. Caruso¹. ¹CRA, Istituto Sperimentale per l'Agricoltura, Corso Savoia 190, 95024 Acireale (CT), Italy. ²Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi, Via Amendola 165/A, 70126 Bari, Italy. E-mail: maria.guardo@entecra.it

Citrus leaf blotch virus (CLBV, type species of the genus *Citrovirus*) causes a bud union disorder of 'Nagami' kumquat and Calamodin grafted on Trifoliate orange, Citrange or Citrumelo rootstocks. After reports from Sicilian nurserymen, monitoring in different nurseries was initiated. We tested leaves collected from 'Nagami' kumquat (50 symptomless grafted on sour orange and 50 symptomatic grafted on Troyer citrange in a nursery near Messina (Sicily) and from 30 Calamondin grafted on Troyer citrange collected in a nursery near Mazara del Vallo (Trapani, Sicily). Primers from a published CLBV sequence, were used in RT-PCR assays to amplify the polymerase and the coat protein genes. All kumquat samples yielded amplicons of the expected size, 456 bp and 438 bp, respectively. Consensus sequences of the two amplicons (Genbank Accession No. EF203229 and EF203230) had 96% and 97% nucleotide sequence identity, respectively, and both had 99% amino acid identity with CLBV. RT-PCR of Calamondin samples yielded amplicons of the expected size. Test herbaceous plants were mechanically inoculated with leaf extracts from a symptomatic kumquat. No symptoms were observed on these plants but two months after inoculation, they were tested by RT-PCR obtaining again the two specific bands. The two sequenced amplicons had 100% identity with sequences of the CLBV isolate used for inoculation. To our knowledge this is the first record of CLBV in Italy.

DOSE-DEPENDENT EFFECTS OF EXOGENOUS MELATONIN ON THE PLANT RESPONSE TO BIOTIC AND ABIOTIC STRESSES. M. Iriti and F. Faoro. Istituto di Patologia Vegetale, Università degli Studi e Istituto di Virologia Vegetale del CNR, Sezione di Milano, Via Celoria 2, 20133 Milano, Italy. E-mail: marcello.iriti@unimi.it

Melatonin was supposed to be an animal hormone, until its recent discovery in plant. It occurs in many species of angiosperms, where it regulates circadian rhythms, photoperiodic reactions, growth, cell oxidative homeostasis and its synthesis responds to priming with plant activators. In this work, different concentrations of exogenous melatonin have been assayed in order to investigate its role in plant response to stresses. In tobacco, melatonin concentrations ranging from 1 to 10 mM, with a 24 h induction time, increased the number of TNV foliar lesions up to 28% vs. untreated inoculated plants, whereas melatonin concentrations lower than 0.5 mM had no effect on infection spreading. Furthermore, 15 mM ascorbic acid (AA) greatly enhanced TNV infection, 10-50 mM H₂O₂ significantly reduced the number and size of lesions, whereas both AA and 0.5 mM melatonin, supplied 1 h before treatment with the plant activator benzothiadiazole, reduced its efficacy against TNV. In beans and currant tomatoes exposed for 45 min to UVC, pretreatment with a low melatonin concentration, 250 μM and 1mM, respectively, worsened the radiation injury, increasing the number of dead cells vs. the untreated irradiated controls as assessed by the Evans blue staining. With higher concentrations, 500 μM and 10 mM, respectively, plants were more protected against UVC. In conclusion, these preliminary results indicate indirectly that melatonin may balance the cell redox status, by scavenging, at some concentrations, the reactive oxygen species involved in plant defence against pathogens and UVC, whereas lower concentrations seem to have an unknown prooxidant activity.

CLONING AND EXPRESSION OF *PSEUDOMONAS CORRUGATA* PHA SYNTHASES FOR USEFUL APPLICATIONS. A. Lombardo¹, P. Bella^{1,2} and V. Catara². *Parco Scientifico e Tecnologico della Sicilia, Zona Industriale, Stradale G. Agnelli angolo V. Lancia, 95030 Catania.* ²*Dipartimento di Scienze e Tecnologie Fitosanitarie, Università degli Studi, Via S. Sofia 100, 95123 Catania, Italy. E-mail: vcatara@unict.it*

In a previous study possible differences were detected by PCR in the genetic locus involved in polyhydroxyalkanoates (PHAs) production by *Pseudomonas corrugata*. PHAs are intracellular inclusions which usually occur when carbon is in excess and at least another essential growth factor (i.e. nitrogen, phosphorus) is limited. In nature, these inclusions are thought to play a strategic role in survival and overcoming environmental stress. Once extracted from the cells, they have the same properties as some common plastics, thus attracting attention as biodegradable counterparts to petroleum-based polymers. *P. corrugata* produces PHA_{MCL} with monomer-unit-repeats of 6-14 carbon atoms. Following the screening of a large collection of strains, a *P. corrugata* strain was selected being an optimum producer of PHAs both from fatty acids and used cooking oil. The PHA biosynthesis locus was detected in an *Escherichia coli* cosmid clone from a genomic library and subcloned in the pUC18 vector. A sequence of about 5.8 kb spanning the PHA locus of *P. corrugata* was determined. A class II PHA genetic system consisting of two synthase genes (*phaC*, *phaC2*) separated by a gene coding for an intracellular PHA depolymerase (*phaZ*) was found, as well as the putative promoters. The PHA synthase sequences were independently cloned from a wild type *P. corrugata* strain and expressed in *E. coli*. The functional expression of *phaC1* and *phaC2* in *E. coli* was confirmed using decanoate and oleic acid as carbon sources. With the aim of producing PHA_{MCL} in plant, two *Agrobacterium*-mediated transfection vectors carrying *phaC1* under the control of *nos* regulation sequences were built.

MONITORING SOIL STERILIZATION BY STEAM TREATMENT IN GREENHOUSE CULTIVATED WITH CHRYSANTHEMUM. F. Lupo¹, F. Campanile², M. Carucci³ and M. Zaccardelli². ¹*Dipartimento di Scienze dei Sistemi Colturali, Forestali e dell'Ambiente, Università degli Studi della Basilicata, Via dell'Ateneo Lucano 10, 85100 Potenza, Italy.* ²*CRA, Istituto Sperimentale per le Colture Industriali, Strada Statale 18 n. 204, 84091 Battipaglia (SA), Italy.* ³*Termotecnica Industriale s.r.l. Via Brodolini, 84091 Battipaglia (SA), Italy. E-mail: lupo@unibas.it*

Soil sterilization in greenhouses by methyl-bromide is progressively substituted by other methods because this fumigant contribute to the ozone hole. Steam treatment seems the most promising methods but is expensive. In this work a preliminary study on soil sterilization by steam was performed in greenhouses cultivated with chrysanthemum. The goal was to set up possible strategies to optimize soil sterilization and to reduce the cost of treatments. During sterilization, soil temperatures were monitored at different depth. Soil samples before and after sterilization were collected at 0-20 and 20-40 cm to count colourable bacteria and fungi and measure dehydrogenase and hydrolase activity. During the 6 h-long treatment, soil temperature rose to 100°C at a depth of 10 cm after about 5 h, while temperatures were 70 and 20°C, respectively, at 20 and 30 cm. When steam treatment was stopped, temperature in the first 20 cm progressively decreased, whereas at 30 and 40 cm temperature increased up to 50 and 40°C, respectively. Higher temperatures were registered in the first 20 cm for many hours after the treatment. No significant difference were observed in the number of culturable

microorganism and dehydrogenase activity before and after steam treatment, suggesting no change in the composition of soil microbial community. Conversely, hydrolase activity significantly decreased after sterilization. These results suggest that steam treatment increases significantly soil temperature in the first 20 cm only and that potentially it is possible to reduce the duration of treatment. Hydrolase activity is proposed as the best method to test the efficiency of soil sterilization.

INVESTIGATING THE MOLECULAR FACTORS INVOLVED IN THE COMPLEX INTERACTION BETWEEN THE BIOCONTROL AGENT *TRICHODERMA*, THE PLANT AND THE FUNGAL PATHOGENS. R. Marra¹, M. Ruocco², F. Vinale¹, S. L. Woo¹, S. Lanzuise¹, D. Turrà¹, V. Aloj¹, F. Scala¹ and M. Lorigio¹. ¹*Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Sezione di Patologia Vegetale, Università degli Studi di Napoli "Federico II", Via Università 100, 80055 Portici (NA), Italy.* ²*Istituto per la Protezione delle Piante del CNR, Via Università 130, 80055 Portici (NA), Italy. E-mail: robmarra@unina.it*

Trichoderma spp. are fungal biocontrol agents that have been largely studied and used in agriculture to enhance crop productivity and control pathogen populations. Nevertheless, the complex multi-partner interactions between these fungal antagonists, the plant and the microbial pathogens have been little studied in detail. In this work we investigated the changes of proteome profiles during the interactions between plants (bean, tomato or lettuce), fungal pathogens (*Botrytis cinerea*, *Rhizoctonia solani*, *Sclerotinia sclerotiorum* or *Pythium ultimum*) and antagonists (*T. atroviride* strain P1 or *T. harzianum* strain T22). Numerous differential proteins were identified by mass spectrometry. Among these, members of the cyclophilin family appeared to accumulate differentially in both *T. atroviride* and *B. cinerea* proteomes in the presence of the plant. Since these proteins are known to act as virulence factors during infection by *Magnaporthe grisea*, *B. cinerea* and *Cryptococcus neoformans*, their possible role in *Trichoderma* biocontrol mechanisms was investigated. EST libraries obtained by growing different *Trichoderma* in conditions related to biocontrol were screened for putative cyclophilin-like sequences. The highest number of cyclophilins (9 unique sequences and 4 contigs) was found in *T. atroviride* strain P1. To characterize the function of cyclophilins in this microorganism, we synthesised primers corresponding to highly conserved regions, based on the information obtained by both proteomic and EST analyses. BlastX analysis confirmed that a cloned PCR product encodes a cyclophilin-like protein. This fragment was used as a probe in Southern analysis and to study by RT-PCR the functional expression of the related gene in different growth conditions.

PARTIAL CHARACTERIZATION OF THE *FOWI* GENE IN *VERTICILLIUM DAHLIAE* KLEB. ISOLATES FROM OLIVE TREES. F. Nigro, I. Pentimone, H. Barham and A. Lorigio. *Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi, Via Amendola 165/A, 70126 Bari, Italy. E-mail: nigro@agr.uniba.it*

Verticillium dahliae Kleb., the causal agent of a vascular wilt disease, is an economically important plant pathogen with worldwide distribution, severely affecting young olive trees. To date, the molecular mechanisms of pathogenicity and symptom induction by *V. dahliae* in olive trees remain largely unexplored, although some pathogenicity genes of the fungus have been recently identified and characterized. Improving knowledge on the *V.*

dabliae pathogenicity could be useful for epidemiological studies and for developing effective control strategies. By using two primers (giFow1F-giFowR), designed on a conserved hypothetical mitochondrial protein of *Gibberella zeae* (XM381597), a 550 bp DNA fragment (VerFow1) was amplified from DNA extracted from several *V. dabliae* isolates, cloned and sequenced. Basic local alignment search tool (BLAST) of the fragment VerFow1 showed a considerable homology with a yeast and fungal hypothetical protein related to a mitochondrial carrier protein (MCPs). This protein is known for its role in the communication between the matrix and the cytosol, being essential in the eukaryotic metabolism. Particularly, sequence analysis revealed a 96% identity with the corresponding region of *Fusarium oxysporum* FOW1 gene (AB078975), encoding a mitochondrial protein that was identified and characterized as a virulence determinant, specifically required for the colonization of melon plants. Trials to determine the role of the fragment VerFow1 in the pathogenesis of *V. dabliae* on olive trees are in progress. To the best of our knowledge this is the first report on the characterization of a fungal hypothetical protein related to mitochondrial carriers in *V. dabliae*.

CHARACTERIZATION OF POLYKETIDE SYNTHASE GENE KNOCK-OUT MUTANTS OF *DIAPORTE HELIANTHI* C. Pane¹, B.A. Maimone Mancarello², S.O. Cacciola², G. Firrao³, G. Magnano di San Lio⁴, R. Vergara⁵ and F. Scala¹. ¹Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Sezione di Patologia Vegetale, Università degli Studi di Napoli "Federico II", Via Università 100, 80055, Portici (NA), Italy. ²Dipartimento di Scienze Entomologiche, Fitopatologiche, Microbiologiche e Zootecniche, Sezione di Patologia Vegetale e Microbiologia Agraria, Università degli Studi, Viale delle Scienze 2, 90128 Palermo, Italy. ³Dipartimento di Coltivazione e Difesa delle Specie Legnose, Sezione di Patologia Vegetale, Università degli Studi, Via del Borghetto 80, 56124 Pisa, Italy. ⁴Dipartimento di Biologia Applicata alla Difesa delle Piante, Università degli Studi, Via delle Scienze 208, 33100 Udine, Italy. ⁵Dipartimento di Agrochimica e Agrobiologia, Università Mediterranea, Località Feo di Vito, 89061 Reggio Calabria, Italy. catello.pane@libero.it

Diaporthe helianthi is the causal agent of sunflower stem canker. A polyketide phytotoxin, named phomozin, is produced during the early phases of *Diaporthe* infection in sunflower plants, and probably opens the way to host colonization. RAPD analysis allowed the detection of a polymorphism among isolates with different virulence degrees. The sequence of a 580 bp polymorphic band, cloned from a virulent French fungal isolate and, successively, extended to 2309 bp, revealed a high homology with fungal genes encoding polyketide synthases in several species of filamentous fungi (*i.e.* *mlcA* and *mlcB* of *Penicillium citrinum*, *lovB* of *Aspergillus terreus*, *fum5* of *Giberella moniliformis*, *pkS1* of *Colletotrichum heterostrophus*, *pkS1* of *Giberella fujikuroi*). This fragment was named *Dhpks1* (*D. helianthi* polyketide synthase 1). The possibility that virulence of *D. helianthi* could depend on the ability to produce a polyketide toxin has been studied in mutants selectively disrupted in *Dhpks1* by an *Agrobacterium*-mediated transformation. *In vitro* assays showed that mutant cultural filtrates were not phytotoxic to sunflower seedlings. Moreover, mass spectrometry analyses revealed that phomozin was absent in these filtrates. The virulence of mutants was significantly reduced compared to that of the parental strain in pathogenicity tests carried out on 4-5-month-old plants.

MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF GRAPEVINE DEFENCE GENES INDUCED DURING THE FIRST STAGES OF *ARMILLARIA MELLEAE* ROOT INFECTION. M. Perazzolli¹, F. Bampi², S. Faccin¹, I. Pertot¹, C. Gessler¹ and C. Moser². ¹Centro SafeCrop, Istituto Agrario di S. Michele all'Adige, Via E. Mach 1, 38010 S. Michele all'Adige (TN), Italy. ²Istituto Agrario di S. Michele all'Adige, Dipartimento di Genetica e Biologia Molecolare, Via E. Mach 1, 38010 S. Michele all'Adige (TN), Italy. E-mail: michele.perazzolli@iasma.it

Grapevine root rot, caused by the fungus *Armillaria mellea*, is a serious disease of increasing relevance in some important grape-growing areas. *A. mellea* attacks grape roots causing a decline in vigour and productivity and, finally, the death of the plant. Up to now, no resistant *Vitis* rootstocks have been identified and the existing pesticides are ineffective in its control. Field observations indicate that the young plants do not show symptoms of *A. mellea* infection during the first two years and the young roots are not infected by the pathogen, suggesting the activation defence mechanisms. In order to study this defence reaction at the molecular level, the suppression subtractive hybridisation approach was used and specific genes induced 24 h after *A. mellea* treatment were identified in the widely used Kober 5BB rootstock. Bioinformatics analyses revealed the induction of several defence-related genes, such as thaumatin-like, antimicrobial peptides and protease inhibitors. The induction of these genes during *A. mellea* challenge was also validated by real time RT-PCR in different infection experiments, confirming the activation of an active response in the young roots. However, the specific functions of these defence-related genes in the grape resistance response remain unknown. In order to elucidate their role, the full-length coding sequences were obtained and cloned in a vector suitable for heterologous expression in bacteria. The assay of the antifungal properties of the recombinant proteins will clarify which are the key players in the grape defence reaction against *A. mellea* infection.

EFFICACY OF NATURAL EXTRACTS IN TOMATO SPECK BIOCONTROL. A. Quattrucci and G.M. Balestra. Dipartimento di Protezione delle Piante, Università degli Studi della Tuscia, Via S. Camillo de Lellis, 01100 Viterbo, Italy. E-mail: balestra@unitus.it

Amongst bacteria of tomato plants, *Pseudomonas syringae* pv. *tomato* (PST), causal agent of tomato speck, is the most widespread and often damaging to greenhouse and open field tomato crops. To control PST, appropriate cultural practices and preventive copper treatments, are suggested. Due to the EU restriction of copper use in agriculture, the importance of organic tomato crops and the damages caused by this pathogen, new PST organic control strategies are required. The efficacy in PST biocontrol by natural extracts from plants of the families *Liliaceae* (*Allium sativum*) and *Moraceae* (*Ficus carica*) were studied. A highly virulent PST strain was used at 10⁶ and 10⁸ cfu/ml and at 10⁵ and 10⁸ cfu/ml concentrations, in *in vitro* and *in vivo* tests, respectively. The *A. sativum* extract was tested at 10 g l⁻¹ and that from *F. carica* at 300 g l⁻¹. *In vitro* tests with both plant extracts showed activity inhibiting PST growth. In *in vivo* tests, both extracts confirmed their antimicrobial activity towards the speck agent reducing disease incidence and disease severity when alone or in combination. Moreover, these natural extracts were able to control PST in tomato phyllosphere at least for 10 days, even in the presence of high bacterial contamination (10⁸ cfu/ml).

CELL REDOX BALANCE, LIPID METABOLISM AND AFLATOXIN BIOSYNTHESIS IN *ASPERGILLUS* SECT. *FLAVI*. M. Reverberi¹, M. Punelli¹, C. Smith³, F. Punelli¹, S. Zjalic¹, A. Ricelli², G. Payne³, A.A. Fabbri¹ and C. Fanelli¹. ¹Dipartimento di Biologia Vegetale, Università "La Sapienza", Largo Cristina di Svezia 24, 00165 Roma, Italy. ²Istituto di Scienze delle Produzioni Alimentari del CNR, Via Amendola 122/O, 70126 Bari, Italy. ³Center for Integrated Fungal Research, North Carolina State University, Raleigh, NC, USA. E-mail: massimo.reverberi@uniroma1.it

In *Aspergillus parasiticus*, oxidative stress produced during conidia germination and fungal growth, regulate aflatoxin synthesis via the expression of the oxidative stress-related transcription factor Apyap1 which promotes the antioxidant defence response of the fungal cell. In *A. flavus* and *A. parasiticus* a gene cluster for sugar utilisation (SU) has been found which is co-regulated together with the aflatoxin gene cluster. Many fungal toxins, among which aflatoxins, are members of a large, diversified class of compounds known collectively as polyketides. Acetyl-CoA, both as polyketide starter unit and extender unit via malonyl-CoA formation, is the fundamental building block of most fungal polyketides. The *nadA* gene, present in the SU cluster, encodes NADH oxidase, an enzyme involved in the conversion of pyruvate to Acetyl-CoA and controlling the NAD⁺/NADH ratio through the utilisation of O₂ present in the cell. Since *A. flavus* infects primarily the embryo and aleurone layer of maize seeds, which are known to house the majority of seed lipids in this plant, it can be argued that the β -oxidation of seed fatty acids, activated during the infection processes, can supply the aflatoxin building blocks, i.e. the Acetyl-CoA. Further, during maize infection, ROS seems to drive aflatoxin synthesis in *A. parasiticus*, generating an endogenous hyperoxidant state. A scenario emerges that leads us to hypothesise that the Acetyl-CoA present in the cell, controlled also by NADH oxidase, supplies the building blocks necessary for toxin synthesis that occurs only in the presence of a hyperoxidant state of the cell.

ENDOPHYTIC FUNGI IN NURSERY POPLAR PLANTS. E. Rocco¹, M. Gennaro², A. Giorcelli² and N. Anselmi¹. ¹Dipartimento Protezione delle Piante, Università degli Studi della Toscana, Via S. Camillo de Lellis, 01100 Viterbo, Italy. ²CRA, Istituto di Sperimentazione per la Pioppicoltura, Gruppo di Ricerca di Patologia Vegetale e Fitoiatria. Strada per Frassineto Po 35, 15033 Casale Monferrato (AL), Italy. E-mail: anselmi@unitus.it

The aim of this research was to assess the frequency of endophytic fungi, with particular reference to the pathogenic ones, in symptomless nursery poplar plants. Sampling was carried out in autumn 2006 in 1-year-old *Populus × canadensis* ('I-214' clone) nurseries both in Viterbo (Central Italy) and Casale Monferrato (Northern Italy). In each nursery 10 plants were randomly selected and from each 20 cuttings and buds were collected. After surface sterilisation, cutting and bud fragments were transferred to proper nutrient media. The fungal isolates obtained were subcultured and identified by morphological and molecular characters. Pathogenic and non pathogenic endophytic fungi were found in symptomless poplar tissues. Among latent pathogens remarkable was the presence of important agents of bark necrosis like *Cytospora* sp. and *Phomopsis* sp. *Discosporium populeum* (Sacc.) Sutton was detected exclusively in Casale Monferrato. This is in agreement with the high incidence of this pathogen in 1- or 2-year-old plants during transplanting in Northern Italy, whereas in Central and Southern Italy it is usually absent. The possible establishment of nurseries in areas deprived of dangerous pathogens is discussed.

CYMBIDIUM RINGSPOT VIRUS REPLICASE PROTEINS ARE TARGETED TO ENDOPLASMIC RETICULUM-DERIVED MEMBRANES IN THE ABSENCE OF PEROXISOMES IN YEAST CELLS. L. Rubino and M. Russo. Istituto di Virologia Vegetale del CNR, Sezione di Bari, Via Amendola 165/A, 70126 Bari, Italy. E-mail: l.rubino@ba.ivi.cnr.it

The replication of *Cymbidium ringspot virus* (CymRSV) defective interfering (DI) RNA in cells of the yeast *Saccharomyces cerevisiae* normally takes place in association with the peroxisomal membrane, thus paralleling the replication events in infected plant cells. However, by using the peroxisome-deficient yeast strain YPH499, it was shown that the presence of peroxisomes is not a strict requirement for CymRSV DI RNA replication. In fact, in the absence of peroxisomes, CymRSV replicase proteins p33 and p92 are targeted to the endoplasmic reticulum (ER) and support a robust replication of DI RNA. Membrane floatation gradient analysis of fractionated protein extracts showed that both proteins were present in the membrane-enriched fraction, thus indicating a true association with ER rather than protein aggregation. The proteins were not released by high-salt and high-pH treatment, suggesting an integrated association with membranes. However, since no protease-resistant fragment was detected, it is suggested that the proteins are peripherally associated to the cytoplasmic side of the ER with no luminal protrusion. The specific function of the different domains contained in the CymRSV p33 protein was investigated using a series of mutants, either fused to GFP or to the Myc tag. It was shown that the ER-targeting signal is composed by multiple recognition sequences in the N-terminal region of p33, including an amphipathic helix upstream of two transmembrane domains.

HYTRA1 FROM THE BENEFICIAL FUNGUS *TRICHODERMA HARZIANUM* T22 IS AN ELICITOR OF DEFENCE RESPONSES IN PLANT. M. Ruocco¹, S. Lanzuise², D. Turrà², F. Vinale², R. Marra², S.L. Woo² and M. Lorito². ¹Istituto per la Protezione delle Piante del CNR, Sezione di Portici, 80055 Portici (NA), Italy. ²Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Sezione di Patologia Vegetale, Università degli Studi di Napoli "Federico II", Via Università 100, 80055 Portici (NA) Italy. E-mail: miruocco@unina.it

Trichoderma harzianum T22 is one of the fungal isolates more used as active ingredient in commercial bio-fungicide and bio-fertilizer preparations. Mechanisms that regulate *Trichoderma* mycoparasitic activity have been widely studied and many of the active components involved have been found and characterised. In addition to their mycoparasitic capabilities, many *Trichoderma* strains are able to colonize roots and to grow in association with them, which may result in a significant increase of the plant growth and development and systemic resistance to pathogen attacks. A complete understanding of the *Trichoderma* spp.– plant recognition and communication process is lacking. We have studied the role of a protein named Hytra1 secreted by *T. harzianum* T22, and describe the molecular and biochemical characterization of this novel factor. This protein was purified both from culture filtrates and a heterologous expression system. The pure protein was tested for its ability to induce hypersensitivity response (HR), SAR, or ISR in tomato plants. Hytra1 infiltration elicits a strong HR on tomato leaves and triggers plant defence reactions both locally and systemically. Our results indicate that Hytra1 is an elicitor produced by the biocontrol fungus and probably a key component of the molecular dialog between *Trichoderma* T22 and tomato roots.

NEW FINDINGS ON THE STRUCTURE AND FUNCTIONS OF CERATO-PLATANIN AND ITS STRUCTURAL HOMOLOGS. A. Scala¹, C. Comparini¹, L. Carresi¹, E. Gemmi¹, L. Pazzagli², C. Zoppi², G. Cappugi², R. Bernardi³, M. Durante³, and A. Santini⁴. ¹Dipartimento di Biotecnologie Agrarie, Laboratorio di Patologia Vegetale Molecolare, Università degli Studi di Firenze, Via della Lastruccia 12, 50019 Sesto Fiorentino (FI), Italy. ²Dipartimento di Scienze Biochimiche, Università degli Studi, Viale Morgagni 50, 50134 Firenze, Italy. ³Dipartimento di Biologia delle Piante Agrarie, Sezione di Genetica, Università degli Studi, Via Matteotti 1/B, 56124 Pisa, Italy. ⁴Istituto per la Protezione delle Piante del CNR, Via Madonna del Piano 10, 50019 Sesto Fiorentino (FI), Italy. E-mail: aniello.scala@unifi.it

Cerato-platanin (CP), a 120 amino acid protein produced by the ascomycete *Ceratocystis platani* (Cep), the causal agent of plane canker stain, is the founder member of the recently identified cerato-platanin protein family [pfam07249]. CP is located in the cell walls of Cep mycelium, is secreted early in culture, contains four cysteines (S-S bridged), is moderately hydrophobic and is able to self-aggregate according to a hierarchical aggregation model. The DNA sequence of the CP gene contains an intron of 59 bp. CP elicits phytoalexin synthesis and/or cell necrosis in host and in non-host plant tissues. The new data presented in the present work include: (i) plane leaves pre-treated with 5×10^{-5} M CP restrict Cep growth and overexpress numerous defense-related genes, such as Rar1 protein, β -1,3 glucanase, thaumatin, lipid transfer protein, etc.; (ii) the sequences of the CP-homologs encoded by *C. populicola*, *C. cacaofunesta* and *C. fimbriata* (cloned from *Coffea arabica*, *Mangifera indica*, *Crotalaria juncea*, *Fagus* sp.) were obtained on the basis of the sequences of the homologs of the CP gene; (iii) the CP-homolog from *C. populicola*, named "cerato-populin" was purified and characterized with regard to both structure and resistance-inducing activity; (iv) the 3D structure of CP was determined by multidimensional NMR (Oliveira *et al.*, 2006; Spisni *et al.*, 2007, unpublished information).

IDENTIFICATION AND CHARACTERIZATION OF SINGLE SEQUENCE REPEATS FOR THE STUDY OF PHYTOPHTHORA SPECIES. L. Schena¹ and D.E.L. Cooke². ¹Dipartimento di Gestione dei Sistemi Agrari e Forestali, Università Mediterranea, Località Feo di Vito, 89060 Reggio Calabria, Italy. ²Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA Scotland, UK. Email: lschena@unirc.it

Microsatellites or single sequence repeats (SSR's) have recently emerged as one of the most powerful choice in the study of *Phytophthora* population biology, epidemiology, ecology, genetics and evolution. In the present study a SSR's database for species of the genera *Phytophthora* was developed taking advantage of publicly available sequences of *P. infestans* (<http://www.sanger.ac.uk/Projects/Fungi/>) and *P. sojae* and *P. ramorum* (<http://genome.jgi-psf.org/>). A first approach was aimed at the identification of useful loci common to many *Phytophthora* species and yielded 171 reliable sequences containing 211 SSR's. Identified microsatellites belonged to 16 target species representative of the breadth of diversity across the genus and ranged in the number of repeats from 3 to 16. Most of them showed seven repeats or less being four the most common repeat number and (AAG)_n, (AGG)_n and (AGC)_n the most common motifs. Trinucleotide repeats were largely the majority followed by pentanucleotide, tetranucleotide and dinucleotide repeats. A second approach was aimed at the identification of useful loci common to a restricted number of species closely related to *P. sojae* (*P. alni*, *P. cambivora*, *P. europaea* and *P. fragariae*). This analysis yielded 10 trinucleotide and 2 tetranucleotide SSR's which were repeated 4, 5 or 6 times. Although key

studies on inter- and intra-specific variation remain, the comprehensive dataset of SSR's already offer great potential for the study of *Phytophthora* species. Furthermore, independently from the presence of microsatellites, some of the amplified regions represent a valuable instrument as new targets to develop high specific molecular markers and for phylogenetic studies.

IDENTIFICATION OF GENES DIFFERENTIALLY EXPRESSED DURING THE INTERACTION BETWEEN TRICHODERMA HARZIANUM AND RHIZOCTONIA SOLANI BY RAPID SUBTRACTION HYBRIDIZATION. B. Scherm¹, M.A. Demontis¹, M. Schmoll², C. P. Kubicek² and Q. Migheli¹. ¹Dipartimento di Protezione delle Piante, Centro interdisciplinare di eccellenza per lo sviluppo della ricerca biotecnologica e per lo studio della biodiversità della Sardegna e dell'area mediterranea, Università degli Studi, Via E. De Nicola 9, 07100 Sassari, Italy. ²Vienna University of Technology, Institute of Chemical Engineering, Division Applied Biochemistry and Gene Technology, Getreidemarkt 9/E1665, 1060 Vienna, Austria. E-mail: scherm@uniss.it

To find key genes of *T. harzianum* expressed during the antagonistic interaction with *Rhizoctonia solani* a cDNA subtraction library technique (Rapid Subtraction Hybridization, RaSH) was used. cDNA from *T. harzianum* grown on complete medium was subtracted from cDNA derived from *T. harzianum* grown as antagonist in confrontation with *R. solani*. Differential gene expression of 200 clones was verified by Reverse Northern blot analysis. About 50 clones were sequenced and estimated for similarities by BLAST search of the JGI *T. reesei* genome database (<http://genome.jgi-psf.org/Trire2/Trire2.home.html>). A total of 13 fragments showed no hits on the databases; for 37 gene fragments, representing 25 different genes, corresponding counterpart sequences were found with similarities to *T. reesei*. The 14 most interesting genes were characterised for their differential expression patterns during the time course of interaction between *T. harzianum* and *R. solani* by Northern Blot analysis. Four genes seem to be significantly up-regulated during antagonism between *T. harzianum* and *R. solani*. These include a gene similar to the endoglucanase Cel61b of *H. jecorina*, a dehydrogenase with similarity to the *Fusarium* NUBM-NEUCR NADH ubiquinone oxidoreductase, a gene similar to *T. reesei* AXE1 (acetyl xylan esterase) and genes involved in general sugar transport. Present research focuses on the characterisation of the complete gene sequences and their regulatory targets in order to get a detailed knowledge of the gene role in metabolism during the antagonistic phase of *Trichoderma*. These data will permit the development of new molecular markers for a rapid and efficient identification of new biocontrol agents.

Work funded by the Italian Ministry of Foreign Affairs, Büro für Akademische Kooperation und Mobilität des Österreichischen Austauschdienstes, Fondazione Banco di Sardegna, University of Sassari.

DIVERSITY OF PHYTOPHTHORA COMMUNITIES IN SCOTTISH NATURAL ECOSYSTEMS. S. Scibetta¹, A. Chimento², L. Schena¹, S.O. Cacciola² and D.E.L. Cooke³. ¹Dipartimento di Gestione dei Sistemi Agrari e Forestali, Università Mediterranea, Località Feo di Vito, 89060 Reggio Calabria, Italy. ²Dipartimento di Scienze Entomologiche, Fitopatologiche, Microbiologiche e Zootecniche, Università degli Studi, Viale delle Scienze 2, 90128 Palermo, Italy. ³Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA, Scotland, UK. E-mail: cacciola@unipa.it

The presence and activity of *Phytophthora* species in natural

ecosystems is probably underestimated since our current knowledge is mainly based on traditional methods of detection (selective media and baiting techniques) and morphological identification which have several shortcomings. In the present study the diversity of *Phytophthora* communities in a range of Scottish natural sites was assessed by a molecular approach, based on sequencing cloned ITS regions obtained by nested-PCR amplification with genus-specific primers. This study provided the first molecular experimental procedure suitable to monitor *Phytophthora* species present in environmental samples. Total DNA was recovered from water and soil samples. An in-field system for filtering *Phytophthora* zoospores from water samples and subsequent DNA extraction from the filter proved to be rapid and efficient for large scale screening. The results of these surveys demonstrated a great diversity of *Phytophthora* species present in a range of different forest ecosystems. A comparative analysis with the baiting assay showed a much greater genetic diversity for each of the cloned libraries. The analysis of genetic distance revealed the presence of over 20 known species of *Phytophthora*, from all main ITS-clades of the genus. The range and type of species varied from sample to sample and up to five different *Phytophthora* species were detected in a single sample. The most frequently detected ITS sequences were related to the ITS-clade 6 (i.e. *P. gonapodyides*-like). Novel unreported ITS sequences were also identified, suggesting the presence of yet undescribed species.

EFFICACY, MECHANISM OF ACTION, MOLECULAR CHARACTERIZATION AND PRODUCTION METHODS: DIFFERENT APPROACHES TO DEVELOP A BIOFUNGICIDE FOR POSTHARVEST DISEASE CONTROL. D. Spadaro, S. Duraisamy, C. Annalisa, A. Garibaldi and M.L. Gullino. *AGROINNOVA, Centro di Competenza per l'Innovazione in Campo Agroambientale, Università degli Studi di Torino, Via L. da Vinci 44, 10095 Grugliasco (TO), Italy. E-mail: davide.spadaro@unito.it*

Metschnikowia pulcherrima occurs naturally on fruits and floral parts of apple trees. A strain (MACH1) of *M. pulcherrima* was studied for its efficacy as biocontrol agent against *Botrytis cinerea*, *Penicillium expansum* and *Alternaria alternata* on apples stored for 8 months at 1°C, providing interesting results. The strain was investigated for its competition for iron against postharvest pathogens of apple. *In vitro*, in the coloured inhibition zone, *B. cinerea* and *A. alternata* conidia did not germinate and mycelial degeneration was observed. On apple, a high reduction of both pathogen growth was recorded when fruits were treated with *M. pulcherrima*. Further, production of cell wall degrading enzymes was investigated with positive results for chitinase and beta-1,3-glucanase activities. The enhanced activity of defence enzymes, such as peroxidase, polyphenoloxidase and phenylalanine ammonia-lyase, were measured in yeast-treated apples. To develop industrially another strain (BIO126) of *M. pulcherrima*, molecular characterization to track the microorganism was carried out and production and formulation protocols were developed. Amplified fragment length polymorphism (AFLP) patterns clearly distinguished 26 strains of *M. pulcherrima*, isolated from different substrates in different geographical regions. A concentration of 10⁹ cfu/ml after 32 h fermentation was obtained in a substrate containing an organic nitrogen source and two carbon sources. Moreover, by adding several sugar solutions at different concentrations to the centrifuged yeast cell suspension, the best protective agent for freeze-drying was a 25% (v/v) maltose solution. No significant loss of viability on the formulated freeze-dried cells was recorded after 3 months of storage at 4°C.

CHARACTERIZATION OF THE HELPER COMPONENT-PROTEINASE GENE IN PLUM POX VIRUS ISOLATES. P. Spadone, P. Casati, N. Costa and P.A. Bianco. *Istituto di Patologia Vegetale, Università degli Studi, Via Celoria 2, 20133 Milano, Italy. E-mail: paola.spadone@unimi.it*

Typing of isolates of *Plum pox virus* strain M (PPV-M) was done using the RT-PCR assays followed by RFLP analysis of found polymorphism. Sequence data of PPV-M isolate SK68 (M92280) was used to design HC1 and HC2 primers. The 1526 bp long PCR fragment was amplified, which includes the 3' terminal part of protein P1 and the 5' terminal part of protein P3. PCR products of primers HC1/HC2 were sequenced. Data obtained from RFLP analysis and sequencing showed that same polymorphisms were present in PPV isolates. In particular an isolate from Stark Red Gold (BS 03) was similar to PPV-M PS. Other PPV-M isolates were similar to PPV-M SK68. Sequence analysis showed that the percentage of nucleotide identity among PPV isolates ranged from 98.1 to 99.2% for PPV-M SK68 and PPV-M PS (AJ 243957), respectively. The analysis showed also that the percentage of amino acid identity ranged from 99.2 to 100% for PPV-M SK68 and PPV-M PS, respectively. The regions so far known to be involved in aphid-mediated transmission, potyvirus genome amplification, or in the ability to suppress RNA silencing (Maia *et al.*, 1996. *J. Gen. Virol.* 77: 1335-1341; Urcuqui-Inchima *et al.*, 2000. *Virology* 268: 104-111) were conserved.

PATHOGENESIS OF FUSARIUM OXYSPORUM F. SP. MELO-NIS: A TRANSCRIPTOMIC APPROACH. K. Szafranska¹, F. Fusari², L. Luongo³, A. Polverari⁴, M. Delledonne¹, N. Ficcardenti² and A. Belisario³. ¹Dipartimento Scientifico e Tecnologico, Strada Le Grazie 15, 37134 Verona, Italy. ²CRA, Istituto Sperimentale per l'Orticoltura, Via Salaria 1, 63030 Monsampolo del Tronto (AP), Italy. ³CRA, Istituto Sperimentale per la Patologia Vegetale, Via C.G. Bertero, 22, 00156 Roma, Italy. ⁴Dipartimento di Scienze, Tecnologie e Mercati della Vite e del Vino, Villa Ottolini-Lebrecht, 37029 San Floriano di Valpolicella (VR) Italy. E-mail: massimo.delledonne@univr.it

Fusarium oxysporum f. sp. *melonis* is the agent of the most destructive disease of melon in Italy and worldwide. Four races are presently known (0, 1, 2, and 1,2), one of which, race 1,2, is able to overcome the resistance of commonly cultivated varieties. No genes have been identified in muskmelon that confer high levels of resistance to race 1,2. Thus, it would be important to understand the molecular basis of resistance and susceptibility in melon, and of virulence in the pathogen. A transcriptomic approach was undertaken by cDNA-AFLP on melon plants cv. Charentais-Fom2 infected with race 1 (avirulent) and race 1,2 (virulent), at 2, 4, 8 and 21 days after inoculation. RNA from fungal colonies of the two races was also included in the analysis, to identify possible fungal transcripts expressed in the plants during infection. A total of 1,376 differentially expressed bands were detected by running 128 primer combinations. All these bands were clustered in expression profiles as follows: (i) genes modulated in the incompatible interaction or (ii) in the compatible interaction only; (iii) genes modulated in both interactions with different profiles; (iv) genes expressed in plant, but showing a band of similar size also in fungal samples, which might be of fungal origin. All cDNA fragments have been eluted from the gels and will be sequenced for homology search in databases. Few differences in gene expression were detected between virulent and avirulent races grown in culture, which will be the basis for race characterization.

RISK OF ESTABLISHMENT AND SPREAD OF *PHYTOPHTHORA RAMORUM* IN THE MEDITERRANEAN AND FOREST AREAS IN ITALY AND SPAIN. A.M. Vettrai¹, E Moralejo², B. Ceccarelli¹ and A. Vannini¹ ¹Dipartimento di Patologia Vegetale, Università degli Studi della Toscana, Via S. Camillo de Lellis, 01100 Viterbo, Italy. ²Istituto Mediterraneo de Estudios Avanzados, Maiorca, Spain. E-mail:vettrain@unitus.it

Phytophthora ramorum is the causal agent of sudden oak death in the USA. Initially observed in Europe in container-grown rhododendron and viburnum plants in nurseries, is now known to infect a wide range of hosts. Symptoms vary on different hosts and are often restricted to leaf spots, stem and twig blight or, occasionally, seedling blight or tip dieback and bleeding cankers. The aim of this work was to assess the susceptibility of Mediterranean and forestry species and to predict the risk of establishment and spread of the pathogen in Italy, based on the potential host distribution and susceptibility. We tested the reaction of 74 coniferous and broad-leaved tree species to stem and leaf inoculation with this quarantine pathogen. Leaves were inoculated with 4×10^7 zoospores ml⁻¹. Stems were inoculated by placing a plug of mycelium in a wound. Foliar and stems lesions were examined after 10 days. The asexual sporulation *in planta* was examined. A significant variation on the leaf and stem susceptibility was observed among species. Disease did not develop on 6 species of 74 examined. The results indicated that Mediterranean species are less susceptible following artificial inoculation of leaves and stems, but they are characterized by a higher sporulation rate. Production of sporangia occurred also on symptomless leaves. The possible implications of sporulation potential and symptom development in *P. ramorum* epidemiology are discussed.

PATULIN DEGRADATION BY THE BIOCONTROL YEAST *RHODOTORULA GLUTINIS* STRAIN LS11. S.A.I. Wright¹, D. De Felice¹, A. Idnurm², J. Heitman² and R. Castoria¹. ¹Dipartimento di Scienze Animali, Vegetali e dell'Ambiente, Università degli Studi del Molise, 86100 Campobasso, Italy. ²Department of Molecular Genetics and Microbiology, Duke University, Durham, NC 27710, USA. E-mail: castoria@unimol.it

Postharvest rots in stored apples caused by *Penicillium expansum* are accompanied by contamination of the mycotoxin patulin. These rots can effectively be controlled through the application of biocontrol agents. One example is the basidiomycetous yeast *Rhodotorula glutinis* strain LS11 that not only inhibits the pathogen, but also lowers the level of patulin contamination of *P. expansum*-infected apples, and degrades it. The present study has focused on delineating the degradative pathway for patulin by strain LS11, using a combination of chemical and molecular bio-

logical approaches. *In vitro*, LS11 effectively degrades patulin to a compound, identified as desoxyapatulinic acid by NMR analyses, which was reported to be non-toxic to different microorganisms that are inhibited by patulin. An example of an organism that is highly sensitive to patulin but insensitive to desoxyapatulinic acid is *Escherichia coli*. This information has been used to develop a high-throughput assay for screening LS11 mutants for the degradation of patulin. Molecular work on LS11 has been initiated. *R. glutinis* LS11 was found haploid by FACS analysis. Uracil auxotrophs were created as another tool for molecular biological work, and are currently being characterized. LS11 was transformed by three different methods (electroporation, *Agrobacterium*-mediated transformation and the gene gun), and two sets of insertion mutants were generated. Work is underway to clone and characterize the genes involved in patulin degradation.

FREQUENCES AND EVOLUTION OF EFFECTOR GENES IN STRAINS OF *PSEUDOMONAS SYRINGAE* pv. *TOMATO*. M. Zaccardelli¹, F. Campanile¹, S. Yan² and B.A. Vinatzer². ¹CRA, Istituto Sperimentale per le Colture Industriali, Strada Statale 18, n. 204, 84091 Battipaglia (SA), Italy. ²Department of Plant Pathology, Physiology, and Weed Science, Virginia Tech, Blacksburg, USA. E-mail: massimo.zaccardelli@entecra.it

Effector genes encode proteins secreted into host cells through a type III secretion system by Gram negative pathogens bacteria. The main role of effectors is the suppression of host defenses. In this work, 38 isolates of *Pseudomonas syringae* pv. *tomato* (*Pto*), the causal agent of bacterial speck of tomato, were characterized by PCR, for the frequency of the effector genes *avrPto* (new denomination *avrPto1_{PtoDC3000}*), *avrPtoB* (*hopAB2_{PtoDC3000}*), *avrRpt2* (*AvrRpt2_{PtoJL1065}*), *hopPtoA2* (*hopAA1-2_{PtoDC3000}*), *hopPtoF* (*hopF2_{PtoDC3000}*) and *hopPtoG* (*hopG1_{PtoDC3000}*). *Pto* strains analysed in this study came from different locations in Italy (23 strains), France (3 strains), Spain (1 strain), Great Britain (1 strain), North America (6 strains) and Tanzania (4 strains). All *Pto* isolates contained *avrPto* except two from North America. All isolates, except one from France, gave amplicons for *avrPtoB*, *hopPtoF* and *hopPtoG*. For *hopPtoA2*, all isolates gave the amplicon except for one strain from France and one from Northern Italy. *AvrRpt2* was present in all *Pto* isolates except in one from Sicily (Italy). These results suggest that all effector genes tested are very common in *Pto* populations all over the world. To get additional insight into the pathogenesis of *Pto* strains, a draft genome sequence of one *Pto* strain was obtained by 454 pyrosequencing and compared to the sequenced *Arabidopsis thaliana* and tomato strain *PtoDC3000*. Interestingly, several effectors present in *PtoDC3000* are either completely absent in *PtoT1* or have early stop codons. The importance of these deletions and mutations for host range will be discussed.