INVITED LECTURE

GENE-FOR-GENE RECOGNITION IN THE *CLADOSPORIUM FULVUM*-TOMATO INTERACTION. P.J.G.M. de Wit, B.F. Brandwagt, H.A. van den Burg, S.H.E.J. Gabriëls, M.J.D. de Kock, R.A.L. van der Hoorn, C.F. de Jong, J.W. van't Klooster, M. Kruijt, R. Luderer, N. Westerink and M.H.A.J. Joosten. *Laboratory of Phytopathology, Department of Plant Sciences, Wageningen University, Binnenhaven 5, 6709 PD Wageningen, The Netherlands. E-mail: pierre.dewit@wur.nl*

Cladosporium fulvum secretes many cysteine-rich peptide elicitors during infection, which are encoded by avirulence (*Avr)* genes. Recognition of these elicitors is mediated by *Cf* resistance genes and leads to a hypersensitive response (HR). Although a number of *Avr* and *Cf* genes have been cloned, the molecular mechanism of recognition remains largely unknown. So far, no direct interaction between Avr and Cf proteins could be found. For recognition of the Avr2 elicitor, in addition to Cf-2, the tomato Rcr3 protein is required. In a search for possible virulence functions of Avr proteins we discovered that Avr4 contains an invertebrate chitin-binding domain that might protect *C. fulvum* against plant chitinases. We also study genes involved in signal transduction pathways leading to HR. Induction of HR in tomato by the Avr4 elicitor is temperature-sensitive. Tomato seedlings expressing both the *Avr4* gene and the matching *Cf-4* gene, quickly develop systemic HR at 23°C, but grow normally at 33°C. Thus, when the seedlings are grown at 33°C and are subsequently incubated at 23°C they synchronously start a cell death program within minutes after the temperature shift. We have identified mRNAs, either up- or down-regulated during the cell death program, by cDNA-AFLP analysis. This approach resulted in the identification of a few hundred cDNAs of which ca. 50% has no homology to known genes and 50% has homology to genes or ESTs from tomato and *Arabidopsis*. We now try to uncover the function of these tomato genes in HR and/or resistance by high throughput, virus-induced gene silencing (VIGS).

INVITED LECTURE

DURABLE RESISTANCE: CAN WE PREDICT THE RISK FOR PLANT PATHOGENS? C.C. Linde and B.A. McDonald. *Institute of Plant Sciences, Universitätstrasse 2, ETHZ, Zürich, 8092, Switzerland. Fax: +41.1.6321572; E-mail: celeste.linde@ipw.agrl.ethz.ch*

Pathogens evolve. Well-documented examples abound in human and veterinary medicine, as well as in agriculture. What is driving pathogen evolution? While selection is important in pathogen evolution, it does not act alone. The relative importance of other evolutionary forces in plant agricultural ecosystems is assessed, including migration, reproduction system, and population size. Pathogens that pose the greatest risk of evolving rapidly are those that possess a mixed reproduction system, including both sexual and asexual reproduction, and a high potential for genotype flow involving asexual propagules. The lowest risk pathogens are those with strict asexual reproduction and low potential for gene flow. Migration was found to be the most important factor driving pathogen evolution. The results were similar across different selection pressures (host resistance, pesticides) and pathogenic agents (fungi, nematodes, viruses), suggesting that this finding may be applicable to pathogens involved in veterinary and human medicine as well as insect pests. Knowledge of the population genetic structure of the pathogen may offer insight into the best breeding strategy for durable resistance.

ARE NITRIC OXIDE OR ITS DERIVATIVES THE PHLOEMATIC SYSTEMIC SIGNALS RESPONSIBLE FOR SYSTEMIC ACQUIRED RESISTANCE IN *ARABIDOPSIS THALIANA*?. R. Buonaurio[1], C. Moretti[1], G. Arienti[2] and C.A. Palmerini[2]. [1]*Dipartimento di Arboricoltura e Protezione delle Piante, Università degli Studi di Perugia, Borgo XX Giugno 74, 06121 Perugia, Italy.* [2]*Dipartimento di Scienze Biochimiche e Biotecnologie Molecolari, Via del Giochetto, 06122 Perugia, Italy. Fax: +39.075.5856482; E-mail: buonaurio@unipg.it*

Using a specific and sensitive solid-state amperometric sensor assay for NO and its derivatives, we observed significant increases of nitrite in phloem sap collected from *Arabidopsis thaliana* (ecotype Columbia) plants in which SAR had been induced with an avirulent strain of *Pseudomonas syringae* pv. *maculicola*. The accumulation was detected in both inoculated and non-inoculated leaves from 3 to 12 hours post-inoculation. Phloematic increases of nitrite were also detected in plants treated with the NO-releasing compound NOC-18 [2,2'-(hydroxynitrosohydrazino)bis-ethanamine], which systemically protected them against a virulent strain of *Pseudomonas syringae* pv. *maculicola*. The protection was detected as reduction in bacterial growth *in planta*. Further investigations are in progress to verify whether NOC-18 treatment induces transcript changes in SAR genes and to establish whether NO derivatives are the systemic signals of SAR.

RESPONSES INDUCED BY WOUNDING IN POTATO TUBERS: ANALYSIS OF OXIDATIVE STRESS - RELATED COMPOUNDS PRODUCTION UNDERNEATH THE BUDS. M. Reverberi[1], A.A. Fabbri[1], S. Zjalic[1], S. Briganti[2] and C. Fanelli[1]. [1]*Università degli Studi "La Sapienza" di Roma, Largo Cristina di Svezia 24, 00165 Roma, Italy.* [2]*Istituto Dermatologico San Gallicano, IRCCS, Via San Gallicano 25, 00153 Rome, Italy. E-mail: massimo.reverberi@uniroma1.it*

Endogenous lipid peroxidation could be involved in biotic and abiotic stresses (wounding and cold storage). Previous studies showed that wounding stress in potato tubers determines the hydrolysis of polyunsaturated fatty acids and subsequently the generation of lipoperoxides (LOOH) from endogenous linoleic and linolenic acid by the activity of lipoxygenase (LOX). Furthermore, LOOH induces indole-3-acetic acid (IAA) formation underneath potato buds. LOOH and IAA accumulation were also found after prolonged cold stress (e.g. after storage at 4°C) and, in both kinds of stresses, reactive species are responsible for cytological events such as mitosis, periderm formation, starch hydrolysis and budding initiation. In the present study, considering the close relationship between LOOH and IAA, LOOH and Jasmonates (JAs) production, we investigated the existence of correlation between JAs and IAA formation. The experiments were carried out on potato tuber slices at different times after wounding (0 up to 24h). The role of different LOXs in these events was studied by molecular and spectrophotometric approaches and IAA, MJ and JAs were monitored by GC-MS analyses. Furthermore, to investigate interrelationships, these compounds were also added separately and exogenously to potato slices. The results obtained showed that JAs and IAA affect each other, since the addition of JA and MJ to potato slices enhanced IAA formation late after wounding, whereas the addition of IAA stimulated an early production of JA and MJ, probably by a regulation of lox and allene oxide cyclase (aoc1).

MOLECULAR AND STRUCTURAL STUDIES ON FUNGAL POLYGALACTURONASES AND THEIR INHIBITORS PGIPs. **F. Cervone, D. Bellincampi, C. Caprari, G. De Lorenzo, A. Di Matteo, L. Federici, S. Ferrari, R. Galletti, C. Manfredini, B. Mattei, A. Raiola, D. Pontiggia, G. Salvi, S. Spadoni, D. Tsernoglou and D. Vairo.** *Dipartimento di Biologia Vegetale, Università di Roma "La Sapienza", Piazzale Aldo Moro, 00185 Roma, Italy. Fax +39.06.49912446*

Polygalacturonase-inhibiting proteins (PGIPs) counteract fungal polygalacturonases (PGs) and reduce fungal disease symptoms by hampering the invasion process and the release of nutrients necessary for fungal growth. PGIPs are encoded by gene families, the members of which are not merely functionally redundant. Polymorphism of their sequences is associated to both a different regulation and a different recognition specificity, likely to be instrumental against different phytopathogenic fungi. The recognition ability of PGIPs resides in their leucine-rich repeat (LRR) protein structure. We have performed structural studies and determined the crystal structure of both PG and PGIP. The interesting features of the PGIP LRR structure may help elucidate the interactive properties and the mechanism of recognition of PGIPs, as well as of other plant LRR proteins. The interaction between fungal PGs and plant PGIPs is thought to favour the accumulation of oligogalacturonides (OGs). The ability of OGs of up-regulating a wide number of defense genes has been analysed by full genome mRNA expression profiling. Many of the OG-regulated genes are also induced during fungal infection, including the expression of PGIPs, suggesting that OGs mediate part of the expression changes occurring in response to pathogen attack.

ANALYZING THE IMPACT OF TRANSGENE-DERIVED siRNAs ON *TOMATO YELLOW LEAF CURL SARDINIA VIRUS* INFECTION. **A. Lucioli[1], E. Noris[2], R. Tavazza[1], P. Caciagli[2], G. P. Accotto[2] and M. Tavazza[1].** *[1]ENEA CR Casaccia, Settore Biotec, Via Anguillarese 301, 00060 Roma, Italy. [2]Istituto di Virologia Vegetale del CNR, Strada delle Cacce 73, 10135 Torino, Italy. E-mail: alessandra.lucioli@casaccia.enea.it*

We have recently shown that *Tomato yellow leaf curl Sardinia virus* (TYLCSV) is able to overcome Rep-210-mediated resistance by shutting-off transgene expression by a post-transcriptional homology-dependent mechanism, suggesting that TYLCSV would be able to evade to some extent transgene-mediated silencing of the essential viral Rep gene (Lucioli *et al.*, 2003, *J. Virol.* 77: 6785). In this report we directly analysed the impact of silencing on TYLCSV infection, using two classes of post-transcriptionally silenced Rep-210 transgenic tomato plants. One class consisted of a sense x antisense Rep-210 hybrid while the other was the selfed-progeny of tomato lines that integrated multiple copies of the sense Rep-210 transgene. In both classes, post-transcriptionally silenced plants accumulated low or undetectable amount of Rep-210 protein and mRNA, but high amount of Rep-210 transgene-specific siRNAs. Interestingly, all silenced plants were susceptible to TYLCSV when challenged by agroinoculation. However, when these plants were challenged with viruliferous *Bemisia tabaci,* the ratio between susceptible versus resistant plants was dependent on the inoculum load. At high viral vector inoculum, all the silenced plants were susceptible. Interestingly, and according to the dose effect, some plants showed delayed infection, indicating that TYLCSV exerts an active role in overcoming transgene-derived siRNA-targeting of its Rep gene. The overall data suggest a threshold model in which transgene-derived siRNAs interfere with virus infection in the initially inoculated cells but, if viral replication/expression reaches a threshold, virus spreading in the silenced tissue cannot be any more prevented.

INDUCTION OF POST-TRANSCRIPTIONAL GENE SILENCING IN PHLOEM TISSUES CONFERS SYSTEMIC RESISTANCE TO *PLUM POX VIRUS* IN *NICOTIANA BENTHAMIANA*. **T. Pandolfini, B. Molesini, L. Avesani, A. Spena and A. Polverari.** *Dipartimento Scientifico-Tecnologico, Università degli Studi di Verona, Strada Le Grazie 15, 37134 Verona, Italy. Fax: +39.045.8027929; E-mail: angelo.spena@univr.it*

Post-transcriptional gene silencing (PTGS) is involved in several biological phenomena, including adaptive defence against viruses. *Plum pox virus* (PPV), the etiological agent of the "sharka" disease of stone fruits, causes serious crop losses in most growing areas. PPV is transmitted by aphids and moves from cell to cell via plasmodesmata and systemically via phloem. In this work, we aimed at conferring systemic resistance against PPV triggering PTGS in phloem tissues. *Nicotiana benthamiana* plants were transformed with a 197 bp sequence of the PPV genome, placed as two inverted repeats under the control of the *rolC* promoter, obtaining the expression of a self-complementary hairpin RNA specifically in phloem cells. The progenies from 5 independent transgenic plants were mechanically inoculated and tested for PPV infection by ELISA and immunocapture-RT-PCR. Local infection was unaffected. Most plants (80%) showed systemic resistance to PPV, remaining symptomless and virus-free till the end of their life cycle. In a few plants, PPV was detected 1 or 6 weeks post-inoculation with only late mild mottling of a few leaves. All plants grew and produced seeds normally. Transgenic plants contained small interfering RNAs of 23-25 nt homologous to the PPV sequence used and to other PPV genome portions (transitivity). This approach should exert a rather low selective pressure on viral populations and could be used to confer PPV resistance to fruit trees.

INDUCTION OF GENE SILENCING TO *GRAPEVINE VIRUS A* AND *GRAPEVINE VIRUS B* THROUGH MARKER FREE TRANSFORMATION OF *NICOTIANA* AND *VITIS* SPP.. **C. Turturo[1], P. Saldarelli[1], G. Bottalico[1], M. Dell'Orco[1], A. Minafra[1], I. Gribaudo[2], V. Savino[1] and G.P. Martelli[1].** *[1]Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi and Istituto di Virologia Vegetale del CNR, sezione di Bari, Via Amendola 165/A, 70126 Bari, Italy. [2]Istituto di Virologia Vegetale del CNR, sezione di Torino, Via Leonardo da Vinci 44, 10095 Gruglisco (TO), Italy. Fax: +39.080.544291; E-mail: csvvps04@area.ba.cnr.it*

Grapevine virus A (GVA) and *Grapevine virus B* (GVB) are associated to the "rugose wood" complex, a severe grapevine disease decreasing the yield and survival of infected plants. Due to the lack of natural sources of resistance, the possibility to induce resistance to these viruses by post-transcriptional gene silencing (PTGS) was investigated as prerequisite for the generation of marker-free tobacco and *Vitis* plants. Highly conserved GVA and GVB sequences were cloned in sense and antisense orientation in the pKannibal vector to obtain the pKcpGVA and pKcpGVB plasmids. These cassettes direct the expression in cells of dsRNA molecules for triggering PTGS. Biolistic delivery of pKcpGVA and pKcpGVB in *N. benthamiana* and *N. occidentalis* leaves, partially protected plants from viral infection, as showed by a delay in symptoms appearance for GVA, or by escape from infection and apparent lack of viral RNA synthesis for GVB. pKcpGVA and pKcpGVB cassettes were transferred to pX6, a binary vector known to direct a site-specific DNA excision in transgenic *Arabidospis* plants through Cre/loxP-mediated recombination. This system should determine the excision of a DNA fragment containing the neomycin *NPTII* gene, thus resulting in transgenic marker-free plants. Leaf discs of *N. benthamiana* and *N. occidentalis* and secondary embryos from different grapevine varieties (Grignolino, Nebbiolo and the rootstock 110R) were Agro-trans-

formed. Preliminary molecular tests showed integration of T-DNAs in transgenic *N. benthamiana* and *N. occidentalis* plantlets and excision of the kanamicin-containing DNA on inductive medium. Grapevine transformation is under way.

THE REPLICATION OF TOMBUSVIRUSES IN *SACCHAROMYCES CEREVISIAE*. **L. Rubino, V. Pantaleo, B. Navarro and M. Russo.** *Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi and Istituto di Virologia Vegetale del CNR, Sezione di Bari, Via Amendola 165/A, 70126 Bari, Italy. Fax: +39.080.544291; E-mail: l.rubino@area.ba.cnr.it*

Carnation Italian ringspot virus (CIRV) and *Cymbidium ringspot virus* (CymRSV), two species in the genus *Tombusvirus*, family *Tombusviridae,* have a monopartite, linear, (+)stranded RNA genome *c.* 4800 nucleotides in length. Genomic RNA contains five functional open reading frames (ORFs). ORF1 encodes a 33- (p33) (CymRSV) or 36-kDa (p36) (CIRV) protein and ORF2 a 92- (p92) or 95-kDa (p95) protein translated by readthrough of the ORF1 amber stop codon. The CIRV p36 and p95 were expressed in *Saccharomyces cerevisiae* and shown to be targeted to the outer membrane of mitochondria. The targeting signal and membrane insertion are mediated by a signal-anchor mechanism. CIRV and CymRSV DI RNAs replicated in yeast cells expressing the p36 and p95 replicase proteins. The replication complex was localized by fluorescence microscopy and fractionation of cell extracts. Newly synthesized and accumulating DI RNA were associated with mitochondria as shown by incorporation of Br-UTP and *in situ* hybridization. Purified mitochondria contained positive and negative strand DI RNA together with the CIRV-encoded replicase proteins. The CymRSV p33 protein was also expressed in yeast cells in native form or fused to the green fluorescent protein (33KGFP). Fluorescence microscopy showed that the 33KGFP fusion protein concentrated in a few large bodies colocalizing clearly with the peroxisomal marker and slightly with the mitochondrial signal. These bodies were shown by electron microscopy to be composed by aggregates of peroxisomes, a few mitochondria and endoplasmic reticulum strands. In immunoelectron microscopy, antibodies to p33 labeled the peroxisomal clumps.

PCR-BASED GENOME SUBTRACTIVE HYBRIDIZATION ON *CURTOBACTERIUM FLACCUMFACIENS* PV. *FLACCUMFACIENS* FOR THE DETECTION OF PATHOGENICITY AND VIRULENCE DETERMINANTS. **S. Tegli, R. Bernardi, C. Nocentini and A. Scala.** *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*

Curtobacterium flaccumfaciens pv. *flaccumfaciens* (*Cff*) is a Gram-positive bacterium which causes "bacterial wilt disease" of *Phaseolus* spp.. Natural infections occur also in *Vigna* spp., *Glycine max* and *Pisum sativum. Cff* is a seed-borne bacterium of EPPO A2 quarantine list and infections of seeds are very often asymptomatic. The disease caused by *Cff* has economical importance and no effective chemical control methods are known. The identification of pathogenicity/virulence genes in *Cff* would allow to better understand the interaction with its hosts and to develop control methods. To this aim we used the Suppressive Subtractive Hybridization (SSH) method to *Cff*, for comparing its genome with that of *C. flaccumfaciens* pv. *betae* (*Cfb*), a very closely related species with a different host range. Using the *Rsa*I digested genomic DNA of *Cff* and of *Cfb* as tester and driver DNA, respectively, 71 subtractive hybridization products (0.2-2 kb) were obtained and cloned. These clones were tested in dot blot hybridization experiments with the DIG-labeled *Rsa*I digested genomes of *Cff* and *Cfb*: among these clones, 8 were judged to be *Cff*-specific. Three of the 18 DNAs had no database match to genes of known function. Two of the 18 clones closely matched the nucleotide sequences of sugar ABC transporters of *Mycobacterium tubercolosis* and *Micrococcus* spp., and 3 other clones exhibited significant protein homologies with transposase-like proteins of the same bacteria, and with the neurotoxin of *Clostridium botulinum.* The nature of these homologies in closely related microrganisms suggests the association of these clones with the pathogenicity/virulence of *Cff.*

ISOLATION OF A NEW *ARABIDOPSIS* MUTANT WITH ENHANCED DISEASE TOLERANCE TO *SCLEROTINIA SCLEROTIORUM*. **C. Boccongelli[1], G. Chilosi[1], P. Magro[1] and R.A. Bressan[2].** *[1]Dipartimento di Protezione delle Piante, Università degli Studi della Tuscia, Via S Camillo de Lellis, 01100 Viterbo, Italy. [2]Horticulture and Landscape Architecture Department, Purdue University, West Lafayette, 625 Agriculture Mall Drive, Indiana 47907-2010, USA.*

Insertional mutagenesis of *Arabidopsis thaliana* was used to identify a mutant that shows enhanced disease tolerance to *Sclerotinia sclerotiorum*. The activation T-DNA vector pSKI015 was used to generate an insertion mutant population (T_1) in the genetic background of *A. thaliana* (L.) Heynold ecotype C24 as in Weigel *et al.* (2002, *Plant Physiol.*, 122: 1003-1013), at Purdue University (West Lafayette, Indiana, USA). We screened 12,000 lines, at the first stage of plant germination, for mutants having enhanced tolerance to the widespread fungal pathogen *Sclerotinia sclerotiorum*. We selected a mutant that displays a higher level of germination in the presence of the pathogen, compared to the wild type. The mutant shows also an increased level of tolerance when inoculated with the pathogen on adult leaves. Preliminary genetic analysis seemed to show that this phenotype was caused by an activation insert in the only intron of the gene encoding for the alpha-3 subunit of 20S proteasome. Further genetic analyses are needed to confirm the linkage between this insertion and the predicted phenotype.

FLUX, PHYTOTOXICITY AND CARBOHYDRATE COMPOSITION OF XYLEM SAP FROM ESCA-AFFECTED SANGIOVESE GRAPEVINES DURING BLEEDING. **G. Bruno[1], A. Forabosco[2], F. Delben[2], G. Liut[2] and L. Sparapano[1].** *[1]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: sparlor@agr.uniba.it. [2]Dipartimento di Biochimica, Biofisica e Chimica delle Macromolecole, Università degli Studi di Trieste, Via L. Giorgieri 1, 34127 Trieste, Italy. Fax: +39.040.558369*

Xylem sap was collected in spring from grapevine (*Vitis vinifera*) cv. Sangiovese for four consecutive years in order to record bleeding rate and duration, phytotoxicity and carbohydrate content. Sixty-five per cent of the vines examined were naturally infected by three fungi, *Phaeomoniella chlamydospora*, *Phaeoacremonium aleophilum* and *Fomitiporia mediterranea,* commonly associated with the esca syndrome. Samples of sap were collected from vines showing symptoms of brown streakings or both brown-streakings and white rot, and from healthy controls. Sap samples were collected from bleeding vines weekly from the end of March to the end of April over a period of five days. The collected samples were filtered and their pH was determined before freezing at –20°C. There were significant differences among data on maximum daily discharge (ml/day/plant) in each year by diseased vines (153 in 2000; 81 in 2001; 132 in 2002; 279 in 2003)

and that by healthy vines (up to 20 ml/day/plant). The maximum bleeding rate was observed at budburst; subsequently it decreased or stopped just prior to bloom. Absorption of 3 ml samples of undiluted and diluted xylem sap by detached leaves of grapevines 'Italia' and 'Sangiovese' caused foliar symptoms (yellowing or reddening of large parts of the lamina, marginal leaf necrosis and "tiger-stripes") within 21 days. Six to nine per cent in weight of the lyophilized xylem sap was shown to contain both polysaccharides, mainly pullulan, and proteins. In some cases, heteropolysaccharides containing galactose, mannose, arabinose, xylose, ribose, fucose and rhamnose, in addition to glucose, were also detected.

EFFECT OF FUNGICIDES ON DEOXYNIVALENOL BIOSYNTHESIS *IN VITRO* AND EXPRESSION OF *TRI* GENES IN THE HEAD BLIGHT PATHOGEN *FUSARIUM CULMORUM*. **L. Covarelli[1], A.S. Turner[2] and P. Nicholson[3].** *[1]Dipartimento di Arboricoltura e Protezione delle Piante, Università degli Studi di Perugia, Italy. Email: cova70@libero.it. [2]Department of Crop Genetics, John Innes Centre, Norwich, UK. [3]Department of Disease and Stress Biology, John Innes Centre, Norwich, UK.*

Liquid media containing different nitrogen sources (1.65 gl^{-1} ammonium sulphate, 0.75 gl^{-1} urea, 2.125 gl^{-1} sodium nitrate), 10 gl^{-1} of glucose and nitrogen free Vogel's medium, were assayed in order to identify a defined medium for optimal production of the trichothecene mycotoxin deoxynivalenol (DON) *in vitro* by *F. culmorum* and to investigate, in such a medium, the effect of some fungicides on DON production and on the regulation of the trichothecene biosynthesis pathway by studying *Tri5* and *Tri6* gene expression. The effect of sub-lethal concentrations of the fungicides azoxystrobin, trifloxystrobin, kresoxim-methyl (strobilurines) and tebuconazole (a triazole), on biosynthesis (DON) by *Fusarium culmorum* was studied. Trifloxystrobin and azoxystrobin significantly reduced the accumulation of DON in the culture medium. Kresoxim-methyl and tebuconazole did not significantly reduce the accumulation of DON although levels were lower than those in non-amended cultures. Post-inoculation addition of trifloxystrobin significantly reduced the accumulation of DON when added to cultures prior to the onset of trichothecene biosynthesis. The expression of *Tri6* and *Tri5* genes, assayed by RT-PCR, showed that trifloxystrobin was acting through inhibiting the initiation of trichothecene biosynthesis. This study provides new information on the regulation of DON biosynthesis and the effect of selected fungicides on mycotoxin production in *F. culmorum.* However, care must be taken in extrapolating the information from such *in vitro* studies to field situations.

ENDOSPORE BIOCONTROL ACTIVITY OF TWO *BACILLUS* STRAINS AGAINST BROWN ROT OF STONE FRUITS CAUSED BY *MONILINIA LAXA*. **L. Casalini, M. Mari and G.C. Pratella.** *Dipartimento di Protezione e Valorizzazione Agroalimentare, Università degli Studi di Bologna, Sezione CRIOF, Via Gandolfi 19, Cadriano 40057 Bologna, Italy. Fax: 051765049; E-mail: casalinicia@yahoo.com*

As biological control agent, Gram-positive bacteria have a natural advantage over their Gram-negative counterparts; i.e. the presence of spores which are resistant to heat and desiccation. Sporulating Gram-positive micro-organisms, such as non-pathogenic species belonging to the genus *Bacillus*, can be formulated into stable products due to the ability, under certain condition such as nutrient depletion, to undergo a differentiation process resulting in the formation of a highly resistant dormant endospore. These spores can then persist in the environment for prolonged periods until a sensitive response mechanism detects specific environmental conditions, initiating the process of germination and outgrowth. Post-harvest fruit treatments with sporulating Gram-positive microorganisms could be supplemented with various formulation additives in order to enhance their viability and performance and to increase the number of propagules applied to the wound surface. The activity of laboratory-produced endospores of two *Bacillus* spp. strains on *M. laxa* was investigated. Treatment with pure spore suspensions did not prevent the growth of the pathogen. Heat activation of spores followed by the contact with nutrient and non nutrient germinants such as L-alanine, AGFK (L-asparagine, D-glucose, D-fructose and KCl) and Calcium Dipicolinate (CaDPA) enhanced spore germination in *in vitro* spectrophotometer assay; only heat-activated spores mixed with CaDPA (50 mM) reduced significantly ($P \leq 0.05$) the incidence of *M. laxa* on peaches cv Flaminia by 30% (2ORB strain) and 43% (7ORC strain) with respect to the untreated control.

CHITINASE AND PEROXIDASE ISOENZYMES IN SUGAR BEET LEAVES TREATED WITH *TRICHODERMA* AGAINST *CERCOSPORA BETICOLA*. **R. Roberti[1], S. Marinello[1], C. Cerato[2], P.L. Burzi[2] and A. Cesari[1].** *[1]Dipartimento di Protezione e Valorizzazione agroalimentare, Sez. Fitoiatria, Università di Bologna, Via Fanin 46, 40127 Bologna, Italy. Fax: +39.051.2096544; E-mail: roberta.roberti@unibo.it. [2]Istituto Sperimentale per le Colture Industriali, Via di Corticella 133, 40129 Bologna, Italy.*

The effect of the antagonistic fungus *Trichoderma* on sugar beet defence response has been studied. Sugar beet plants at 6 leaves stage were treated with 10-day old liquid culture (diluted 5 times in PDB, 16.6×10^6 cfu/ml) of the *Trichoderma* isolate BA12/86 (ISCI collection). The treatment was applied on one leaf of each plant, while *C. beticola* (1×10^5 conidia/ml) was inoculated on untreated leaves of the same plants, two days after. Untreated – non inoculated plants and untreated – inoculated plants served as controls. The experiment was carried out with the cultivars Aaron (susceptible to *C. beticola*) and Faro (partially resistant), on 9 plants per treatment, under greenhouse conditions and was repeated twice. Three, 5 and 7 days after the inoculation, untreated leaves were excised and proteins were extracted, separated by SDS-PAGE and IEF and their chitinase and peroxidase enzymatic activities were determined. *Trichoderma* treatment induced an increase of the expression of chitinase isoforms with a MW of 50 Kda and a high expression of peroxidase activity in plants treated and inoculated with the pathogen, compared to inoculated untreated plants. Peroxidase isoenzymes showed a 4-5 PI range. The antagonist induced a higher enzymatic activity in Faro than in Aaron cultivar. Furthermore, the induction of the enzymatic activities persisted at a steady level during all the time of the treatment.

FLUORESCENT PROTEINS AS A TOOL FOR HISTOPATHOLOGICAL STUDIES OF SCLEROTIA PARASITIZED BY ANTAGONISTIC *TRICHODERMA* SPP. USED AS BIOLOGICAL CONTROL AGENTS. **S. Sarrocco[1], D.F. Jensen[2] and G. Vannacci[1].** *[1]Department of Fruit Science and Plant Protection "G. Scaramuzzi", Plant Pathology Section, University of Pisa, Via del Borghetto 80, 56124 Pisa, Italy. Fax: +39.050.543564; E-mail: gvann@agr.unipi.it. [2]Department of Plant Biology, Plant Pathology Section, The Royal Veterinary and Agricultural University, 40 Thorvaldsensvej, DK-1871 Frederiksberg C, Denmark.*

The use of fluorescent proteins as reporters has proved an outstanding tool for studying various biological systems. In this work the *gfp* gene from the jellyfish *Aequorea victoria* encoding the green fluorescent protein (GFP) and, for the first time, the *DsRed* gene from a reef coral *Discosoma* sp., encoding the DsRed fluorescent protein (DsRedFP) were used for transformation of

two *Trichoderma* isolates (*T. harzianum* I252 and *T. virens* I10) both characterized by a promising biocontrol activity. A new plasmid construction containing the *DsRed* gene was successfully used as the vector resulting in constitutive expression of DsRed in both fungi (Mikkelsen *et al*., 2003. *FEMS Microb. Lett.*, 223: 135-139). The genetic transformation of these two antagonists allowed an *in vivo* evaluation of their mycoparasitic competence against sclerotia of *Sclerotium rolfsii, Sclerotinia minor* and *S. sclerotiorum.* The parasitic competence of wild type and transformed antagonists was determined by quantifying the decay of pathogen sclerotia after inoculation with *Trichoderma* strains in a plate assay. Colonization of sclerotia was assessed by detecting the presence of fluorescent hyphae in sclerotia sections using fluorescent- and confocal-microscopy.

A NEW EUROPEAN PROJECT ON BIOLOGICAL CONTROL. **M. Vurro.** *Istituto di Scienze delle Produzioni Alimentari (ISPA), CNR, Viale Einaudi 51, 70125 Bari, Italy. Fax: +39.0805486063; E-mail: maurizio.vurro@ispa.cnr.it*

An European Project entitled "Enhancement and Exploitation of Soil Biocontrol Agents for Bio-Constraint Management in Crops" has been recently accepted by EU, within the VI EU Framework Programme for Research and Technological Development, Priority 5, Food Quality and Safety. Using a combination of strategies, the main aim of the project is to study some of the already available or the most promising biocontrol microorganisms (i.e. *Fusarium, Trichoderma* or *Coniothyrium* sp.) against plant diseases, such as those caused by *Sclerotinia*, *Fusarium* or *Pythium* spp., or some noxious plants, such as parasitic and perennial weeds. Studying the genetic and physiological enhancement strategies, the ecological fitness of the agents, the production, formulation and application methods, the integration with other organisms and with control methods, and assessing their quality and the risk of release into the environment, it will be possible to improve the efficacy of fungal biocontrol agents, allowing their wider use at the European level, and giving new important tools to support the production of safer and healthier foods. The three-year project, coordinated by ISPA, includes 9 different work-packages, with the involvement of 9 partners (7 scientific institutions and 2 industries) from 7 countries. The structure of the project, as well as its objectives, work-plans and the role of partners are described.

ASSESSMENT OF GENETIC AND PATHOGENICITY DIVERSITY AMONG *ERWINIA AMYLOVORA* STRAINS ISOLATED FROM DIFFERENT HOST PLANTS AFFECTED WITH FIRE BLIGHT IN THE EMILIA ROMAGNA REGION. **P. Minardi, M. Morbio and U. Mazzucchi.** *Dipartimento Territorio e Sistemi Agro-Forestali, Università di Padova, Viale dell'Università 16, 35020 Legnaro, Italy. Fax: +39.049.8272747; E-mail: paola.minardi@unipd.it*

Host plants exercise a selective pressure on their endophytic bacteria. Host plant changes and passages on the new species may modify gene frequencies and generate aplotypes distinct from the population on the original host plant. In the Emilia-Romagna region, after the 1997 epidemics on pear, there has been a large increase in the officially certified cases of fire blight in different host plants. The clonal lineage of *Erwinia amylovora* introduced in 1994 might have diversified for the selective pressure on new host plants or another clonal lineage might have arised. To test this hypothesis, genetic variations among 30 strains of *E. amylovora* isolated in the Emilia-Romagna region from apple, hawthorn, quince, azarole and other host plants during 1997-2001 were analyzed with amplified fragment length polymorphism (AFLP). The *Eco*RI/*Mse*I and E03(+G)/M02(+C) pairs were used as restriction enzymes and selective amplification primers, respectively. The virulent strain *E. amylovora* OMP-BO 1077/7, associated with primary fireblight foci on pear in 1994, was used as a reference strain. A mean of 76 monomorphic and polymorphic AFLP fragments was scored as discrete characters and analyzed by cluster analysis using the software package NTSYS-pc version 2.02h (1997). The genetic distance between isolates was determined using the Dice similarity coefficient. AFLP fingerprints revealed that all the strains belong to the same clonal lineage indistinguishable from the original strain introduced in 1994. Virulence diversity among 47 *E. amylovora* strains isolated from pear and other hosts, was studied by pathogenicity tests on pear fruits measuring the minimum and the maximum axis of the induced lesion. For each strain the virulence index was calculated and statistical analysis was used to determine the significant difference with the reference strain.

DIVERSITY OF LOCAL *PHYTOPHTHORA CAMBIVORA* POPULATIONS BASED ON AFLP MARKERS. **A.M. Vettraino and A. Vannini.** *Dipartimento di Protezione delle Piante, Università degli Studi della Tuscia, Via S.Camillo de Lellis, 01100 Viterbo, Italy. Fax: +39.0761.357473; E-mail: vannini@unitus.it*

Phytophthora cambivora is the main pathogen involved in the "ink disease" of *Castanea sativa* in Italy. *P. cambivora* is a diploid, heterothallic oomycete with two mating types, designed A1 and A2. Fifty-seven isolated of *P. cambivora* were collected between 1998 and 2000 from three diseased chestnut stands in the Monti Cimini (Central Italy). All these isolates belonged to mating type A2 (Vettraino *et al*., 2001). In order to verify the phenotypic and genotypic diversity of the three local populations of *P. cambivora*, the isolates were analysed for amplified fragment length polymorphism (AFLP) marker profile, growth rate at different temperature, morphology of the colony on different media, size and morphology of sporangia and oogonia, metalaxyl sensitivity and pathogenicity on chestnut shoots. *P. cambivora* populations resulted highly variable and nearly each isolate represented a unique genotype based on the analysis of 145 AFLP marker loci. A dendrogram based on UPGMA with arithmetic average cluster analysis showed discrete clusters based on location. Most of the isolates within the same area showed a clustering based on the chestnut tree from which they were sampled. A large phenotypic variation among isolates was detected, even if less variable quantitative traits were identified such as pathogenicity. This is the first investigation on phenotypic and genotypic variation of *P. cambivora* populations.

MOLECULAR FINGERPRINTING OF ANTAGONIST *AUREOBASIDIUM PULLULANS* ISOLATES BY FLUORESCENT AMPLIFIED FRAGMENT LENGTH POLYMORPHISM. **F. De Curtis[1], L. Caputo[2], R. Castoria[1], G. Lima[1] and V. De Cicco[1].** *[1] Department of Animal, Plant and Environmental Sciences, University of Molise, Via De Sanctis, 86100 Campobasso, Italy. Fax: +39.0874.404678; E-mail: decurtis@unimol.it. [2] Institute of Sciences of Food Productions (ISPA), CNR Viale Einaudi, 51 Bari, Italy.*

The epiphytic yeast-like fungus *Aureobasidium pullulans* includes strains showing biocontrol activity on different stored crops against the major postharvest pathogens. Molecular fingerprinting of biocontrol agents is pivotal both for environmental monitoring and registration purposes. Several strains of *A. pullulans* with different levels of antagonistic activity were analysed by fAFLP (fluorescent Amplified Fragment Length Polymorphism). Total genomic DNA was digested with two restriction endonucleases, and compatible oligonucleotide adapters were ligated to the

resulting fragments. Subsets of fragments from the total pool of cleaved DNA were then amplified by four selective sets of primers (AC/CC, AT/CG, AC/CA, G/CT) extending beyond the adapter and restriction site sequences. In each set, one of the primers was labelled with a fluorescent dye, thus enabling amplified fragments to be detected and sized with an automated DNA sequencer. This procedure generated 150 to 280 fragments, 35 to 500 bp in size. A dendrogram was obtained by analysing the four fAFLP patterns by using Dice similarity coefficient (S_D) and clustering of fingerprints performed with the unweighted pair groups (UPGMA). All isolates fell into four clusters with a level of similarity ranging from 0.02 to 0.98. Only two isolates displayed high similarity under each fAFLP condition. fAFLP appears to be a fast and reproducible molecular tool for characterising intraspecific genetic variability between *A. pullulans* strains. Further, it could pave the way to the identification of genetic marker(s) to be used in ecological studies for monitoring the environmental fate of these microorganisms when applied for biocontrol purposes.

HOST RANGE OF GALL-FORMING *PSEUDOMONAS SAVASTANOI* STRAINS. P. Bella[1], C. Guarino[2], V. Catara[2] and G. Cirvilleri[2]. *[1]Parco Scientifico e Tecnologico della Sicilia S.c.p.a., Catania, Italy. [2]Dipartimento di Scienze e Tecnologie Fitosanitarie, Università degli Studi di Catania, Via S. Sofia, 102, 95100 Catania, Italy. Fax: +39.095.292730; E-mail: patrizia.bella@tiscali.it*

Pathogenicity tests of *Pseudomonas savastanoi* strains causing hyperplasia in various genera of *Oleaceae*, i.e. *Nerium oleander*, *Myrthus* sp., and *Retama sphaerocarpa* are often done only on host they were isolated from, or, sometimes, also on olive, oleander and ash. This investigation tested eleven strains of *P. savastanoi* pv. *nerii*, chosen according to the results of a study on nutritional profile variability, REP-PCR fingerprint, and virulence on oleander, included also a set of strains from other hosts (olive, ash, jasmine, privet, *Phillyrea* sp., *Myrthus* sp., and *R. sphaerocarpa*) which had been subjected to numerical analysis of nutritional profile. Cross-pathogenicity tests showed that all strains were pathogenic to their specific host. *P. savastanoi* isolated from the other hosts caused no symptoms in oleander. Nine of the eleven oleander strains induced typical knots on olive, ash and jasmine, whereas two strains induced symptoms only in olive and oleander. Olive strains caused disease in ash and jasmine; ash strains were pathogenic also to olive. Strains isolated from privet induced symptoms in olive, jasmine and *Myrthus* sp. Except for pathogenicity to jasmine, strains isolated from privet, jasmine, *Myrthus* sp. and *Phillyrea* sp. showed a variable host range. The results of cross-pathogenicity tests and numerical analysis of phenotypic characters were compared. No difference in pathogenicity was observed between oleander strains clustered in different phena, except for two strains that were pathogenic only to oleander and olive and segregated in a different phenon. Three strains from privet and three from *R. sphaerocarpa* showed the same host range although they clustered in different phena.

MOLECULAR ANALYSIS OF *PLUM POX VIRUS* M ISOLATES FROM NORTHERN ITALY. P.A. Bianco[1], A. Fanigliulo[2], I. Aliverti[1], S. Comes[2], P. Casati[1], A. Crescenzi[2] and G. Belli[1]. *[1]Istituto di Patologia Vegetale, Università degli Studi and Istituto di Virologia Vegetale, CNR, Via Celoria 2, 20133 Milano, Italy. E-mail: piero.bianco@unimi.it. [2]Dipartimento di Biologia, Difesa e Biotecnologie Agro-forestali, Università degli Studi della Basilicata, Campus Macchia Romana 3A310, I85100 Potenza Italy. E-mail: crescenzi@unibas.it*

Plum pox virus (PPV), the causal agent of Sharka disease of stone fruits, is widely spread in Europe, the Mediterranean basin, and America. In Italy, after the first record in Alto Adige in 1974, PPV was found in different regions. Recently, severe outbreaks of Sharka disease have occurred in peach orchards of north-eastern Italy. In order to establish the PPV sub-group responsible of the outbreaks, leaf samples were collected from symptomatic plants and analysed. Preliminary characterisation using monoclonal antibodies specific to PPV-M, D, EA and C showed that the more widely spread isolates belong to PPV-M group. The most virulent isolates were then selected for molecular characterisation. Two PPV isolates, recovered from peach orchards in Brescia and Verona areas, were propagated in *Nicotiana benthamiana* and purified according to Crescenzi *et al.* (1997, *J. Virol. Methods* 69: 181-189). Genomic RNA, extracted for purified virions, was used for cloning the coat protein and part of the Nib gene by RT-PCR. Amplified products of the expected size were cloned into pCRII vector using TATM cloning kit (Invitrogen) and sequenced. Pairwise analysis confirmed preliminary biological and serological data, showing that the two isolates effectively belong to PPV-M group. The isolates, named PPV-Stark Red Gold and PPV-Big-Top are strictly related and share a high percentage of similarity with the PPV-SK68 isolate.

EPIDEMIC OUTBREAKS OF OLIVE ANTHRACNOSE IN CENTRAL ITALY. G.E. Agosteo, C. Macrì, R. Faedda, A.M. Pennisi, S.O. Cacciola and G. Magnano di San Lio. *Dipartimento di Agrochimica ed Agrobiologia, Università Mediterranea di Reggio Calabria, Piazza S. Francesco di Sales 2, I-89061 Gallina di Reggio Calabria, Italy. Fax: +39.0965.689049; E-mail: gmagnano@unirc.it*

In autumn 2001, episodic but severe outbreaks of olive anthracnose were observed in Umbria (central Italy). After an epidemic burst in the early 1950s, olive anthracnose has remained endemic in a few southern regions, including Apulia, Calabria and Sardinia. The causal agent of this disease, first referred to as *Gloeosporium olivarum* Alm., was subsequently transferred to the species complex *Colletotrichum gloeosporioides* (Penz.) Penz. *et* Sacc.. Another *Colletotrichum* species, *C. acutatum* J. H. Simmonds, was reported as the major causal agent of olive anthracnose in Spain and Portugal. Conversely, on the basis of biochemical and molecular data, we have recently concluded that the causal agent of olive anthracnose in southern Italy is a *Colletotrichum* sp. distinct from both *C. gloeosporioides* and *C. acutatum*. In this study, RAPD-PCR analysis and polyacrylamide gel elecphoresis of mycelial isozymes were used to compare olive isolates from Umbria with reference isolates of various *Colletotrichum* species from olive and other hosts. Eight isozymes were tested and 16 decamer primers (Operon Technologies, Alameda, CA, USA) were used for RAPD-PCR. Olive isolates from Umbria showed electrophoretic phenotypes and RAPD-PCR banding patterns very similar to those of the *Colletotrichum* sp. responsible for olive anthracnose in southern Italy. Moreover, like this *Colletotrichum* sp., they were benomyl-resistant (MIC > 10^2 µg ml^{-1}) and grew slowly in culture (radial growth rate on potato-dextrose agar at 24°C 7-8 mm day^{-1}), with an optimum growth temperature at 24°C. This host-specific *Colletotrichum* sp. is reported for the first time in central Italy.

PCR REAL TIME IDENTIFICATION OF A HIGHLY PATHOGENIC GROUP OF *FUSARIUM OXYSPORUM* F.SP. *CHRYSANTHEMI* ON *ARGYRANTHEMUM FRUTESCENS*. M. Pasquali, L. Marena, E. Fiora, P. Piatti, M.L. Gullino and A. Garibaldi. *Centre of Competence for the Innovation in the Agro-environmental Field (AGRINNOVA), University of Torino, Via Leonardo da Vinci*

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A real time PCR system based on Taqman chemistry was developed in order to identify a new group of *Fusarium oxysporum* f.sp. *chrysanthemi* highly pathogenic to Paris daisy. Sensitivity of traditional PCR and real time PCR were compared on genomic DNA and on DNA extracted from infected plants with the use of a selective substrate. Real time PCR allowed the identification of infected plants on the fifth day after infection from stem tissue and on the second day from root tissue, even if the plants remained asymptomatic until the 13th day from infection. The sensitivity of real time PCR allowed the identification of 300 fg of genomic DNA. The possibility of automation of the process and the rapidity in the analysis is discussed and real time PCR is proposed as an elective system for diagnosis of this new epidemic disease.

WINE CONTAMINATION BY OCHRATOXIN A IN SOUTH ITALY: CAUSES AND PREVENTIVE ACTIONS. S. Pollastro[1], C. Dongiovanni[2], A. Abbatecola[1], G. Tauro[2], P. Natale[1], M. Pascale[3], A. Visconti[3] and F. Faretra[1]. *[1]Dipartimento di Protezione delle Piante e Microbiologia Applicata, Università degli Studi, Via Amendola 165/a, 70126 Bari. Fax: +39.080.5442911; E-mail: faretra@agr.uniba.it. [2]Centro di Ricerca e Sperimentazione in Agricoltura "Basile Caramia", Via Cisternino 281, 70010 Locorotondo (BA) Fax: +39.080.431307; E-mail: crsa@libero.it. [3]Istituto di Scienze delle Produzioni Alimentari del CNR, Viale Einaudi 51, 70126 Bari, Italy. Fax: +39.080.5486063; E-mail: angelo.visconti@ispa.cnr.it.*

Secondary bunch rots of grapevine are caused by saprotrophic or emipathogenic fungi, including species of *Aspergillus* and *Penicillium* known for their ability to produce mycotoxins. In particular, ochratoxin A (OTA) has been recently detected in grape derivatives, such us grape juice, raisin, and wine. A four-year monitoring programme was carried out on 24 cultivars grown in over 100 vineyards in Southern Italy. Bunch rots caused by fungi belonging to *Aspergillus* section *Nigri* prevailed. Must samples plated on DYGS or MEAS showed that the genus *Aspergillus* (*A. niger* 84%, *A. carbonarius* 14%, *A. wentii* 1.6%, *A. aculeatus* 0.4%) was largely prevalent on *Penicillium* (*P. variable* 43%, *P. paxilli* 27%, *P. janthinellum* 14%, *P. implicatum* 12%, *P. purpurogenum* 3%, *P. brevicompactum* 1%). Among these fungal species, only *A. carbonarius* proved be an OTA producer. OTA was found in 87% of the must samples at concentrations up to 18.7 ng ml^{-1} (average: 1.5 ng ml^{-1}). Several groups of fungicides were tested *in vitro* and in field trials against *A. carbonarius*. Anilinopyrimidines and phenylpirroles, were the most effective. Two-three sprays according to the spray schedule used against grey mould reduced *A. carbonarius* contamination in must and OTA contamination in wine up to 50%. SCAR (Sequence Characterized Amplified Regions) primers specific for *A. carbonarius* were designed starting from species-specific RAPD markers. The primer pair $OPA3_{519}C$ proved specific for *A. carbonarius*. These primers are presently used for deriving Scorpion primers since Real-Time PCR should allow quantification of *A. carbonarius* directly in field samples.

OCHRATOXIN A - PRODUCING STRAINS OF *PENICILLIUM* SPP. ISOLATED FROM GRAPES. E. Torelli, E. Gobbi, G. Firrao and R. Locci. *Dipartimento di Biologia Applicata alla Difesa delle Piante, Università degli Studi di Udine, Via delle Scienze 208, 33100 Udine, Italy. Fax: +39.0432.558501; E-mail: torelli@pldef.uniud.it*

Ochratoxin A (OTA) is a carcinogenic, teratogenic, immunotoxic mycotoxin produced by several species of the genera *Aspergillus* and *Penicillium*, detected in different commodities including wine and grape juice. Drying of grapes for the production of "passito" wines is a major risk, because to reduce the water content, bunches are kept for 90-120 days in wooden boxes or on iron grids, i.e. conditions which favour contamination by moulds. In this work, the micoflora of dried grapes of five wine grape cultivars (Picolit, Verduzzo, Ribolla nera, Refosco and Riesling Renano) was sampled and examined with particular reference to OTA-producing strains of *Penicillium* spp.. In order to assess the presence of OTA producing fungi, 63 grape samples were examined. Strains of *Penicillium* spp. (379 isolates) were obtained and the morphology of the conidiophores determined. The isolates were tested for the production of secondary metabolites by growing on coconut milk agar. *Penicillium* spp. Strains, which produced fluorescent metabolites as detected under UV light, were analysed by thin-layer chromatography. Four strains were positive and were tested for ochratoxigenic activity by HPLC after cleaning-up in an OchraTest™ column. Two strains of *P. puberulum* and 1 strain of *P. variabile* produced OTA *in vitro*, the remaining strain were negative.

CHARACTERIZATION OF THE DIVERSITY OF *PSEUDOMONAS SAVASTANOI* POPULATIONS FROM OLIVE TREES IN CENTRAL ITALY. G. Marchi, L. Giovanetti, C. Viti, and G. Surico. *Dipartimento di Biotecnologie Agrarie, Sezione Patologia Vegetale, Università degli Studi, P.le delle Cascine 28, I-50144 Firenze, Italy. Fax: +39.055.3288279; E-mail: guido.marchi@unifi.it*

Pseudomonas savastanoi pv. *savastanoi*, a typically levan-negative bacterium that is fluorescent on the B medium of King *et al.*, is the causal agent of olive knot disease, a disease characterized by the production of hyperplastic growth (knots) on the woody tissues of the host. In surveys carried out on infected olive trees from different cultivars in various parts of Tuscany, isolates from within the tubercles were often found to produce levan-producing colonies and did not produce fluorescent pigments on King's B medium. These isolates were provisionally assigned to the species *Pseudomonas savastanoi* on the basis of pathogenicity tests on olive. Subsequently, to confirm this taxonomic allocation, various isolates were characterized phenotypically (LOPAT tests) and genotypically (16S ARDRA). All the isolates tested were oxidase and arginin dihydrolase negative, did not rot potato tuber slices, and induced a hypersensitive response in tobacco leaves. The ARDRA technique, performed with three different restriction enzymes, showed that the restriction patterns generated from the DNA of all the levan-positive isolates, three levan-negative reference strains, and from the levan-negative strain of *P. savastanoi* (NCPPB639) were identical. All the *P. savastanoi* isolates were screened for the gene *IscC,* encoding the levansucrase enzyme C, by PCR amplification of a 550 bp fragment. As expected, the amplicon was not observed in the levan-negative strains. The effect of an extracellular polysaccharidic layer on resistance to possible stress factors such as dehydration and UV radiation is being investigated.

OCCURRENCE OF FUNGAL ENDOPHYTES IN HEALTHY AND DECLINING PLANTS OF *PINUS RADIATA.* B.T. Linaldeddu, A. Franceschini and P. Marongiu. *Dipartimento di Protezione delle Piante, Sezione di Patologia vegetale, Università degli Studi di Sassari, Via E. De Nicola 9, 07100 Sassari, Italy. Fax: +39.079.229316; E-mail: afran@uniss.it*

Recently several cases of decline and death were observed in Sardinia in *Pinus radiata* stands close to commercial maturity. As endophytic fungal pathogens are often involved in the etiology of

this type of disorders, preliminary studies were carried out on the composition of the endophytic fungal communities in healthy and declining plants of *P. radiata*. The results showed that there is no significant difference in the qualitative-quantitative composition of mycocenoses in the two types of plants. In both branches and needles examined, the model of colonisation always included: a single dominant *taxon*, some less widespread species which occurred constantly, and a variable number of other occasional species, mainly *mycelia sterilia*. Dominant *taxa* were identified by biomolecular methods, comparing the internal transcribed spacer (ITS) sequences of ribosomal subunits. They were represented in the branches by *Sydowia polyspora* (Bref. *et* Tavel) E. Müll. (=*Hormonema dematioides* Lagerb. *et* Melin), a common endophyte of conifers which produces secondary metabolites with antimicrobial properties, and in the needles by a fungus phylogenetically related to species of the genus *Bartalinia* Tassi. Among the other species constantly isolated, *Sphaeropsis sapinea* (Fr.:Fr) Dyko *et* Sutton, and *Biscogniauxia mediterranea* (De Not.) O. Kuntze deserve a special mention. The former is a well-known pathogen of conifers, which can survive in latent form in asymptomatic hosts for a long time, whilst the latter is a pathogen frequently associated with decline of *Quercus* species in the Mediterranean area.

THE DIEBACK OF HAZELNUT IN THE PROVINCE OF VITERBO: A STUDY ON THE CORRELATION AMONG THE DISTRIBUTION OF THE DISEASE, LAND CHARACTERISTICS, AND CLIMATIC PARAMETERS BY USING GPS/GIS TECHNOLOGY. A. Fabi, G.M. Balestra, G. Vuono and L. Varvaro. *Dipartimento di Protezione delle Piante, Università degli Studi della Tuscia, Via S. Camillo de' Lellis, 01100 Viterbo, Italy. Fax: +39.0761.357473;E-mail: fabi@unitus.it*

Since the 80s, in the province of Viterbo (north Latium, central Italy) a dieback of hazelnut is causing a progressive decline of the plants. To analyze the spatial distribution of the disease that affect hazelnut production in this area and the relationships among different territorial compartments, a Global Positioning System (GPS) monitoring has been set up to record diseased plants, in association with Geographic Information System (GIS) spatial databases based on Mapinfo® software. The hazelnut areas affected by dieback were monitored during 2003 and were correlated with historical data on the incidence of dieback in the last 5 years. Furthermore, data acquired with the ground activity were compared with the multiple sets of data furnished by the Latium Region and local farmer associations (meteorological, geological, chemical, hydrografic, agronomical data and land use), were analyzed by GIS software, and digital maps were drawn. The results show a certain correlation between the incidence of the disease and some agronomical practices (such as irrigation), the environmental conditions (relative humidity and temperature) and soil characteristics. Site-monitoring will continue in association with aerial photos taken on the entire territory, in order to correlate infrared foliage emission with the early symptomatology to find out a possible forecasting method by using flight monitoring.

DYNAMIC MODELLING OF *PLASMOPARA VITICOLA* PRIMARY INFECTIONS. V. Rossi[1], S. Giosuè[1], T. Caffi[1], R. Bugiani[1], A. Brunelli[2] and M. Collina[2]. *[1]Istituto di Entomologia e Patologia vegetale. Università Cattolica del Sacro Cuore, Via Emilia Parmense 84, 29100 Piacenza, Italy. E-mail: ist.patologia-pc@unicatt.it. [2]Dipartimento di Protezione e Valorizzazione Agroalimentare, Università degli Studi di Bologna, Viale G. Fanin, 40127 Bologna (Italy). Fax: +39.051.4640127; E-mail: mcollina@agrsci.unibo.it*

A dynamic model simulating the life cycle of *Plasmopara viticola* from overwintering oospores to the appearance of primary symptoms on the leaves was elaborated in order to obtain precise information on primary infections of downy mildew on grape. The model was elaborated according to the principles of 'systems analysis' and takes into account the following stages of the infection cycle: oospore maturation and germination, survival of sporangia and zoospore ejection, zoospore survival and dispersal, infection and incubation. For this reason, this model differs from other available models. The effect of meteorological factors (temperature, relative humidity, rain, leaf wetness) on each stage was described by mathematical equations using data from both literature and specific experiments. The model allows to evaluate, hourly, the progress of the infection process and to forecast the time period of symptom onset. The model was validated against data not used in model elaboration. In a first step, simulations of oospore maturation and germination were compared with data from laboratory assays on detached grapevine leaf disks. Then, hourly meteorological data were collected under different epidemiological conditions (several locations and years) in northern Italy (Piedmont, Oltrepo Pavese and Emilia-Romagna) and simulations were compared with the actual time of disease appearance. In all cases considered, there was a good agreement between observed data and simulations. Compared to the "3 x 10 rule", which is largely used, this model provides more accurate information for both early and late periods of downy mildew onset.