

**MOLECULAR CLONING OF THE HARPIN GENE OF PSEUDOMONAS SYRINGAE PV. APTATA.** A.R. Musa, Paola Minardi\* and U. Mazzucchi. \*Dipartimento TESAF, Università di Padova, Via Romea, I-35020 Legnaro (Padova), Italy. Fax: +39.049.8272686; e-mail: pminardi@agripolis.unipd.it

In heterologous plant-bacteria interactions the hypersensitive response (HR) is a rapid programmed death of plant cells. Bacterial *hrp* genes are directly related to HR and several of them encode components of a type-III protein secretion system that transfer virulence factors into eukaryotic cells. In *Pseudomonas syringae* pv. *syringae* (Pss), the *hrpZ* gene encodes a harpin protein (HrpZ<sub>Pss</sub>) that is secreted by the type-III pathway and can elicit an HR-like response when infiltrated (> 0.1 µM) into tobacco leaves. The relationship between HrpZ and the HR is complex, and understanding the role of the protein could lead to improved strategies for disease resistance. *P. syringae* pv. *aptata* (Psa), the causal agent of bacterial blight of sugar beet, triggers HR in nonhost plants and harbours *hrp* genes that are similar to *hrp* genes of Pss. Initial analyses using the *hrpZ*<sub>Pss</sub> as a DNA probe revealed the presence of an *hrp* gene homologous to the *hrpZ*<sub>Pss</sub>, while polyclonal antibodies against HrpZ<sub>Pss</sub> revealed a harpin-like protein in Psa and in *E. coli* DH10B (pCPP1069) (Psa *hrp* gene cluster). In this study, we cloned and expressed the harpin gene from Psa in a suitable vector. Oligonucleotides based on the *hrpZ*<sub>Pss</sub> sequence were used as PCR primers with the total DNA of Psa as template. A single product, corresponding in size to the *hrpZ*<sub>Pss</sub> gene, was obtained and its hybridization to *hrpZ*<sub>Pss</sub> was confirmed. The product was cloned into the pMAL<sup>®</sup> expression system and HrpZ<sub>Psa</sub> was purified by affinity chromatography.

**PURIFICATION AND MOLECULAR CHARACTERIZATION OF TOXIN PC-f FROM PHYTOPHTHORA CACTORUM.** G. Orsomando, M. Dalla Rizza, M. Lorenzi, L. Landi, B. Mezzetti and Silverio Ruggieri\*. \*Dipartimento di Biotecnologie Agrarie ed Ambientali, Università di Ancona, Via Breccie Bianche, I-60131 Ancona, Italy. E-mail: ruggieri@popcsi.unian.it

During plant-pathogen interactions one possible result is the hypersensitivity response (HR), eventually leading to resistance. HR involves plant cell death and tissue necrosis at the site of infection, which has been related to the appearance of a so-called 'oxidative burst', i.e. the release of active oxygen species (AOS) by elicited plant cells. To study the biochemical mechanisms involved in the toxicity and/or defence transduction cascade in the *Phytophthora cactorum*-strawberry system, we purified to electrophoretic homogeneity a toxic protein released by the pathogen into the culture medium. A 38-amino acid N-terminal sequence determined for the toxin showed no similarity with known protein sequences in data-bases. A molecular weight of about 17,000 daltons was obtained under natural and denaturing conditions, indicating a monomeric structure for the toxin. Chromatofocusing experiments revealed an isoelectric point of 4.4. The amino acid composition showed a relatively higher content of acidic amino acids in correspondance with the isoelectric point, the absence of methionine and the presence of at least 12 cysteine residues. The UV spectrum showed a maximum at 272 nm, with an  $\epsilon_{\text{mm}}$  value of 35.4. Incubation of cultured strawberry cells with the pure toxin at 80 ng ml<sup>-1</sup> (5 pmol ml<sup>-1</sup>) showed a 50% cell death and concomitant release of H<sub>2</sub>O<sub>2</sub> in the culture medium. Since the toxin did not appear similar to known toxins, we have named it toxin PC-f (*P. cactorum*-fragaria).

**TRANSFORMATION OF THE POSTHARVEST BIOCONTROL AGENT METSCHNIKOWIA PULCHERRIMA WITH THE GREEN-FLUORESCENT PROTEIN GENE, GFP.** Franco Nigro\*, M. Finetti-Sialer and D. Gallitelli. \*Dipartimento di Protezione delle Piante dalle Malattie, Università di Bari, Via G. Amendola 165/a, I-70126 Bari, Italy. Fax: +39.080.5442911; e-mail: nigrof@agr.uniba.it

*Metschnikowia pulcherrima* (Pitt) M.W. Miller (anamorph: *Candida pulcherrima*) isolate 320, showing antagonistic activity against *Botrytis* storage rot of table grapes, strawberries and kiwifruit, was transformed with the yeast-enhanced green-fluorescent protein (yEGFP) gene, using plasmid pACT2.yEGFP. The plasmid was constructed by subcloning in the *Sma*I site of pACT2 a yEGFP fragment obtained from *Pst*I/*Hind*III digested pUC19.yEGFP. The resulting construct contained the coding region for GFP downstream the promoter sequence for the *Saccharomyces cerevisiae* alcohol-dehydrogenase 1 (*ADH1*). Cells of *M. pulcherrima* were transformed using the lithium acetate protocol and the chimaeric colonies expressing yEGFP selected by means of an epifluorescence microscope. Biocontrol activity of five GFP transformants (GFP1, GFP2 GFP3, GFP4 and GFP5) was evaluated on table grape, cvs 'Matilde' and 'Italia', and compared to that of the *M. pulcherrima* 320 wild type. Biocontrol activity of GFP4 and GFP5 transformants was indistinguishable from that of the wild type, providing a significant ( $P=0.001$ ) reduction of *Botrytis* rot. GFP1, GFP2 and GFP3 transformants lost the antagonistic activity completely. Factors affecting stability of the transformants phenotype are under investigation. To date this is the first report of *M. pulcherrima* transformation with GFP.

**X-DISEASE-RELATED PHYTOPLASMAS IN ORNAMENTAL TREES AND SHRUBS WITH WITCHES' BROOM AND MALFORMATION SYMPTOMS.** S. Paltrinieri, M. Martini, M. Pondrelli and Assunta Bertaccini\*. \*U.C.I. "S.T.A.A.", Istituto di Patologia Vegetale, Università di Bologna, Via F. Re 8, I-40126 Bologna, Italy. Fax: +39.051.351451; e-mail: Bertaccini\_A@biblio.cib.unibo.it

During surveys carried out in urban areas, witches' broom, stunting and fasciation were observed in cypress and willow. Malformations were also present in forsythia and spiraea growing in nurseries. Samples from symptomatic and symptomless plants were collected, and DNA was extracted for use in direct and nested PCR using universal primers R16F1/R0 or R16F2/R2 and specific primers R16(I)F1/R1, R16(III)F2/R1, R16(V)F1/R1 and R16(X)F1/R1. The presence of phytoplasma DNA bands of 1.2 kbp was observed after PCR with R16F2/R2 in a few symptomatic willow and spiraea plants, while nested-PCR using R16(III) F2/R1 gave specific 0.8 kbp products for the majority of the symptomatic samples of all four species tested. No amplification was obtained from reaction mixtures without nucleic acid template, or those containing nucleic acid from symptomless plants, and when the PCR products obtained with general primers were reamplified with the other phytoplasma-specific primers. Patterns obtained after RFLP with *Mse*I and *Rsa*I of the amplified 0.8 kbp sequences showed some differences among isolates from the four species and CX and poinsettia isolates used as control for the 16SrIII phytoplasma group; the phytoplasmas from forsythia and spiraea closely resembled each other. This is the first report of phytoplasma detection in cypress and the first detection in Italy of phytoplasmas in the other species. Studies are in progress to verify genetic relationships among the phytoplasmas detected and to clarify their role in the associated diseases.

**FUNCTIONAL ANALYSIS OF THE GENOME OF OLIVE LATENT VIRUS 1.** V. Pantaleo, F. Grieco, M.A. Castellano and Giovanni P. Martelli\*. \*Dipartimento di Protezione delle Piante, Università degli Studi e Centro di Studio del CNR sui Virus e le Virosi delle Colture Mediterranee, Via G. Amendola 165/a, I-70126 Bari, Italy. Fax:+39.080.5442911; e-mail: martelli@agr.uniba.it

Olive latent virus 1 (OLV-1) (genus *Necrovirus*, family *Tombusviridae*) has isometric particles about 30 nm in diameter, a monopartite ssRNA genome (3699 nt) and a coat protein (CP) of Mr 23 kDa. The genome contains five open reading frames (ORFs) encoding proteins of 23, 82, 8, 6 and 30 kDa. A full length cDNA copy of the RNA was cloned in the vector pMUC-19 downstream of a phage T7 promoter and was denoted pMUC-OLV. Transcripts of this template were infectious in local and systemic hosts, and crude extracts of infected tissues contained virus-like particles that could be decorated by a polyclonal antiserum to wild type OLV-1. Site-directed and deletion mutants of pMUC-OLV were used to map the viral genes required for replication and movement. Mutants in ORFs 1 and 2 were not viable suggesting that the 23 and 82 kDa proteins are required for replication. The products of ORF 3 (8K) and ORF 4 (6K) were dispensable for RNA replication, but mutations in them prevented infection, indicating that they played a role in cell-to-cell movement. CP gene mutants replicated locally but did not become systemic, indicating that the CP gene is involved in long-distance translocation of the virus. A frameshift CP mutant in which the last 30 amino acids were replaced induced synthesis of apparently intact virus-like particles which did not spread systemically. This suggests the presence in the CP gene of a domain involved in long-distance movement.

**THE EFFECT OF NITROGEN NUTRITION ON BOTRYTIS STEM-END ROT OF KIWIFRUIT.** Ilaria Pertot\* and L. Perin. \*Istituto Agrario di San Michele all'Adige, Via E. Mach 1, I-38010 San Michele all'Adige (Trento), Italy. Fax: +39.0461.650872; e-mail: Ilaria.Pertot@ismaa.it

Storage rot, caused by *Botrytis cinerea* Pers., is a major problem for the kiwifruit industry. At present *Botrytis* stem-end rot can be prevented by applications of fungicide immediately after harvest or by treating fruits, holding them at room temperature for a few days before cold storage, but little is known about the factors that increase the incidence of the disease. Recent investigations have shown that there could be a correlation between plant nutrition and susceptibility of kiwifruit to *Botrytis* rot. In a three-year study, in northern Italy, nitrogen application rates were increased in a part of a kiwifruit plantation. A total amount of 10.5 Kg/plant of urea was applied in June, July and August. At harvest fruits were sprayed with conidial suspensions of *B. cinerea*. At the end of incubation period fruit rot was assessed. The incidence of gray mold decay was recorded both in the year of treatment and in the following one. Results show that excessive nitrogen application can significantly increase the incidence of *Botrytis* rot during cold storage. The same effect, though at a lower degree, was also observed in the following year. Thus excess nitrogen can increase the level of postharvest gray mold decay of kiwifruit, possibly by increasing the susceptibility of the fruit to the disease. However other factors seem to influence the incidence of *B. cinerea* during cold storage.

**EFFICIENCY OF RESISTANCE TO ALFALFA MOSAIC VIRUS IN *LYCOPERSICON HIRSUTUM* PI 134417 UNDER DIFFERENT ENVIRONMENTAL AND BIOLOGICAL CONDITIONS.** Giuseppe Parrella\*, H. Laterrot, K.G. Selassie and G. Marchoux. \*Centro di Studio del CNR sui Virus e le Virosi delle Colture Mediterranee, Via G. Amendola 165/a, I-70126 Bari, Italy. Fax:+39.080.5442911; e-mail: csvvgp06@area.ba.cnr.it.

Identification of natural resistance genes and their introduction into commercial varieties is still an appealing and often winning strategy for controlling virus diseases. To be useful in practice, resistance must remain effective under a wide range of environmental and biological conditions. Stability of resistance to alfalfa mosaic virus (AMV) derived from *Lycopersicon hirsutum* accession PI 134417 was therefore studied in relation to the following variables: (i) constant temperature of 20°C, 30°C, and 35°C; (ii) concentration of virus inoculum (1 µg, 5 µg, 10 µg, 50 µg, 100 µg, and 1000 mg of purified virus); (iii) plant age at the time of inoculation (11, 20, 30, and 40 days from sowing); (iv) virus isolates inducing necrosis in tomato or not; (v) mixed infections of AMV with cucumber mosaic (CMV), tomato spotted wilt virus (TSWV) tobacco mosaic virus (TMV) and potato virus Y (PVY); (vi) graft inoculation with different combinations (resistant on susceptible, susceptible on resistant, susceptible-resistant-susceptible). The kinetics of AMV infection in susceptible controls and resistant accessions was also studied. The results were highly satisfactory suggesting a mechanism of 'extreme resistance' probably due to inability of the virus to move from cell to cell (resistance to short distance movement). The resistance was efficient under all conditions tested, confirming that accession PI 134417 is a very promising source of resistance to a AMV, whose economic importance is increasing in field tomatoes in the south of Italy and France.

**FEMALE FERTILITY IN SOUTH AFRICAN AND ITALIAN ISOLATES OF *CYLINDROCLADIUM CANDELABRUM* GROUP 1.** G. Polizzi, C.L. Schoch and Pedro W. Crous\*. \*Department of Plant Pathology, University of Stellenbosch, P. Bag X1, Matieland 7602, South Africa. Fax: +27.21.808.4336; e-mail: pwc@maties.sun.ac.za

Mating studies on *Cylindrocladium candelabrum* have shown the species to be heterothallic and have indicated the presence of four genetically mating groups/populations. Although published records indicate that *C. candelabrum* gp 1 is associated with diseases of plants in South Africa (as *C. scoparium*), it has only recently been reported in Italy. *C. candelabrum* is self sterile, and female structures consist of protoperithecia which can be spermated by conidia or hyphae from an opposite mating type. Hermaphrodite isolates can form female structures and perform spermatization. In order to compare the population structure of *C. candelabrum* gp. 1, 50 isolates from both Italy and South Africa were paired in various combinations with tester strains. Although the South African population indicated an equal distribution of the two mating types, the observed percentage of hermaphrodites reduced the effective mating population to 76%. The Italian population had a mating type distribution of 1:4, but a high percentage of hermaphrodites, which again increased the effective mating population to 98%. We hypothesise that the uneven distribution of mating types of *C. candelabrum* gp. 1 in Italy can be explained by its recent introduction into the country. When conditions are favourable for mating, mating type distribution of the population should show a trend towards a 1:1 ratio as more mating occurs. Otherwise a lower percentage of hermaphrodites should be expected.

**IMPATIENS NECROTIC SPOT VIRUS IN VEGETABLE CROPS IN APULIA.** O. Potere and Crisostomo Vovlas\*. \*Dipartimento di Protezione delle Piante dalle Malattie, Università degli Studi e Centro di Studio del CNR sui Virus e le Virosi delle Colture Mediterranee, Via G. Amendola 165/a, I-70126 Bari, Italy. Fax: +39.080.5442911; e-mail: dipprotp@agr.uniba.it

In 1997 impatiens necrotic spot virus (INSV) was detected in severely diseased crops of artichoke (*Cynara scolymus*), lettuce (*Lactuca sativa*) and chicory (*Cichorium intybus*) in the Apulian provinces of Brindisi and Lecce and in Basilicata (southern Italy). The symptoms were of extensive necrosis of young leaves (lettuce and chicory) and stems and heads (artichoke). Disease incidence was up to 35 % in artichoke, 50 % in lettuce and 5 % in chicory. Mechanical inoculation with extracts from symptomatic plants induced necrotic local lesions within three days in *Nicotiana glauca* and *N. rustica*. The causal agent was identified by electron microscopy and ELISA using commercial monoclonal antibodies to INSV (Loewe Biochimica) and a mixture of specific monoclonal antibodies raised in Bari to a local isolate of tomato spotted wilt virus (TSWV) from chicory. More than 100 infected plants tested by TAS-ELISA were shown to be infected with INSV, and in 25 % of these TSWV was also present. This is the first report of INSV in vegetable crops in Apulia. In 1996 INSV was detected in lisianthus (*Eustoma grandiflorum*) in a small area north of Bari, and it would not be surprising if INSV was introduced into the region with infected lisianthus, and rapidly spread by *Frankliniella occidentalis*, the only natural vector of INSV, whose populations were especially high in 1997.

**MOLECULAR FINGERPRINTING OF PLEUROTUS AND AGARICUS GERMLASMS.** Pompilio Rapanà\*, B. Laddomada, F. Marras, G.L. Rana, D. Sisto and U. Tomati. \*Istituto di Biochimica ed Ecofisiologia Vegetali, Servizio di Micoteca dell'Area della Ricerca di Roma, CNR, Monterotondo scalo (Roma), Italy. Fax: +39.06.9064492; e-mail: toma@nserv.icmat.mlib.cnr.it

Molecular markers are very efficient tools for evaluating inter- and intraspecific genetic differences among fungi. Results obtained using Random Amplified Polymorphic DNA markers (RAPDs) for the characterisation of about 100 species of edible mushrooms commercially important in Italy, i.e. *Pleurotus eryngii*, *P. ostreatus*, *P. nebrodensis*, *P. cornucopiae*, *P. opuntiae*, *Agaricus bisporus* and *A. bitorquis* are reported. The analysis of polymorphism has been carried out on total DNA isolated from mycelia grown in submerged culture. Specific fragments of mitochondrial rDNA small- and large subunits were successfully amplified by PCR. The analysis of mit-rDNA seems to be a promising technique for the molecular systematics of fungi, since different levels of polymorphism of amplified regions of small and large subunits of mitochondrial rDNA can be revealed. The MS1-MS2 region is rather conservative and could serve as a molecular marker for genus-level phylogenetic studies, while the ML regions allow discrimination at interspecific level. Results concerning the MS1-MS2 region reveal the specific patterns for the genera *Pleurotus*, *Agrocybe* and *Lentinula*, while those concerning the ML1-ML4 region permit the separation of the *Pleurotus* species into 3 groups: 1) *P. eryngii* and *P. ostreatus*; 2) *P. cornucopiae*, SMR0120, *P. pulmonarius*, *P. sajor-caju* and *P. sapidus*; 3) *P. cornucopiae*, SMR0237, *P. ulmarius* and *P. citrinopileatus*.

**STRUCTURAL CHANGES IN QUERCUS ROBUR SEEDLINGS EXPOSED TO SALT STRESS.** Alessandro Ragazzi\*, S. Moricca and I. Dellavalle. \*Istituto di Patologia e Zoologia Forestale e Agraria, Università di Firenze, P.le delle Cascine 28, I-50144 Firenze, Italy. Fax: +39.055.354786; e-mail: ragazzi@ipaf.fi.cnr.it

The connection between oaks and salt stress in the context of oak decline was studied on seed-grown *Quercus robur*. After fifteen days of growth, 20 seedlings were placed in tanks with nutrient solution added to 0.05, 0.1, 0.2 M NaCl respectively to obtain saline nutrient solutions of Electrical Conductivity (EC) 5, 10 and 20 mS cm<sup>-1</sup>. A fourth control tank had no salt added. Visual characteristics recorded were: seedling height, number of nodes and defoliation index. Microscopic parameters, examined by SEM on cross sections of the stems, included: the average lumen diameter of 100 xylem vessels and the number of xylem vessels per mm<sup>2</sup>. After 45 days of treatment there were significant differences in height, number of nodes, and defoliation index between the controls and seedlings grown with EC 10 and EC 20 mS cm<sup>-1</sup> solutions. The average lumen diameter of xylem vessels from seedlings in nutrient solutions with EC 10 and 20 mS cm<sup>-1</sup> was significantly smaller than that of the controls. However, the number of xylem vessels increased proportionately with increasing EC values. An increase in NaCl levels therefore lowered the osmotic potential and created a state of physiological drought that led to a reduction in the diameter of the vessels. This smaller vessel diameter increased the difficulty of translocating sap, resulting in physiological stress, only partly compensated for by the increase in number of vessels.

**TRANSIENT EXPRESSION OF THE TOMBUSVIRUS REPLICASE IN PLANT PROTOPLASTS AND IN YEAST.** Luisa Rubino. Dipartimento di Protezione delle Piante, Università degli Studi and Centro di Studio del CNR sui Virus e le Virosi delle Colture Mediterranee, Via G. Amendola 165/a, I-70126 Bari, Italy. Fax: +39.080.5442911; e-mail: csvvlr02@area.ba.cnr.it

Cytopathological studies have identified in cells infected by toombusviruses vesiculated structures termed multivesicular bodies (MVBs). These vesicles originate from the proliferation of the limiting membrane of mitochondria or peroxisomes. The organelle targeting signal, responsible for the development of MVBs, resides in the protein encoded by the 5' most-proximal open reading frame (ORF 1). This protein has a size of 33 kDa (33K) or 36 kDa (36K), depending on the virus species, and constitutes the pre-readthrough portion of the virus replicase. Constructs were prepared expressing the protein encoded by ORF 1 in plant protoplasts and yeast. The sequence encoding the 33K or 36K protein was cloned into a vector containing the CaMV 35S promoter and a polyadenylation signal. *Nicotiana benthamiana* protoplasts were transfected with these constructs and expression of the viral proteins was monitored by immunofluorescence. Other constructs contained the gene for the 33K or 36K protein fused to the sequence encoding the green fluorescent protein (GFP) to allow monitoring of the expressed proteins. To express the virus proteins in *Saccharomyces cerevisiae*, the viral genes were cloned into an episomal vector carrying the GAL1 promoter for high-level inducible expression. The constructs could express either the native viral sequence or a fusion product with a GFP sequence especially designed for expression in yeast. Plasmids were first amplified in *Escherichia coli*, then cloned in yeast and their expression monitored as above.

**ARE FUNGAL HYDROPHOBINS INVOLVED IN PLANT PATHOGENESIS?** Aniello Scala\*, G. Parrella, G. Camici, G. Cappugi, G. Manao, L. Pazzagli, G. Del Sorbo and F. Scala.

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Hydrophobins are small hydrophobic proteins produced by both saprophytic and phytopathogenic fungi and constitute a subgroup of cysteine-rich proteins. They are between 75 and 150 amino acids in length and contain eight cysteine residues in conserved positions. Some of their known functions suggest that they are potentially involved in different phases of plant pathogenesis. Hydrophobins produced by phytopathogenic fungi include cerato-ulmin and its related structural homologues from *Ophiostoma* spp., the MPG1 protein from *Magnaporthe grisea* and cryparin from *Cryphonectria parasitica*. *Claviceps purpurea* also expresses genes which encode typical hydrophobin domains and repeated stretches of amino acids. Moreover, it has been suggested that there is a nonrodlet-forming member of the hydrophobins present on conidia of *Botrytis cinerea*. Among the proteins mentioned, cerato-ulmin is the most studied hydrophobin, but its role in pathogenesis and symptom expression of Dutch elm disease is still debated. In this work we present the results of physiological and molecular studies on these aspects. Moreover, we show that *Ceratocystis fimbriata* f.sp. *platani*, the causal agent of plane canker stain, in axenic culture produces cerato-platanin, a protein able to induce synthesis of fluorescent compounds by plane leaves and necrosis of tobacco leaf cell tissue. Cerato-platanin has been purified; and its complete amino acid sequence determined. On the basis of the sequence and hydrophobic profile it has been shown to be a novel hydrophobin.

**GENETIC IMPROVEMENT OF PEPPER FOR THE RESISTANCE TO *PHYTOPHTHORA CAPSICI* LEON.: STATUS AND PERSPECTIVES.** Giacomo Tamietti\*, G. Nervo and D. Valentino.

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Sources of resistance to *Phytophthora capsici* have been found in accessions of *Capsicum annuum* L. such as 'PI201234' and 'Serano Criollo de Morelos 334' (SCM). Resistance of 'PI201234', at first used in breeding programs, seems to be quite complex and conditioned by both environmental factors and physiological plant status, and can be overcome by some strains of the pathogen. Resistance of 'SCM' is also under investigation, at our Institute. Results of some experiments carried out on the progenies obtained by crossing 'SCM' with different Italian varieties suggest the polygenic nature of resistance even if a contribution by the susceptible parent to expression was observed. Our objective is the constitution of pepper lines with resistance genes of different origin. To achieve this some lines with satisfactory agronomic characters have been obtained by crossing 'PI201234' and 'SCM' with several commercial pepper lines. These lines have been stabilised by self-pollination and by *in vitro* androgenesis and, at present, some of them perform well. In fact stem colonisation rate at 25° C ranges from 0.59 to 2.25 mm day<sup>-1</sup>, 95.9-84.5% lower than that shown by the susceptible parent 'Quadrato d'Asti'. These lines produce 9.7-12 fruit per plant, measuring 95-105 x 78-81 mm and weighing about 180 g. They need to be further improved as regard fruit productivity and morphology.

**MOLECULAR DIVERSITY AT THE MAJOR CLUSTER OF DISEASE RESISTANCE GENES IN CULTIVATED AND WILD *LACTUCA* SPP.** Delphine Sicard\*, O. Ochoa, S.S. Woo,

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Variation was analyzed in wild and cultivated *Lactuca* spp. germplasm using molecular markers derived from resistance genes of the NBS-LRR type. Three molecular markers, one microsatellite marker and two SCAR markers that amplified LRR-encoding regions, were developed from sequences of resistance gene homologous at the main resistance gene cluster in lettuce. Variation for these markers was assessed in germplasm including accessions of cultivated lettuce (*Lactuca sativa*) and three wild spp., *L. serriola*, *L. saligna* and *L. virosa*. Diversity was also studied within and between natural populations of *L. serriola* from Israel and California; the former is a part of the center of diversity for *Lactuca* spp. while the latter is an area of more recent colonization. Amplification was successful from nearly all accessions. Large numbers of haplotypes were detected indicating the presence of numerous resistance genes in wild species. The variation in haplotypes provided evidence for gene duplication and unequal crossing over during the evolution of this cluster of resistance genes. However, there was no evidence for duplications and deletions within the LRR-encoding regions studied. The three markers were highly correlated with resistance phenotypes in *L. sativa*. They were able to discriminate between accessions that had previously been shown to be resistant to all known isolates of *Bremia lactucae*. These markers will therefore, be highly informative for establishment of core collections and marker-aided selection. A hierarchical analysis of the population structure of *L. serriola* showed that countries as well as locations were significantly differentiated. These differences may reflect local founder effects and/or divergent selection.

**ADVANCES IN GENOMIC MAPPING OF *PHYTOPHTHORA INFESTANS*.** Antonino Testa\*, T. van der Lee, G. Cristinzio and F. Govers.

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Previously a genetic linkage map of the heterothallic oomycetous plant pathogen *Phytophthora infestans* was constructed using only one cross from two Dutch field isolates (cross 71) and 73 F1 progeny. The map is based on polymorphic DNA markers generated by the DNA fingerprinting technique AFLP. The 183 AFLP markers and the 7 RFLP markers were comprised in 10 major and 7 minor linkage groups covering a total of 827 cM. Non Mendelian segregation ratios were found for the mating type locus and for 13 AFLP markers, all of which are located on the same linkage group as the mating type locus. A new genetic map was constructed using 56 individuals derived from a cross between two Mexican isolates (cross 68). The Mexican parental isolates are genetically more diverse than the Dutch ones. Approximately 300 segregating markers were scored. Although the number of markers present in the map of the cross 68 and cross 71 is limited, preliminary analysis indicate that parts of genome are co-linear. To further investigate whether we can construct an integrated map, we are increasing the number of the markers in both cross 71 and cross 68.

**NON-CONVENTIONAL RESISTANCE TO CUCUMBER MOSAIC VIRUS IN CANNING TOMATO.** L. Tomassoli and Marina Barba\*. \*Istituto Sperimentale per la Patologia Vegetale, Via C.G. Bertero, 22, I-00156 Roma, Italy. Fax: +39.06.86802296; e-mail: mc0540@mclink.it

Agricultural practices and vector control are not sufficient to contain virus diseases, and traditional breeding to produce resistant varieties and hybrids is often unsuccessful for lack of resistance genes in nature. Biotechnology has overcome this difficulty, and "transgene-mediated resistance" can be obtained in several crops by introducing a segment of viral genome via *Agrobacterium*. Since 1986, Italian tomato crops have been severely affected by cucumber mosaic virus (CMV), and in 1992 our Institute started a programme aimed at introducing coat protein-mediated resistance to CMV in the tomato cv. 'UC82B'. Transformation constructs contained the coat-protein (CP) gene of different CMV isolates belonging to subgroups Tors and DTL. 442 R0 plants were regenerated on a selective medium containing kanamycin and tested for CP expression. The R1 progeny was tested for CMV resistance under artificial infection conditions. Only single transgene copy lines were selected to produce R2 generation lines. Twenty three homozygous resistant lines were finally released in the field to investigate their behaviour towards CMV infection under conditions of natural infection. Over a period of five years, nine experimental fields were established in three different areas of Italy where tomato crops are severely affected by CMV. All but three transgenic lines showed good agronomic traits and satisfactory levels of resistance as compared with untransformed controls. The resistance has been durable, maintaining the same level up R6, the last progeny tested.

**POTATO TUBER FUNGAL MICROORGANISMS DURING STORAGE.** S. Vitale, V. Balmas and Luciana Corazza\*. \*Istituto Sperimentale per la Patologia Vegetale, Via C.G. Bertero 22, I-00156 Roma, Italy. Fax: +39.06.86802296; e-mail: mc\_ispave@www.inea.it

During the storage of potato tubers several pathological problems, for example dry rot, water rot and scab, can damage seed tubers or those for industry or consumption. The main fungal agents belong to the genera *Phytophthora*, *Phoma*, *Fusarium*, *Rhizoctonia*, *Alternaria* and bacteria to *Erwinia* spp. A mycological survey was carried out on potato tubers during storage; the cultivars analysed were mainly 'Hermes', 'Monalisa' and 'Spunta'. The tubers were collected from northern Italy (51% of the samples), central Italy (32% of the samples) and from southern Italy (16% of the samples). Most of fungal species isolates (81%) were *Fusarium* species. Among these, *F. oxysporum* was the most frequent (26%), isolated mainly from northern Italy, followed by *F. sambucinum* (19%), especially from central Italy and by *F. solani* (11%), from northern Italy. Most of *F. solani* isolates were classified as *F. solani* var. *coeruleum*, according to Booth description. The other fungal species isolates were represented by: *F. equiseti* (10%), *F. avenaceum* (5%), *F. culmorum* (4%), *Rhizoctonia solani* (3%), *Colletotrichum coccodes* (3%), *Alternaria* spp. (3%), *F. proliferatum* (2%), *F. crookwellense* (2%), *Spondylocladium atroviens* (sin.: *Helminthosporium solani*) (2%), *Cylindrocarpon* spp. (2%), *F. acuminatum* (1%), *F. compactum* (1%), *Botrytis* sp. (1%), *Curvularia* sp. (1%), *Acrostalagmus cinnabarinus* (1%). Most of the microorganisms isolated are important phytopathogenic and toxigenic fungi.

**NUCLEOTIDE SEQUENCE OF ORF-A OF ITALIAN HYPOVIRUS PURIFIED FROM HYPOVIRULENT ISOLATES OF C. PARASITICA.** Andrea Vannini\*, V. Ponzio, A. Mazzaglia and A. Gasbarri. \*Dipartimento di Protezione delle Piante, Università della Tuscia di Viterbo, Via S. Camillo de Lellis, I-01100 Viterbo, Italy. Fax: +39.0761.357473; e-mail: vannini@unitus.it

The genus *Hypovirus*, with 4 known species, is a hyperparasite of the *Ascomycota* fungus *Cryphonectria parasitica*, responsible for the chestnut blight. The viruses of this genus cause a loss of virulence in *C. parasitica*, best known as 'exclusive hypovirulence'. In the present work the complete sequence analysis of the ORF-A for 9 hypoviruses collected in Italy was carried out for the first time. For this purpose, a region of the viral dsRNA, corresponding to the first 2560 nucleotides in 5'-3' direction of the coding strand, was amplified in RT-PCR with two couples of primers deduced from the sequence of French *Hypovirus* CHV1-713. Amplified fragments were cloned into pGEMT and sequenced using universal primers T7 and SP6 and, for the region 424-1178, a couple of primers (HG1 and HF30) deduced from sequence of *Hypovirus* isolated from strain TR48/b from Umbria. This work points out differences in sequences among Italian *Hypovirus*, even from the same area but never exceeding 3%. However, they showed 11% base difference with the French CHV1-713, for the sequenced fragment, and more than 50% with the North American *Hypovirus* CHV2-NB58. ORF-A genetic organisation was very similar in all the hypoviruses, particularly in regard to the position of the start and termination codons. Analysis of ORF-A, responsible for fungal phenotypic modifications, permitted to notice the presence of two high variable regions, representative of complete coding region's variability, that can be used for virus population studies at local or wider scale.

**ROLE OF THE ECH42 (ENDOCHITINASE) GENE IN THE BIOCONTROL BY T. HARZIANUM ASSESSED BY GENE DISRUPTION.** S.L. Woo, B. Donzelli, F. Scala, G. Del Sorbo and Matteo Lorito\*. \*Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università di Napoli "Federico II", Via Università 100, I-80055 Portici (Napoli), Italy. Fax: +39.081.7755129; e-mail: lorito@unina.it

The potential of *Trichoderma* spp. as biocontrol agents has not been fully exploited because their mechanisms of action are not completely understood. Cell wall degrading enzymes (CWDEs) and the encoding genes are considered key factors in biocontrol, and have been studied for their antifungal properties. However, the molecular evidence to assess their role in mycoparasitism or antagonism, obtained for instance by disrupting the encoding genes, has not been reported. We prepared mutants of a biocontrol *T. harzianum* strain (P1) having the endochitinase encoding gene *ech42* disrupted. These mutants were unable to digest colloidal chitin but retained all the other CWDE activities. Fitness was not affected in the mutants, as indicated by *in vivo* biocontrol tests against *Pythium ultimum*. The *in vitro* antifungal activity of the mutant culture filtrates against *Botrytis cinerea* was reduced and could be restored by adding small amounts of purified endochitinase. *In vivo* experiments also indicated that the biocontrol activity of the *ech42* mutants against *B. cinerea* on bean leaves was significantly reduced. Surprisingly, the biocontrol activity of mutants in *Rhizoctonia* infested soil was substantially enhanced compared to the wild type strain P1. Additional experiments indicated that the disruption of the endochitinase gene in *T. harzianum* strain P1 may have improved its ability to colonise seeds and plant roots and, therefore, to control *Rhizoctonia*.

**VARIABILITY IN AFLP GENOMIC FINGERPRINTS OF *XANTHOMONAS ARBORICOLA* PV. *PRUNI*.** Massimo Zaccardelli\*, P. Ceroni and U. Mazzucchi. \*Istituto di Patologia Vegetale, Università di Bologna, Via F. Re 8, I-40126 Bologna, Italy. Fax: +39.051.351438; e-mail: umazzucc@agrsci.unibo.it.

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The AFLP (amplified fragment length polymorphism) technique was used to produce genomic fingerprints of 109 strains of *Xanthomonas arboricola* pv. *pruni* (previously called *Xanthomonas campestris* pv. *pruni*), isolated in Italy and abroad from different host plants. Of numerous strains isolated in the province of Verona, 40 were associated with fruit spots in the same peach orchard. Restriction enzymes *EcoR* I and *Mse*I were used to generate the fragments to be bonded to the adapters and amplified with PCR. Comparison of the genomic fingerprints revealed 12 homogenous groups of profiles, designated with different letters. The majority of strains fell within groups A (65 strains) and B (29 strains). Groups C, D and E included 4, 2 and 2 strains. The remaining 7 groups (F-N) consisted of only one strain each. Analysis of the similarity indices revealed the existence of at least 4 composite groups (A,C,D), (B,I), (H,L), (E,G) with intragroup indices higher than 0.98. Strains with different geographical origins and host plants came within the same group or composite group. In contrast, strains isolated from peach fruits in the same orchard fell within different groups. The variability of the genomic fingerprints of strains from the same peach orchard was comparable to that between strains with distinct geographic origins and host plants.

**VIRULENCE OF *XANTHOMONAS ARBORICOLA* PV. *PRUNI* AND ITS RESISTANCE TO BACTERIOPHAGES.**

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The resistance to bacteriophages of *Xanthomonas arboricola* pv. *pruni* (*X.a.p.*) (previously called *X. campestris* pv. *pruni*) has in the past been attributed to lysogeny, according to some authors associated with a reduction in virulence. Eighteen phage-resistant *X.a.p.* strains (16 from a plate of the wild virulent phage-resistant strain VR 69, 1 from an international collection and 1 from a Verona peach orchard treated with a bacteriophage suspension) were investigated for lysogeny. None of them released phage particles after prolonged growth in broth or exposure to UV light and no hybridisation occurred between their DNA and radioactive phage DNA probes. AFLP profiles of the 16 phage-resistant strains did not differ greatly from that of the VR 69 strain. Virulence of the 18 phage-resistant strains was tested on young peach fruits, cv. 'Elegant Lady' collected at the fruitlet stage. Symptoms were examined after one week (diameter of the spots around the inoculation holes and intensity of water-soaked areas), an analysis of variance was done, and a comparison (Dunnett Test) was made between the mean diameter of the spots of each phage-resistant strain and that for the control strain VR 69. Comparison showed that only 29% of the strains resistant to the bacteriophage showed a significant reduction in virulence. These results indicate that resistance to bacteriophages shown by the *X.a.p.* strains studied was not due to lysogeny and that resistance to bacteriophages does not necessarily lead to a reduction in virulence.

