INDUCTION OF RESISTANCE IN MELON AGAINST FUNGAL PATHOGENS ASSOCIATED TO COLLAPSE BY TREATMENTS WITH CHEMICAL RESISTANCE ACTIVATORS. M.P. Aleandri, R. Reda, P. Magro and G. Chilosi. *Dipartimento di Protezione delle Piante, Università degli Studi della Tuscia, Via S. Camillo de Lellis, 01100 Viterbo, Italy. E-mail: chilosi@unitus.it*

Soil-borne fungal pathogens causing collapse of melon, such as *Monosporascus cannonballus,* induce great economic losses since most of the melon cultivars are susceptible to this disease. A potentially effective prevention method of these diseases may be represented by induced systemic resistance by chemicals, which can provide an alternative, sustainable approach to protection, especially for problems that are not satisfactorily controlled by conventional methods. In this study the plant resistance elicitors acybenzolar-*S*-methyl (BTH), DL-beta-aminobutyric acid (BABA), potassium phosphonate (K_2HPO_3), methyl jasmonate (MeJA), hydrogen peroxide (H_2O_2) applied to seeds were studied as inducers of resistance on melon seedlings and adult plants against *M. cannonballus.* On seedlings and adult plants symptoms by *M. cannonballus* were restricted by treatments with both MeJA, K_2HPO_3 and BTH in terms of percentage and length of root lesions compared with inoculated control plants. The augmented level of resistance of tissues by seed soaking treatments was associated with rapid increases in the activity of various pathogenesis related proteins, including chitinase and peroxidase. MeJA elicited also a rapid and transient accumulation of lipoxygenase. Moreover, BTH and MeJA treatments determined the differential induction of particular *de novo* synthesised isoenzymes of these proteins. If confirmed by field trials, chemically induced resistance through seed application has the potential for controlling soil-borne fungal pathogens of melon, possibly in combination with the existing crop protection practices.

THE USE OF CONVENTIONAL AND REAL-TIME PCR FOR DETECTING AND QUANTIFYING *PYRENOCHAETA LYCOPERSICI* IN SOIL. M. Aragona, N. Pucci and A. Infantino. *CR.A., Istituto Sperimentale per la Patologia Vegetale, Via C.G. Bertero 22, 00156 Rome, Italy. E-mail: alessandro.infantino@entecra.it*

Pyrenochaeta lycopersici is a soil-borne fungus causing "corky root" of tomato. In our laboratory, a conventional PCR method has been successfully developed for the detection of the fungus in the soil. The same primer pair has been tested for quantitative amplification of *P. lycopersici* DNA by Real-Time PCR based on the use of SYBR Green dye for fluorescence monitoring. DNA was extracted and amplified from soils artificially infested with increasing concentrations of inoculum. Preliminary results have shown the accuracy of the method to quantify fungal DNA only when it was diluted after extraction, probably because of the presence of soil inhibitors or of an inhibiting excess of DNA in the undiluted extracts. Analysis is in progress of the relationship among conventional procedure by CFU counting on selective media, molecular quantification of the fungal biomass in the soil, and inoculum threshold able to cause disease in inoculated plants. The quantitative detection of *P. lycopersici* in natural soils will avoid the utilization of the heavily infested ones for tomato cropping and will favour the adoption of the most suited control strategies.

Work supported by ISPESL, project B102/DIPIA/03: "Utilizzo di tecniche molecolari per la diagnosi precoce di patogeni radicali in colture intensive, al fine di limitare l'impiego di composti chimici tossici".

ELISA: A NEW TOOL FOR THE LARGE SCALE DETECTION OF PLUM BARK NECROSIS STEM PITTING-ASSOCIATED VIRUS. D. Boscia, O. Potere, M.A. Castellano and V. Savino. *Istituto di Virologia Vegetale del CNR, Sezione di Bari, e Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università di Bari, Via Amendola, 165/A, 70126 Bari, Italy. E-mail: d.boscia@ba.ivv.cnr.it*

Plum bark necrosis stem pitting-associated virus (PBNSPaV) is routinely detected by molecular hybridization or RT-PCR. The recent production of antisera raised against virus particles of an Italian isolate partially purified from bark scrapings of woody cuttings and its successful utilization for virus detection by ELISA in crude plant extracts opens the possibility to extend this diagnostic tools for PBNSPaV identification. To this aim, PBNSPaV distribution in the canopy of an infected field-grown tree of *Prunus salicina* cv. Black Beaut, and the variation of its titer throughout the vegetating season were investigated to define a sampling protocol for routine ELISA. From July 2005 to May 2006, 36 samples equally distributed in the basal, central and apical part of the tree canopy were collected monthly. Data so far obtained indicate spring or summer leaves as the best material for reliable ELISA. This behaviour is somewhat surprising if compared with the variation of virus titer determined for other members of the genus *Ampelovirus* (e.g. most of the grapevine leafroll-associated viruses, GLRaVs) to which PBNSPaV is tentatively assigned. In fact, all protocols currently used for GLRaVs detection suggest the use of cortical tissues from dormant canes or, as second choice, the main veins and/or petioles of mature basal leaves. Preliminary tests carried out on other *Prunus* species seem, however, to indicate that, at least in the case of apricot, the distribution of PNBSPaV in the plant could be different from that in *P. salicina*. Therefore, investigation needs to be extended to other *Prunus* species.

BIOLOGICAL AND CHEMICAL CONTROL OF TOMATO CORKY ROOT AND EGGPLANT VERTICILLIUM WILT. G. Bubici, M. Amenduni, C. Colella, M. D'Amico and M. Cirulli. *Dipartimento di Biologia e Patologia vegetale, Università degli Studi di Bari, Via Amendola 165/A, 70126 Bari, Italy. E-mail: cirullim@agr.uniba.it*

Applications of antagonistic streptomycetes, fungicides (strobilurins) and a synthetic inducer of systemic acquired resistance (acibenzolar-S-methyl, ASM), alone or in combination, were evaluated for controlling corky root of tomato and Verticillium wilt of eggplant under greenhouse conditions. Antagonistic activity of 27 streptomycete isolates was evaluated *in vitro* against *Pyrenochaeta lycopersici* and *Verticillium dahliae*. Growth of colonies (diameter) of *P. lycopersici* and *V. dahliae* were reduced up to 20% and 31%, respectively, compared with controls. Two biological control trials were conducted in greenhouse using the 27 streptomycetes. Corky root and Verticillium wilt severity were reduced up to 46% and 61%, respectively, in the first trial, and up to 66% and 74%, respectively, in the second trial. Based on obtained results, St-B 3, St-B 6, St-B 11 and St-B 12 were considered as the most promising isolates in controlling tomato corky root and eggplant Verticillium wilt. Integrated control trials were carried out in greenhouse. Streptomycetes (St-B 3, St-B 12 or St-B 19), strobilurins (azoxystrobin or trifloxystrobin) and ASM, were applied alone or in combination. Azoxystrobin provided the best control of corky root of tomato and Verticillium wilt of eggplant. Three ASM foliar applications induced resistance of tomato and eggplant to corky root and Verticillium wilt, respectively, however they yielded only a partial disease control. Soil applications of antagonistic streptomycetes provided a disease severity reduction similar to that of ASM.

IDENTIFICATION IN *PSEUDOMONAS SAVASTANOI* OF A NEW SECRETION SYSTEM FOR THE TRANSFER OF EFFECTOR PROTEIN SUBSTRATES INTO HOST CELLS. M. Cerboneschi, E. Santilli, B. Babbini, and S. Tegli. *Dipartimento di Biotecnologie Agrarie, Sezione di Patologia Vegetale, Laboratorio di Patologia Vegetale Molecolare, Università degli Studi di Firenze, Via della Lastruccia 10, 50019 Sesto Fiorentino, Firenze, Italy. E-mail: stefania.tegli@unifi.it*

Type IV Secretion System (TFSS) is a multi-protein and energy-driven mechanism for the delivery of very different substrates from a bacterial donor cell to a recipient cell. It is auto-assembled starting from about a dozen membrane proteins in response to many different biotic and abiotic environmental signals. It is is used by Gram-negative bacteria to translocate proteins/peptides into eukaryotic cells or to mediate conjugative transfer of broad-host range plasmids between bacteria, and it is crucial for the survival of these bacteria in widely different habitats. TFSS is also an essential determinant of host-pathogen interactions, because it enables bacteria to deliver virulence factors and/or toxic proteins into the cytoplasm of the eukaryotic potential host cells, as shown for humans and animal pathogens like *Helicobacter pylori, Legionella pneumophila, Brucella* spp. and *Bartonella* spp. Here we describe the discovery of the TFSS in another plant pathogenic bacterium, *Pseudomonas savastanoi* (*Ps*). Starting from genomic libraries in the cosmid pLAFR3 of two isolates belonging to the pathovars *savastanoi* (*Psv*) and *nerii* (*Psn*), attacking olive and oleander respectively, some clones were identified that contained TFSS genes, using a PCR-strategy to screen the libraries. Data collected until now show that both in *Psv* and *Psn* the TFSS is located in a plasmid. Substantial differences were found between the genes coding for *Psv* and *Psn* TFSSs. Moreover, research is in progress to elucidate the role of TFSS in the diseases caused by *Ps* pathovars, using a knocking-out strategy.

POPULATION STRUCTURE AND HOST MOLECULAR INTERACTION OF *BOTRYOTINIA FUCKELIANA* INFERRED FROM COMPARATIVE SEQUENCE ANALYSIS OF ENDOPOLYGALACTURONASE GENES. E. Cettul, D. Rekab, R. Locci and G. Firrao. *Dipartimento di Biologia Applicata alla Difesa delle Piante, Università di Udine, Via Scienze 208, 33100 Udine, Italy. E-mail: emanuele.cettul@uniud.it*

The necrotrophic ascomycete fungus *Botryotinia fuckeliana* (anamorph: *Botrytis cinerea*) encodes at least six endopolygalacturonases known as BcPG that are used to degrade host cell wall. A collection of 32 strains of *B. cinerea* isolated from different hosts in various European countries was analyzed for the sequence of genes *Bcpg1*, *Bcpg2*, *Bcpg 3* and *Bcpg5*. Analysis of the nucleotide sequences showed a lack of congruence of the phylogenies constructed on the different genes and therefore did not support previous claims of speciation. No association was detected of phylogenetic grouping with the source plant host, geographic origin, or the presence of transposable elements *Boty* and *Flipper*. As far as the deduced aminoacidic sequences were concerned, BcPG3 and BcPG5 were completely conserved in all strains, while BcPG1and BcPG2 were variable. Using the program Codeml (PAML 3.15), the Bayes test (that calculated the posterior probability to be under positive selection for each site class) detected positively selected sites in BcPG2, thus suggesting a relevant role of BcPG2 in recognition by the host. This is consistent with expression analysis results reported by others. Interestingly, one of the protein region subjected to positive selection was not previously identified as a potential site of interaction.

SOIL-BORNE FUNGI ASSOCIATED WITH MELON COLLAPSE IN ITALY. Chilosi G.[1], R. Reda[1], M.P. Aleandri[1], I. Camele[2], C. Marcone[2], L. Altieri[2], C. Montuschi[3], V. Rossi[4], A. Carlucci[5], F. Lops[5], L. Colatruglio[5], M.L. Raimondo[5], S. Frisullo[5]. *[1]Dipartimento di Protezione delle Piante, Università degli Studi della Tuscia, 01100 Viterbo, Italy. [2]Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali, Università degli Studi della Basilicata, Viale dell'Ateneo Lucano 10, 85100 Potenza, Italy. [3]Servizio Fitosanitario Regione Emilia-Romagna, 40128 Bologna, Italy. [4]Istituto di Entomologia e Patologia Vegetale, Università Cattolica S. Cuore, 29100 Piacenza, Italy. [5] Dipartimento di Scienze Agro-ambientali, Chimica e Difesa Vegetale, Università degli Studi di Foggia, 71100 Foggia, Italy*

Collapse of melon represents one of most destructive disease worldwide. In Italy this disease has been reported in several melon-growing areas. The disease is caused by a variety of soil-borne fungal pathogens. *Monosporascus cannonballus* induces collapse in hot arid and semiarid regions. Other fungi associated with the disease are *Acremonium cucurbitacearum, Plectosporium tabacinum* and *Rhizopycnis vagum*. Aboveground symptoms appear just before harvest resulting in rapid wilt of plants, premature fruit ripening, and low sugar content. Typical symptoms of the disease are on the root system, consisting of necrotic lesions, root rots and loss of smaller feeder roots. As consequence of the rapid spread of melon collapse in Italy, a constant monitoring of the disease, as well as investigations on its epidemiology, prevention and control were carried out. This note reports the results on the morphological and molecular identification of isolates collected and on pathogenicity tests on melon and other cucurbits. Taken together, results indicate that melon collapse in the last few years has become one of the yield-limiting factor of this crop in Italy due to its complex aetiology. The frequency of isolation of fungal species varied with locations. *M. cannonballus* was the most frequent and aggressive species as confirmed by pathogenicity tests. *A. cucurbitacearum* and *P. tabacinum* were frequently recovered from symptomatic roots in the cucurbit-growing areas of Apulia. Their pathogenicity was experimentally proven. *R. vagum* was commonly isolated in Emilia-Romagna and Apulia, but it appeared to be less important as single pathogen due to its low virulence. In this case, there may be concomitant factors that determine the occurrence of the disease.

Work supported by the Ministero dell'Università e della Ricerca Scientifica, COFIN 2004.

CHARACTERIZATION OF SYNERGY BETWEEN *CUCUMBER MOSAIC VIRUS* AND *POTATO VIRUS Y* IN TOMATO. F. Cillo, T. Mascia, V. Fanelli, A. De Stradis, M.M. Finetti-Sialer, 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. E-mail: f.cillo@ba.ivv.cnr.it*

We investigated some biological and molecular characteristics of the synergistic effects shown by *Cucumber mosaic virus* (CMV) and *Potato virus Y* (PVY) in tomato. Plants inoculated with CMV-Fny exhibited the typical symptoms leaf surface reduction. We generated two pseudorecombinant strains with different pathogenetic properties inoculating CMV-Fny RNA1 and RNA3 in addition to, respectively, CMV-LS RNA2 (CMV-FLF) and the CMV-Fny RNA2 mutant 209m3D2b (CMV-D2b). CMV-FLF, like the RNA2 donor strain CMV-LS, induced very mild symptoms on tomato, whereas CMV-D2b, a modified CMV-Fny that cannot translate the 2b protein, did not infect tomato systemical-

ly. Tomato plants infected with PVY-SON41 showed symptomless infection. CMV-D2b spread systemically when inoculated in PVY-infected tomato plants, suggesting that the loss of movement functions of the 2b protein mutant was complemented by PVY analogue functions, as shown also by electron microscopy and immunogold labeling. With the exception of PVY + CMV-D2b, which induced a mild phenotype, symptoms were exacerbated in mixed infections, confirming a role for the 2b protein as an enhancer of symptom severity. Symptom severity in mixed infections correlated with increased viral spread and RNA accumulation levels. This was more evident at 60 days post-infection, both in the case of CMV and PVY, although the latter virus showed heterogeneity in the distribution throughout the plant and sample-to-sample variation. Small interfering RNA (siRNA) accumulation, the hallmark of RNA silencing in virus-infected plants, varied in different PVY/CMV combinations showing positive correlation with the corresponding viral RNA levels. This suggests that the CMV and PVY RNA silencing suppressors, 2b and HC-Pro proteins respectively have no role in preventing RNA silencing directed against both viruses in natural infections.

A NEW SPECIES OF TOSPOVIRUS FROM PIEDMONT, ITALY. M. Ciuffo, D. Pacifico, V. Masenga and M. Turina. *Istituto di Virologia Vegetale, CNR, Strada delle Cacce 73, 10135 Torino, Italy. E-mail: m.turina@ivv.cnr.it*

During surveys of weeds in the vineyards in Piedmont carried out in order to identify possible reservoirs of infection for the phytoplasma disease Bois Noir, *Polygonum convolvolus* plants showing ringspot symptoms were observed. Electron microscopy of homogenates of these plants showed the presence of a virus with the morphological features of a tospovirus. The viral isolate (called Plg3) was mechanically transmitted to several host plants. Serological tests showed no reaction to any tospovirus so far characterized, with the exception of a very faint reaction in DAS-ELISA with an Iranian isolate of *Tomato yellow fruit ring virus* (TYFRV). We purified nucleocapsids from Plg3-infected *Nicotiana benthamiana* and, running these in SDS-PAGE, detected a difference from TSWV isolates in the coat protein size. An antiserum against purified nucleocapsids was prepared and used to specifically detect Plg3 by DAS and ACP ELISA and by Western blotting. Lateral flow devices using gold-conjugated antiserum also detected the virus in crude sap extracts. Cloning and sequencing the full length of segment S and part of the M segment of Plg3 allowed comparison with tospovirus sequences in the databases, indicating that Plg3 is a new tospovirus species most closely related to TYFRV and *Iris yellow spot virus* (IYSV). Transmission experiments with thrips species in a controlled environment are currently under way.

MOLECULAR VARIABILITY OF *CITRUS VARIEGATION VIRUS* ISOLATES FROM CAMPANIA. A. Damiano[1], M. Malfitano[1], N. Duran-Vila[2] and D. Alioto[1]. *[1]Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università degli Studi di Napoli, Facoltà di Agraria, Via Università 100, 80055 Portici (NA), Italy. [2]Instituto Valenciano de Investigaciones Agrarias, Moncada, Valencia, Spain. E-mail: alioto@unina.it*

Citrus variegation virus (CVV) is a member of the subgroup 2 of *Ilarviruses*. Two strains of the virus are known, which induce different diseases in citrus denoted infectious variegation and crinkly leaf. During a survey conducted in Campania (southern Italy) in 2004, CVV was detected in different lemon and orange cultivars showing crinkling of the leaves or no symptoms at all. Partial molecular characterization of twelve CVV isolates from different lemon and orange cultivars was therefore carried out. When the genome of the CVV isolates was analyzed amplifying a fragment of the coat protein gene and part of the intergenic region by RT-PCR, divergence values ranging from 0.2 to 3.7% at the nucleotide level and from 0 to 3.6% at the amino acid level were found. Phylogenetic analysis showed that the sequences of CVV isolates from Campania did not cluster with those in databanks, forming a new group.

DYNAMICS OF *VACUMA* AND *TRANSPOSA* SUB-POPULATIONS OF *BOTRYOTINIA FUCKELIANA* IN VINEYARDS. R.M. De Miccolis Angelini[1], S. Pollastro[1], M.A. De Guido[1], F. Faretra[1]. *[1]Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi di Bari, Via Amendola 165/a, 70126 Bari, Italy. E-mail: faretra@agr.uniba.it*

Botryotinia fuckeliana (de Bary) Whetzel (anamorph: *Botrytis cinerea* Pers.:Fr.) is the causal agent of grey mould on well over 200 host plants including grapevine. Two coexisting sympatric populations of the fungus, characterized for the presence/absence of two transposable elements (TE), Boty and Flipper, fungicide response and other molecular markers have recently been identifieid. The dynamics in the field of the sub-populations *transposa* (Boty$^+$Flipper$^+$; Boty$^+$Flipper$^-$; Boty$^-$Flipper$^+$) and *vacuma* (Boty$^-$Flipper$^-$) during the grapevine-growing season and their relationship with fungicide resistance was investigated. Samples of leaves, shoots, and bunches were collected in 8 vineyards from flowering to ripening, and more than 550 monoconidial isolates were obtained. The presence of TE in single isolates was assessed by PCR with specific primers carried out adding a few conidia directly to the reaction mixture. On the whole, 85% of the tested isolates were identified as *transposa* (Boty$^+$Flipper$^+$ 78%; Boty$^+$Flipper$^-$ 20%; Boty$^-$Flipper$^+$ 2%), while the remaining 15% were classified as *vacuma* (Boty$^-$Flipper$^-$). Different ratios *vacuma*:*transposa* sub-populations were found from flowering to ripening. On shoots and leaves, early in the season, the ratio was 1:4, while later, on bunches, the ratio became 1:17. The response of representative isolates to several fungicides used against grey mould (benzimidazoles, dicarboximides, anilinopyrimidines, phenylpyrroles, and fenhexamid) was evaluated in colony growth tests. *Transposa* isolates were often resistant to one or more of the tested fungicides, while *vacuma* isolates showed more frequently the sensitive "wild-type" phenotype. Hence, the dynamics of *vacuma* vs. *transposa* sub-populations was associated to changes in the frequency of fungicide-resistant individuals in fungal populations. It remains to be clarified if the different response to fungicides is a cause or an effect of the dynamics of the two sub-populations.

SILENCING OF *PLUM POX VIRUS* 5' UTR/P1 SEQUENCES CONFERS RESISTANCE TO A WIDE RANGE OF VIRUS ISOLATES. E. Di Nicola-Negri and V. Ilardi. *C.R.A., 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 considered the most serious disease of stone fruit. Six PPV strains are known, of which D and M are of major agronomical importance. In many countries the virus has quarantine status and its elimination by eradication programs has been envisaged. The production of plant resistant to a wide range of PPV isolates is essential for the effective control of the virus. In

a previous work we transformed *Nicotiana benthamiana* plants with four PPV sequences, comprising the 5' UTR, P1 and HC-Pro sequences, arranged so as to express a self-complementary "hairpin" RNA. The sequences were cloned from PPV-ISPaVe44, an Italian isolate of strain M i. Fully PPV-ISPaVe44 resistant transgenic plants were obtained for each construct. One single transgenic locus line for each construct was further selected for a wide PPV resistance screening analysis. R1 transgenic plants were challenged with PPV isolates belonging to strains M, D, C and El Amar collected from *Prunus* species in different geographic areas (Italy, Hungary, Greece and Egypt). All the lines were resistant to the PPV-D and M isolates. Moreover, line 6 transformed with the 5' UTR/P1 sequence was also resistant to the distantly related strain PPV-C. Our data strongly suggest that the UTR/P1 construct is of particular practical interest to obtain transgenic stone fruit plants resistant to a broad range of PPV strains.

STOMATAL UPTAKE, GATEWAY TO OZONE INJURY. F. Faoro[1,2], F. Derghi[3], G. Gerosa[4], S. Cieslik[4], M. Saracchi[2], R. Marzuoli[4], A. Zorloni[2] and M. Iriti[1,2]. *[1]CNR, Istituto di Virologia Vegetale, U.O. di Milano e [2]Istituto di Patologia Vegetale, Università di Milano, Via Celoria 2, 20133, Milano Italy, [3]European Commission, Joint Research Centre, 21020 Ispra (VA) Italy, [4]Università del Sacro Cuore, Brescia, Via Musei 2, 25121 Brescia, Italy. E-mail: f.faoro@ivv.cnr.it*

Crop yield losses due to ozone are very difficult to assess, in term of productivity and quality of plant food-stuffs, as the generally asymptomatic chronic exposures in open field mostly depends on the real O_3 uptake by the leaves. This is not necessarily correlated with O_3 external concentration because of the determinant influence of stomatal conductance. In fact, environmental variables, such as sunlight, air temperature, relative humidity, and soil nature, affect evapotranspiration and, in turn, gas uptake. During summer 2003, a measuring campaign was conducted over an onion field near Voghera (Northern Italy), correlating O_3 stomatal flux with stomatal uptake in term of cell and tissue damage. Plants were monitored from the 3-leaf stage (20th of May) to harvest (9th of July). Leaves were sampled during the first 3 weeks, and examined microscopically with a histo-cytochemical method to evaluate O_3-induced oxidative stress and cell death. These phenomena were observed in stomata guard cells, peristomatal epidermis and stomatal cavities, the primary sites where O_3 reacts, before the appearance of the typical tipburns symptoms in the leaves, that occurred during the second week of June, in correspondence with high O_3 stomatal fluxes. Afterwards, fluxes decreased, in spite of the still high level of atmospheric O_3. Thus, stomatal uptake of the pollutant, as assessed by histo-cytochemistry and leaf lesions, validated the data concerning stomatal fluxes, confirming that O_3 injury strictly depends on stomatal conductance, rather than atmospheric concentration of the pollutant.

TRIALS TESTING THE *IN VITRO* ANTIBIOTIC ACTIVITY OF A RESVERATROL-LIKE TRANS-PICEID VERSUS SOME FUNGAL PARASITES OF POPLAR. M. Gennaro[1], F. Mattivi[2], D. Carbonera[3] and A. Giorcelli[1]. *[1]C.R.A.-I.S.P., Istituto di Sperimentazione per la Pioppicoltura, Strada Frassineto 35, 15033 Casale Monferrato (AL), Italy. [2]Istituto Agrario di S. Michele all'Adige, Via E. Mach 1, 38010 S. Michele all'Adige (TN), Italy. [3]Università degli Studi di Pavia, Dipartimento di Genetica e Microbiologia, Via Ferrata 1, 27100 Pavia, Italy. E-mail: gennaro@populus.it*

In the context of a study for testing the response of some white poplar transgenic lines, characterized by the insertion of a stilbene synthase gene (*StSy*), against recurring leaf fungal diseases, a series of *in vitro* trials was collaterally programmed to assess a possible direct activit of the purified metabolite on axenic colonies of the pathogens. A trans-piceid compound, chemically derived from trans-resveratrol, was extracted by flash chromatography and stored at 4°C in crystalline form. Different amounts of trans-piceid (previously dissolved in acetone, in the order of milligrams per liter) were added to potato-dextrose agar before pouring in 90 mm Petri dishes which were subsequently inoculated with the tested fungus. Pathogens both of white poplar and of the more extensively cultivated *Populus×canadensis* were considered in the trials. Hitherto responses to the various concentrations of trans-piceid in the medium have been contradictory but sometimes significant. The growing of *Venturia populina* colonies was stopped at 500 mg/l, whereas it was not affected by lower concentrations of active principle (50 and 5 mg/l). As to the preliminary trials with *Discosporium populeum*, the isolates were sometimes inhibited towards the end of the growing period ($P<0.05$) but not at the highest concentration of metabolite as it could have been expected. Instead, one isolate of *Rosellinia necatrix* was significantly highly inhibited at 500 mg/l active principle during all its growing period. Further experiments are needed especially in order to calibrate the concentration of trans-piceid and to assess its activity versus the conidia of the pathogens.

PRELIMINARY RESULTS ON THE USE OF ESSENTIAL OILS FOR SEED SANITATION. N.S. Iacobellis and P. Lo Cantore. *Dipartimento di Biologia, Difesa e Biotecnologie Agro Forestali, Università degli Studi della Basilicata, Viale dell'Ateneo Lucano 10, 85100 Potenza, Italy. E-mail: iacobellis@unibas.it.*

The control of bacterial diseases of plants is a considerable problem in agricultural practice because of the limited availability of bactericides. Furthermore, the control of bacterial diseases is complicated by the fact that a large number of phytopathogenic bacteria are spread, also over long distance, by contaminated and/or infected seeds. The presence of a few contaminated/infected seeds in a seed lot may lead to highly damaging epidemic outbreaks. The above consideration prompts the need for the development of alternative active compounds and/or methods for the control of plant bacterial diseases and, in particular, for the eradication of seed-borne pathogens. Recently, our studies showed the significant antibacterial activity of essential oils of coriander, cumin, caraway against gram-positive and gram-negative bacteria belonging to *Clavibacter, Curtobacterium* and *Rhodococcus*, and *Erwinia, Xanthomonas*, *Ralstonia* and *Agrobacterium* genera, responsible for several plant and mushrooms diseases. Furthermore, assays with the main components ($\geq 0.7\%$) of the above essential oils showed the reamarkable antibacterial activity of oxygenated monoterpenes with phenol and alcohol functions. Only a reduced activity was observed in the case of other oxygenated monoterpenes with aldehydes, ketons, ethers, and esters function, as well as of non oxygenated monoterpenes and sesquiterpenes. Preliminary results showed the potential use of the above essential oils for seed sanitation. In fact, the application of eugenol emulsions to bean seeds artificially contaminated with *Xanthomonas campestris* pv. *phaseoli* var. *fuscans*, significantly reduced the bacterial population. At the effective assay concentrations, negligible or limited effects on seed germination were observed.

IDENTIFICATION OF THE QUORUM-SENSING SYSTEM IN *PSEUDOMONAS CORRUGATA*. G. Licciardello[1,3], I. Bertani[2], L. Steindler[2], P. Bella[1], V. Venturi[2] and V. Catara[3]. *[1]Parco Scientifico e Tecnologico della Sicilia, z.i. Blocco Palma I, Stradale V. Lancia, 95131 Catania, Italy. [2]Bacteriology Group, International Centre for Genetic Engineering and Biotechnology, Area Science Park, Padriciano 99, 34012 Trieste, Italy. [3]Dipartimento di Scienze e Tecnologie Fitosanitarie, Università di Catania, Via S. Sofia 100, 95123 Catania, Italy. E-mail: vcatara@unict.it*

Quorum sensing (QS) is a cell-density dependent regulatory system, which in gram-negative bacteria usually involves the production of *N*-acyl homoserine lactones (AHLs) as signal molecules. In *Pseudomonas,* QS is known to control the expression of genes involved in virulence, biocontrol and ecological fitness. We investigated the production of AHLs in *Pseudomonas corrugata*, causal agent of tomato pith necrosis. Thirty *P. corrugata* strains were screened in a cross-streak plate assay using three different bacterial AHL biosensors, which respond to a different range of AHLs. Since AHL signal molecules were produced by all the strains, the AHLs produced were further characterized by thin-layer chromatography (TLC) with extracts from spent supernatants. Although bacterial strains had different geographic origin they all produced *in vitro* the same *N*-hexanoyl AHL (C6-AHL) signal molecule. In order to identify the molecular determinants of the QS system, a cosmid genomic library of *P. corrugata* was screened by triparental mating with the AHL sensor *Chromobacterium violaceum* CV026. Two cosmid clones could restore purple pigmentation on strain CV026 indicating that they contained gene(s) responsible for the synthesis of AHLs. In fact, TLC analysis showed that C6-AHL was synthesised. Transposon Tn5 mutagenesis was performed within one of the recombinant cosmid clones and Tn5 insertions were mapped. Subcloning and sequencing revealed that the quorum-sensing system of *P. corrugata*, consists of *pcoI/R* genes coding for the LuxR transcriptional activator PcoR and PcoI autoinducer synthase (LuxI family). An AHL knock-out mutant in the *pcoI* synthase in *P. corrugata* was constructed and is currently being investigated.

INNOVATIVE APPROACHES FOR THE INTEGRATED MANAGEMENT OF POSTHARVEST DISEASES. G. Lima, F. De Curtis and V. De Cicco. *Dipartimento di Scienze Animali, Vegetali e dell'Ambiente, Sezione di Patologia Vegetale, Via De Sanctis, 86100 Campobasso, Italy. E-mail: lima@unimol.it*

Postharvest storability of fruit and vegetables is heavily affected by fungal decays. Some pathogens are also responsible for the production of toxic metabolites (i.e. mycotoxins). Strategies of decay control often focused on single intervention technology (e.g., genetic, physical, chemical or biological means). Little attention has been paid to the integration of various available technologies. Despite of the risks and restrictions of the use of synthetic fungicides, chemical substances are still the most widely-known and used. In recent years, the search for safer alternatives has produced a large number of candidate biocontrol agents displaying antagonistic activity against various postharvest pathogens of several fruit crops. The most promising biocontrol agents (yeasts, yeast-like fungi and bacteria) have been studied for their antagonistic activity, physiological and biochemical aspects and different mechanisms of action. Unfortunately, when applied alone under commercial conditions, biocontrol agents rarely yield satisfactory results and are unlikely to completely replace applications of synthetic fungicides without new strategies aimed at enhancing and stabilizing their efficacy. It has been shown that integrated approaches involving combined applications of antagonists with alternative control means can provide disease control rates comparable or higher than those obtained with synthetic fungicides without negative risks due to the extensive use of chemicals. Integrated strategies improve efficacy of biocontrol agents and/or stabilize variability in their performance with additive or synergistic effects. The potential of integrated approaches is briefly discussed along with future prospects for a more effective and safer management of postharvest fungal pathogens.

PHYSIOLOGICAL SPECIALIZATION OF *FUSARIUM OXYSPORUM* ISOLATED FROM ORNAMENTALS BELONGING TO THE FAMILY *COMPOSITAE*. A. Minuto, P. Pensa, D. Berretti, M.L. Gullino, A. Garibaldi. *Centre of Competence for the innovation in the agro-environmental sector (AGROINNOVA), University of Torino, Via L. Da Vinci 44, 10095 Grugliasco (TO), Italy. E-mail: andrea.minuto@unito.it*

Paris daisy (*Argyranthemum frutescens*), gerbera (*Gerbera jamesonii*) and African daisy (*Osteospermum* sp.) were found to be infected by *Fusarium oxysporum* respectively in late 1997, early 2002 and late 2002 in Liguria (northern Italy). Diseased plants of Paris daisy and African daisy were observed in commercial farms producing potted plants, while wilted plants of gerbera were present in a soilless system for cut flower production. In 2004 and 2005 two experimental trials were carried out in order to identify the relationships within the causal agents of the three Fusarium wilts. Strains of *F. oxysporum* isolated from Paris daisy, gerbera, African daisy and chrysanthemum and strains of *Fusarium oxysporum* f.sp. *chrysanthemi* obtained from chrysanthemum (American Type Culture Collection ATCC 52422, ATCC 66279) were compared in "*in vivo*" tests. Paris daisy, gerbera, African daisy and chrysanthemum plants were separately inoculated with all tested strains by directly dipping into the inoculum (conidia, mycelium and chlamidospores) the roots of plants immediately before transplanting. Plants of Paris daisy, African daisy, chrysanthemum and gerbera were infected by strains isolated from African daisy, Paris Daisy, chrysanthemum, gerbera, and by ATCC strains of *F. oxysporum* f.sp. *chrysanthemi*. The data collected in the two experimental trials clearly show that all the strains of *Fusarium oxysporum* isolated from Paris daisy, gerbera and African daisy may be considered as belonging to the *forma specialis F.o. chrysanthemi*.

BIOLOGICAL AND MOLECULAR CHARACTERIZATION OF *FUSARIUM* SPECIES ISOLATED FROM BAKANAE DISEASED RICE PLANTS. A. Moretti[1], A.M. Picco[2], M. Rodolfi[2] and S. Somma[1]. *[1]ISPA, CNR, Via Amendola 122/0, 70126 Bari, Italy. [2]Dipartimento di Ecologia del Territorio e Ambienti Terrestri, Sezione di Micologia, Università degli Studi di Pavia, Via S. Epifanio 14, 27100 Pavia, Italy. E-mail: apicco@et.unipv.it*

In the last few years bakanae disease (BD) of rice, caused by *Fusarium* species, has emerged as a major disease in Northern Italy. Despite the ready identification of affected plants (i.e. abnormal growth of the stems, yellowish green leaves and pale green flag leaves), distribution and organization of the *Fusarium* species involved in BD aetiology are not well understood, although experimental evidence shows that only *F. fujikuroi* can cause the typical symptoms of BD. Additional species of *Fusarium* morphologically very similar to *F. fujikuroi* are frequently associated with BD. The identity of the main *Fusarium* species isolated from plants affected by BD cultivated in different sites of Northern Italy and belonging to different *Oryza sativa* L. vari-

eties, during the last two vegetative seasons, was firstly assessed morphologically. It was subsequentially confirmed by testing their sexual fertility and by using DNA analysis, in particular sequencing ß-tubulin and elongation factor loci. Around 150 strains of *Fusarium* species were isolated from stems and roots of the rice plants with BD symptoms. The main species isolated was *F. fujikuroi.* followed by *F. proliferatum* and, to a lesser extent, *F. oxysporum*. These data show that *F. fujikuroi* is the prevailing pathogen in Italian rice crops and that BD could become endemic in Northern Italy. Finally, the alleged capability of *F. fujikuroi* and *F. proliferatum* to produce toxic metabolites such as fumonisins, moniliformin, beauvericin and gibberellins is alarming, as it represents a potential risk for rice consumption.

MOLECULAR VARIABILITY OF *GRAPEVINE VIRUS A* COAT PROTEIN GENE IN GRAPEVINE FROM THE MARCHE REGION OF ITALY. S. Murolo[1], G. Romanazzi[1], A. Minafra[2], P. Saldarelli[2], M.B. Branzanti[1] and V. Savino[2]. *[1]Dipartimento di Scienze Ambientali e delle Produzioni Vegetali, Università Politecnica delle Marche, Via Brecce Bianche 10, 60131, Ancona, Italy. [2]Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi di Bari e Istituto di Virologia Vegetale, CNR, Sezione di Bari, Via Amendola, 165/A, 70126 Bari, Italy. E-mail: s.murolo@univpm.it*

Grapevine (*Vitis vinifera*), most important perennial crop in the Marche (Central-eastern Italy) is grown on about 20,000 ha, 35% of which are for production of DOC and DOCG wines. Rugose wood (RW), a complex disease to which *Grapevine virus A* (GVA) is associated, affects about 38% of the vines and it is one of the most economically important diseases of this crop. The aim of the work was the evaluation of the molecular variability of GVA isolates from typical local cultivars (Verdicchio, Lacrima nera, and Garofanata). Genetic diversity and population structure were studied in the entire coat protein (CP) gene by PCR-RFLP analysis using three restriction enzymes (*Mse*I, *Alu*I, and *Aci*I). RFLP with *Aci*I detected the highest polymorphism among GVA isolates, showing 6 different "simple" profiles (A, B, C, D, E, and F). "Complex" profiles were also found, and the most common variant combination was A+B, found in 48% of the viral isolates. The analysis of cloned GVA sequences confirmed the presence of mixed infections by different variants, suggesting that PCR-RFLP is suitable to estimate the population structure of GVA and to carry out a screening of different haplotypes. Moreover, a newly designed primer pair CP1F (5'-TGAAGACAAATGGCACACTACGC-3') and CP1R (5'-GATGGGTCATCCATCTATATCT-3') showed a higher efficiency of amplification, as compared with the primers Ah587/Ac995 currently used for GVA diagnosis. Distribution of RFLP profiles and phylogenetic analysiswere not correlated with the position of infected plants in the same vineyard, nor with the cultivar, supporting the notion that GVA occurs as a single and undifferentiated population.

BIOFUMIGATION WITH *TRANS*-2-HEXENAL FOR CONTROLLING *PENICILLIUM EXPANSUM* INFECTIONS IN POME FRUITS. F. Neri, M. Mari, A.M. Menniti, S. Brigati and P. Bertolini. *Criof, Dipartimento di Protezione e Valorizzazione Agroalimentare, Alma Mater Studiorum, Università di Bologna, Via Gandolfi, 19, 40057 Cadriano Bologna, Italy. E-mail: fiorella.neri@unibo.it*

Penicillium expansum Link, the agent of blue mould, is one of the main causes of postharvest losses in pome fruits and is of particular concern because it produces the mycotoxin patulin. The selection of thiabendazole-resistant strains of *P. expansum* and the restrictions in the use of synthetic fungicides have stimulated research in alternative control measures. Among these, *trans*-2-hexenal, a volatile compound naturally occurring in many fruits and vegetables, known to be involved in plant defence responses to wounds and pathogen attack, was tested for its effect on blue mould, patulin content, and fruit quality in several cultivars of pears and apples. Fruit, wound-inoculated or non-inoculated, were treated at 20°C with different concentrations of *trans*-2-hexenal over a 24 h period. Blue mould control, ranging from 50 to 98% (depending on the cultivar), and reduction of patulin content were achieved when fruit were exposed to vapours of *trans*-2-hexenal at a concentration of 12.5 mL L^{-1} 24 h after pathogen inoculation. *Trans*-2-Hexenal (12.5 mL L^{-1}) did not affect the physical-chemical characteristics of the fruits. Only in 'Abate Fetel' pears phytotoxic symptoms developed within 3 days of treatment. Off-odour developed just after treatment, but the intensity decreased or disappeared during shelf-life. After 7 days at 20°C following the treatment no significant difference in *trans*-2-hexenal content or in sensory traits was detected by a trained panel between untreated and treated 'Golden Delicious' apples, while off-flavour persistence was perceived in the fruis of 'Conference', 'Bartlett', 'Abate Fetel' pears and 'Royal Gala' apples.

IGS SEQUENCING FOR THE DETECTION AND CHARACTERIZATION OF *CYLINDROCLADIUM PAUCIRAMOSUM* ISOLATES. F. Nigro, I. Pentimone, L. Schena, A. Ligorio, A. Ippolito and M.G. Salerno. *Dipartimento di Protezione delle Piante e Microbiologia Applicata, Via Amendola 165/A, 70126 Bari, Italy. E-mail: nigrof@agr.uniba.it*

During the last three years a severe and recurrent disease of milkwort (*Polygala myrtifolia L.*) was observed in Apulia (Southeastern Italy). Plants showed wilting, stunting, chlorosis, and, often, loss of foliage. Extensive necrotic areas in the crown developed into basal cankers. A *Cylindrocladium* sp. was consistently isolated from diseased plants. To identify the fungal species the 5'terminus of the ß-tubulin gene was amplified and sequenced as reported in literature. The sequence was identical to that of C. *pauciramosum* DISTEF-G 192, available in GenBank and showed 98% sequence homology with *C. pauciramosum* DISTEF-G 196, both isolated from *P. myrtifolia* in Italy. The IGS region of rDNA and fragments of 28S and 18S rDNA were amplified with universal primers and sequenced. The total length of the amplified region was 2298 bp, which constitutes the first complete IGS sequence of *C. pauciramosum*. Based on IGS sequence, a primer set was designed into the more variable region (600-1550nt). Primer pairs were used in PCR assays, using as template DNA extracted from closely related species (*C. scoparium* var *scoparium*, *C. insulare*, *C. clavatum*) and fungi belonging to 65 different taxa. The expected amplicon (218bp) was obtained exclusively using *C. pauciramosum* DNA as template. Primers also allowed the detection of the pathogen in contaminated soil. As the pathogen survives also through the production of microsclerotia, the detection system developed in the present work could represent a useful diagnostic tool to avoid the spread of *C. pauciramosum* and to control the disease.

STRUCTURE AND ACCUMULATION OF A DEFECTIVE RNA OF *GRAPEVINE VIRUS A* SYNTHESIZED *DE NOVO* DURING VIRUS REPLICATION IN INFECTED *NICOTIANA BENTHAMIANA*. **J. Obreque[2], A. Minafra[1], M. Dell'Orco[1], P. Saldarelli[1], M. Jashes[2] and G.P. Martelli[1].** *[1]Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi e Istituto di Virologia Vegetale, CNR, sezione di Bari, Via Amendola, 165/A, 70126 Bari, Italy. [2]Laboratorio de Virologia Vegetal, Departamento de Biología, Facultad de Química y Biología, Universidad de Santiago de Chile, Alameda 3363 Santiago de Chile, Chile. E-mail: a.minafra@ba.ivv.cnr.it*

Grapevine virus A (GVA), a RNA virus of the family *Flexiviridae*. is associated with rugose wood of grapevine. GVA can be transmitted mechanically to *Nicotiana benthamiana* in which bands of smaller-than-genomic RNAs were detected by RT-PCR and molecular hybridization. Oligo-dT primed cDNA, amplified by primers at the 5' and 3' end of genomic RNA, gave PCR products of about 0.8 kbp, made up of a fragment of 459 nt from the 5' sequence fused to a fragment of 341 nt, comprising the whole ORF5. An internal set of primers across the fusion site was designed to verify the variability of the 0.8 kb amplicon, representing a putative defective RNA (dRNA). cRNA probes from the 5' end and the 3' end of the viral genome were used to hybridize total RNA extracts from infected plants 8 to 25 days post infection. RNA molecules 0.8 to 1.4 kb in size were recognized by the probes confirming the existance of dRNAs *in vivo.* These molecules comprised the conserved 5' and 3' viral RNA termini with wide internal deletions and a variable junction site. The coding sequence of the 3' most gene (ORF5) was conserved, with its ATG codon immediately after the stop codon of the CP gene. dRNAs accumulate only in single-stranded polyadenilated form, at a late stage of infection and do not apparently interfere with symptom expression or genomic RNA replication. Whereas the biological function of dRNAs is unknown, template switching of the replicase complex along the genome during viral RNA synthesis can be hypothesized for their origin.

PHOTOSYNTHETIC RESPONSES OF TWO *TRITICUM DURUM* VARIETIES EXPOSED TO CHRONIC OZONE FUMIGATION. **V. Picchi, A. Francini, C. Nali and G. Lorenzini.** *Dipartimento di Coltivazione e Difesa delle Specie Vegetali "G. Scaramuzzi", Università di Pisa, Via del Borghetto 80, 56124 Pisa, Italy. E-mail: vpicchi@agr.unipi.it*

Two Italian wheat (*Triticum durum*) varieties, Mongibello and Claudio, were grown in fumigation chambers from emergence to maturity, where they were exposed to 80 ppb O_3 (7 days per week, h 10.00-15.00). From the tillering stage, "Mongibello" displayed a stronger reduction in photosynthetic activity with respect to "Claudio" (-44.7 and -12.7%, respectively). During emergence of the inflorescence, only "Mongibello" showed a significant decrease in photosynthetic activity (-15.6%) and stomatal conductance (-33.7%), while "Claudio" showed a consistent decrease in chlorophyll (*a* and *b*) and β-carotene content. In "Mongibello", O_3 decreased the relative growth rate between inflorescence emission and maturity of caryopsis. As a result, the overall growth was clearly reduced with respect to controls. Assessment of yield at harvest revealed a stronger O_3-induced decrease in the number of grains per ear in "Mongibello" with respect to "Claudio" (-40.0 and -28.6%, respectively). Only in "Mongibello" there was a decrease of the dry weight of grains per plant and of spikelets per ear. No negative effects on the dry weight of leaves and stems or in the number of ears per plant were found. Our findings highlight the different response of the wheat varieties under study. The higher ozone sensitivity of "Mongibello" may be due to: (i) greater sensitivity in terms of growth during the critical period between inflorescence emission and maturity; (ii) decrease in photosynthetic activity from tillering to inflorescence emission; (iii) no exploitation as antioxidants of photosynthetic pigments.

IDENTIFICATION OF DIFFERENTIALLY EXPRESSED GENES IN GRAPEVINE FOLLOWING INFECTION WITH *PLASMOPARA VITICOLA*. **M. Polesani[1], F. Desario[1], J. Knörzer[2], N. Regier[2], A. Kortekamp[2] and A. Polverari[1].** *[1]Dipartimento Scientifico e Tecnologico, Università degli Studi di Verona, Strada Le Grazie 15, Cà Vignal 1, 37134 Verona, Italy. [2]Institute for Phytomedicine, Department of Phytopathology, University of Hohenheim, 70593 Stuttgart, Germany. E-mail: annalisa.polverari@univr.it*

Plasmopara viticola is an obligate biotrophic pathogen that obtains nutritional resources from grapevine living cells through haustoria. The haustorium-host cell interface is thought to involve a largely unknown, extensive molecular traffic. A comprehensive analysis of transcriptional changes associated to the infection process of *P. viticola* in susceptible grapevine (cv. Riesling) has been undertaken by cDNA-AFLP, with the aim of identifying differentially expressed genes from the host and the pathogen, in infected leaves at the oil spot stage. RNA was extracted from leaves of *in vitro* grapevine plants either infected with *P. viticola* or healthy, as well as from sporangia. Amplifications with 128 primer combinations allowed the visualization of about 7000 transcripts, with 1653 differentially expressed fragments characterizing the interaction. Most of them are predicted to be of plant origin, also in consideration of the very low concentration of *P. viticola* RNA in infected leaf samples. However, a small percentage of *Plasmopara* genes are also expected to be identified. Up-to-date, about 500 differentially expressed fragments of cDNA corresponding to putative grapevine transcripts have been successfully sequenced. After homology search in databases, all transcripts are being classified into functional categories. Selected genes, possibly involved in signal transduction, will be the object of further investigations. Additionally, about 2000 cDNA fragments from sporangia have been identified, that are going to represent a possible wide integration of the presently scarce knowledge on expressed *P. viticola* sequences.

***FUSARIUM VERTICILLIOIDES* AND RELATED SPECIES OF THE *GIBBERELLA FUJIKUROI* COMPLEX SECRETE AN ENDOPOLYGALACTURONASE NOT INHIBITED BY MONOCOT PGIPs.** **A. Raiola[1], L. Sella[1], C. Castiglioni[1], V. Balmas[2], A. Tomassini[1] and F. Favaron[1].** *[1]Dipartimento Territorio e Sistemi Agro-Forestali, Università degli Studi di Padova, Viale dell'Università 16, 35020 Legnaro, Italy. [2]Dipartimento di Protezione delle Piante, Università degli Studi di Sassari, Viale E. De Nicola, 07100 Sassari, Italy. E-mail: francesco.favaron@unipd.it*

Fusarium verticillioides, like others fungal species of the *Gibberella fujikuroi* complex, is a toxigenic and pathogenic species able to induce disease on important monocotyledonous crops. In order to understand the involvement of the endopolygalacturonase (endo-PG) of *F. verticillioides* in the infection process we have purified the endo-PG produced in culture by four different isolates of this fungus and cloned the corresponding genes. The purified endo-PGs showed similar biochemical and molecular properties. In particular, all endo-PGs were not inhibited by polygalacturonase-inhibiting proteins (PGIPs) extracted from

monocot host plants (like asparagus or maize) and were partially inhibited by bean PGIP, but only at an extraordinarily high dose. Deduced amino acid sequences of endo-PGs from *F. verticillioides* were similar to those obtained from endo-PGs of seven other species of the *G. fujikuroi* complex (*F. sacchari, F. fujikuroi, F. proliferatum, F. subglutinans, F. thapsinum, F. nygamai, F. circinatum*). These endo-PGs, like that of *F. verticillioides*, were not inhibited by monocot PGIP whilst were inhibited at various degrees by the bean PGIP. Thus, since many species of the *G. fujikuroi* complex are pathogens of monocot plants, their endo-PG appears particularly suitable to overcome the hindrance of the host PGIPs. Multiple alignments of the endo-PG sequences allowed the identification the few amino acid substitutions of the different fungal enzymes that are likely involved in the binding of the bean PGIP.

YAP1-LIKE GENE MODULATES AFLATOXIN BIOSYNTHESIS IN _ASPERGILLUS PARASITICUS_. **M. Reverberi[1], S. Zjalic[1], A. Ricelli[2], F. Punelli[1], A.A. Fabbri[1], and C. Fanelli[1].** *[1]Dipartimento di Biologia Vegetale, Università "La Sapienza", Largo Cristina di Svezia 24, 00165 Roma, Italy. [2]ISPA-CNR, Via Amendola 122/o, 70126 Bari, Italy. E-mail: massimo.reverberi@uniroma1.it*

In *Aspergillus parasiticus* Speare, NRRL 2999, lipoperoxide promotes aflatoxin biosynthesis in media favouring and not favouring their biosynthesis (potato dextrose broth -PDB- and czapek dox broth -CD-, respectively). To stimulate oxidative stress in CD, cumene hydroperoxide (CH, 1 mM), a lipoperoxidation inducer, was used. The two regioisomers of the hydroperoxides of linoleic acid (9-HODE and 13-HODE) were analysed in *A. parasiticus* mycelia by liquid chromatography-mass spectrometry (LC-MS). Some oxidative stress-related transcription factors such as *yap1*-like, *skn7*-like and *hsf2*-like appeared to drive, following their activation, the correlation between oxidative stress in the mycelia and aflatoxin biosynthesis. Expression of these factors led to the activation of defence responses of *A. parasiticus* cells such as antioxidant enzyme activities: superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPX). In accordance with the role played by antioxidant machinery in the cell and toxin biosynthesis, the well-known antioxidants caffeic acid and BHA, were able to inhibit lipoperoxide formation and aflatoxin biosynthesis. The *A. parasiticus* mutant Δ*yap1*-like grown in an aflatoxin conducive medium (PDB) anticipated and increased aflatoxin biosynthesis in comparison to wild type and, at the same time, markedly inhibited SOD, CAT and GPX activity. Probably in Δ*yap1*-like strain the cell environment is more suitable to support earlier and higher toxin formation. Reactive species as lipoperoxides formed during fungal growth create a stressing environment in front of which fungal cell activates oxidative stress transcription factors and aflatoxin biosynthesis. *Yap1*-like appears to play a modulating role in metabolic events leading to aflatoxin biosynthesis in *A. parasiticus*.

ALTERNATIVE REPLICATION SITES IN TOMBUSVIRUS INFECTIONS. **L. Rubino, B. Navarro and M. Russo.** *Istituto di Virologia Vegetale del CNR, Sezione di Bari, Via Amendola 165/A, 70126 Bari, Italy. E-mail: l.rubino@ba.ivv.cnr.it*

Positive-strand RNA viruses replicate in association with intracellular membranes of host cells, rearranged or *de novo* synthesized to form vesicles. The membranes can derive from diverse organelles, i.e. endoplasmic reticulum (ER), tonoplast, mitochondria, chloroplasts, or peroxisomes. The confined environment protects the viral replication machinery from host cell defence. Targeting of the viral proteins required for replication to a specific membrane is determined by signals encoded in the viral genome. For instance, tombusviruses evolved to direct their replication machinery either to mitochondrial or peroxisomal membranes. However, it is matter of debate whether the requirement for a specific membrane is an absolute need for a given virus, or alternative sites of replication are possible under particular host cell conditions. This issue is relevant not only for comprehension of the basic mechanism of plus-strand virus replication, but also for envisaging possible antiviral strategies based on altering the composition of a particular host membrane. The tombusvirus *Cymbidium ringspot virus* (CymRSV) replicase proteins normally associate with the peroxisomal membrane both in plant and yeast (*Saccharomyces cerevisiae*) cells where genomic or DI RNA replication takes place. Taking advantage of the availability of yeast strains defective for peroxisome biogenesis, CymRSV replicase proteins were expressed along with the replication template DI RNA in one of these strains (YPH499), which contains only primordial forms of peroxisomes unable to import any peroxisomal or viral proteins Biochemical, immunofluorescence and immunoelectron microscopy investigations demonstrated that replication takes place on ER-derived membrane, thus confirming the adaptability of plus-strand viruses to changes of the membranous environment of host cells.

IS IT POSSIBLE TO AVOID DUTCH ELM DISEASE? **A. Santini[1], L. Ghelardini[1], F. Ferrini[1], A. Dahmani[1] and M. Falusi[2].** *[1]Istituto per la Protezione delle Piante, CNR, Via Madonna del Piano, 10, 50019 Sesto fiorentino (FI), Italy. [2]Dipartimento di Biologia Vegetale, Università di Firenze, P.le Cascine 28, 50144 Firenze, Italy. E-mail: a.santini@ipp.cnr.it*

The fate of European elms is linked to Dutch elm disease (DED): as a result of two epidemics (in the 1930s and 1970s), caused by *Ophiostoma ulmi* (Buisman) Nannfeldt and *Ophiostoma novo-ulmi* Brasier respectively, the great majority of mature field elms (*Ulmus minor* Mill.) in Europe has been killed. Since a robust source of resistance could not be found within the European elm population, breeding programs for DED resistance involve cross-breeding of Asian with other indigenous species, but these clones cannot met the increasing demand for indigenous material arising in these last years. For this reason the "resistance to DED" character of the European elm species has been re-considered. A statistically significant relation between resistance and latitude of origin of field elms grown in the same experimental field was found, southerly clones showing less disease than the more northerly ones. Relationships were also found between disease severity and timing to bud burst, being the early flushers less susceptible. Southern clones flushed significantly earlier than those from the north in 2 years characterized by dramatically different thermal trends. A strongly significant regression between days to bud burst and the latitude of origin of the clones even in clonal banks located in different environmental conditions was highlighted. Clones with different origin were, then, inoculated at different dates. The results showed that the date of higher susceptibility varies according to the bud-burst phenology. Southern clones, as a matter of fact, could avoid DED if planted far northern than their origin.

EFFECTS OF ALLOPURINOL ON GREEN FLUORESCENT PROTEIN-EXPRESSING *TOBACCO MOSAIC VIRUS* IN *NICOTIANA TABACUM*. **S. Silvestri[1], A.M. Murphy[2], J.P. Carr[2] and R. Buonaurio[1].** *[1]Dipartimento di Scienze Agrarie ed Ambientali, Università degli Studi di Perugia, Via Borgo XX Giugno 74, 06121 Perugia, Italy. [2]Department of Plant Sciences, University of Cambridge, Downing Street, Cambridge CB2 3EA, United Kingdom. E-mail: simonaaidasilvestri@hotmail.com*

Allopurinol [4-hydroxypyrazolo (3,4-*d*) pyrimidine] is a specific and potent *in vitro* and *in vivo* inhibitor of xanthine oxidase. Tobacco plants of the cultivar Xanthi, which is susceptible to *Tobacco mosaic virus* (TMV), were watered with solutions of allopurinol prior to challenge inoculation with a TMV strain engineered to express the green fluorescent protein (TMV-GFP). TMV-GFP infection was monitored by examination of inoculated leaves under UV light and detailed examination of infection sites was done by confocal scanning laser microscopy and/or epifluorescence microscopy. The replication and movement of TMV-GFP was enhanced in allopurinol-treated plants. This was seen as a statistically significant increase in the number of fluorescent infection sites per leaf (1.3-2.3 fold increase in TMV-GFP infection foci compared with control plants) and the number of cells showing GFP fluorescence per infection site (1.3 fold increase) in allopurinol-treated plants. Inhibition of xanthine oxidase by allopurinol decreases production of O_2^-, a defensive signal, and this may explain the apparent increased susceptibility of allopurinol-treated plants to infection and spread of the virus. Our results are consistent with those obtained by Montalbini (*J. Phytopathology* 139: 177-186, 1993) with TMV-resistant (*NN* genotype) tobacco. In these plants, allopurinol promoted escape of TMV from hypersensitive local lesions, resulting in systemic spread of the virus and the appearance of necrotic lesions on non-inoculated leaves. Interestingly, allopurinol inhibited the spread of a different virus, *Tobacco necrosis virus* (Montalbini, *Riv. Pat. Veg*., 4: 81-91, 1994). Taken together, our results and the earlier data indicate that the production of O_2^- plays a role in determining the outcome (resistance or susceptibility) of plant-virus interactions.

SPREAD OF *CITRUS TRISTEZA VIRUS* IN AN AREA OF SICILY. **G. Sorrentino[1], S. Davino[2], M. Guardo[1], A. Caruso[1], G. Iacono[2] and M. Davino[2].** *[1]C.R.A., Istituto Sperimentale per l'Agrumicoltura di Acireale, Corso Savoia 190, 95024 Acireale, Italy. [2]Dipartimento di Scienze e Tecnologie Fitosanitarie (DISTEF), Sezione di Patologia vegetale, Università degli Studi di Catania, Via S. Sofia 100, 95123 Catania, Italy. E-mail: wdavino@unict.it*

A study was carried out in a citrus-growing area of Sicily (Southern Italy) with the purpose of monitoring the spread of *Citrus tristeza virus* (CTV). The site of the investigation was in the area of Baè (Catania) where thousand of 'Tarocco comune' sweet orange trees grafted on sour orange of different age are infected by a severe strain of CTV. Two blocks of 400 plants each, chosen at different distance from the initial CTV focus were tested every 3 months by imunoenzymatic assays (DAS-ELISA and DTBIA) using a mixture of two monoclonal antibody. Samples were collected weekly during the months of March, April, May, June, September and October 2001 through 2005, for identifying the species constituting aphid populations of the area. Some of the collected aphids were used for CTV transmission trials in rearing cages placed in a constant temperature cabinet whereas others were checked for the presence of CTV in their body. About 90% of the aphid population in the area under study was made up of the cotton aphid (*Aphis gossypii*). CTV was present in the body of the aphids that were able to transmit the virus. In five years infected trees increased from 5 to 88%.

CHARACTERISATION OF *PSEUDOMONAS SAVASTANOI* pv. *SAVASTANOI* STRAINS ISOLATED FROM DIFFERENT HOST PLANTS BY F-AFLP. **A. Sisto[1], M.G. Cipriani[1], M. Cerboneschi[2], E. Santilli[2], G. Stea[1] and S. Tegli[2].** *[1]Istituto di Scienze delle Produzioni Alimentari, C.N.R., Via Amendola 122/O, 70126, Bari, Italy. [2]Dipartimento di Biotecnologie Agrarie, Laboratorio di Patologia Vegetale Molecolare, Università degli Studi di Firenze, Via della Lastruccia 10, 50019 Sesto Fiorentino, Italy. E-mail: angelo.sisto@ispa.cnr.it*

The genetic diversity of 71 *Pseudomonas savastanoi* pv. *savastanoi* strains was determined by fluorescent amplified fragment length polymorphism (f-AFLP) using three different selective primer combinations. *P. s.* pv. *savastanoi* strains isolated from different host plants and diverse geographical regions were compared with 5 outgroup strains including *P. amygdali*, *P. syringae* pv. *syringae*, *P. s.* pv. *phaseolicola*, *P. s.* pv. *glycinea* and *P. s.* pv. *tagetis*. Based on cluster analysis of AFLP data, all *P. s.* pv. *savastanoi* strains showed a high degree of similarity grouping in a cluster and forming a single taxon that was clearly differentiated from outgroup strains. Moreover, some subclusters were formed that clearly correlated with the host plant of isolation. All *P. s.* pv. *savastanoi* strains from oleander were differentiated from all strains from ash and were included in two diverse subclusters. *P. s.* pv. *savastanoi* strains isolated from olive were split into two additional subclusters differing from subclusters that comprised strains from oleander or ash. Three strains isolated from jasmine were also differentiated from those isolated from different host plants. By contrast, two strains from privet were similar to those isolated from olive and were included in the same subcluster. Further subclusters could be identified within the aforementioned clusters and they seemed mainly related to geographical origin of strains. The results of this study indicate that the use of AFLP markers has the potential to play a major role in the accurate study of *P. s.* pv. *savastanoi* populations.

TOXIGENICITY OF *PENICILLIUM EXPANSUM* ISOLATED FROM APPLES AND PEARS AND BIOTRANSFORMATION OF PATULIN BY YEASTS. **M. Solfrizzo[1], A. Ricelli[1], L. Caputo[1], I. Marcellino[2], A. Carlucci[2] and S. Frisullo[2].** *[1]CNR, Istituto di Scienze delle Produzioni Alimentari, Bari, Italy. [2]Dipartimento di Scienze Agro-Ambientali, Chimica e Difesa Vegetale, Università degli Studi di Foggia, 71100 Foggia, Italy. E-mail: michele.solfrizzo@isp.cnr.it*

Sixty-three samples of apples and pears with rot symptoms were collected from local markets in Southern Italy. Forty isolates of *Penicillium expansum* Link and 94 isolates of yeasts were isolated from these samples. The toxigenicity of *Penicillium* isolates was checked by culturing them on autoclaved mashed pulp of apple or pear fruits and analysing the relevant water extracts by HPLC. Five selected isolates of yeasts were tested for their attitude to degrade patulin in apple juice and identified by partial rDNA sequencing. The water extract of fruit samples with rot symptoms were analysed by HPLC for their patulin content whereas water extracts of *Penicillium* cultures and apple juice artificially contaminated with 15 µg/ml of patulin and inoculated with yeasts were analysed by HPLC for patulin, desoxypatulinic acid and ascladiols. The latter have been reported as precursors and degradation products of patulin, respectively. Of the 32 apple samples 16 were contaminated by patulin at concentrations ranging from 0.08 to 125.59 µg/g whereas 25 out of 30 pear samples were contaminated with 0.12-317.26 µg/g of patulin. Thirty-seven out of 40 isolates of *P. expansum* produced patulin and desoxypatulinic acid, one isolate produced only patulin, one isolate produced patulin, desoxypatulinic acid and ascladiols whereas

two isolates were not toxigenic. The five isolates of yeasts were identified as *Pichia spp., Debaryomyces spp., Rhodotorula spp., Candida spp.* and *Discosphaerina spp.*. Four out of the five tested yeasts reduced the concentration of patulin added to apple juice. *Candida spp.* reduced patulin concentration by 99% with subsequent formation of ascladiols.

TESTING GENETICALLY ENGINEERED PHYTOCHROME A CHERRY PLANTS FOR RHIZOSPHERE AND PHYLLOSPHERE BENEFICIAL BACTERIA AND FOR SUSCEPTIBILITY TO BACTERIAL CANKER. S. Spina[1], R. Muleo[2], C. Iacona[3], A. Catara[4], G. Cirvilleri[4]. *[1]Parco Scientifico e Tecnologico della Sicilia S.c.p.a, Viale V. Lancia Z.I., Catania, Italy. [2]Dipartimento di Produzioni Vegetali, Via S.C. DeLellis s.n.c, Università della Tuscia, 01100 Viterbo, Italy. [3]Dipartimento di Coltivazione e Difesa della Specie Legnose, Via del Borghetto 80, Università di Pisa, 56124 Pisa, Italy. [4]Dipartimento di Scienze e Tecnologie Fitosanitarie, Università di Catania, Via S. Sofia 100, 95123 Catania, Italy. E-mail: gcirvil@unict.it*

Somatic tissues of the cherry rootstock Colt (*Prunus avium* x *Prunus cerasus*) were transformed with phytochrome A gene to modify the incoming light perception. Three lines were chosen: PD3, with increased phytomer formation, branching and plant growth; PO1, with reduced phytomer formation; and PA, with unchanged morphology and development. Molecular analyses did not reveal any difference in the number of inserted copies of the exogenous gene. Over the course of one year the total bacterial population from rhizosphere of PA line (log 7.54) was significantly higher than the population recovered from PO1, PD3 and WT lines. Moreover, more fluorescent pseudomonads were recovered from PA and PO1 lines (log 6.50 and 6.65, respectively) than from PD3 and WT lines. No significant difference was detected in the number of fluorescent *Pseudomonas* from the phyllosphere of transgenic and non-transgenic lines. Resistance to kanamycin of rhizosphere and phyllosphere strains was not affected by plant lines. A total of 143 rhizosphere *Pseudomonas fluorescens* strains were tested against *Phytophthora nicotianae* and no difference in antagonistic activity was recorded among lines. Only *P. fluorescens* strains from phyllosphere of PA and PO1 lines showed antagonistic activity against *P. syringae* pv. *syringae*, whereas no difference among lines was detected when bacterial strains were tested against *P. syringae* pv. *morsprunorum* and *Monilinia laxa*. Symptoms on leaf, midrib and petiole first appeared on WT line three days after *P. syringae* pv. *syringae* inoculation. On the whole, PO1 line showed the lowest and PD3 the highest number of infected sites. Four days after *P. syringae* pv. *morsprunorum* inoculation, WT and PA lines showed the first symptoms. PD3 and PO1 lines showed the lowest number of infected sites.

DEVELOPMENT OF THE FUSARIUM HEAD BLIGHT COMPLEX AND ACCUMULATION OF ASSOCIATED MYCOTOXINS IN FIELD CONDITIONS IN ITALY IN COMPARISON WITH THREE EUROPEAN COUNTRIES. A. Susca[1], G. Mule[1], A. Ritieni[2] and A. Moretti[1]. *[1]ISPA-CNR, Via Amendola 122/0, 70126 Bari, Italy. [2]Università di Napoli "Federico II", Dipartimento di Scienza degli Alimenti, Parco Gussone, 80055 Portici (NA) Italy. E-mail: antonio.moretti@ispa.cnr.it*

Fusarium head blight (FHB) is caused by a complex of *Fusarium* species producing harmful metabolites for plants, humans and animals. From 2001 to 2004 we have monitored the field development of FHB outbreaks in Italy comparing our data with those collected by other research groups in three European countries (Ireland, UK, and Hungary), characterized by different environmental conditions. In Italy data were collected from 60 sites. For each sample, we evaluated at the flowering (GS 69) and milky stage (GS 77) disease symptoms and the presence and amount of FHB pathogen DNA (based on diagnostic PCR by using species specific primers and quantitative PCR, respectively). At harvest, both the presence and amount of FHB pathogen DNA in the kernels were evaluated, together with the mycotoxin contamination (in particular, trichothecenes, beauvericin, and enniatins). Finally the climatic data from anthesis to harvest were collected every hour. Key findings of our study were: (i) *Fusarium poae* was the most frequent species detected: (ii) in cooler regions the frequency of *Fusarium graminearum* was higher, while in Italy *Fusarium culmorum* was rarely encountered; (iii) FHB pathogens appeared to be positively correlated with each other in their presence or absence. Overall, *F. graminearum* showed fewest interactions with other pathogens. Mycotoxin contamination was detected in 42% of samples from Italy. In general, however, the amount of toxin was very low, well below the EU regulatory level, with the exception of two samples from central Italy containing high deoxynivalenol levels (up to 15 ppm). Finally, field data also showed that, in general, the level of mycotoxins was not related to disease incidence and fungal biomass (measured as total fungal DNA) with the exception of *F. graminearum* the presence of which and of its associated mycotoxins varied greatly within a single field.

PREDICTION OF FUMONISIN CONTAMINATION OF MAIZE AT HARVEST. E. Torelli[1], E. Gobbi[1,2], R. Gubiani[3], R. Locci[1] and G. Firrao[1]. *[1]Dipartimento di Biologia Applicata alla Difesa delle Piante, Università di Udine, Via Scienze 208, 33100 Udine, Italy. [2]Biodiversity s.r.l., Via Corfù 71, 25100 Brescia, Italy. [3]Dipartimento di Scienze Agrarie ed Ambientali, Università di Udine, Via Scienze 208, 33100 Udine, Italy. E-mail: torelli@uniud.it*

Fumonisins are a family of mycotoxins produced mainly by *Fusarium verticillioides* and *Fusarium proliferatum*. Maize production in North-east Italy is frequently contaminated by high levels of fumonisin B1 (FB1). The early identification and isolation of heavily contaminated lots for use in energy production is an effective way to prevent contamination and reduction of the economic value of the bulk. Methods for the detection of FB1 contamination are therefore urgently needed. We developed and evaluated methods for the early assessment of contamination risk at the time of delivery of the grains to drying and storage services. Aereobiological sampling of fungal spores was performed during the harvest of maize using a cyclone-type air sampler. Spores were filter blocked and subsequently analysed. About 60 fungal spore samples were collected and diluted for the determination of colony forming units (CFU) and extraction of fungal extracellular polysaccharides (EPS), later used in immunological assays with *Fusarium* specific EPS-antibodies (rabbit IgG). In addition, PCR and real-time PCR assay were done after bead-beating extraction of fungal DNA. Grain samples were also collected and surveyed for the determination of CFU and the occurrence of FB1 by HPLC. Finally image analysis was also carried out to record kernel damage. Preliminary results of this work showed that an effective model can be developed that predicts the amount of FB1 from location/weather data and the estimated amount of toxigenic fungal spores. In the present work, the method accounted for about 85% of variability of FB1 contamination. Fungal spore enumeration in air samples as an alternative to the chemical determination of fumonisin in collected grains is a potentially effective solution

to mass sampling procedures and time consuming chemical analyses, which are not compatible with early risk detection.

PHYTOSANITARY MONITORING OF FORESTS: NEW METHODOLOGIES OF REMOTE SENSING AND GEOSTATISTIC APPLICATIONS. A. Vannini[1], S. Noce[1], G. Natili[1], C. Belli[2] and A.M. Vettraino[1]. *[1]Dipartimento di Protezione delle Piante, Università degli Studi della Tuscia, Via S. Camillo de Lellis, 01100 Viterbo, Italy. [2]Terrasystem s.r.l., Via Pacinotti 5, 01100 Viterbo, Italy. E-mail: vannini@unitus.it*

An efficient phytosanitary monitoring system is a pre-requisite for a correct management of forests in order to forecast pathological events and elaborate adequate strategies of control. The traditional utilization of ground activities to collect information and data on the phytosanitary status of forests is time consuming and does not guarantee covering of the whole area. These disadvantages can be overcome by combining target ground activities with remote sensing. The airborne multispectral system ASPIS, for high radiometric resolution and digital image acquisition was used to identify ink disease foci over a surface of 2500 ha of natural and cultivated chestnut forests in Central Italy in 2003 and 2004. The system was able to recognize single declining trees and different degree of crown decline. Validation of remote sensing activities was done by target ground activities. The multispectral images, once georeferenced and ortho-projected, were reported in a GIS and elaborated with additional territorial layers including DEM, land use, slope and exposition. The elaborated maps allowed estimating the pattern of spread of ink disease from existing foci and the localization of new foci. In addition, a strong association was found between spread of the disease and density of roads per area unit. In particular most of the infected areas and of the new foci were localized within 25 meters from the roadside. These findings confirm the importance of the roads for soil-borne Phytophthoras spread across areas, as previously reported for other binomials. Pathological data introduced in GIS systems can be further elaborated with geostatistic modules in order to produce spatialized risk maps. Ordinary kriging was applied to georeferenced ink disease incidence data. Semivariograms elaborated for the year 2002 and 2003 showed a spatial correlation of data. The kriged maps, properly validated and overlapped with landform layers, showed a preferential pattern of spread of the disease and a strong association between incidence and the distance from natural drainages. Remote sensing integrated to GIS technology and eventually to geostatistic modules confirmed to be powerful tools for epidemiological studies and for the elaboration of accurate disease maps and predictions.

ROLE OF FUNGAL SECONDARY METABOLITES IN THE INTERACTION OF *TRICHODERMA* SPP. WITH PLANTS AND OTHER MICROORGANISMS. F. Vinale[1], K. Sivasithamparam[2], E.L. Ghisalberti[2], M. Barbetti[2], H. Li[2], R. Marra[1], F. Scala[1], S.L. Woo[1] and M. Lorito[1]. *[1]Dipartimento di Arboricoltura Botanica e Patologia Vegetale, Università degli Studi di Napoli Federico II, Via Università 100, 80055 Portici (NA), Italy. [2]University of Western Australia, 35 Stirling Highway, Crawley, Perth, WA, Australia. E-mail: frvinale@unina.it*

Secondary metabolites play an important role in biocontrol, although their modes of action towards pathogens and plants have not been fully elucidated. In this work, we isolated major secondary metabolites produced by three *Trichoderma harzianum* (T22, T39 and A6) and one *T. atroviride* (P1) isolates. Seven compounds obtained from fungal culture filtrates were extracted and characterised: two new molecules [T22 azaphilone and T39 butenolide] and five already known secondary metabolites [1-hydroxy-3-methyl-anthraquinone, 1,8-dihydroxy-3-methyl-anthraquinone, harzianolide, 6-n-pentyl-6H-pyran-2-one and harzianopyridone]. Different levels of antibiotic activity against various pathogens were detected. Since these compounds may be produced also during plant colonization, their ISR-inducing ability was evaluated. We obtained a reduction of disease symptoms on tomato and canola seedlings inoculated with *Botrytis cinerea* and *Leptosphaeria maculans*, respectively and treated with the fungal metabolites applied on the cotyledons at a concentration ranging from 1 to 10 ppm. RT-PCR analysis (chitinase IV, PR1 from *Brassica napus* and an endochitinase from *Lycopersicum esculentum*) indicated that treatments with the purified fungal compounds induced over-expression of the tested PR proteins *in planta*, thus indicating a role of *Trichoderma* secondary metabolites in the activation of plant defence genes. Moreover, a possible involvement of these fungal compounds in canola and wheat growth promotion was evaluated. Purified harzianolide and T39 butenolide induced a significant increase development of seedlings when applied directly on the roots.

PLANT-DERIVED VACCINES. A. Vitti, M. Lapelosa, M. Nuzzaci and P. Piazzolla. *Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali, Università degli Studi della Basilicata, Viale dell'Ateneo Lucano 10, 85100 Potenza, Italy. E-mail: piazzolla@unibas.it*

The production of plants expressing foreign antigens is a particularly promising approach to produce vaccines. Antigen (Ag) expression either in transgenic plants or in plants infected with genetically engineered chimeric viruses to display potentially immunogenic peptides on their outer surfaces offers several advantages. First of all, plants can be eaten, thus providing an easy and not expensive route of Ag administration. In such a way, plant viruses carrying on their coat protein peptides of medical interest can be considered, in association with their hosts, right partners of biological systems devoted to pursue the goal of functioning as medical molecular farming. Thus, plants may behave as heterologous expression vectors, but unlike the transgenic strategy, which necessarily needs the manipulation of plant genomes, the chimeric virus strategy requires the manipulation of virus genomes. So, the first strategy has a genetically modified organism as a final product, whereas the second strategy generates a genetically modified virus, which is an elementary biosystem possessing some of the properties of living organisms, such as having a genome and being able to adapt to changing environments. We have been able to use as a carrier *Cucumber mosaic virus* (CMV), a tripartite genome isodiametric plant virus about 30 nm in diameter, with both a worldwide distribution and an extremely wide host range. The viral CP gene was successfully engineered to express peptides deriving from the *Hepatitis C virus* (HCV) envelope and the amyloid-beta protein (A-beta) of Alzheimer's disease (AD).

CHARACTERIZATION OF *PSEUDOMONAS SYRINGAE* PV. *TOMATO* STRAINS FOR FIVE DIFFERENTIAL EFFECTOR GENES. M. Zaccardelli[1], F. Campanile[1] and B.A. Vinatzer[2]. *[1]CRA, Istituto Sperimentale per le Colture Industriali, SS. 18 204, 84091 Battipaglia (SA), Italy. [2]Department of Plant Pathology, Physiology, and Weed Science, Virginia Polytechnic Institute and State University, Fralin Biotechnology Center, Blacksburg, USA. E-mail: m.zaccardelli@tiscali.it*

Gram-negative bacteria are equipped with genes that encode

effector proteins secreted into the host cell by a Type III secretion system. The precise function of the majority of effectors is unknown. However, they interact with plant cells so as to create an optimal environment for bacterial growth. In the last few years effector genes have been studied in *Pseudomonas syringae* pv. *tomato* (*Pst*), the causal agent of bacterial speck of tomato. Characterization of *Pst* strains done previously, showed that some Italian isolates did not hybridize twelve effector genes, all present in the reference strains DC3000. In this work, twenty-three isolates of *Pst*, fourteen from different Italian regions, four from Tanzania, three from France, one from Spain and the reference strain DC3000 from Great Britain, were characterized for five of these twelve differential effector genes. All the isolates, except for one from France, gave amplicons for $hopAB2_{PtoDC3000}$ (ex *avrPtoB*), $hopF2_{PtoDC3000}$ (ex *hopPtoF*) and $hopG1_{PtoDC3000}$ (ex *hopPtoG*). For $hopAA1\text{-}2_{PtoDC3000}$ (ex *hopPtoA2*), all the isolates yielded an amplicon except for one strain from France and one from Northern Italy. Only three strains from Italy, one from France and DC 3000, amplified *avrB3*. These results suggest that effector genes $hopAB2_{PtoDC3000}$, $hopF2_{PtoDC3000}$, $hopG1_{PtoDC3000}$ and $hopAA1\text{-}2_{PtoDC3000}$, are widespread in *Pst* populations all over the world whereas *avrB3* is rarely present. The absence of all five effector genes in one isolate from France requires further investigations.