

**PROTEOMIC ANALYSIS OF *TRICHODERMA HARZIANUM* SECRETED PROTEINS BY PEPTIDE MASS FINGERPRINT AND EXPRESSED SEQUENCE TAG APPROACHES.** P. Ambrosino<sup>1</sup>, R. Marra<sup>1</sup>, V. Carbone<sup>2</sup>, M. Ruocco<sup>1</sup>, S.L. Woo<sup>1</sup>, F. Scala<sup>1</sup> and M. Lorito<sup>1</sup>. <sup>1</sup>Dipartimento Ar.Bo.Pa.Ve., Sezione di Patologia Vegetale, Università degli Studi di Napoli, CNR-IPP, Via Università 100, Portici (NA) 80055, Italy. <sup>2</sup>ISA-CNR, Istituto di Scienze dell'Alimentazione, Via Roma 52, 83100 Avellino, Italy. Fax: +39.081.2539339; E-mail: lorito@unina.it.

*Trichoderma harzianum* is an antagonist able to parasitize phytopathogenic fungi. It secretes proteins that play a pivotal role in the penetration of the host fungus cell wall and in the induction of systemic disease resistance in plant. Two-dimensional gel electrophoresis was used to display the secretome of *T. harzianum* T22 grown on media containing different inducers of mycoparasitic activities. Twenty-eight differentially expressed proteins were identified by using MALDI-TOF and MS/MS analyses and by searching the Expressed Sequence Tag (EST) database of *Trichoderma* spp.. Identified up-regulated proteins include: (i) different cell wall-degrading enzymes, such as endo-polygalacturonases, chitin deacetylases, xylanases, cellulases, proteases etc; (ii) proteins with conserved domains involved in pathogen-host interactions such as Ras or LRRs, and (iii) ABC transporter proteins required to regulate the efflux/afflux of molecules across the cell membrane. Water-soluble fractions of extracellular proteins inhibiting tobacco infection by *Botrytis cinerea* include an endopolygalacturonase and a chitin deacetylase. Future work will address cloning of the genes coding for these two proteins, their disruption and overexpression in *Trichoderma*, and their expression in vectors to obtain large quantities of proteins.

**TRANSCRIPTIONAL PROFILING OF TOMATO PLANTS INFECTED WITH THE TOMATO YELLOW LEAF CURL SARDINIA VIRUS BY LONG SERIAL ANALYSIS OF GENE EXPRESSION.** A. Berardi, A. Luciola, F. Gatti, G. Giuliano and M. Tavazza. Biotec Gen ENEA C.R. Casaccia, Via Anguillarese 301, 00060 Rome, Italy. Fax: +39.06.30484203; E-mail: alessandra.luciola@casaccia.enea.it.

Tomato yellow leaf curl sardinia virus (TYLCSV) (genus *Begomovirus*, family *Geminiviridae*) is one of the agents of the devastating tomato yellow leaf curl disease. Although a huge amount of molecular data exist on TYLCSV, little is known about its interaction with the host plant. To gain insight into TYLCSV-tomato interactions, the transcriptional profile of TYLCSV-infected tomato plants was investigated by Long-Serial Analysis of Gene Expression (Long SAGE). This technique, based on the identification of 20 nt long sequences (TAGs), allows the quantitative analysis of transcripts without the need of any prior knowledge of their sequences. Two Long SAGE libraries from TYLCSV-infected and healthy tomato plants were constructed. About 41,000 TAGs were produced for each library, representing a total of 9,433 genes, among which 5,809 differed in their expression by at least two-fold. A preliminary analysis was made on TAGs whose number differed at least seven-fold between infected and healthy samples. In TYLCSV infection, 62 and 50 TAGs were up- and down-represented, respectively. Among the up-represented TAGs, besides viral transcripts, 39 matched tomato unigenes and 15 were not found in the SGN data bank, representing possible novel genes. In the down-represented group, only 20 TAGs matched tomato unigenes. Moreover, 10 out of 59 TAGs matching tomato unigenes were in antisense orientation. As to the function of up- and down-regulated genes, TYLCSV infection stimulates expression of genes involved in defense and stress responses, signal transduction, and protein metabolism, but reduces the expression of genes involved in photosynthesis.

**DIFFERENT APPROACHES TO STUDY THE INTERACTION *CITRUS/PHOMA TRACHEIPHILA* REVEAL AN IMPORTANT ROLE PLAYED BY OXIDATIVE STRESS.** C. Betti, M. Reverberi, S. Zjalic, B. Mattei, A.A. Fabbri and C. Fanelli. Dipartimento di Biologia Vegetale, Università di Roma "La Sapienza", Largo Cristina di Svezia 24, 00165 Roma, Italy. Fax: +39.06.6833878; E-mail: corrado.fanelli@uniroma1.it.

Mal secco is a disease of *Citrus* caused by the mitosporic ascomycete *Phoma tracheiphila*, a pathogen that produces a phytotoxic complex known as "malseccin". Based on a six peptides sequence identified by Fogliano *et al.* (1998) several couples of degenerated primers were designed for attempting the isolation of a unique gene sequence coding for malseccin, either from genomic DNA or cDNA. A number of fragments obtained by PCR amplification of both templates was cloned and the conceptual translation of the obtained sequences showed significant homologies (>50-60%) with iron membrane transporter, NADPH-dependent reductase, and a monoamine oxidase. Considering these results it is impossible to assign a unique nucleotide sequence corresponding to malseccin. A MALDI-TOF/TOF analysis of extracellular proteins of *P. tracheiphila* was carried out to evaluate the possible presence of more than a single protein in the 60 kDa band found by Fogliano *et al.* (1998). It was found that a complex of proteins with different functions is present in the single 60 kDa band, suggesting that more than one toxic component is involved in the genesis of mal secco disease, even though also this analysis detected the presence of a monoamine oxidase in this mixture. Physiological experiments have disclosed the activity of different enzymes related to the oxidative burst in different filtrates of *P. tracheiphila*, indicating that, in the tolerant lemon cv Monachello this fungus undergoes oxidative stress that affects its ability to invade the host.

**POPULATION DYNAMICS OF *PENICILLIUM EXPANSUM* STRAINS ON PEARS.** L. Casalini, E. Baraldi and M. Mari. Criofo - Dipartimento di Protezione e Valorizzazione Agroalimentare, Università degli Studi di Bologna, Via Gandolfi 19, 40057 Cadriano, Bologna, Italy. Fax: +39.051.765049; E-mail: fiorella.neri@unibo.it.

Blue mould caused by *Penicillium expansum* is the most common disease of stored pears. In the past, *P. expansum* has been controlled by postharvest chemical treatments, mainly thiabendazole (TBZ) and benomyl, but the occurrence of resistant strains to these fungicides has reduced the effectiveness of such treatments. This study examined 120 isolates from stored pears showing typical symptoms of blue mould. Morphological and genetical characterization showed that all isolates belong to *P. expansum*, confirming that this is the prevailing agent of blue mould in pears. Artificial inoculation of wounded pears by two fungal isolates showed different types of interaction: (i) a synergic effect when the lesions produced were wider than those produced by a single strain; (ii) a competition effect when the lesions were smaller and (iii) a neutral effect when lesions were the same as those produced by a single strain. These relations were not influenced by the isolates resistant or susceptible to thiabendazole. *In vitro* trials showed that competition for nutrients and toxic metabolites production have a deep influence on lesion diameter, although a physical interaction between both strains cannot be excluded. A significant reduction of pH was observed in media where the strains were grown. Toxic metabolites produced by different fungal strains were investigated using TLC technique. Patulin production was observed only in 60% of the tested strains, other extracellular metabolites were found in competitive strains. Further investigations are required to identify metabolites that can influence the interactions of the various *P. expansum* isolates.

**FURTHER DATA ON MOLECULAR CHARACTERIZATION OF PPV-M IN NORTHERN ITALY.** P. Casati<sup>1</sup>, P.A. Bianco<sup>1</sup>, A. Fanigliulo<sup>2</sup>, S. Comes<sup>2</sup>, A. Crescenzi<sup>2</sup> and G. Belli<sup>1</sup>. <sup>1</sup>Istituto di Patologia Vegetale, Università degli Studi e Istituto di Virologia Vegetale - CNR, Via Celoria 2, 20133 Milano, Italy. <sup>2</sup>Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali, Università degli Studi della Basilicata, Campus Macchia Romana 3A310, 85100 Potenza, Italy. Fax: +39.097.1205700; E-mail: crescenzi@unibass.it.

Sharka, a disease induced by *Plum pox virus* (PPV), is becoming endemic in some important stone fruit-growing areas. Despite the enforcement in Italy of a mandatory control scheme for PPV, the successful elimination of sharka was reported only from Apulia (south-east Italy). In the last decade the quick spreading of a PPV-M strain very similar to the Hungarian isolate SK68 (GenBank, M92280) has been observed in Northern and Southern Italy, supporting the notion of a unique origin of the epidemics. Previous information based on PPV coat protein (CP) sequence data obtained from a selected diseased peach plant, disclosed the existence of diverse isolates of PPV-M in the provinces of Verona and Brescia (northern Italy) and in Basilicata (southern Italy). In this work the results are reported of the molecular characterization of 35 PPV-M isolates collected in two areas of northern Italy. PCR/RFLP analyses were carried out on three PPV genomic fragments corresponding to the helper component (HC) gene, the P3+6K<sub>1</sub> and the CP regions. The results confirmed the prevalence of the isolate PPV-M/SK68 and showed the presence of six sequence variants of PPV-M, identified on the basis of the RFLP tests. In particular, one of these isolates showed a pattern very similar to that of the Serbian strain PPV-M/PS (accession n° AJ243957). Further studies should be made to evaluate the frequency and distribution of these sequence variants in peach orchards and nurseries.

**IN VITRO DEGRADATION OF OCHRATOXIN A TO LESS TOXIC OCHRATOXIN BY AUREOBASIDIUM PULLULANS BIOCONTROL STRAINS.** R. Castoria<sup>1</sup>, D.V. De Felice<sup>1</sup>, M. Solfrizzo<sup>2</sup>, V. Lattanzio<sup>2</sup>, F. De Curtis<sup>1</sup> and V. De Cicco<sup>1</sup>. <sup>1</sup>Dipartimento di Scienze Animali, Vegetali e dell'Ambiente, Sezione di Patologia Vegetale, Università degli Studi del Molise, Via De Sanctis, 86100 Campobasso, Italy. <sup>2</sup>Istituto di Scienze delle Produzioni Alimentari, CNR - Bari, Via Amendola 122/O, 70126 Bari, Italy. Fax: +39.0874.404855; E-mail: castoria@unimol.it.

Ochratoxin A (OTA) is a possibly carcinogenic mycotoxin consisting of two moieties linked by a peptide bond: 7-carboxy-5-chloro-8-hydroxy-4,4-dihydro-3R-methylisocoumarin (i.e. the less toxic ochratoxin  $\alpha$ , OT- $\alpha$ ) and L- $\beta$ -phenylalanine. OTA is produced by *Aspergillus* and *Penicillium* spp. and is frequently detected as a contaminant of different crops (e.g. cereals) and beverages (coffee, cocoa and wine). Wine contamination derived from the attack of *A. carbonarius* to wine grapes. Preventive measures cannot eliminate OTA contamination, whose highest tolerable levels have recently been established by the European Community for food commodities, including wine and grape juices [Reg. (CE) No. 123/2005]. Therefore, possible methods for the decontamination/detoxification of this mycotoxin to less toxic compounds are worth of investigation. In this study, we assessed the ability of *in vitro* OTA degradation by four strains of the yeast-like fungus *Aureobasidium pullulans* (LS30, AU14-3-1, AU34-2, AU18-3B), displaying biocontrol activity against *A. carbonarius*. These strains were grown for six days in the presence of OTA and mycotoxin decrease was monitored by HPLC analysis. Strains AU34-2 and AU14-3-1 were the most effective in reducing OTA concentration. OTA decrease started from the 4th day of incubation and reached its maximum on the 6th day, when recovery of the mycotoxin was lower than 9% for AU34-2, about 10% for AU14-3-1, 17% for

LS30, and 23% for AU18-3B, as compared to respective controls. Liquid chromatography-mass spectrometry analyses of culture media enabled us to identify and quantify OT- $\alpha$  as the main degradation product of OTA by the four biocontrol fungal strains.

**DETECTION OF PSEUDOMONAS SAVASTANOI PV NERII BY PCR AND REAL-TIME PCR.** V. Catara<sup>1</sup>, G. Licciardello<sup>2</sup>, P. Bella<sup>2</sup>, L. Raciti<sup>2</sup> and M. Tessitori<sup>1</sup>. <sup>1</sup>Dipartimento di Scienze e Tecnologie Fitosanitarie, Università degli Studi di Catania, Via Santa Sofia 100, 95123 Catania, Italy. <sup>2</sup>Parco Scientifico e Tecnologico della Sicilia, Z.I. Blocco Palma 1, 95131 Catania, Italy. Fax: +39.095.7147379; E-mail: vcatara@unict.it.

Oleander knot disease control is based on preventive measures as the use of pathogen-free propagating material. Since fast and reproducible detection methods are needed and available PCR protocols for *P. savastanoi* pv *savastanoi* detection in olive are not fully satisfactory for processing oleander samples, we developed a modified sample processing method and a new primer pair targeted to the *iaaL* gene. The 101 bp DNA amplicon was also detected with a TaqMan real-time PCR assay. Fifty *P. savastanoi* strains from eight different hosts were positive by PCR tests, whereas no positive results were obtained with any of the other 15 pseudomonad strains tested. Real-time PCR reactions utilizing the rapid cycling SmartCycler TD II (Cepheid) coupled with tissue extraction with Generation Capture Column kit (Gentra, Minneapolis, MN, USA) allowed to complete a diagnostic identification from symptomatic olive and oleander samples in 1 h. In pure culture, detection of *P. savastanoi* pv *nerii* to a concentration of as little as 20 cells per reaction was achieved. Sensitivity was similar when PCR was made with DNA extracted from oleander tissue with known bacterial amounts. A 24-h enrichment step either on PVF-1 or OKAm broth improved the sensitivity of PCR to 10 cells ml<sup>-1</sup>. Cycle threshold values showed a better sensitivity after enrichment in PVF-1 than on OKAm, and in DNA obtained with a Puregene Cell and Tissue kit (Gentra) compared to that obtained with columns. Detection of the bacterium was successful also from symptomless stems of symptomatic plants, and was more effective after bacterial enrichment.

**CONTROL OF TOMATO CORKY-ROOT WITH OZONE FUMIGATION IN OPEN FIELD.** F. Ciccarese, O. Longo, D. Schiavone, A. Ambrico A. and T. Ziadi. Dipartimento di Biologia e Patologia Vegetale, Università degli Studi di Bari, Via G. Amendola 165/A, 70126 Bari, Italy. Fax: +39.080.544290; E-mail: ficcare@agr.uniba.it.

In previous experiments, ozone fumigation of soil yielded good results in controlling corky-root of greenhouse tomato caused by *Pyrenochaeta lycopersici*. In this work, results of trials with ozone soil fumigation on tomato grown in open field naturally infected by *P. lycopersici* are reported. Ozone as ozonising water (at the concentration of 0.750 mg l<sup>-1</sup> of ozone), manufactured on site with a generator, was applied through drip tubing buried at a depth of 200 mm in the soil. The ozonising water was also delivered through drip-tubing set on the mulched soil. Growth of tomato plants, marketable crop, and corky-root severity were recorded. No positive effect was observed in plots where ozonising water was applied through drip-tube set on mulched soil. The treatment with ozonising water applied through drip tubes buried in soil showed a considerable increase in total marketable yield as compared with the untreated control. As to corky-root, ozonising water treatment reduced disease severity, thus confirming the results obtained in previous greenhouse trials.

**CMV- AND PVY-MEDIATED RNA SILENCING IN MIXED INFECTIONS IN TOMATO.** F. Cillo, M. Finetti-Sialer, V. Fanelli and D. Gallitelli. *Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi di Bari and Istituto di Virologia Vegetale del CNR, Via Amendola 165/A, 70126 Bari, Italy.* Fax: +39.080 5442911; E-mail: f.cillo@ba.ivv.cnr.it.

We investigated the role of RNA silencing-based defence mechanisms in tomato plants supporting a double infection with *Cucumber mosaic virus* (CMV) and *Potato virus Y* (PVY). For this purpose we generated *in vitro* transcripts of: (i) two CMV strains (CMV-Fny and CMV-LS); (ii) two pseudorecombinants (CMV-F1LS2F3 and CMV-F1d2bF3) and (iii) the strain SON41 of PVY (PVY-SON41), expressing the strong RNA silencing suppressor HC-Pro protein. CMV-F1LS2F3 and CMV-F1d2bF3 had a different RNA-2, which influenced their ability to induce disease symptoms and to move systemically in the plant. CMV-F1LS2F3, as well as the RNA-2 donor strain CMV-LS, induced very mild symptoms in tomato, while CMV-F1d2bF3, a modified CMV-Fny that cannot translate the 2b protein, was unable to infect tomato plants systemically. The PVY-SON41 infection was symptomless in tomato. All CMV variants, when inoculated to plants at three days post-inoculation with PVY, reduced the level of PVY RNA accumulation. On the other hand, PVY affected the CMV-Fny infection increasing symptom severity and RNA accumulation. PVY effects on CMV-F1LS2F3 led to the appearance of CMV-Fny-like symptoms, but were not significant on the accumulation of CMV RNAs. Additionally, CMV-F1d2bF3 was able to spread systemically in PVY-infected tomato plants. Small interfering RNA (siRNA) accumulation varied in the different PVY/CMV combinations, suggesting no role of CMV 2b protein in preventing RNA silencing directed against PVY. The role of RNA silencing and silencing suppression on both the increased CMV accumulation and severity of disease phenotypes observed in mixed infections remains to be cleared.

**SOURCES OF VERTICILLIUM WILT RESISTANCE IN WILD OLIVE GERMLASM FROM THE MEDITERRANEAN REGION.** C. Colella, C. Miacola, M. Amenduni, M. D'Amico, G. Bubici and M. Cirulli. *Dipartimento di Biologia e Patologia Vegetale, Università degli Studi di Bari, Via G. Amendola 165/A, 70126 Bari, Italy.* Fax: +39.080.5442906; E-mail: cirullim@agr.uniba.it.

Fifty-seven wild olive accessions collected from the Mediterranean basin were screened for resistance to *Verticillium* wilt. One defoliating and one non-defoliating *Verticillium dahliae* isolate, both obtained from diseased plants in southern Italy, were used. Disease reaction of tested accessions was evaluated on the basis of external symptoms, vascular browning and by calculating the area under disease progress curve (AUDPC). On the basis of AUDPC values and severity of external symptoms, the tested accessions were grouped into four phenotypic groups: highly resistant, moderately resistant, susceptible, and highly susceptible. Most accessions showed different levels of resistance/susceptibility to both *V. dahliae* pathotypes. A minor part was resistant/susceptible to one of the two pathotypes only. Three accessions showed high type resistance to both *V. dahliae* pathotypes. Forty resistant plants were selected from accessions that had shown the highest levels of resistance. From each of these plants, clones were obtained by *in vitro* micro-propagation. The M-1 clones were inoculated with the defoliating pathotype using the same procedures adopted to test the original accessions. Ten M-1 clones, showed the high type resistance characteristic of their original mother plants, while the others showed different levels of disease severity. This study provided the identification of new olive root-

stocks highly resistant to *Verticillium* wilt, which could be included in breeding programs for resistance of olive to *V. dahliae*.

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**ENHANCEMENT OF ANTAGONIST EFFICACY IN CONTROLLING CITRUS GREEN MOLD WITH CARBONIC ACID SALTS SOLUTIONS: INVOLVEMENT OF HOST DEFENCE MECHANISMS.** G. D'hallewin, G. Arras, M.G. Molinu, A. Dore and T. Venditti. *ISPA-CNR Sezione di Sassari, Via Dei Mille 48, 07100 Sassari, Italy.* Fax: +39.079.2320047; E-mail: Guy.dhallewin@ispa.cnr.it.

Carbonic acid salts, mainly sodium carbonate-bicarbonate (SC-SBC), have been used for long to control the green mold agent (*Penicillium digitatum*) on Citrus fruits, but their mode of action is still incompletely understood. SC and SBC were also used together with antagonist bacteria or yeasts and the results obtained indicate synergistic interactions with a significant increase of their efficacy. To improve the effectiveness of these treatments studies were carried out to understand the mode of action of SC and SBC solutions when used alone or combined with the yeast 5A, an isolate of *Pichia guilliermondii*. The experiments carried out using inoculated viable and nonviable albedo with *P. digitatum* showed that SC-SBC salt solutions induced natural resistance and enhanced growth of the antagonist. The incidence of decay in nonviable albedo showed a clear-cut role of the induced natural resistance in containing *P. digitatum* infection. A comparative *in vitro* growth experiments, using the yeast 5A, in amended (SC-SBC) and non amended liquid media showed a faster increase of cfu by two log units in the amended media after 2 days of incubation. This significant difference of cfu may account for the synergistic increase of the efficacy when the isolate 5A was combined with SC or SBC solutions.

**MECHANISMS UNDERLYING INDUCED RESISTANCE IN BARLEY AGAINST POWDERY MILDEW.** F. Faoro<sup>1</sup>, D. Maffi<sup>2</sup>, D. Cantu<sup>1</sup> and M. Iriti<sup>2</sup>. <sup>1</sup>CNR, Istituto di Virologia Vegetale and <sup>2</sup>Istituto di Patologia Vegetale, Università degli Studi di Milano, Via Celoria 2, 20133 Milano, Italy. Fax: +39.02.503.16781; E-mail: f.faoro@ivv.cnr.it.

Chitosan (CHT), a deacetylated chitin derivative, and benzo-(1,2,3)-thiadiazole-7-carbothioic acid S-methyl ester (BTH), a non toxic synthetic functional analogue of salicylic acid, were applied as foliar spray to barley plants (*Hordeum vulgare* L.), to compare their effectiveness in inducing resistance against *Blumeria graminis* f.sp. *hordei* and to investigate the mechanisms underlying the establishment of acquired resistance. In CHT-treated plants, a significant reduction of the infected areas (-49%) was observed, compared to the untreated controls, while in plants sprayed with BTH the reduction was even greater (-76%). In BTH- and CHT-treated plants conidia germination was not inhibited, whereas 4 and 8 days post inoculation (dpi), respectively, the haustorial growth was slowed down by the apposition of phenolic compounds in the epidermal cells neighboring the haustorium, as assessed by cytochemical assays. On the contrary, callose deposition in papillae did not impair fungal penetration in all treated plants, as shown also when its synthesis was impaired by the callose synthesis inhibitor 2-deoxy-D-glucose (DGG). Hydrogen peroxide accumulated in the epidermal cell walls adjacent to mesophyll cells at 0.25 dpi, with a maximum at 1-2 dpi, but no cell

death was detected in the same treated tissues. These results, besides confirming the effectiveness of both CHT and BTH as resistance inducers in barley, suggest that the resulting quantitative resistance is a consequence of H<sub>2</sub>O<sub>2</sub> accumulation and phenolic compound deposition, rather than callose apposition to papillae.

**USE OF TARGETED DISRUPTION AND GFP MUTANTS TO STUDY THE ROLE OF THE GENE CODING FOR AN ABC TRANSPORTER IN *TRICHODERMA ATROVIRIDE*.** S. Gigante, M. Ruocco, S. Lanzuise, C. Analeria, F. Scala, S.L. Woo and M. Lorito. *Dipartimento Ar.Bo.Pa.Ve., Sezione di Patologia Vegetale, Università degli Studi di Napoli and CNR-IPP, Via Università 100, Portici (NA) 80055, Italy. Fax: +39.081.2539339; E-mail: lorito@unina.it.*

The role of the ABC transporter proteins in *Trichoderma* is still unknown. This work is a preliminary study on the functions of the *TABC2* gene of *T. atroviride* through the targeted gene disruption and the GFP expression under the promoter region of the gene. Hygromycin resistant progeny have been selected and analysed to verify the disruption of the gene and the GFP expression. Disruptant and GFP mutants were tested by molecular analysis (PCR, Southern and northern blotting) to verify the correctness of transformation events. *In vitro* assays were done to determine the role of the gene during fungal growth and biocontrol activity. The disruptants were grown on PDA to which four different carbon sources (glucose 1%, glycerol 1%, colloidal chitin 0,05% and fructose 1%) were added. As a result, we observed a better growth of the transformants on fructose, while by comparison with the wild type strain, the growth was slow and difficult on glucose and chitin. This was confirmed when all the strains were grown in the presence of culture filtrates of *Botrytis cinerea*, *Rhizoctonia solani*, and *Pythium ultimum* added to SM containing glucose or fructose. The presence of the culture filtrates was not discriminating, while the transformants showed a good rate of growth on fructose and a difficult growth on glucose, as in the previous assay. Finally, we tested all transformant strains for the production of some enzymes. They all showed a lower concentration of all enzymes tested except for  $\beta$ -1,3-glucanase, in comparison with the wild type strain. The results obtained with the disruptants were confirmed by the use of GFP mutants.

**CHARACTERIZATION OF ATYPICAL STRAINS OF *PSEUDOMONAS SAVASTANOI* PV *SAVASTANOI*.** N.S. Iacobellis<sup>1</sup>, P. Lo Cantore<sup>1</sup>, G. Mastronuzzi<sup>1</sup> and A. Sisto<sup>2</sup>. <sup>1</sup>*Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali, Università degli Studi della Basilicata, Viale dell'Ateneo Lucano 10, 85100 Potenza, Italy.* <sup>2</sup>*Istituto di Scienze delle Produzioni Alimentari - CNR - Bari, Via Amendola 122/O, 70126 Bari, Italy. Fax: +39. 0971.205702; E-mail: iacobellis@unibas.it.*

Bacteria isolated from olive and oleander knots or from hyperplastic cankers on ash were recently classified as *Pseudomonas savastanoi* pv *savastanoi*, *P.s.* pv *nerii*, and *P.s.* pv *fraxini*, respectively. In particular, several investigations showed that oleander strains in artificial infections caused overgrowths in oleander, olive, and ash, whilst olive and ash strains were avirulent in oleander. The above behavior suggested that bacteria evading from diseased oleander plants could represent an inoculum source for olive knot disease. However, previous findings indicated that oleander strains do not apparently infect olive plants in nature. More recent investigations confirmed this. However, during our investigations a representative number of strains, isolated from

knots and the phylloplane of olives that were in physical contact with oleander plants, did not produce bacteriocins, a typical feature of olive strains, but infected oleander, causing typical knots. RFLP analysis using the BATT-1 probe involved in the bacteriocin biosynthesis in *P.s.* pv *savastanoi* showed that, contrary to bacteriocin-producing strains, the strains under study lacked a 10.3 kb *Hind*III DNA fragment hybridizing with the probe. This DNA sequence appears important for bacteriocin biosynthesis. Further RFLP analysis using the D2 probe, which discriminates olive and oleander strains, showed that the strains in question are apparently atypical olive strains since, like typical strains and contrary to oleander strains, they presented a 28 kb *Hind*III DNA fragment hybridising with the probe.

**ALTERED PATHOGENICITY OF THE OLIVE ANTHRACNOSE PATHOGEN BY *IMPALA* TRANSPOSON TAGGING.** M.G. Li Destri Nicosia<sup>1</sup>, S.O. Cacciola<sup>2</sup> and G.E. Agosteo<sup>1</sup>. <sup>1</sup>*Dipartimento di Agrochimica ed Agrobiologia, Università Mediterranea di Reggio Calabria, Piazza S. Francesco di Sales 2, I-89061 Gallina (RC), Italy.* <sup>2</sup>*Dipartimento S.En.Fi.Mi.Zo, Università degli Studi di Palermo, Viale delle Scienze 2, I-90128 Palermo, Italy. Fax: +39.091.7028855; E-mail: cacciola@unipa.it.*

The causal agent of olive anthracnose in Italy, formerly referred to as *Gloeosporium olivarum*, has been recently demonstrated to be a *Colletotrichum* sp. distinct from both *C. gloeosporioides* and *C. acutatum* (Agosteo *et al.*, 2003, *J. Plant Pathology* 85: 280). A stable *niaD* mutant of this pathogen was transformed with an autonomous copy of *impala*, a DNA transposable element isolated from *Fusarium oxysporum*, by using a construct carrying the transposon inserted into the promoter region of the *Aspergillus nidulans* nitrate reductase (*niaD*) gene. Transposition events were selected by using a phenotypic excision assay. Excision and reinsertion events were demonstrated at the molecular level by southern blotting analysis, and a high frequency of reinsertion events was recorded. Further demonstration of *impala* excision was obtained by sequencing the footprint left by the transposon at the excision site. Typical *impala* footprints of two or three additional nucleotides were found. Two revertants (R31 and R39), among 24 analysed, showed an altered ability to cause necrotic lesion on apples and olives as compared with the original transformant as well as other revertants. Revertant R31 was significantly more virulent, whereas R39 was less virulent than the other strains. Southern blotting analysis showed that *impala* excised from the *niaD* gene, was reinserted in a different position in the genome of both revertants, thus suggesting that this transposon could have affected the expression of gene (s) involved in pathogenicity.

**REAL-TIME PCR: A MOLECULAR TOOL FOR THE EARLY DETECTION OF FUNGAL DISEASES IN FOREST TREES.** N. Luchi<sup>1</sup>, P. Capretti<sup>1</sup>, P. Pinzani<sup>2</sup>, C. Orlando<sup>2</sup>, M. Pazzagli<sup>2</sup>, A. Vettraino<sup>3</sup> and A. Vannini<sup>3</sup>. <sup>1</sup>*Dipartimento Biotecnologie Agrarie, Sezione di Patologia Vegetale, Università degli Studi di Firenze, Piazzale delle Cascine 28, Firenze, Italy.* <sup>2</sup>*Dipartimento Fisiopatologia Clinica, Sezione di Biochimica Clinica, Università degli Studi di Firenze, Viale Pieraccini 6, Firenze, Italy.* <sup>3</sup>*Dipartimento di Protezione delle Piante, Università della Tuscia, Via San Camillo de Lellis, Viterbo, Italy. Fax: +39.0761.357473; E-mail: vettrain@unitus.it.*

During the last few years, molecular detection techniques like real-time PCR have become important tools for the identification of pathogens in asymptomatic plants and, more recently, for the study of fungal diseases in forest trees and for assessing and quantifying mycoflora fluctuations in latent phase. Some of these

pathogens are difficult to isolate on agar media, while others are masked by endophytic micro-organisms that overgrow them *in vitro*. In this study a real-time PCR assay was developed for detection of fungi in symptomless tissues of three pathosystems: *Sphaeropsis sapinea*/*Pinus nigra*, *Biscogniauxia mediterranea*/*Quercus cerris* or *Quercus ilex*, and *Biscogniauxia nummularia*/*Fagus sylvatica*. Samples collected from one-year-old shoots of asymptomatic trees were divided in two portions: one for isolations on PDA, as control, and the other for DNA extraction. Real-time PCR was done using TaqMan™ chemistry after designing specific primers and probes for each fungal species and testing them on axenic cultures (Luchi *et al.*, 2005, LAM 41: 61-68). The detection threshold of the assay ranged from 0.001 to 0.01 pg of DNA. Pathogenic fungi were detected by real-time PCR in symptomless shoots with percentages ranging from 75% to 95%. This study confirmed the endophytic habit of the investigated fungi, which may affect trees subjected to environmental stresses.

**A GENE ENCODING A POLYKETIDE SYNTHETASE MAY AFFECT THE VIRULENCE OF *DIAPORTHE HELIANTHI*.** B.A. Maimone Mancarello<sup>1,4</sup>, R. Vergara<sup>2</sup>, G. Firrao<sup>3</sup>, S.O. Cacciola<sup>4</sup>, G. Magnano di San Lio<sup>5</sup>, F. Scala<sup>1</sup> and G. Del Sorbo<sup>1</sup>. <sup>1</sup>Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università degli Studi di Napoli, Via Università 100, 80055 Portici (NA), Italy. <sup>2</sup>Dipartimento di Coltivazione e Difesa delle Specie Legnose, Sezione di Patologia Vegetale, Università degli Studi di Pisa, Via del Borghetto 80, Pisa, Italy. <sup>3</sup>Dipartimento di Biologia Applicata alla Difesa delle Piante, Università degli Studi di Udine, Via delle Scienze 208, 33100 Udine, Italy. <sup>4</sup>Dipartimento S.En.Fi.Mi.Zo., Sezione di Patologia Vegetale e Microbiologia Agraria, Università degli Studi di Palermo, Viale delle Scienze 2, 90129 Palermo, Italy. <sup>5</sup>Dipartimento di Agrochimica e Agrobiologia, Università Mediterranea di Reggio Calabria, Piazza S. Francesco di Sales 4, 89061 Reggio Calabria, Italy. Fax: +39.081.7755320; E-mail: bartolo.m@libero.it.

*Diaporthe helianthi* Munt.-Cvet. (anamorph = *Phomopsis helianthi* Munt.-Cvet.) is a phytopathogenic fungus that causes canker and withering of the stem and death of the leaves of sunflower. The early phases of *D. helianthi* pathogenesis are characterized by the production of phytotoxins that probably open the way to host colonization. Two phytotoxins of polyketidic nature were isolated and purified from culture filtrates of the most virulent strains of *D. helianthi* (the so called "French strains"). One of the two toxins, phomozin, was also found *in planta*. A sequence of 580 bp, named *Dhpk1*, showing a high level of similarity with genes encoding polyketide synthases of other 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*) was cloned from the above strains of *D. helianthi*. In order to analyze the role of the gene *Dhpk1* in the virulence of *D. helianthi*, mutants in which this gene was inactivated by *Agrobacterium*-mediated transformation were selected by hygromycin resistance.

**FUSARIUM WILT OF SOILLESS GROWN GERBERA: POSSIBILITY OF SPREAD AND NON-CHEMICAL CONTROL.** A. Minuto, D. Bertetti, M.L. Gullino and A. Garibaldi. Centre of Competence for the Innovation in the Agro-Environmental Sector (AGROINNOVA) – Università degli Studi di Torino, Via L. Da Vinci 44, 10095 Grugliasco Torino, Italy. Fax: + 39.0182.554949; E-mail: minuto.andrea@tiscali.it.

In 2002, gerbera (*Gerbera jamesonii* cv. Kaliki) plants were

observed in a soilless cultivation system in Northern Italy exhibiting symptoms of wilting caused by *Fusarium oxysporum*. Affected plants were stunted, had yellowish leaves and showed brown streaks in the vascular system that eventually turned black. A wilt of gerbera was previously described in the Netherlands in 1948, but its presence was not confirmed with further observations. During 2003 and 2004 two trials were carried out to investigate the possibility of *F. oxysporum* diffusion in a closed soilless system, and to verify the effects of slow sand filtration to reduce the risk of *F. oxysporum* spread within soilless-grown gerbera crops. Results confirmed the possibility of *F. oxysporum* spreading via recycled nutrient solution drained from infected plants. Moreover, the efficacy was demonstrated of slow sand filtration to limit the spread of *Fusarium* wilt. In conclusion, the use of slow sand filtration represents a viable strategy for the contemporary control of *Phytophthora cryptogea*, as already well known, and *F. oxysporum* in soilless-grown gerberas.

**INTERACTIONS BETWEEN THE ENDOPHYTE FUNGUS *ALTERNARIA ALTERNATA* AND *PLASMOPARA VITICOLA* IN GRAPEVINE LEAF TISSUES.** R. Musetti<sup>1</sup>, S. Borselli<sup>1</sup>, A. Vecchione<sup>2</sup>, L. Zulini<sup>2</sup>, M. D'Ambrosio<sup>3</sup> and I. Pertot<sup>2</sup>. <sup>1</sup>Dipartimento di Biologia Applicata alla Difesa delle Piante, Via delle Scienze 208, 33100 Udine, Italy. <sup>2</sup>Istituto Agrario San Michele all'Adige, Via Mach 1, San Michele all'Adige, 38000 Trento, Italy. <sup>3</sup>Dipartimento di Fisica-Laboratorio di Chimica Bioorganica, Università degli Studi di Trento, Via Sommarive 14, 38050 Povo (TN), Italy. Fax: +39.0432.558501; E-mail: Rita.Musetti@uniud.it.

*Plasmopara viticola* (Berk. et Curt.) Berl. et de Toni, the grapevine downy mildew pathogen, causes an economically important disease. Several American grape varieties show considerable resistance to downy mildew, but most European varieties are quite susceptible, requiring chemical protection that, over the years, can cause negative effects such as pollution and development of resistant strains of the fungus. Thus, organic control and the use of biocontrol agents could be important to solve the problems caused by conventional methods. We have isolated a fungal endophyte of grapevine, *Alternaria alternata*, which inhibits the sporulation of *P. viticola* on leaf disk. An ultrastructural study was carried out to investigate cellular interactions between pathogen and endophyte in grapevine leaf tissue. Cytological observations showed that, even without close contact with *A. alternata*, *P. viticola* mycelium exhibited ultrastructural alterations, such as the presence of vacuoles that were enlarged or contained electron-dense precipitates. Haustoria were necrotic and with an irregular shape or were enclosed in callose-like substances. In liquid culture, *A. alternata* produced three main metabolites belonging to the family of diketopiperazines, namely cyclo(L-phenylalanine-trans-4-hydroxy-L-proline), cyclo(L-leucine-trans-4-hydroxy-L-proline), and cyclo(L-alanine-trans-4-hydroxy-L-proline). These compounds were tested at different concentrations on grapevine leaf disks previously inoculated with a suspension of *P. viticola* sporangia, showing a good capacity of inhibiting pathogen sporulation.

**DIFFERENTIAL ANTIVIRAL ACTIVITY OF SOME IMPDH INHIBITORS.** A. Panattoni, F. D'Anna and E. Triolo. Dipartimento di Coltivazione e Difesa delle Specie Legnose 'G. Scaramuzzi', Sezione di Patologia Vegetale, Università degli Studi di Pisa, Via del Borghetto 80, Pisa, Italy. Fax: +39.050.960622; E-mail: fdanna@agr.unipi.it.

Chemical therapy, one of the experimental approaches used

for restoring health of infected plants, has been evaluated for its ability to interfere with virus replication in host tissue. Few chemicals proved capable of eliminating or substantially reducing replication of phytoviruses as compared to the broad selection of therapeutic chemicals available against human viruses. With reference to inhibitors of the inosine monophosphate dehydrogenase (IMPDH) group, two types of compounds endowed with antiviral activity have been discovered: synthetic nucleoside inhibitors, like tiazofurin (2-D-ribofuranosylthiazole-4-carboxamide), benzamide-riboside [3-(1-deoxy-D-ribofuranosyl)benzamide], and non-nucleoside inhibitors, like mycophenolic-acid (6-(4-hydroxy-6-methoxy-7-methyl-3-oxo-1,3-dihydroisobenzofuran-5-yl)-4-methyl-hex-4-enoic acid). One of our projects, carried out to investigate the antiviral activity of several IMPDH inhibitors, and designed for verifying their action in an *in vitro* system (explants of *Nicotiana tabacum* cv Xanthi infected by *Cucumber mosaic virus*), yielded a rapid technique for the identification of suitable compounds against plant viruses. The appropriate drug concentration was evaluated by preliminary screening on healthy explants. ELISA and biological assays were used to identify the sanitary conditions of treated explants after each therapeutic cycle. After healing treatments, ELISA-negative explants were bio-assayed on *N. benthamiana* plants. ELISA readings and biological assays showed that 66.6%, 60.0% and 27.2% of the explants were apparently sanitized following mycophenolic-acid, benzamide-riboside and tiazofurin treatments, respectively. The same chemicals were tested against *Tobacco mosaic virus* infection, but no virus eradication was obtained with any of them. These results represent the starting point for an in-depth analysis of the potential action of IMPDH inhibitors also against plant viruses.

**TABLE GRAPE BERRY COLONISATION AND ENVIRONMENTAL FATE OF THE BIOCONTROL AGENT *AUREOBASIDIUM PULLULANS*, STRAIN L47. I. Pentimone, L. Schena, A. Ligorio, A. Ippolito and F. Nigro.** *Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi di Bari, Via Amendola 165/A, 70126 Bari, Italy. Fax: +39.080.5442911; E-mail: nigrof@agr.uniba.it.*

The colonization and environmental fate of *Aureobasidium pullulans*, strain L47, applied before harvest to control Botrytis storage rot of table grapes, were studied. The antagonist was applied once to table grape bunches cv Italia by spraying a suspension containing  $5 \cdot 10^8$  cells  $\text{ml}^{-1}$ . The ability of strain L47 to colonize plant and fruit surfaces, and to disperse and persist in the field was monitored using classic and molecular techniques. Microbial epiphytic population was periodically assessed on the leaves and bunches of treated plants and the surrounding untreated vines by means of dilution plating method. Scorpion highly specific primers were used in an automated PCR-based assay for real-time identification of strain L47. *A. pullulans* L47 colonizes bunches and leaves of both treated and untreated adjacent plants, with frequency of isolation declining with time. Two years after application, strain L47 was either not present or significantly reduced on both the leaf and fruit surface of treated plants. However, it was detected also on untreated plant organs, which may have resulted from either natural colonization or handling contamination. There were no significant alterations of the leaf and berry microbial community. On the whole, these results suggest that strain L47 of *A. pullulans* used for the preharvest biological control of Botrytis storage rot, would have minimal environmental impact due to its limited mobility and weak persistence over time.

**ADDITIONAL FUNGAL SPECIES ASSOCIATED WITH ELEPHANTIASIS OF KIWIFRUIT. A. Prodi, S. Sandalo, S. Tonti and P. Nipoti.** *Dipartimento di Scienze e Tecnologie Agroambientali, "Alma Mater Studiorum" Università di Bologna, Viale Fanin 40, 40127 Bologna, Italy. Fax: +39.051.2096722; E-mail: paola.nipoti@unibo.it.*

Elephantiasis of kiwifruit is characterized by a hypertrophy of the trunk. Diseased plants show also typical wood browning starting from the medullar area. Studies on the aetiology of this disease and its monitoring were initiated in 2000 in some kiwifruit-growing areas of Emilia-Romagna (northern Italy). The main fungal genera isolated were: *Fusarium* spp., *Phaeoacremonium* like (Phm-like, including the genera *Phaeoacremonium*, *Phialophora*, *Cadophora*, and *Lecythophora*), *Cylindrocarpon*, *Phomopsis*, and some basidiomycetes. Among all fungal genera, the Phm-like group was accurately studied since its members could play a "precursor" role in disease onset. Some species within the different genera were identified by molecular methods, using sequence data from the nuclear ribosomal internal transcribed spacer regions 1 and 2 (ITS1 and ITS2). The *in vitro* pathogenic activity of all species identified within each genus was also investigated. Of the three species of genus *Phaeoacremonium* (*P. aleophilum*, *P. inflatipes* and *P. parasiticum*) that were found, *P. aleophilum* showed a higher pathogenicity than *P. parasiticum* towards semi-woody tissues of kiwifruit, while *P. inflatipes* was apparently avirulent. Two species of genus *Cadophora* were identified, of which *C. luteo olivacea* had a medium pathogenicity whilst *C. malorum* showed a higher capacity of tissue colonization and deterioration. *Lecythophora luteoviridis*, the only recovered representative of genus *Lecythophora*, had a good pathogenic activity. The results obtained indicate that the majority of the members of the Phm-like group of fungi are good candidates for being precursors of other mycetes, especially *F. solani*.

**INTERACTIONS BETWEEN PEACH LATENT MOSAIC VIROID VARIANTS CAUSING PEACH CALICO DISEASE AND THE NATURAL HOST: CELLULAR AND MOLECULAR STUDIES. M.E. Rodio<sup>1</sup>, A. De Stradis<sup>1</sup>, S. Delgado<sup>2</sup>, R. Flores<sup>2</sup> and F. Di Serio<sup>1</sup>.** <sup>1</sup>*Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi di Bari, and Istituto di Virologia Vegetale del CNR, Sezione di Bari, Via Amendola 165/A, 70126 Bari, Italy.* <sup>2</sup>*Instituto de Biología Molecular y Celular de Plantas (UPV-CSIC), Universidad Politécnica de Valencia, 40022 Valencia, Spain. Fax: +34.963877859; E-mail: rflores@ibmcp.upv.es.*

Peach calico, a disease characterized by a severe chlorosis (albinism) of leaves, stems and fruits, is caused by *Peach latent mosaic viroid* (PLMVd) variants containing a molecular determinant located in a 12-13 nt insertion that adopts a hairpin conformation capped by a U-rich loop. Histological, cytological and molecular alterations of peach leaves expressing PC symptoms are described. Albino leaf areas lack a differentiated mesophyll cell layer and contain cells with altered proplastids instead of fully developed chloroplasts. Because PLMVd replicates actively in PC symptomatic areas, our findings suggest that PLMVd variants inducing PC block an early step of chloroplast biogenesis impairing plastid-to-nucleus signaling pathways involved in leaf differentiation, a condition resembling that of some albino mutants. The discovery that PLMVd invades the shoot apex, the site of chloroplast biogenesis, and that proplastids from the apex of PC-expressing shoots are structurally altered, strongly support this hypothesis. Because the discolored areas had reduced accumulation of chloroplast ribosomal RNAs and plastid-encoded proteins, the inhibition of chloroplast differentiation could result from impaired protein translation in proplastids caused by the

viroid. An important implication of these findings is that all host proteins mediating PLMVd chloroplastic replication must be nucleus-encoded.

**SIRBINT, A SIMULATION MODEL OF THE INTERACTION ORYZA SATIVA-PYRICULARIA GRISEA.** M. Rodolfi<sup>1</sup>, M. Biloni<sup>2</sup> and A.M. Picco<sup>1</sup>. <sup>1</sup>Dipartimento di Ecologia del Territorio e degli Ambienti Terrestri, Sezione di Micologia, Università degli Studi di Pavia, Via Sant'Epifanio 14, 27100 Pavia, Italy. <sup>2</sup>SA.PI.SE. Cooperativa Agricola Sardo Piemontese Sementi, Via Mameli 7, 13100 Vercelli, Italy. Fax: +39.0382.34240; E-mail: apicco@et.unipv.it.

Blast, caused by *Pyricularia grisea* (Cooke) Sacc., is generally considered as the main disease of rice worldwide. In Italy, its occurrence and severity vary with the year, location, and field depending on the environmental conditions and crop management practices. Even if the disease can be very destructive, no strategies in current use are based on the dynamics of airborne conidia, the most important means of dissemination of the pathogen. After a three-year study, a dynamic crop growth model (SiRBInt) simulating rice-blast interactions and including both crop and pathogen growth pattern dependent on meteorological conditions was developed. The following items were considered: four potential blast damage mechanisms (reduction of photosynthetic rate, increase in respiration, loss of carbohydrates, acceleration of leaf aging); some rice physiological processes (such as phenology, biomass development, CO<sub>2</sub> assimilation, maintenance and growth respiration); daily climatic data (maximum temperature, day-night temperature range, maximum relative humidity, day-night relative humidity range, rainfall); specific traits of cultivars. Aerobiological and agricultural results demonstrate that the model may be useful to simulate both leaf and neck blast appearance in the field and to plan fungicide application. Work is in progress to validate the model and to verify the effect of meteorological data both on rice growth and blast development during a higher number of years.

**FILM-FORMING, ANTIMICROBIAL, AND ELICITING PROPERTIES OF CHITOSAN.** G. Romanazzi<sup>1</sup>, F. Mlikota Gabler<sup>2</sup>, D. Margosan<sup>3</sup> and J.L. Smilanick<sup>3</sup>. <sup>1</sup>Department of Environmental and Crop Sciences, Marche Polytechnic University, Via Breccia Bianche, 60131 Ancona, Italy. <sup>2</sup>Institute for Adriatic Crops, Put Duiilova 11, 21000 Split, Croatia. <sup>3</sup>USDA – Agricultural Research Service, San Joaquin Valley Agricultural Sciences Center, 9611 South Riverbend Ave. Parlier, CA 93648, USA. Fax: +39.071.2204856; E-mail: g.romanazzi@univpm.it.

Chitosan, N-acetylated derivative of the polysaccharide chitin, is a natural biopolymer with numerous applications in agriculture and agroindustries. It is a polycationic compound, the only natural substance with this property, and it interacts with negatively charged substances such as proteins, fatty acids, and phospholipids as a result of the high density of amino groups it contains. Chitosan has antimicrobial activity against a wide range of pathogens and induces host resistance to fungal, bacterial, and viral infections. The aim of our investigation was to test the film-forming, antimicrobial, and eliciting properties of chitosan on table grapes. The biopolymer needs to be dissolved in an acid to exhibit its properties. When single table grape berries were immersed in a chitosan solution, it formed a film on the treated surface upon drying, regardless of the acid used for dissolving. Chitosan dissolved in each of the acids tested reduced postharvest gray mold (caused by *Botrytis cinerea*) in table grape berries; the

highest effectiveness was observed when acetic acid was used. Preharvest chitosan treatment and postharvest UV-C irradiation were synergistic in reducing gray mold and blue mold (caused by *Penicillium* spp.) infections to grape berries. Chitosan did not induce catechin or trans-resveratrol. However, a higher production of both was induced in berries after a combined treatment of preharvest chitosan and postharvest UV-C irradiation than after a UV-C treatment alone.

**FUNCTIONAL INVOLVEMENT OF THE TRANSCRIPTION FACTOR ANAC102 IN PATHOGENESIS-RELATED PROTEIN EXPRESSION.** E. Rossignoli, G. Malacarne, E. Mica, E. Zago, M. Polesani and A. Polverari. Dipartimento Scientifico-Tecnologico, Università di Verona, Ca' Vignal 1, Strada Le Grazie 15, 37134 Verona, Italy. Fax: +39.045.8027929; E-mail: annalisa.polverari@univr.it.

A functional genomic approach was undertaken to investigate the role of ANAC102 in resistance to pathogens in *Arabidopsis thaliana*. The gene product belongs to the NAC protein family, which contains a number of transcription factors unique to plants, associated with development and stress response. No experimental research has apparently been made until now on ANAC102. Previous work in our laboratory indicates that ANAC102 expression is induced by nitric oxide (NO) treatment and in the hypersensitive reaction (HR) of *A. thaliana* to avirulent *Pseudomonas syringae* pv. *tomato* and to *Alternaria brassicicola*. *Arabidopsis* Col-0 insertional mutants, carrying a single homozygous T-DNA insertion in the coding region of ANAC102, were obtained. The T-DNA insertion mutants produced undetectable levels of ANAC102 transcript as assessed by Real-Time RT-PCR, even in strongly inducing conditions (treatment with NO donors). These plants were studied to understand whether, and to what extent, resistance could be compromised by the loss-of-function of ANAC102. Experiments performed included observation of macroscopic and microscopic cell death, measurements of bacterial growth in plants, Northern analysis of the expression of pathogenesis related protein-1 (PR-1) and defensin PFD1.2, both in wild type and mutant plants. Results suggest that resistance against *Ps.* pv. *tomato* and *A. brassicicola* in the mutants is not compromised, but also indicate that ANAC102 is an important positive regulator of PR-1 gene expression. Plants overexpressing ANAC102 have also been produced and will be analysed in relation to resistance and hypersensitive cell death.

**CYTOLOGICAL ANALYSIS OF SACCHAROMYCES CEREVISIAE CELLS SUPPORTING CYMBIDIUM RINGSPOT VIRUS DEFECTIVE INTERFERING RNA REPLICATION.** M. Russo, B. Navarro, V. Pantaleo and L. Rubino. Istituto di Virologia Vegetale del CNR, Sezione di Bari, c/o Dipartimento di Protezione delle Piante e Microbiologia Applicata Università degli Studi, Via Amendola 165/A, 70126 Bari, Italy. Fax: +39.080.5442911; E-mail: l.rubino@area.ba.cnr.it.

Positive-strand RNA [(+)RNA] viruses replication takes place in association with host cell membranes, but different viruses recruit diverse intracellular membranes for the assembly of their replication complexes. The study of targeting signals of viral proteins interacting with host cell membranes and of the formation, maintenance and mode of action of the viral replication complex is important for the comprehension of a virus life cycle, also in view of the possible development of an efficient therapy. The use of simple viruses, like tombusviruses, in a genetically character-

ized host like the yeast *Saccharomyces cerevisiae*, constitutes a valuable model system to study (+)RNA virus-host cell interactions. In this system the replicase proteins p33 and p92 of *Cymbidium ringspot virus* (CymRSV) supported the replication of defective interfering (DI) RNA. Three yeast strains were used that differ in the biogenesis of peroxisomes, i.e. the organelles that supply the membranous vesicular environment in which CymRSV RNA replication takes place in infected plant cells. Replication occurred only in yeast cells containing either well-developed or immature peroxisomes, but not in mutant cells where peroxisome biogenesis is impaired. Double-label immunofluorescence showed that both p33 and p92 proteins localized to peroxisomes, independently of one another. It is suggested that these proteins move initially from the cytosol to the endoplasmic reticulum, then to peroxisomes. The expression of p33, but not p92, increased the peroxisome number and induced membrane proliferation. BrUTP incorporation confirmed that newly synthesized DI RNA accumulates predominantly in association with peroxisomes, whereas DI RNA progeny is diffused in the cytoplasm.

**A GENERAL ANTISILENCING STRATEGY FOR ENHANCING PATHOGEN-DERIVED RESISTANCE TO GEMINIVIRUSES.** D. Sallustio, A. Berardi, D. Barboni, V. Lucioli, A. Papacchioli and M. Tavazza. *Biotech Gen, ENEA CR Casaccia, Via Anguillarese 301, 00060 Rome, Italy. Fax: +39.06.30484203; E-mail: alessandra.lucioli@casaccia.enea.it.*

Tomato yellow leaf curl geminiviruses (TYLCVs) are the cause of a devastating disease of cultivated tomato. Several laboratories have tried to confer resistance to TYLCVs by transforming plants with viral-derived sequences. Nevertheless, only partial resistance has been obtained, suggesting that pathogen-derived resistances (PDRs) cannot be applied "tout court" to TYLCVs. We have previously shown that: (i) transgenic expression of a truncated form of TYLCV-Sardinia (TYLCSV) replication-associated protein (Rep210) confers resistance to the homologous virus but not to the Spanish isolate (TYLCSV-ES1); (ii) TYLCSV overcomes with time Rep210-mediated resistance by silencing the transgene and being able to spread in silenced transgenic plants; (iii) TYLCSV is able to infect Rep-210-silenced transgenic plants suggesting that it can accommodate to some extent RNAi-mediated targeting of an essential viral gene; (iv) the susceptibility to TYLCSV-ES1 isolate could be the result of an early activation of the virus-induced gene silencing (VIGS). The above data prompted us to develop a new strategy to escape VIGS of transgenic viral sequences for enhancing PDRs to geminiviruses. We have built a synthetic Rep210 transgene that is an inefficient target of the TYLCSV-induced transgene silencing. Transgenic plants expressing the synthetic Rep210 transgene showed an enhanced and broader resistance to viral infection.

**DETECTION AND IDENTIFICATION OF *PSEUDOMONAS SAVASTANOI* BY PCR-BASED DIAGNOSTICS.** E. Santilli, M. Carboneschi and S. Tegli. *Dipartimento di Biotecnologie Agrarie, Sezione di Patologia Vegetale, Università degli Studi di Firenze, Via della Lastruccia 10, 50019 Sesto Fiorentino, Firenze, Italy. Fax: +39.055.4573232; E-mail: stefania.tegli@unifi.it.*

*Pseudomonas savastanoi* (*Ps*) attacks olive, oleander, and ash, with the pathovars *savastanoi* (*Psv*), *nerii* (*Psn*), *fraxini* (*Psf*), respectively. The development of reliable and rapid procedures for the identification/detection of these pathogens in symptomless materials for vegetative propagation would be important. Here

we describe new approaches that might be useful for this purpose. A conventional PCR-based method was optimized, starting from the specific bands obtained in Rep-PCR experiments with ERIC1R and ERIC2 primers using *Psv*, *Psn* and *Psf* representative strains. These DNA fragments were isolated, cloned and sequenced. Alignments and comparisons of these sequences allowed the identification of conserved regions common to all strains belonging to the same *Ps* pathovar, upon which three different primer pairs were designed. Sequences of these primers were compared with those present in EMBL/GenBank, but no significant matches were found. Using these primer pairs and after having optimized PCR conditions, amplification experiments gave rise to single products of 627, 382 and 412 bp for *Psv*, *Psn* and *Psf*, respectively, whose length tallied with the expected size. No amplification was detected using as template DNA extracted from several bacterial strains belonging to different species of the genus *Pseudomonas* and from the epiphytic bacterial microflora isolated from the host plants of *Psv*, *Psn* *Psf*. Sequencing confirmed the pathovar-specificity of amplifications. Moreover a Real-Time PCR-based assay was developed, the preliminary results of which indicate that it is efficient for the quantitative estimation of *Psv*, *Psn* and *Psf* on plant matrices.

**A MULTIDISCIPLINARY APPROACH TO STUDY THE ROLE OF CERATO-PLATANIN, A SMALL PROTEIN FROM *CERATOCYSTIS FIMBRIATA* F.SP. *PLATANI*, IN THE PATHOGENESIS OF THE PLANE CANKER STAIN.** A. Scala<sup>1</sup>, C. Comparini<sup>1</sup>, L. Carresi<sup>1</sup>, N. Luchi<sup>1</sup>, F. Sebastiani<sup>1</sup>, I. Petroncini<sup>1</sup>, L. Pazzagli<sup>2</sup>, B. Pantera<sup>2</sup>, C. Zoppi<sup>2</sup>, G. Cappugi<sup>2</sup>, A. Spisni<sup>3</sup>, T.A. Perthinhez<sup>3</sup>, R. Bernardi<sup>4</sup>, F. Fontana<sup>4</sup>, M. Salvini<sup>4</sup>, M. Durante<sup>4</sup>, C. Pane<sup>5</sup> and F. Scala<sup>5</sup>. <sup>1</sup>Dipartimento di Biotecnologie Vegetali, Laboratorio di Patologia Vegetale, and Laboratorio GENEXPRESS, Università di Firenze, Via della Lastruccia 10, 50019 Sesto Fiorentino, Italy. <sup>2</sup>Dipartimento di Scienze Biochimiche, Università di Firenze, Viale Morgagni 50, 50142 Firenze, Italy. <sup>3</sup>Centro de Biologia Molecular Estrutural-LNLS, Campinas, Brazil. <sup>4</sup>Dipartimento di Agricultural Plant Biology, Genetics Section, Università di Pisa, Via Matteotti 1/B, 56124 Pisa, Italy. <sup>5</sup>Dipartimento Ar.Bo.Pa.Ve., Sezione di Patologia Vegetale, Università di Napoli "Federico II", Via Università 100, 80055 Portici (NA), Italy. Fax: +39. 055.4573232; E-mail: aniello.scala@unifi.it.

Cerato-platanin (CP) is a 12.4 kDa protein purified from the culture filtrate of *Ceratocystis fimbriata* f.sp. *platani* (*Cfp*), the causal agent of plane canker stain (Pazzagli *et al.*, 1999, *J. Biol. Chem.* 274: 24959-24964). CP is 120 amino acid in length, contains 4 cysteines forming two S-S bridges, Cys20-57 and Cys60-115, and has a high percentage (40%) of hydrophobic residues. According to databases, CP is the founder member of the "cerato-platanin family" and shares some of its chemical and physical characteristics with the hydrophobins and with a few other hydrophobic cell wall proteins. CP is located in the *Cfp* cell walls, is able to self-aggregate and elicits defense responses in host and in non-host plant tissues (Bennici *et al.*, 2005, *Phytopathol. Medit.* 44: in press; Boddi *et al.*, 2004, *FEMS Microbiol. Letters* 233: 341-346; Pazzagli *et al.*, 2005, *Cell. Biochem. Biophys.* 42: in press; Scala *et al.* 2004, *J. Plant. Pathol.* 86: 23-29). To understand the role of CP in pathogenesis, a multidisciplinary approach was undertaken, and some of the studies in progress concern: (i) isolation of genes homologous to CP; (ii) determination of the three-dimensional structure of CP; (iii) aggregation of cerato-platanin, and its effect on plane cells; (iv) characterization of *Cfp* CP non-producer mutants; (v) screening of two suppressive subtractive hybridization (SSH) cDNA libraries constructed from RNAs extracted from plane leaves treated with CP or *Cfp*; (vi) regulation of the isolated gene sequences.

**NEW TARGET GENES FOR THE IDENTIFICATION AND DETECTION OF *PHYTOPHTHORA* SPECIES.** L. Schena<sup>1</sup>, A. Ippolito<sup>1</sup> and D.E.L. Cooke<sup>2</sup>. <sup>1</sup>Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi di Bari, Via Amendola 165/A, 70126, Bari, Italy. <sup>2</sup>Scottish Crop Research Institute, Invergowrie, Dundee, DD2 5DA, Scotland, UK. Fax: +39.080.5442911; E-mail: leonardo.schena@agr.uniba.it.

In recent years a large number of new *Phytophthora* species damaging forests and other natural ecosystems have been identified, whose knowledge is increasing with the use of molecular methods mainly based on the analysis of ITS regions of rDNA. However, these regions do not always resolve at a sufficiently fine level for the discrimination of closely related taxa. To overcome these limitations, four different intergenic spacers in mitochondrial DNA, a fragment of the intergenic spacer (IGS) region and a fragment of the ras-related protein gene were amplified and sequenced from 31 *Phytophthora* species representing the diversity of the genus. Non-coding regions of the ras-related protein gene showed high levels of polymorphism suitable for the development of specific primers for 15 different species: *P. cactorum*, *P. cambivora*, *P. cinnamomi*, *P. citricola*, *P. europaea*, *P. inundata*, *P. lateralis*, *P. megasperma*, *P. nemorosa*, *P. kernoviae*, *P. pseudosyringae*, *P. psychrophila*, *P. quercina*, *P. ramorum*, and *P. ilicis*. The more conserved flanking coding regions were appropriate for the design of *Phytophthora*-genus specific primers. No intraspecific variability was observed. The organization of the ras-related protein gene is suited to a nested-PCR in which amplified products from genus-specific primers could be used as a common template for second round reactions with species-specific primers. The detection limit of a single amplification ranged between 1 and 10 pg of target DNA and was increased 100-fold in nested PCR assays.

**PRELIMINARY ASSESSMENT OF *PSEUDOMONAS SYRINGAE* ISOLATES USING FLUORESCENT AMPLIFIED FRAGMENT LENGTH POLYMORPHISM.** G. Scuderi<sup>1</sup>, A. Bonaccorsi<sup>1</sup>, M. Scortichini<sup>2</sup> and G. Cirvilleri<sup>1</sup>. <sup>1</sup>Dipartimento di Scienze e Tecnologie Fitosanitarie, Sezione di Patologia Vegetale, Università degli Studi di Catania, Via S. Sofia 102, 95100 Catania, Italy. <sup>2</sup>Istituto Sperimentale per la Frutticoltura, Via di Fioranello 52, I-00040 Ciampino aeroporto, Roma, Italy. Fax: +39.095.7147287; E-mail: gcirvil@unict.it.

Eighty-six isolates of *Pseudomonas syringae*, obtained from healthy and diseased tissues of herbaceous and woody plant species, were molecularly typed using fluorescent amplified fragment length polymorphism (fAFLP). Total genomic DNA was digested with *Mse*I and *Eco*RI restriction endonucleases and compatible oligonucleotide adapters (*Mse*I: 5'-GACGATGAGTCCT-GAG-3', 5'-TACTCAGGACTCAT-3'; *Eco*RI: 5'-CTCGTAGA-CTGCGTACC-3', 5'-AATTGGTACGACAGTCTAC-3') were ligated to the resulting fragments. Subsets of fragments from the total pool of cleaved DNA were amplified and this procedure generated 255 fAFLP polymorphic markers in the range of 100 to 600 nt using just one primer set (*Mse*I+C, *Eco*RI+A). A binary data

matrix was generated reflecting the presence or the absence of fragments obtained by fAFLP from different isolates. Cluster analysis was done according to the unweighted pair-group method with average linkages (UPGMA). Analysis of electropherograms allowed the identification of strain-specific discriminative peaks. Distinct fAFLP clusters with similarity percentage from 55 to ca. 95% were identified. The resulting dendrogram showed also that bacterial isolates obtained from the same host plant were not always allocated in the same cluster. In this paper, fAFLP analysis was used for the first time as a molecular approach to assess the intraspecific variability of *P. syringae* isolates obtained from different hosts. Such a technique proved sensitive, rapid, easy to use, and reproducible. All these features make fAFLP an excellent tool for molecular and phylogenetic analyses, which can be tailored to the level of typing the required discrimination.

**DIFFUSION OF AVIRULENCE GENES *AVRPTO* AND *AVRRPT2* IN POPULATIONS OF *PSEUDOMONAS SYRINGAE* pv. *TOMATO*.** M. Zaccardelli<sup>1</sup>, F. Campanile<sup>1</sup>, B.A. Vinatzer<sup>2</sup> and J.T. Greenberg<sup>3</sup>. <sup>1</sup>C.R.A.-Istituto Sperimentale per le Colture Industriali, SS 18 204, 84091, Battipaglia, Salerno, Italy. <sup>2</sup>Department of Plant Pathology, Physiology and Weed Science, Virginia Polytechnic Institute and State University, Fralin Biotechnology Center, VA-24061 Blacksburg, USA. <sup>3</sup>Department of Molecular Genetics and Cell Biology, The University of Chicago, IL 60637 Chicago, U.S.A. Fax: +39.0828.340169; E-mail: m.zaccardelli@tiscali.it.

When phytopathogenic bacteria interact with plants they introduce proteins, named effectors, into plant host cell via the type III secretion system (T3SS). The effector genes *avrPto* and *avrRpt2* are avirulence genes of *Pseudomonas syringae* pv *tomato* (PST) that encode effector proteins with a well known role. The avirulence gene *avrPto* interact in a "gene for gene" manner with the tomato resistant gene *Pto*. The avirulence gene *avrRpt2* promotes virulence of *P. syringae* strains inoculated to *Arabidopsis thaliana* and, recently, was shown to contribute to virulence of PST in tomato plants. This gene is widely represented in *P. syringae* pv *syringae* populations isolated from different herbaceous and woody plants. With this work, the distribution of *avrPto* and *avrRpt2* genes in populations of PST was investigated. Seventy-six isolates of PST, mainly from Southern Italy, were analysed by PCR for the presence of *avrPto* and *avrRpt2*. The typical amplification product of *avrPto* was obtained from fifty-nine isolates whereas no amplicons were obtained from twelve isolates, and five isolates gave very weak amplicons. The typical amplification product of *avrRpt2* was obtained from seventy isolates whereas no amplicons were obtained from five isolates; only one isolate gave a very weak amplification product. These results show that the avirulence genes *avrPto* and *avrRpt2* are widely present in PST. However, *avrRpt2* seems to be the most common (92% of the analysed isolates) and, therefore, more conserved with respect to *avrPto* (78% of the analysed PST isolates). This agrees with the virulent role of *avrRpt2* with respect to the avirulence of *avrPto* in tomato.