RECENT ADVANCES IN STUDIES ON SPECIES OF *PHYTOPHTHORA* ASSOCIATED WITH INK DISEASE IN ITALY. N. Anselmi, A.M. Vettraino, G. Natili and A. Vannini*. **Dipartimento di Protezione delle Piante, Università della Tuscia di Viterbo, Via S. Camillo de Lellis, I-01100 Viterbo, Italy. Fax +39.761.357473; e-mail: vannini@unitus.it*

A serious recrudescence of 'ink disease' has been reported in the recent years in a number chestnut stands in Italy. Two species have been suggested as causal agents of the disease in these areas, *Phytophthora cambivora* and to a less extent *P. cinnamomi*; furthermore it cannot be excluded that other species could contribute to the development of the disease. A large number of soil sample has been collected under diseased and healthy looking trees in two chestnut areas in central Italy with the aim of determining the number of species of *Phytophthora* associated with 'ink disease' in Italy. The identification of the isolates has been carried out with classical morphological methods and through RFLP analysis of the ITS1-5,8S-ITS2 region. Five species were isolated from soil in infected areas: *P. cambivora*, *P. cactorum*, *P. citricola*, *P.gonapodyides* and *P. cryptogea*. However only *P. cambivora* has been found always associated with diseased trees and isolated from symptomatic tissues. Pathogenicity tests with inoculated cuts and through soil infestation, confirmed the high virulence of *P. cambivora*. However all the species resulted pathogenic on inoculated chestnut cuts but only *P. cambivora* and *P. citricola* were able to kill one-year seedlings following soil inoculation. These preliminary results would suggest that more than one species of *Phytophthora* could be responsible of 'ink disease' in Italy.

CELL POLAR LIPID (PL) FATTY ACIDS WITH HIGH PRESENCE OF *PSEUDOMONAS SYRINGAE* PV. *TOMATO* IN THE PHYLLOSPHERE AND IN THE RHIZOSPHERE OF TOMATO PLANTS. Giorgio Mariano Balestra*, M. Antonelli, A. Fabi and L. Varvaro. **Dipartimento di Protezione delle Piante, Università della Tuscia di Viterbo, Via S. Camillo De Lellis, I-01100 Viterbo, Italy.*

Bacterial tomato speck caused by *Pseudomonas syringae* pv. *tomato* (Okabe) Young *et al.* is one of the main diseases of tomato. The bacterium is able to survive and multiply at an epiphytic level on the host and to live in the soil where tomatoes are cultivated. The aim of this study was to verify the effect of an organic cropping system on the presence of *P. syringae* pv. *tomato* in the phyllosphere and rhizosphere throughout the tomato vegetative season. When a conventional cropping system was used, *P. syringae* pv. *tomato* survived on the tomato phylloplane for the entire vegetative cycle especially on plants fertilised with mineral compounds. With the organic cropping system the bacterium was occasionally recorded but with values lower than 10^3 cfu cm^{-2} leaf. The pathogen was not found in the rhizosphere in plots cultivated with organic methods. *P. syringae* pv. *tomato* presence was also low (10^1-10^2 cfu g^{-1} soil) in conventional plots. Moreover, in the field cultivated with organic methods, a relevant microbial presence (10^6 cfu g^{-1} soil) was recorded with a high percentage of bacterial species known to be natural antagonists of the pathogen (*e.g. P. fluorescens* and *Bacillus* spp.). Biological aspects and the possible epidemiological implications are discussed.

MECHANISMS OF ACTION OF *CANDIDA OLEOPHILA* IN RELATION TO THE BIOSYNTHESIS OF β-1,3-GLUCANASE ON HYPHAE OF *BOTRYTIS CINEREA*. Giovanni Arras*, S. Arru and V. Astone. **Istituto per la Fisiologia della Maturazione e della Conservazione del Frutto delle Specie Arboree Mediterranee, Via dei Mille 48, I-07100 Sassari, Italy. Fax: +39.079.232047; e-mail: G.Arras@imfpp.ss.cnr.it*

This study was undertaken to evaluate the inhibitory activity of several antagonistic yeasts and to characterise the mode of action of *Candida oleophila* (13L) against *Botrytis cinerea.* Yeasts *C. oleophila* 13L, *Rhodotorula minuta* 7L and *Pichia guilliermondii* 5A showed inhibitory values ranging between 87 and 100% on mandarin fruits. According to scanning electron microscope observations, yeast 13L colonised the exocarp of the fruit, the wounds and the hyphae of *B. cinerea*, causing alterations to the cell wall of the latter. *In vitro C. oleophila* 13L degraded laminarin and *B. cinerea* cell walls, more effectively than *R. minuta* 7L and *Pichia guilliermondii* 5A. A fragment of the β-1,3-glucanase gene was amplified by PCR, using primers selected on the basis of the β-1,3-glucanase from *Candida albicans*. 13L did not reveal any amplicons, which suggests deep differences in the genic sequence of the two micro-organisms. The results could indicate the presence of peculiar features of 13L, which would make this microorganism an ideal antagonist to control postharvest microbial fruit alterations.

VARIABILITY OF *CERCOSPORA BETICOLA* POPULATION IN RELATION TO HOST RESISTANCE. Paola Battilani*, V. Rossi, G. Chiusa and L. Languasco. **Istituto di Entomologia e Patologia Vegetale, Università Cattolica S. Cuore, Via E. Parmense 84, I-29100 Piacenza, Italy. Fax:+39.0523.599254; e-mail: patolo@pc.unicatt.it*

Cercospora beticola has been widely studied to verify the existence of races, in relation to possible interaction between races and sugarbeet resistance. Notwithstanding this, the problem is still debated. Several studies found differences in the morphology or ecology of the fungus, whereas other researches showed that isolates from different geographical areas caused different disease severity when artificially inoculated onto plants, but only in a few cases they demonstrated an interaction between isolate and cultivar. In the USA, 3 different races were described on differential varieties. In the present work, interaction between sugarbeet cultivar and location was studied, under the assumption that different pathogen populations exist in different areas. Two 3 year trials were carried out with several sugarbeet genotypes, showing different resistance ratings, in several locations in the Mediterranean area. The results showed that the rating of the genotypes was not significantly influenced by location. Fungal isolates were collected in several countries and characterised for ecological and nutrimental needs, for toxin production, for genetic characters (by RAPD) and for several aspects of pathogenicity. The results confirmed the variability between *C. beticola* populations isolated from different areas. Therefore, variability of *C. beticola* populations does not significantly influence the rating of resistant genotypes.

CHARACTERIZATION OF PATHOGENIC POPULATIONS OF *FUSARIUM OXYSPORUM* F.SP. *MELONIS*. A. Belisario, M. Zaccardelli 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*

Fusarium wilt of melon, caused by *Fusarium oxysporum* f.sp. *melonis* Snyder and Hans. (*Fom*), is considered one of the major constraints to melon cultivation worldwide. Four races of *Fom* have been designated 0, 1, 2, and 1,2, and they are all present in Italy. Race 1,2, which is further subdivided into wilting (1,2w) and yellows (1,2y) strains, represents the emerging problem for melon-growing areas because of its rapid diffusion, and for the lack of completely resistant cultivars. Race determination tests take at least 30 days to complete moreover, differences in virulence within race and the influence of temperature and age of the host plant on symptom development can make this determination ambiguous. The use molecular markers could make race determination more rapid and reliable giving insights on genetic relatedness. This study compared 19 isolates of *Fom* for genetic diversity among 1, 2, and 1,2 races by AFLP, by RFLP-ITS and ITS sequencing, amplification of mini and microsatellite regions, or RAPD methods of investigation. Italian and North American isolates were compared. In RAPD analysis race 1, 2 and 1,2 produced a different pattern with 7 primers tested. Microsatellite amplification (GACA)4 distinguished race 1, 2 and 1,2 while (CAA)5 distinguished only race 2. Race 2 isolates gave two different patterns both with microsatellite and RAPD supporting the hypothesis of a multiple origin of this race, while the more similar profiles of race 1 and 1,2 support the hypothesis that one race can give rise to another. This study shows that the three main races of *Fom* can be distinguished by RAPD and microsatellite procedures.

FURTHER DATA ON THE PRESENCE AND SPREAD OF GRAPEVINE YELLOWS IN LOMBARDIA (NORTHERN ITALY). Pier Attilio Bianco*, P. Casati and G. Scattini. **Centro CNR per il Miglioramento Sanitario delle Colture Agrarie c/o Istituto di Patologia Vegetale, Università di Milano, Via Celoria 2, I-20133 Milano, Italy. Fax: +39.02.70631287; e-mail: piero.bianco@unimi.it*

Flavescence dorée (FD) is an epidemic form of grapevine yellows (GY) transmitted by the leafhopper *Scaphoideus titanus* and caused by phytoplasmas belonging to 16SrV group (subgroup C). After the first record in Italy (1973), a severe outbreak of FD was observed at the beginning of the 80s, in several areas of Veneto (northeastern Italy). At the beginning of the 90s, an extensive survey was conducted in some important viticultural areas of Lombardia. Low percentage of grapevine plants showing symptoms of GY was observed and only the presence of phytoplasmas related to 16SrXII-A (Bois noir, BN) was detected. During summer 1998, in some vineyards located in the S. Colombano area, a large number of plants showing typical symptoms of GY was observed. Samples collected from symptomatic plants of cvs 'Barbera', 'Chardonnay' and 'Verdea' were analysed, by PCR and RFLP of nucleic acid extracts. 16SrV-C (FD) and 16SrXII-A (BN) phytoplasmas were detected. In June 1999, new foci of GY were found in Franciacorta and Valtenesi (province of Brescia) and in Oltrepò pavese (province of Pavia). The preliminary data of molecular tests indicate a prevalence of phytoplasmas 16SrV-C (FD) in the grapevine samples collected in the field at the end of June. These results strongly suggest that in the areas surveyed in 1999 spread of FD is in progress.

IDENTIFICATION OF AN ISOLATE OF POTATO VIRUS Y IN *DATURA STRAMONIUM*. Maria Grazia Bellardi*, C. Rubies-Autonell and E. Maffettone. *U.C.I.-S.T.A.A., Istituto di Patologia Vegetale, Università di Bologna, Via F. Re 8, I-40126 Bologna, Italy. E-mail: crublbet@dns.agrsci.unibo.it*

In 1997, during a virus survey carried out at the Herb Garden of Casola-Valsenio (Emilia-Romagna, northern Italy), several plants of *Datura stramonium* were found showing stunting and dark green spots on the leaves. From these plants, potyvirus-like particles were sap transmitted to *Chenopodium amaranticolor* which reacted with local chlorotic-necrotic lesions and *Nicotiana tabacum* cvs 'White Burley' and 'Samsun', which developed systemic veinal necrosis. This virus was serologically identified a strain of potato virus Y (PVY) by immunoelectron microscopy 'decoration' and PAS-ELISA. The polyclonal antisera tested were those to several potyviruses, including PVY and *Tobacco etch virus* (TEV). Indirect ELISA with specific monoclonal antibodies to tobacco veinal necrosis strain group of PVY, revealed that this PVY was an isolate of PVY^N. Seedling of *D. stramonium*, mechanically inoculated with sap from PVY^N infected tobacco leaves, showed stunting and green spots on the leaves, after about 30-40 days. The literature reports that only *Datura innoxia* and *D. metel* are natural hosts of PVY, whereas systemic mottle, distorsion and vein banding occur in *D. stramonium* infected by TEV. From our results it appears now that also *D. stramonium* is a natural host of PVY^N. Preliminary RT-PCR tests with primers specific for a tuber necrotis-inducing isolate of PVY^N (PVY^{NTN}), PVY^N-*D. stramonium* appears to be similar to PVY^{NTN}, the elicitor of serious damage to potato crops in Europe.

***SPHINGOMONAS* SP. ON HONEYDEW MELONS (*CUCUMIS MELO* VAR. *INODORUS*) WITH BROWN SPOT SYMPTOMS. Roberto Buonaurio*, V.M. Stravato and C. Cappelli.** **Dipartimento di Arboricoltura e Protezione delle Piante, Università di Perugia, Via Borgo XX Giugno 74, I-06121 Perugia, Italy. Fax: +39.075.5856482; e-mail: buonaurio@unipg.it*

In November 1997, brown fruit spots were observed on *Cucumis melo* var. *inodorus* Naud. (cv. 'Amarillo Oro') plants cultivated in a greenhouse located in Almeria (Spain). Non-sporing, motile, rod-shaped bacteria were isolated from diseased fruits on nutrient agar, where they produced small lemon-yellow colonies. Two bacterial isolates, selected for the identification, were Gram-negative, catalase positive, weakly oxidase-positive and phenylalanine deaminase negative. They hydrolysed esculin but not gelatin and they oxidised glucose. When the two bacterial isolates were inoculated in honeydew melon fruits they provoked disease symptoms similar to those observed in greenhouse. Fatty acid analysis revealed that both bacterial isolates belong to the *Sphingomonas* genus. In addition, 16S rDNA sequence analysis, performed on one isolate, revealed a significant sequence similarity (more than 97%) with *S. asaccharolytica*, *S. mali* and *S. pruni*, non-phytopathogenic bacteria isolated from plants. Although rep-PCR results suggested that the two honeydew fruit isolates belong to a new *Sphingomonas* species, DNA-DNA hybridisation is necessary to verify this hypothesis. In addition, further investigations are necessary to establish whether the pathogenicity of the *Sphingomonas* sp. isolates is restricted to mature honeydew melon fruits.

TOMATO SPOTTED WILT IN TWO CULTIVATED COMPOSITAE IN BASILICATA (SOUTHERN ITALY). I. Camele and Gian Luigi Rana*. **Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali, Università della Basilicata, Via N. Sauro 85, I-85100 Potenza, Italy. Fax:+39.0971.55748; e-mail: rana@unibas.it*

During a survey of virus diseases of vegetable crops in southern Italy, plants of hybrid artichoke (*Cynara scolymus*) no. 6370 and escarole (*Cichorium endivia* var. *crispum* and var. *latifolium*) with green and red leaves of cvs 'Samoa', 'Klara', 'Ciarda', 'Despa', 'Kublay', 'Concorde', 'Sesam' and 'Circeo' with symptoms probably due to viral infections were observed in horticultural areas of Metapontum and Policoro (Basilicata, southern Italy). Symptomatic artichoke plants were not frequent (1%) and showed severe malformations of leaves, stems and heads, which also exhibited variously extended necrotic areas of the inner bracts. Escarole plants with symptoms were very numerous (50-75%) and exhibited malformed leaves with large chlorotic and necrotic areas. In the most severe cases, the plants withered and died. The lettuce strain of tomato spotted wilt virus (TSWV-L) was consistently isolated from symptomatic plants of both hosts. The identification was made on the basis of the symptomatological responses of herbaceous indicators (*Catharanthus roseus*, *Chenopodium quinoa*, *Nicotiana benthamiana*, *N. glutinosa*, *Petunia hybrida,* etc.) and immunosorbent electron microscopy followed by decoration.

ALTERNATIVES TO SOIL FUMIGATION WITH METHYL BROMIDE FOR THE CONTROL OF SOILBORNE PLANT PATHOGENS IN SOUTHERN ITALY. Girolamo Cartia* and P. Di Primo. **Dipartimento di Agrochimica e Agrobiologia, Università di Reggio Calabria, Piazza S. Francesco di Sales 2, I-89061 Gallina (Reggio Calabria), Italy.*

Methyl bromide (BM) is the most widely used fumigant for soil disinfestation and for commodity and postharvest quarantine treatments due to its broad spectrum activity against soilborne pests. The inclusion of MB as a Class I ozone depleting substance into the Montreal protocol and the phase out by 2005 has prompted a need for alternative methods for soil disinfestation. In recent years, much attention has been devoted at reducing the usage of MB in the Italian agriculture by environmentale safe approaches such as soil solarization and 'biofumigation'. In the present review the results of a three year study on the control of soilborne plant pathogens such as *Fusarium oxysporum* f.sp. *radicis-lycopersici* (FORL), *F. oxysporum* f.sp. *melonis* (FOM), *Sclerotium cepivorum* and weeds by soil solarization and 'biofumigation' in Calabria and in Sicily are reported. Moreover, the approaches for the implementation of soil solarization and 'biofumigation' as soil disinfestation methods into the southern Italy agricultural conditions are discussed. Due to the favorable climatic conditions soil solarization represents a valid, feasible and environmental safe soil disinfestation method for southern Italy.

RESISTANCE TO OXIDATIVE STRESS AND ACTIVITY OF ANTAGONISTIC YEASTS AGAINST POSTHARVEST PATHOGENS. Raffaello Castoria*, L. Caputo, F. De Curtis, G. Lima and V. De Cicco. **Dipartimento di Scienze Animali, Vegetali e dell'Ambiente, Università del Molise, Via De Sanctis, I-86100 Campobasso, Italy. Fax: +39.0874.404678; e-mail: castoria@hp-srv.unimol.it*

Wounds inf horticultural crops are the main penetration sites for postharvest fungal pathogens. Wounding is accompanied by the formation of free radicals such as semiquinones, lipoperoxyl radicals and, possibly, reactive oxygen species (ROS) such as superoxide anion (O^-_2) and hydrogen peroxide (H_2O_2), which are known to be generated in plant tissues following elicitor treatment or challenge by incompatible pathogens. Rapid colonisation of wounds by antagonistic micro-organisms is crucial for efficient prevention of pathogen attack. We compared two yeast isolates with high (LS-28, *Cryptococcus laurentii*) and low (LS-11, *Rhodotorula glutinis*) antagonistic activity to assess (*i*) their ability to colonise apple wounds and (*ii*) for their *in vitro* resistance to oxidative stress caused by H_2O_2 and an O^-_2 generating system. LS-28 was able to colonise apple wounds at a higher level than the less efficient antagonist LS-11 for a time interval of 2 hours to 7 days after application. LS-28 was also more resistant to H_2O_2 treatment. Analogous results were obtained following treatment with an O^-_2 generating system.

EPIDEMIOLOGICAL ASPECTS OF *PUCCINIA RECONDITA* F.SP. *TRITICI* IN ITALY. Fedele Casulli*, D. Pancaldi and M. Pasquini. **Dipartimento di Protezione delle Piante, Via G. Amendola 165/A, I-70126 Bari, Italy. Fax +39.080.5442911; e-mail: casullif@agr.uniba.it*

Leaf rust, caused by *Puccinia recondita* Rob. *ex* Desm. f.sp. *tritici* Eriks. *et* Henn. (*Prt*), is one of the most important wheat diseases in Italy. The use of resistant varieties is a safe and economic way to control this disease. To prevent sudden epidemics, the behaviour of many wheat varieties and the variability of the *Prt* populations were annually studied, since 1973. For that, a 'National Epidemiological Nursery' was annually prepared and located in about 12 of the most important Italian cereal growing areas. The most frequent *Prt* pathotypes were *Pr* 01060, *Pr* 01160, *Pr* 03162, *Pr* 41160 and *Pr* 43162. About the *Prt* virulence, the resistant genes *Lr* 9, *Lr* 19, *Lr* 24, *Lr* 25 and *Lr* 29 were highly effective while the res-genes *Lr* 1, *Lr* 2a, *Lr* 15, *Lr* 17 and *Lr* 28 were 90% effective and the effectiveness of *Lr* 2b, *Lr* 3, and *Lr* 26 varied from 40 to 80%. The other *Lr* genes were ineffective. *Prt* population has a greater variability on bread wheat and shows a higher virulence in the northern regions, nevertheless no significant change occurred in the last years. The Italian *Prt* population is similar to the French one and different from the eastern Countries populations. *Prt* is more frequent in the fields along the coast and in southern Italy. Here, it is often present during winter time and the heavy infections are located on the bottom leaves. Infections are more serious after a rainy summer and where 'set-aside' fields and alternate hosts are present. The development of *Prt* is also influenced by the wheat cultivars resistance and the cultivation schemes.

DIRECT DETECTION OF *PSEUDOMONAS CORRUGATA* IN TOMATO PLANTS WITH PCR. Vittoria Catara*, D.L. Arnold, G. Cirvilleri, P. Bella and A. Vivian. **Dipartimento di Scienze e Tecnologie Fitosanitarie, Sez. Patologia Vegetale, Università di Catania, Via Valdisavoia 5, I-95123 Catania, Italy. Fax: +39.095.350043; e-mail: vittoria@mbox.fagr.unict.it*

The detection and identification of *Pseudomonas corrugata*, the causal agent of tomato pith necrosis, is routinely performed by direct isolation of the organism on nutrient media and performing specific biochemical and pathological tests. Attempts to find rapid methods for the identification and/or detection of *P. corrugata* have encountered problems associated with the high phenotypic and genomic variability observed in this species. Two unique fragments, generated by RAPD-PCR, were used as probes against representative isolates of *P. corrugata*. Each isolate hybridised to only one of the two probes. Fragments were isolated, cloned and sequenced. The resulting sequences were used to design two pairs of oligonucleotide primers, which when used in combination in PCR with *P. corrugata* cells of fifty strains, produced one of the two PCR bands (either 1100 bp or 600 bp). No bands were detected in a range of closely related *Pseudomonas* species following PCR amplification. A PCR protocol for the detection of *P. corrugata* in 'tomato pith necrosis'-infected plants was successfully developed based on the two pairs of primers and a quick alkaline DNA extraction protocol.

TRANSFORMATION OF *PSEUDOMONAS CORRUGATA* WITH RANDOM GENOMIC LUX FUSION. Gabriella Cirvilleri*, P. Bella and V. Catara. **Dipartimento di Scienze e Tecnologie Fitosanitarie, Sez. Patologia Vegetale, Università di Catania,Via Valdisavoia 5, I-95123 Catania, Italy. Fax:+39.095.350043; e-mail: cirville@mbox.fagr.unict.it*

An isolate of *Pseudomonas corrugata*, causal agent of tomato pit necrosis, was modified with *lux* operon DCABE marker gene. Transposon Tn4431 was used to introduce the *lux* operon into the chromosome of *P. corrugata* strain 4.3t, originally isolated from symptomatic tomato plants in Sicily. Light emitted by bioluminescent derivatives was detected with a luminometer and autoradiography. One bioluminescent derivative, designated strain 4.3t lux 18, contained chromosomally integrated *lux* genes and expressed bioluminescence in culture and *in planta*. This strain was identical to its wild-type parent in all aspects studied, including pathogenicity, *in vitro* and *in planta* growth characteristics, genetic characteristics, antimicrobial activity. Growth and survival of wild-type and bioluminescent strains of *P. corrugata* in tomato plants were followed over time with dilution plating techniques and with the luminometer. The populations declined gradually and were not significantly different from each other at each sampling time. The pathogen was consistently recovered from tissue sections emitting bioluminescence. Bacterial movement on symptomatic and asymptomatic tissues were monitored and visualised. The results indicate that the bioluminescent derivative retained its activity in tomato, hence, *lux* appears to be a good genetic marker system for investigation of epidemiology, population dynamics and genetic determinants of the pathogenicity of this bacterium.

EFFECT OF COPPER RESISTANCE LOCUS ON THE EPIPHYTIC FITNESS OF *PSEUDOMONAS SYRINGAE*. Gabriella Cirvilleri* and S. Lindow. **Dipartimento di Scienze e Tecnologie Fitosanitarie, Sez. Patologia Vegetale, Università di Catania, Via Valdisavoia 5, I-95123 Catania, Italy. Fax: +39.095.350043; e-mail: cirville@mbox.fagr.unict.it*

Copper resistant strains of several plant pathogenic bacteria have been detected under field conditions. Failure to control copper resistant strains by standard bactericides complicates the control of the diseases, increasing the cost of management and the pesticide load in the environment. This study was set up to test the hypothesis that copper resistant strains of *Pseudomonas syringae* are less fit than copper-sensitive strains and hence that selection pressure will tend to reduce the frequency of such strains on plants in the absence of copper bactericide. We produced a copper-sensitive isogenic derivate of a copper resistant strain, *P. syringae* Al513, engineered to express xylE, to determine the fitness contribution of the copper resistant locus under various conditions on plants. Epiphytic colonisation and competition experiments with and without copper bactericide were conducted on bean plants. While the relative fitness of the copper-resistant strain was slightly lower than that of the copper-sensitive strain on untreated bean plants, this strain was much fitter on plants treated with even very low amounts of copper hydroxide. Our studies support the hypothesis that under stress conditions the expression of the copper-resistance trait, in the absence of corresponding cell benefits, can substantially reduce the fitness of *P. syringae*.

OVERWINTERING OF *UNCINULA NECATOR* AND EPIDEMICS OF GRAPE POWDERY MILDEW. Paolo Cortesi*, F. Zerbetto, M. Bisiach, M. Miazzi and F. Faretra. **Istituto di Patologia Vegetale, Università di Milano, Via Celoria 2, I-20133 Milano, Italy. E-mail: pcortesi@unimi.it*

Uncinula necator overwinters as ascospores in cleistothecia and as mycelium in dormant infected buds that produce flag-shoots in the spring. The distribution and density of cleistothecia during winter and flag-shoots during spring were assessed in 34 commercial vineyards located in 7 viticultural areas in Tuscany. In each viticultural area the frequency of vineyards where cleistothecia were found on the bark of grapevines was 33 to 86%; during the three years cleistothecia were not observed only in 7% of the vineyards. Inoculum density, as viable cleistothecia, ranged from a few units to about 3000 per kg of dry bark. Flag-shoots were found at low densities, 3-5 per ha, in 33% of the vineyards. Cleistothecia are a more important source of inoculum compared to flag-shoots. In the untreated vineyard Santa Cristina the percent of vines with flag-shoots and the number of flag-shoots/vine increased year by year, regardless the cultivar. However, the frequency of vines that had flag-shoots in consecutive years decreased. The ratios of the two mating types alleles, *MAT*-1 and *MAT*-2, of isolates of *U. necator* representative of 4 populations, two from flag-shoots and ascospores from vineyards in Tuscany, respectively, one from an Apulian vineyard and a collection of isolates from southern Italy, were not different from 1:1. Isolates from flag-shoots mated successfully with isolates from ascospores and molecular markers (PCR-RAPD) and phenogram analysis did not allowed to discriminate two biotypes of the fungus, contrary to what was reported by French Authors.

VIBRATIONAL SPECTROSCOPIC MONITORING OF GLADIOLUS ROOT EXUDATES. Elisabetta Dallavalle*, A. Zechini D'Aulerio and G. Bottura; **Dipartimento di Protezione e Valorizzazione Agroalimentare, Università di Bologna, Via F. Re 8, I-40126 Bologna, Italy. Fax:+39.051.2091414; e-mail: dallaval@pop.agrsci.unibo.it*

The spectroscopic characterisation (FTIR) of gladiolus root exudates and the evaluation of their action on *Fusarium oxysporum* f.s. *gladioli* (Fog.) spore germination are reported. Some cvs of gladioli were compared with different degrees of resistance to the pathogen. The gladiolus exudate FTIR spectra revealed the following absorption bands: 3400 cm^{-1}, stretching vibrations of OH groups of alcohols and phenols; 2925 and 2860 cm^{-1}, stretching modes of CH_2 groups, the most abundant group in vegetables; 1735 cm^{-1}, stretching vibrations of esteric CO groups. The bands at 1650-1500 cm^{-1} define the 'aromatic domain' (phenols): structure modifications of such compounds can be detected by analysing this region. The bands at 1200-900 cm^{-1}, (bending modes of C-O, O-H groups of secondary alcohols) give the spectroscopic fingerprint of carbohydrates. A comparison of the spectra of the different varieties shows differences due to the presence of new bands in only some species or the different intensity of the same bands. The most meaningful difference is observed in the region of the aromatic domain: its components are more numerous and intense in the more resistant cv. ('White Prosperity'), indicating a higher amount of aromatic compounds than aliphatic ones. Carbohydrates show a similar trend. Furthermore, the root exudates of 'White Prosperity' caused extremely high inhibition of Fog. spore germination.

AN ASSOCIATION OF *FUSARIUM* SPP. AND AMBROSIA BEETLES ON DISEASED EUROPEAN WALNUTS. Gabriella Frigimelica*, M. Faccoli and P. Zandigiacomo. **Isituto di Entomologia Agraria, Università di Padova, Via Romea 16/a, I-35020 Legnaro (Padova), Italy. Fax: +39.049.8272810; e-mail: frigimelica@hotmail.com*

At the end of spring 1998, several trees growing in young plantations of European walnut (*Juglans regia* L.) in the Friuli Venezia Giulia region (northeastern Italy), showed several distinct disease symptoms, such as wilting, stem cankers and sprouts near the ground. A large part of diseased trees was also colonised by the ambrosia beetle *Xylosandrus germanus* (Blandford), a world-wide polyphagous species, found for the first time in Italy. Similar symptoms were observed in northern America, where *X. germanus* seems to be associated with fungi of the genus *Fusarium* in causing the American black walnut (*J. nigra* L.) disease. The most frequent fungi isolated from tissue samples were *Fusarium* spp. (*F. merismoides* Corda, *F. lateritium* var. *majus* Wollenweber and *F. oxysporum* Schlechtendahl: Fries). In spring and summer 1999, new fungal infections on large branches and trunks were not observed, although old *Fusarium* cankers were still active. In addition, not every plantation growing in the same region showed signs of beetle attack. The lack of the beetles could partially explain the absence of new fungal infections. However, several new cankers developing on young sprouts were found. As *X. germanus,* like other ambrosia or bark beetles, attacks only well lignified tissues, other vector species are likely involved in both diffusion and inoculation of the fungi in smaller branches.

EPIDEMIOLOGY OF *TAPHRINA DEFORMANS*, CAUSING LEAF CURL ON PEACHES. Simona Giosuè*, G. Spada, V. Rossi, G. Carli and I. Ponti. **Istituto di Patologia Vegetale, U.C.S.C., Via Parmense 84, I-29100 Piacenza, Italy. Fax: +39.0523.599256; e-mail: patolo@pc.unicatt.it*

A model forecasting *Taphrina deformans* infection on peaches, elaborated in Israel, was modified and validated in different locations in Emilia-Romagna (1996-1998). The modified model computes the infection risk daily as a function of: host phenological stage, maximum daily temperature (when it is > 5°C), rainfall (when it is > 10 mm), and it forecasts disease onset after an incubation period. The model does not take into account cultivar susceptibility and inoculum level, as the Israeli model does. Several aspects of the model were validated: beginning and length of the peach susceptibility period, infection establishment and the length of incubation. The model simulated disease onset accurately, when the following aspects were taken into consideration: (*i*) the host becomes susceptible to infection when the first winter buds break and it remains susceptible for at least the following 9 weeks ; (*ii*) thresholds for temperature and rainfall need not be considered rigorously, because infection can occur also at 3.5°C and 9.5 mm; (*iii*) the incubation length is very variable (9 to 33 days), with a mean length of 24 days, and it varies according to the number of days that elapse after bud break and the temperature. Further studies are going to be performed in order to verify the accuracy of the model in forecasting disease appearance, by taking into account the level of the overwintering inoculum and its dynamic over the time of host susceptibility.

APRICOT LEAF CURL IN CAMPANIA AND APULIA. Salvatore Frisullo*, G.L. Rana and A. Crescenzi. **Istituto di Produzioni e Preparazioni Alimentari, Università di Bari, Via Napoli, 25, I–71100 Foggia, Italy. Fax: + 39.0881.740211; e-mail: patoveg.fgagr@isnet.it*

During a survey on diseases of stone fruits in southern Italy, several apricot (*Prunus armeniaca*) plants of cvs 'Tyrinthos' and 'Cafona' with leaf curl or witches' broom symptoms were encountered in provinces of Brindisi and Salerno in spring 1999. Some microscopic observations were accomplished to ascertain the identity of the micromycete infecting the above plants. Measurements of asci and ascospores from symptomatic apricot leaves showed that they were infected by a species of *Taphrina* very like if not identical to *T. armeniacae* whose eventual difference from *T. deformans* must be still demonstrated. So far apricot leaf curl presence was reported in several countries (China, Japan, Australia, India, Romania, Bulgaria, Argentina, ex Soviet Union, ex Jugoslavia, Italy). In Italy, the disease was found in 1999 on plants of cvs 'Buttianese' and 'Tyrinthos' growing in an experimental plot under plastic protection in Campania. Therefore its new finding in the open field in Campania and Apulia, probably due to the atmospheric conditions of spring 1999 characterized by frequent rains, seems worth mentioning.

EYESPOT IN WINTER CEREALS AND CROP SYSTEMS. **Gloria Innocenti*, M. Montanari, A. Marenghi and G. Toderi.** **Dipartimento di Protezione Valorizzazione Agroalimentare, Università di Bologna, Via F. Re ,8 I-40126 Bologna, Italy. Fax:+39.051.2091414, e-mail: innocent@agrsci.unibo.it*

Eyespot caused by *Ramulispora* (*Pseudocercosporella*) *herpotrichoides* (Fron.) v. Arx, anamorph of *Tapesia yallundae* Wallwork and Spooner, is one of the most important components of the foot and root disease complex of winter cereals in temperate countries. Symptoms are eye-shaped, pale lesions with brown margin on basal leaf sheaths and culms. Severely infected stems with one or several lesions may lodge and break. In our country eyespot does not play usuallly an important role in the foot disease complex of cereals, however recently a higher incidence than in the past has been observed in some wheat growing areas of northen Italy. Studies carried out in other countries on the influence of cultivation treatments on the disease have produced conflicting results. The effect on the eyespot incidence of three tillage systems (minimum tillage, 25 and 50 cm ploughing) and different crop rotations (continous wheat, barley, triticale and rotations with spring crops or fallow) was studied in field experiments carried out at the Bologna University farm in 1999. Data obtained indicate that the disease was greatly influenced by tillage techniques and crop rotations. The frequency of eyespot symptoms was significantly lower in ploughed plots than in minimum tilled plots. Wheat was more susceptible than barley and triticale and continous cereals resulted more favourable to eyespot than the rotation with spring crops.

***BOTRYTIS CINEREA* INOCULUM DYNAMICS IN STRAWBERRY FIELD.** **Luca Languasco*, P. Galletta, S. Giosuè and V. Rossi.** **Istituto di Entomologia e Patologia Vegetale, U.C.S.C., Via E. Parmense 84, I-29100 Piacenza, Italy. Fax: +39.0523.599256; e-mail: patolo@pc.unicatt.it*

In vitro and *in vivo* studies were carried out on the dynamics of *Botrytis cinerea* inoculum on strawberries, with particular reference to the rate of spore production and dispersal. The rate of sporulation on strawberry leaves at several temperatures was determined *in vitro*, as well as the effect of rainfall, while the dynamics of spore dispersal was studied *in vivo*, by simulating different rain events. The optimum for sporulation was about 20°C, in agreement with what has been reported in bibliography, with significant spore production also on dry leaves. A non-linear model was fitted to the experimental data showing the relationship between temperature, time of incubation and rate of sporulation. Simulated rains caused detaching of the conidia from the leaf surface, without compromising the possibility of further sporulation at the same seat, which started in a few hours and progressed at an unchanged rate. In the *in vivo* trial, rainfall was the only meteorological parameter correlated in a significant way to the number of trapped conidia; it was responsible for spore dispersal from the sporulating surfaces on the floor to the strawberry flowers. An exponential model fitted the relationship between rain intensity and the number of trapped conidia accurately.

APPLE POSTHARVEST DECAY CONTROL WITH *AUREOBASIDIUM PULLULANS* AND INDUCTION OF DEFENCE RESPONSES. **Antonio Ippolito*, C.L. Wilson and A. El Ghaouth.** **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: antonio.ippolito@agr.uniba.it*

Knowledge of the mode of action of postharvest biocontrol agents is still meagre. Studies on pathogen, antagonist and host interactions at the site of infection may facilitate the development of biocontrol strategies. The activity of *Aureobasidium pullulans* (de Bary) Arnaud, isolate L47, on apple fruit decay caused by *Botrytis cinerea* Pers. and *Penicillium expansum* Link and its ability to induce biochemical defence responses in apple tissue were investigated. In apple wounds *A. pullulans* multiplied rapidly and controlled decay caused by either *B. cinerea* or *P. expansum*. At the end of the storage period, *A. pullulans* reduced the incidence of apple grey and blue mould by 89 and 67%, respectively, compared to the water-treated control. In addition to controlling decay, *A. pullulans* caused a transient increase in host β-1,3-glucanase, chitinase and peroxidase activities starting after 24 h and reaching maximum levels 48 and 96 h after treatment. An increase in β-1,3-glucanase, chitinase and peroxidase activity was also observed in water treated control, however, the increase was markedly lower than that detected in *A. pullulans*-treated fruit. The ability of *A. pullulans* to induce β-1,3-glucanase, chitinase and peroxidase accumulation in addition to its known capacity to outcompete the pathogen for nutrients and space may be at the basis of its biocontrol activity.

VARIABILITY OF POPULATIONS OF *FUSARIUM OXYSPORUM* F.SP. *MELONIS*. **Luca Languasco*, S. Giosuè and V. Rossi.** **Istituto di Entomologia e Patologia Vegetale, U.C.S.C., Via E. Parmense 84, I-29100 Piacenza, Italy. Fax: +39.0523.599256; e-mail: patolo@pc.unicatt.it*

A study was undertaken to investigate the variability among isolates of *Fusarium oxysporum* f.sp. *melonis* in their pathogenetic relationship with the host. 36 *Fom* isolates were submitted to pathogenicity tests to determine the race to which they belonged and the disease incidence, using a set of differential genotypes and the susceptible 'Supermarket', respectively. For each isolate, a non-linear regression was estimated according to the logistic model and, afterwards, the day on which disease incidence reached 10, 50 and 90% of affected plants (Di) was calculated. To obtain groups of homogeneous isolates for the development of disease incidence, a cluster analysis was performed using D10, D50 and D90 as separating variables. To find the probability that an isolate belonged to the group assigned by the cluster analysis, a discriminant analysis was performed, which, moreover, allowed us to identify the most important variables in the separation of isolates. The mean progress of disease incidence in each cluster of isolates was then calculated as a logistic regression analysis. Finally, the degree of similarity between the two clusters of *Fom* isolates (the former based on the physiological races, the latter on the disease incidence) was evaluated with the Pearson similarity correlation coefficient. Significant differences exist among the *Fom* isolates studied, as regards their rapidity in causing disease symptoms in susceptible hosts, and a significant degree of similarity does not exist between the two clusters of isolates.

BIOLOGICAL AND MOLECULAR CHARACTERISTICS OF A VIROID RESPONSIBLE FOR CITRUS DWARFING. **Rosa La Rosa*, G. Albanese and M. Tessitori.** **Dipartimento di Scienze e Tecnologie Fitosanitarie, Sez. Patologia Vegetale, Università di Catania, Via Val di Savoia 5, I-95123 Catania, Italy. Fax: +39.095.350043; e-mail: larosa@mbox.fagr.unict.it*

Since many years citrus viroids, especially those belonging to group III (CVd-III), are considered a very promising tool for containing citrus tree size so as to obtain high density plantings. In 1983, a viroid (isolate CMC) was found in a clementine plant grafted on alemow rootstock that induced mild epinasty and size reduction in Etrog citron. These symptoms were later shown to be characteristic of the CVd-III group. This viroid isolate, inoculated to different citrus species grafted on citrange and trifoliata rootstocks, causes tree size reduction of variable extent without other apparent alterations. A preliminary molecular characterization of CMC showed that the viroid RNA is *ca* 293 nucleotide in size, is not homologous to citrus exocortis viroid (CEVd) or to group II citrus viroids (CVd-II), but belongs to the CVd-III group. Recently, CMC cloning and sequencing demonstrated that this molecule is 290 nucleotide in size and shows extensive intramolecular complementary base pairing (71%) conferring the typical rod-like secondary structure of other viroids. Since CMC has the central sequence which is conserved in the apple scar skin viroid (ASSVd) group, it can be assigned to this group of which is the smallest member. CMC shows a high sequence identity (98%) with CVdIIIA, a citrus viroid isolated and sequenced in Australia, suggesting that they are variants of the same species.

CHARACTERIZATION BY RAPD-PCR OF YEASTS AND YEAST-LIKE FUNGI POTENTIAL ANTAGONISTS OF POSTHARVEST PATHOGENS. **Giuseppe Lima*, S. Pacifico, F. De Curtis, R. Castoria and V. De Cicco.** **Dipartimento di Scienze Animali, Vegetali e dell'Ambiente, Università del Molise, Via De Sanctis, I-86100 Campobasso, Italy. Fax: +39.0874.404678; e-mail: lima@hpsrv.unimol.it*

Biological control of postharvest disease of fruits and vegetables by antagonistic microorganisms appears as a promising strategy to replace or integrate synthetic fungicides. Yeasts, including yeast-like fungi, are considered the most suitable microorganisms for postharvest use. In our laboratory researches on the selection of yeasts from aerial parts of different crops growing in southern Italy are in progress. Among the most frequently isolated microrganisms *Aureobasidium pullulans*, *Metschnikowia pulcherrima, Rhodotorula* spp., *Cryptococcus* spp. and *Candida* spp. showed a higher antagonistic activity against postharvest pathogens. However, to optimize the selection and utilization of these antagonists we need to find suitable techniques both for their characterization and monitoring. In this regard, the use of PCR (polimerase chain reaction)-based techniques represents a reliable and time-saving methodology. The aim of this paper was to characterize by RAPD (random amplified polymorphic DNA)-PCR several isolates of the yeasts more frequently detected on fruits and vegetables. DNA from these organisms was amplified using arbitrary oligonucleotide primers. Some of the tested primers generated electrophoretic profiles showing different degrees of genetic polymorphism among species and strains. This genetic diversity represents a useful pre-requisite to develop suitable tools for rapid identification and monitoring of the potential antagonists living on fruits and vegetables.

FURTHER EVIDENCE ON THE ROLE OF SYRINGOMYCINS AND SYRINGOPEPTINS IN THE VIRULENCE OF *PSEUDOMONAS SYRINGAE* PV. *SYRINGAE*. **M. Latorraca, Nicola Sante Iacobellis* and A. De Stradis.** **Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali Università della Basilicata,Via N. Sauro 85, I-85100 Potenza, Italy. Fax: +39.0971.55748; e-mail: iacobellis@unibas.it*

The majority of *Pseudomonas syringae* pv. *syringae* strains produce syringomycins and syringopeptins which are involved in plant tissue necrosis. According to *in vitro* observations, this is due to the alteration of plant cell membranes. To analyse the role of these toxins in pathogenesis, lilac and pear leaves and pear plantlets were inoculated with strains of *P. syringae* pv. *syringae* with different toxin phenotypes. Assays on pear plantlets confirmed that these toxins contributed to bacterial virulence by about 70%. Lilac leaf tissues, observed with TEM 72 hours after inoculation, showed significant alterations of cell ultrastructures which were similar in toxigenic strains and in strains that do not produce toxins. The most relevant ultrastructural changes were observed on plasma membranes. In particular, detachment of the plasmalemma from the walls was apparent in most mesophyl and bundle sheath tissue cells. The effects were of different intensity and in many cases interposition of vesicles and/or electron dense materials were also observed. Significant effects were also seen on other cell ultrastructures. The results confirmed the role of toxins in *P. syringae* pv. *syringae* virulence as well as the importance of another unknown bacterial factor(s). On the basis of the TEM observations, the above factor(s) seems to interact with toxins causing alterations in plant cell membranes.

PRELIMINARY RESULTS ON THE CHARACTERIZATION OF *PSEUDOMONAS 'REACTANS'*. **P. Lo Cantore and Nicola Sante Iacobellis*.** **Dipartimento di Biologia, Difesa e Biotecnologie Agro-Forestali, Università della Basilicata, Via N. Sauro 85, I-85100 Potenza, Italy. Fax: +39.0971.55748 e-mail: iacobellis@unibas.it*

Recent investigations have shown that *Pseudomonas 'reactans',* along with *P. tolaasii* and *Pseudomonas* spp., is responsible for brown and yellow blotch diseases on *Agaricus bisporus* and *Pleurotus ostreatus*, and for yellowing on *P. eryngii*. The finding that *P. 'reactans'*, an unclassified bacterial entity considered a saprophytic form associated with cultivated mushrooms, is also a pathogen of this crop, prompted us to characterise it in comparison with *P. tolaasii*. Our preliminary results are reported here. All the *P. tolaasii* and *P. 'reactans'* isolates had the nutritional pattern already reported for the two bacterial entities. Pathogenicity assays showed that *P. tolaasii* isolates were generally more virulent than those of *P. 'reactans'*. In the antagonistic plate assays, isolates of *P. tolaasii* inhibited the growth of *Bacillus megaterium* and *Rhodotorula pilimanae* and *P. 'reactans'* that of *B. megaterium*. When grown alongside on agar media (white line assay), all isolates of *P. tolaasii* and *P. 'reactans'* formed precipitates. In the last three assays a strong variability was observed among the isolates of both bacteria. This might be due to phenotypic variants which appear to lack the above characters in almost all *P. 'reactans'* and *P. tolaasii* cultures. These results indicate that *P. 'reactans'* is a bacterial entity distinct from *P. tolaasii*, although further studies are necessary for a full characterisation.

PRELIMINARY INVESTIGATION OF HRPZ GENE PRESENCE IN *PSEUDOMONAS AVELLANAE* AND IN A BACTERIUM INDUCING HR ON TOBACCO. Stefania Loreti*, S. Sarrocco and A. Gallelli. **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*

The *Pseudomonas syringae* pv. *syringae* hrpZ gene ($hrpZ_{Pss}$) encodes a harpin that can elicit an HR (hypersensitive response) -like response when infiltrated into tobacco leaf and may have a role in virulence, although the biological role remains elusive. The aim of this study was to investigate the presence of heterologous hrpZ genes in two strains of *P. avellanae* (ISPaVe-B-011 and ISPaVe-B-596) coming from different Italian regions and in *P. syringae* pv. *syringae* (ISPaVe-B-14a) isolated from hazelnut. *P. avellanae* strains have been studied by other authors, who have highlighted differential characteristics. With PCR specific primers from $hrpZ_{Pss}$ amplified, a fragment corresponding in size to the hrpZ gene, from ISPave-B-14a genomic DNA. This PCR product hybridised with the $hrpZ_{Pss}$ cRNA probe and was cloned and sequenced revealing a similarity of 96-97% with $hrpZ_{Pss}$ genes. Moreover, the genomic DNA of the three strains, digested with restriction endonucleases, was hybridised with the $hrpZ_{Pss}$ cRNA probe. ISPaVe-B-14a and ISPaVe-B-596 gave strong hybridisation signals. These results confirm hrpZ- ISPaVe-B-14a homology with $hrpZ_{Pss}$ genes and suggest the presence of a heterologous gene in *P. avellanae* ISPaVe-B-596. PCR, using degenerated primers, amplified a fragment of the presumptive gene in all strains; these products were of the expected size and hybridised with $hrpZ_{Pss}$ cRNA probe. These PCR product are currently being cloned and sequenced to obtain specific probes for the *P. avellanae* hrpZ.

COMPONENTS OF PARTIAL RESISTANCE TO *PYRENOCHAETA LYCOPERSICI* IN PROCESSING TOMATO. Luisa M. Manici*, M. Di Candilo and F. Caputo. **Istituto Sperimentale per le Colture Industriali, Via di Corticella 133, I-40129 Bologna, Italy. Fax: +39.051.374857; e-mail: istsci4@iperbole.bologna.it*

Pyrenochaeta lycopersici Schneider and Gerlach, agent of corky root in tomato *(Lycopersicon esculentum*), causes crop losses in southern Italy, in particularly Campania, in the greenhouse and also in the open field. The disease control strategies, other than genetic resistance, are soil fumigation or solarisation, economically unacceptable in the open field. A study of the *P. lycopersici* partial resistance components was carried out on some old tomato genotypes, an accession of *L. hirsutum* and two breeding lines partially resistant to *P. lycopersici*. F1, F2 and F3 progenies, obtained from resistant parents of two processing tomato varieties (UC82 and Cannery Row) were also evaluated. The study was done in the greenhouse, on artificially infected soil with two infection levels. Assessment of resistance, at the early growth stages of tomato plants, was evaluated on the basis of the following parameters : (*i*) root rot with a scale of 1-5; (*ii*) stunted growth as compared to healthy control; (*iii*) ability to produce secondary roots after necrosis of main root. The role of different partial resistance components can vary according to the soil infection level. F1, F2 and F3 progenies from UC82 crosses with two old resistant tomato genotypes ('Rezzano' and 'Victorio') showed a good resistance, maintaining levels not significantly different from that of resistant parents.

VARIATION OF *PYTHIUM* SPP. IN STRAWBERRY FIELD SOIL ACCORDING TO THE SOIL MANAGEMENT. Luisa M. Manici*, G. Baruzzi and L. Lazzeri. **Istituto Sperimentale per le Colture Industriali, Via di Corticella 133, I-40129 Bologna, Italy. Fax: +39.051.374857; e-mail: istsci4@iperbole.bologna.it*

Pythium spp., one of the most widespread strawberry root pathogens, is almost always present in non-fumigated soils of the most important Po valley strawberry cultivation areas (Cesena, Italy). This area is characterised by clay-loam soil that, under wet conditions, is optimum for *Pythium* growth. A two-year survey of variations in *Pythium* and micoflora in the soil was carried out in two non-fumigated strawberry fields to compare the effect of three different soil management techniques before transplanting (burley green manure, no tillage and fumigation with methyl bromide). The main results of this research were: (*i*) *Pythium* spp. (mainly *P. ultimum* Trow var. *ultimum*) was always present in soil. It increased in August and September (after trasplanting) and in early spring, while it decreased in winter and late spring-early summer; (*ii*) micoflora increased during summer, decreased in winter and then began to increase in the early spring; (*iii*) *Pythium* and micoflora varied according to the soil management techniques adopted before strawberry transplanting: soil fumigation strongly reduced both *Pythium* and micoflora; in the first year, *Pythium* increased with green manure as compared to no tillage, while in the second year, *Pythium* did not differ in a significant way with the green manure treatment or no tillage; there was always an increase in micoflora with green manure as compared to no tillage.

EPIDEMIOLOGICAL ASPECTS OF *MONILINIA LAXA* ON STONE FRUITS. PRELIMINARY FINDINGS. Marta Mari*, G. Dato, L. Casalini and G.C. Pratella. **C.R.I.O.F., Università di Bologna, Via F. Re 8, I-40126 Bologna, Italy. Fax: +39.051.765049; e-mail: mari@agrsci.unibo.it*

Brown rot caused by *Monilinia laxa* (Aderh. and Ruhl.) Honey is one of the most important diseases found on all commercially grown *Prunus* species in Italy. The objective of the present study was to elucidate the effect of fruit maturity on the susceptibility to brown rot of K2 peach cultivar. Fruit samples were harvested at weekly intervals and taken to the laboratory for inoculation beginning from 19 weeks before harvest (shuck fall) and continuing until full ripeness. At each sampling date, 50 fruits were inoculated with a pathogen conidial suspension (10^3 conidia ml^{-1}), stored at 20°C for 7 days and evaluated as percentage of infected fruits. Results have shown three stages of different susceptibility to *M. laxa* infections: the first stage characterized by rapid increase of fruit susceptibility, the second stage where it decreased and during which the pit (pericarp) hardened, finally the third stage was a period of rapid growth of the flesh near maturity and usually began 2 or 3 weeks before full ripeness. In that stage, the fruits increased their susceptibility to *M. laxa*. Similarly the same results were obtained on apricots (cv. 'Tyrinthos'). Determination of tannin content in peaches showed a positive correlation with susceptibility to brown rot.

MOLECULAR ASSAYS TO DIAGNOSE AND DIFFERENTIATE THE AGENTS OF CYPRESS CANKER. **Salvatore Moricca* and P. Raddi.** **CNR, Istituto per la Patologia degli Alberi Forestali, Piazzale delle Cascine 28, I-50144 Firenze, Italy. Fax: +39.055.354786; e-mail: moricca@ipaf.fi.cnr.it*

In addition to *Seiridium cardinale*, another two virulent canker pathogens belonging to the genus *Seiridium*, *S. cupressi* and *S. unicorne*, represent a grave danger for the survival of cypress in the Mediterranean. Absolute priority must be assigned to devising molecular assays for: (*i*) early diagnosis of latent *S. cardinale* infections; (*ii*) prompt differentiation between the three pathogens. The techniques of PCR and SSCP (Single Strand Conformation Polymorphism) were therefore applied, using isolates of *S. cardinale*, *S. cupressi*, *S. unicorne* and *Pestalotiopsis funerea*. Amplification and sequencing of the ITS region of rDNA allowed construction of 2 primers specific for *S. cardinale*, denominated Seir 4 and Seir 7. PCR-primers detected *S. cardinale* DNA but not DNA from congeneric species or from *P. funerea*, whether from pure mycelial DNA or from infected tissue with or without disease symptoms. In the SSCP analysis the rDNA ITS2 region was amplified with primers ITS3 and ITS4. The resulting products were denatured, electrophoresed on a polyacrilamide vertical gel and visualized by silver staining. Subtle sequence variations produced differences in the conformation of single strands. The different mobility in the gel allowed discrimination between *S. cardinale, S. cupressi* and *S. unicorne*. The *Seiridium species* were also clearly differentiated from *P. funerea*. The PCR-based diagnosis made it possible to specifically and effectively detect *S. cardinale* DNA from various sources. The SSCP-method was found to be a rapid and accurate tool to distinguish among related species.

OZONE DISTRIBUTION IN TUSCANY (ITALY) AND ITS IMPACT ON PLANT PRODUCTIVITY. **Cristina Nali* and C. Pucciariello.** **Dipartimento di Coltivazione e Difesa delle Specie Legnose, Sez. Patologia Vegetale, Università di Pisa, Via del Borghetto 80, I-56124 Pisa, Italy. Fax: +39.050.960622; e-mail: cnali@agr.unipi.it*

Eighty-thousand hourly mean ozone concentrations were recorded by ten automatic analysers in the districts of Florence, Pisa, Lucca and Prato, from May to September, 1995 to 1997. The highest daily mean concentrations were reached in Florence, with a maximum hourly average of 197 ppb. In Lucca and Pisa the peaks were close to 100 ppb. Data from Prato were much lower. Long-term critical levels for vegetation, as set by UNECE (United Nations Economic Commission for Europe), were constantly exceeded in Florence and Pisa, occasionally in Lucca, never in Prato. The results were used to fit exposure/yield response relationships proposed by UNECE and U.S. National Crop Loss Assessment Network for some important crops. The estimated yield losses varied in Florence from 8% for corn and alfalfa to 27% for soybean, in Pisa from 5% for corn to 24% for soybean, in Lucca from 3% for corn to 17% for soybean. A preliminary economic estimate for corn, wheat, barley, soybean, tomato and alfalfa, calculated annual damage to be 9 billion ITL in Florence, 1 billion ITL in Lucca and 3 billion ITL in Pisa. The picture must be regarded as only partial, as dose/response functions for important Italian crops (such as grapevine and vegetables) are not available.

***CLADOSPORIUM TENUISSIMUM* ANTAGONISM TO *CRONARTIUM FLACCIDUM* AND *PERIDERMIUM PINI* AND ITS POTENTIAL EPIDEMIOLOGICAL SIGNIFICANCE.** **Salvatore Moricca* and A. Ragazzi.** **CNR, Istituto per la Patologia degli Alberi Forestali, Piazzale delle Cascine 28, I-50144 Firenze, Italy. Fax: +39.055.354786; e-mail: moricca@ipaf.fi.cnr.it*

European isolates of the mycoparasite *Cladosporium tenuissimum* were tested to determine (*i*) capacity to inhibit the germination of *Cronartium flaccidum* and *Peridermium pini* spores *in vitro*; (*ii*) to ascertain whether there was a true nutritional relation between *C. tenuissimum* and the biotrophs; and (*iii*) to assess the efficacy of *C. tenuissimum* in controlling or reducing the incidence of pine rust *in planta.* A variety of tests confirmed the antagonism of *C. tenuissimum* to the rust fungi and revealed a number of aggression mechanisms. The mycoparasite strongly reduced germination of aeciospores on solid medium and aeciospore vitality at different temperatures over time. The culture filtrate inhibited germination, suggesting the secretion of toxic metabolites. Examination under the light microscope and with SEM revealed that parasitisation of rust spores was common and that the antagonist evolved a variety of strategies to destroy the host–cell wall and invade the cell, also confirming that enzymatic action was involved in the disease process. *C. tenuissimum* also strongly reduced disease development under controlled conditions in pine seedlings that had been infected with *C. flaccidum* the year before. The antagonist properties both *in vitro* and *in planta* show that *C. tenuissimum* merits serious consideration as an agent in the biological control of blister Pine rust.

***IN VITRO* BEHAVIOUR OF TOBACCO GERMPLASM WITH MIXED INFECTIONS OF CUCUMBER MOSAIC VIRUS AND POTATO VIRUS Y^N.** **Alessandra Panattoni* and E. Triolo.** **Dipartimento di Coltivazione e Difesa delle Specie Legnose, Sez. Patologia Vegetale, Università di Pisa, Via del Borghetto 80, I-56124 Pisa, Italy. Fax +39.050.960092; e-mail: apanatto@agr.unipi.it*

In necrotic phenomena caused to tobacco crops by mixed infections of cucumber mosaic virus (CMV) and potato virus Y necrotic strain (PVY^N), the possible involvement was hypothesized of two distinct CMV satellite RNAs characterized by a different electrophoretic mobility ('light' and 'heavy'). Healthy and infected explants of both *Nicotiana tabacum* Xanthi and *N. benthamiana* (PVY^N + CMV + CMV satRNA 'light'; PVY^N + CMV + CMV satRNA 'heavy'; CMV + CMV satRNA 'light'; PVY^N) were grown *in vitro* to investigate the possible active involvement of the satellite molecules in the virulence of the above co-infection. *In vitro* culture proved suitable for maintaining indefinitely all infectious combinations, as shown by immunoenzimatic tests and CMV ds-RNA electrophoretic patterns. Differences in growth enabled to distinguish healthy explants from infected explants regardless of the viral combination. The presence of one or the other satRNA did not have any apparente effect on the severity of symptoms *in vitro* culture. However, since no necrotic reaction was shown by the explants, our experimental conditions may not have triggered the mechanisms underlying the pathogenetic process expressed under natural conditions.

OCCURRENCE OF TOMATO SPOTTED WILT VIRUS IN PLANTS AND THRIPS COLLECTED IN APULIA (SOUTHERN ITALY). M. Papanice, V. Lasorella, M.M. Finetti Sialer, A. Di Geronimo, P. Sumerano, A. Di Franco, A. Guario, C. Vovlas and Donato Gallitelli*. **Dipartimento di Protezione delle Piante dalle Malattie, 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: gallitel@agr.uniba.it*

Tomato spotted wilt virus (TSWV) and other plant viruses have emerged as the most serious threat to the economic viability of the farming systems of the Brindisi province (Apulia, southern Italy). The necessity for a better understanding of the epidemiology of these viruses prompted an extensive survey over a period of one year. Within the first five months, more than 2400 plants belonging to 31 species and 6500 thrips were collected and analysed by Digoxigenin-labeled riboprobes. TSWV was detected in artichoke, chicory, pepper, tomato, all of which showing symptoms, and in the following weeds (percent of infection in parentheses) none of which was symptomatic: *Amaranthus retroflexus* (20%), *Calendula officinalis* (2.3%), *Capsella bursa-pastoris* (21%), *Chenopodium foetidum* (4%), *Convolvulus* spp. (15%) *Diplotaxis erucoides* (36%), *Fumaria officinalis* (3%), *Malva parviflora* (7.5%), *Oxalis acetosella* (3.7%), *Papaver rhoeas* (15%), *Portulaca oleracea* (7.6%), *Ranunculus* spp. (2.5%) *Senecio vulgaris* (4%), *Solanum nigrum* (4%) *Sonchus oleraceous* (8%), *Stellaria media* (2.8%), *Trifolium* spp. (2.7%), *Veronica* spp. (1.5%). The virus was recorded in *Frankliniella occidentalis* (30.5%), *Thrips tabaci* (47%), and in other undetermined thrips species (34%) and larvae (6%).

DYNAMICS OF *CRYPHONECTRIA PARASITICA* POPULATIONS IN CALABRIA. Alfio Maria Pennisi*, M. Prigoliti and P. Cortesi. **Dipartimento di Agrochimica e Agrobiologia, Università di Reggio Calabria, Piazza S. Francesco di Sales 2, I-89061 Gallina (Reggio Calabria), Italy. Fax: +39.0965.689049; e-mail: ampennisi@unirc.it*

Isolates of C*ryphonoctria parasitica* were sampled in Calabria (Italy) in 1990 and 1995 from the same chestnut (*Castanea sativa*) coppice forests. Four vegetative compatibility (vc) types, EU-1, EU-2, EU-10 and EU-12 were identified in both years; 80% of all isolates were in two vc types, EU-2 and EU-12, and no difference in vc type frequency was observed between years. The frequency of isolates with white phenotype increased from 25% in 1990 to 39% in 1995, and one or more white isolates were in all vc types. The frequency of hypovirus transmission among the virus-infected and virus-free isolates ranged between 0 and 100%, and was negatively correlated to the number of *vic* alleles different between the two isolates paired. Two mating type alleles, *MAT*-1 and *MAT*-2, at a single locus control sexual compatibility in *C. parasitica*. In the 1990 sample, all isolates had the *MAT*-2 allele, whereas in the 1995 sample both mating types were found; 4% of the isolates had the *MAT*-1 allele. Although 5 *vic* loci were polymorphic in both samples, thus giving the potential of 32 vc types, only 4 vc types were found. Absence of one *MAT* allele or skewed mating type ratio could have prevented sexual reproduction and recombination among *vic* alleles, which could have increased vc type diversity. The limited number of vc types is probably due to a combination of founder effects, restricted gene flow and limited recombination.

BIODIVERSITY OF ITALIAN AND FRENCH STRAINS OF ALFALFA MOSAIC VIRUS AND THEIR DIFFERENTIATION BY RT-PCR RFLP. Giuseppe Parrella*, C. La Nave, G. Marchoux, M.M. Finetti Sialer, A. Di Franco and D. Gallitelli. **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: csvvgp06@area.ba.cnr.it*

Alfalfa mosaic virus (AMV), infects naturally more than 400 plant species, including several vegetable and woody crops. The nucleotide sequence of the coat protein of seven previously uncharacterized AMV strains from Italy and France was determined and aligned with comparable sequences of other AMV strains to infer phylogenetic relationships. The topology of the trees obtained showed that all AMV strains clustered in two monophyletic groups, denoted II and I. Clustering of Italian strains in subgroup I and of French strains in subgroup II seems to suggests a driving effect of geographic distribution on evolutionary dynamics of these AMV strains. This separation did not correlate with differences in host range but, rather, it reflected variations in the amino acid sequence which might be related to structural properties of virus particles. A simple and rapid procedure based on reverse transcriptase-polymerase chain reaction (RT-PCR) followed by enzymatic digestion (RFLP) was developed to identify and classify AMV isolates into the two subgroups.

RELATIONSHIP BETWEEN CONTAMINATION MOMENT OF KIWIFRUIT PEDICEL SCAR AT HARVEST TIME AND BOTRYTIS ROT IN COLD STORAGE. Ilaria Pertot* and L. Perin. **Istituto Agrario di San Michele all'Adige, Dipartimento Produzione Agricola, Via Mach 2, I-38010 San Michele all'Adige (Trento), Italy. Fax:+39.0461.650872; e-mail: Ilaria.Pertot@ismaa.it*

Stem-end rot caused by *Botrytis cinerea* is the major cause of losses during cold storage of kiwifruit. It has been suggested that, during harvest, *B. cinerea* propagules present on the fruit surface are transferred to the picking wounds and, when the conditions are conducive, they can infect fruit. Aim of this study was to determine when infection occurs. At different lengths of time after being harvested, the fruits were sealed with paraffin wax to prevent infection. Most of infections took place immediately after the fruits were harvested and no differences were observed between the number of infections on unsealed fruits and those sealed after 6 days. Effects of the presence of water on the pedicel scar and delays in post-harvest treatment on incidence of *B. cinerea* storage rots in kiwifruit were also investigated. It is possible to delay post-harvest treatments for 48 hours without observing a reduction in its efficacy. Curing is not influenced by fruit wetness. Topical post-harvest treatment on picking wounds with vinclozolin has the same efficacy as dipping treatment, but there are benefits of residue reduction.

EPIDEMIOLOGY OF WHEAT HEAD SCAB. Andrea Ravanetti*, E. Pattori, L. Languasco, S. Giosuè and V. Rossi. **Istituto di Patologia Vegetale, U.C.S.C., Via Parmense 84, I-29100 Piacenza, Italy. Fax: +39 .0523.599256; e-mail: patolo@pc.unicatt.it*

Several experiments on the ecology and epidemiology of the main pathogens causing wheat head scab (*Fusarium graminearum* group 2, *F. culmorum*, *F. avenaceum* and *Microdochium nivale*) were performed. The following stages of the infection cycle were considered: spore yield, dispersal and germination, colony growth and infection. The effect of temperature (in the range 5-35°C), wetness (wet or not) and relative humidity (in the range 100-65%) on the different infection stages – with the exception of spore dispersal – was analysed by *in vitro* experiments. Minimum, maximum and optimal conditions were determined for each fungus and for each factor considered. Mathematical equations showing the relationships between environmental conditions and the rate of pathogen development were elaborated by a non-linear regression procedure: they showed a good agreement with experimental data. Spore dispersal was studied under natural conditions by placing spore traps inside the wheat canopy during a three-year period. Environmental conditions favouring the dispersal of conidia from the basal part of the plant to the head were determined, paying particular attention to rainfall ocurrence, duration and intensity. The information and mathematical equations will be used to elaborate a dynamic model simulating the risk of head scab infection on wheat.

PREHARVEST CHITOSAN TREATMENTS FOR THE CONTROL OF POSTHARVEST DECAY OF SWEET CHERRIES AND TABLE GRAPES. Gianfranco Romanazzi*, L. Schena, F. Nigro and A. Ippolito. **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: gianfranco.romanazzi@agr.uniba.it*

Grey mould, due to *Botrytis cinerea*, is responsible for severe losses of table grapes and sweet cherries, the latter also attacked by *Monilia laxa*, causal agent of brown rot. Chitosan, an animal derived polymer, is reported to be effective in controlling postharvest rot. The results of 2 year preharvest application, at 0.1, 0.5 and 1.0%, on sweet cherries cv. 'Ferrovia' and table grapes cv. 'Italia', are reported. Chitosan activity was compared with an untreated control as well as with fungicides (tebuconazole for sweet cherries; procymidone for table grapes). After harvest, fruits were stored at 0±1°C, followed by 4-7 days shelf-life at 20±1°C. Chitosan-treated sweet cherries showed a significant reduction in infected fruits, compared to the control. The best results (-70%) were obtained at 1%; at this concentration the reduction was also higher or no different from tebuconazole, for grey mould and brown rot, respectively. Table grapes sprayed with 1% chitosan showed a significantly lower grey mould disease index than the control. In the second year, significant activity was also observed with chitosan at low concentrations (0.1 and 0.5%) and with procymidone. Results from preliminary assays showed that 1% chitosan reduces the *in vitro* growth of *B. cinerea* and induced PAL activity in table grape skin. Therefore, the observed reduction in rot could be due to direct activity against the pathogen and to induced resistance.

ACTIVITY OF GLYCOL CHITOSAN ON POSTHARVEST STRAWBERRY ROT. Gianfranco Romanazzi*, A. Ippolito and F. Nigro. **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: gianfranco.romanazzi@agr.uniba.it*

Grey mould, caused by *Botrytis cinerea*, and Rhizopus rot, caused by *Rhizopus stolonifer*, are the most common rots found in stored strawberries. Glycol chitosan, a water soluble polymer, was applied at 0.2% on strawberries (cv. 'Clea') both as a preharvest spray, at three vegetative stages (full bloom, green fruit and whitening fruit), and as a postharvest treatment (by dipping strawberries inoculated or not with *B. cinerea*). Untreated fruits were used as a controls as well as, for preharvest treatments, strawberry plants sprayed with procymidone (at full bloom and green fruit stages) and pyrimethanil (at whitening fruit stage). Strawberries were stored at 3±1°C for 7 days, followed by 7 days shelf-life. Preharvest treatments induced a significant reduction in *B. cinerea* infections as compared to the untreated control; the highest disease reduction was found with strawberries treated at full bloom and whitening fruit stages. However, strawberries treated with fungicides showed the lowest percentage of infected fruits. Postharvest treatments with glycol chitosan reduced the number of rotted fruits, both naturally and artificially infected. This is the first report on the use of glycol chitosan as preharvest treatment on fruits and vegetables to reduce postharvest decay. It seems worth investigating further the possible modes of action and the feasibility of applying it alone or in combination with other biocontrol agents.

RESISTANCE COMPONENTS TO *CERCOSPORA BETICOLA* IN SUGARBEET: EPIDEMIOLOGICAL MEANING. Vittorio Rossi*, P. Battilani, L. Languasco, G. Chiusa, S. Giosuè and P. Racca. **Istituto di Patologia Vegetale, U.C.S.C., Via E. Parmense 84, I-29100 Piacenza, Italy. Fax: +39.523.599256; e-mail: patolo@pc.unicatt.it*

Cercospora leaf spot resistance in sugarbeet is a rate-reducing resistance (rRR), which reduces the rate of epidemic progress. It could be due to a reduced effectiveness of spores in causing infection, to fewer spores being produced per lesion (due to both reduced sporulation capability and reduced lesion size), to a lengthened latent period, and to a shortened infectious period. Therefore, rRR is the result of different resistance components (RCs). To determine the RCs involved in the sugarbeet/*C. beticola* relationship, infection efficiency, incubation length, lesion size, conidiation length, and spore yield were measured in four beet genotypes showing different resistance ratings, by experiments with single infection cycles. All RCs were effective, excluding the conidiation length; infection efficiency, incubation length and spore yield were the most important; they showed a wide variability, being reduced by up to 1/5 compared to susceptible genotypes. Furthermore, their magnitude changed with genotype. To simulate the effect of resistance on the chain of infection cycles, RCs were integrated in a model simulating disease progress over time. Different epidemiological conditions were simulated. RCs reduced disease progress in proportion to their magnitude; when all components were improved by the same amount, they had about the same effectiveness in slowing epidemics. Changing more components simultaneously reduced disease development more than additively.

EPIDEMIOLOGICAL SURVEY ON FOOT ROT OF WINTER CEREALS. **Alberto Santori*, L. Corazza and V. Balmas.** **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*

Foot rot is an important disease of winter cereals, in Italy, durum wheat being particularly susceptible. Foot rot is a complex disease, in which several fungi, saprobes and/or pathogenic, are involved. Furthermore their frequence resulted variable in the different growth stages, *e.g. Fusarium culmorum* was generally more frequent after stem exstension while *Bipolaris sorokiniana* and *Microdochium nivale* were more frequently isolated during tillering and stem exstension. An epidemiological survey was carried out in 1998-99 on durum and bread wheat cultivars grown in 13 localities distributed in northern, central and southern Italy and, in two localities, in Sardinia. Oats and triticale samples, with the typical symptoms, were collected in 5 fields in northern and central Italy, while samples of barley were collected also in southern Italy. *F. culmorum* was constantly isolated from durum wheat grown in central and southern Italy and in Sardinia as well. From barley, triticale and oats, even though less damaged, the most important pathogen causal agents of crown and foot rot, were isolated; *F. culmorum* was frequent on barley, which is also susceptible to *B. sorokiniana*. *F. graminearum* Group 2 *sensu* Francis and Burgess affected triticale grown after maize. Oats, even though less damaged as compared to the other winter cereals, resulted suscettible to the most important causal agents of foot and crown rot (*F. culmorum*, *B. sorokiniana*, *G. graminis* var. *avenae*).

PREPARATION OF MOLECULAR TOOLS TO MONITOR THE L47 STRAIN OF *AUREOBASIDIUM PULLULANS*. **Leonardo Schena*, M.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: leonardo.schena@agr.uniba.it*

The strain L47 of *Aureobasidium pullulans* (de Bary) Arnaud shows antagonistic activity against a number of agents of postharvest diseases of fruits. Field release of this strains on fruits surfaces requires a specific method of monitoring in order to evaluate level of colonisation and dispersal in the environment. RAPD (Random Amplified Polymorphic DNA) was used for a preliminary analysis of the genetic variability of *A. pullulans* and to select amplicons with different specificity. Random amplification of two hundred isolates with primer OpC8 (Operon Technologies, Inc. U.S.A.) allowed selection of a 1.3 Kb fragment (L4) which was present only in two isolates, including L47, and of a second fragment of 1.1 Kb which was uniformly distributed in all isolates. In Southern blot analysis, digoxigenin-labelled L4 amplicon targeted specifically the strain L47 among a number of isolates of *A. pullulans*, whereas amplicon L1 hybridised with the corresponding fragment in all isolates. Both L4 and L1 fragments were ligated to pGEM-T plasmid, cloned in *Escherichia coli*, strains DH5a, and their nucleotide sequence determined. Two SCAR (Sequence Characterised Amplified Regions) primers were synthesised on the basis of L4 sequence. These primers proved highly specific for the L47 strain allowing its accurate identification among other isolates of *A. pullulans*. The possibility to monitor the strain L47 in nature with the molecular tools now available is under way.

POWDER FORMULATION OF *STREPTOMYCES* SP. FOR USE IN AGRICULTURE. **Marco Saracchi* and S. Quaroni**. **Istituto di Patologia Vegetale, Università di Milano e CNR, Centro di Studio per il Miglioramento Sanitario delle Colture Agrarie, Via Celoria 2, I-20133 Milano, Italy. Fax: +39.02.23691122; e-mail: marco.saracchi@unimi.it*

In recent years the use of micro-organisms in agriculture to increase crop production has received considerable attention, especially their formulation and the possibility of large-scale production. The strain IPV-2733, *Streptomyces* sp., used in this research, is a potential biocontrol agent, characterised by wide range of activity against root phytopathogenic fungi as well as plant growth promotion. Early formulation studies examined spore water suspension and peat compost. Recently, clay powder formulations were tested. Streptomycete colonies, grown on solid oatmeal medium, were lyophilised without removing the culture medium and finely ground, to obtain a final concentration of 10^{10} cfu g^{-1}. The powder was then mixed with some different clay carriers to produce the final formulates, containing 10^7 cfu g^{-1}. Bentonite was the most suitable mineral matrix for formulate multipurpose use. Spore viability, tested after nine months storage in airtight containers at room temperature, remained above 95%, decreasing by less than 7% during following months, so that viable spore concentration passed from 10^7 to 10^5 cfu g^{-1}. Microbiological analysis of stored formulate confirmed the lack of contamination in sealed packages. The formulate was assessed to be compatible with the usual agriculture practices, especially the application methods as a dry powder (*i.e.* seed and soil bacterial inoculation) and as a wettable one (*i.e.* root, tuber and bulb dipping).

SPATIAL DISTRIBUTION OF ESCA-DISEASED GRAPEVINE PLANTS IN FIVE VINEYARDS IN TUSCANY (ITALY). **Giuseppe Surico*, G. Marchi, P. Braccini and L. Mugnai.** **Istituto di Patologia e Zoologia Forestale e Agraria, Università di Firenze, Piazzale delle Cascine 28, I-50144 Firenze, Italy. Fax. +39.055.354786; e-mail: surico@ipaf.fi.cnr.it.*

Five vineyards in Tuscany (SCF1 at S. Casciano val di Pesa; GTF1 at Gambassi Terme; CBS1, 2 and 3 at Castelnuovo Berardenga) were examined for Esca over periods of 3 (SCF1), 4 (CBS1, 2 and 3) or 6 years (GTF1). A high level of discontinuity in the symptom expression of each diseased plant was observed from year to year. The overall disease incidence, calculated by counting all plants presenting symptoms at least once during the entire 3 to 6 year test period, was 85.4% at S. Casciano, 50.1% at Gambassi Terme and an average of 13% for the 3 vineyards at Castelnuovo Berardenga. Analysis of the field data by three indices of dispersion (Lloyd's index of patchiness, variance-to-mean ratio and Morisita's index), doublet and ordinary runs tests showed occasional aggregation of diseased vines only in vineyards with a higher disease incidence (SGF1 and CBS3). On the other hand results of Ferrandino's 2DCORR analysis indicated a significant spatial correlation of infected-infected plant pairs in the GTF1 vineyard which extended to a distance of 10 plants in all directions. In the remaining 3 vineyards (SCF1 was excluded from the statistical analyses because of its high disease incidence) a random pattern was consistently found. On the whole, the results obtained suggest that in the vineyard examined esca was spread by airborne spores from distant and/or internal sources rather than by contaminated pruning tools along the vine columns.

MOLECULAR CHARACTERIZATION OF ISOLATES OF *PHAEOACREMONIUM* SPP. FROM ESCA DISEASED GRAPEVINES. Stefania Tegli*, E. Bertelli, E. Santilli and G. Surico. **Istituto di Patologia e Zoologia Forestale e Agraria, Università di Firenze, Piazzale delle Cascine 28, I-50144 Firenze, Italy. Fax: +39.055.354786; e-mail:tegli@ipaf.fi.cnr.it*

Genetic diversity of the species *Phaeoacremonium aleophilum* (*Pal*) and *P. chlamydosporum* (*Pch*) was evaluated on 15 *Pal* and 28 *Pch* isolates from Esca-diseased grapevines in various Italian regions. Strains from the Centraalbureau voor Schimmelcultures (CBS), Baarn, NL (1 *Pal*, 2 *Pch*, 2 *P. angustius,* 2 *P. inflatipes* and 1 *P. rubrigenum*) were tested for comparison. RAPD- and RAMS-PCR were used to measure genetic variation between and within species. No relationships were found between variation and geographic origin of the isolates. A higher level of polymorphism was found with RAPD-PCR, while the level of intraspecific diversity was low with RAMS-PCR, in both species. RAMS-profiles distinguished *Pal* from *Pch*, and both these species from other fungi commonly present in the wood of grapevines. Analysis of the Internal Transcribed Spacers (ITS) of rDNA was performed. The ITS region of the five *Phaeoacremonium* species tested was amplified using the universal primers ITS5 and ITS4. A single band of approximately 620 bp was obtained with all species. Sequencing of the ITS region enabled species-specific primers to be designed. Primer pairs Pal1N-Pal2 and Pch1-Pch2 were able to detect *Pal* and *Pch* genomic DNA, with the amplification of specific fragments of 400 and 360 bp respectively. The sensitivity threshold was 10 pg fungal DNA. The same results were obtained when grapevine wood from plants artificially inoculated with *Pal* and/or *Pch* was examined.

DETECTION OF *BISCOGNIAUXIA MEDITERRANEA* IN ASYMPTOMATIC TISSUES OF *QUERCUS CERRIS* BY SPECIES-SPECIFIC PRIMERS. A. Vannini*, A. Mazzaglia, A. Gasbarri and N. Anselmi. **Dipartimento di Protezione delle Piante, Università della Tuscia di Viterbo, Via S. Camillo de Lellis, I-01100 Viterbo, Italy. Fax +39.761.357473; e-mail: vannini@unitus.it*

Biscogniauxia (= *Hypoxylon*) *mediterranea*, a weakness parasite involved in the oak decline syndrome in Mediterranean area, is able to live in latent phase for long periods in the host tissues before spreading. In order to study properly the habitus of this fungus during the latent phase, the present work aims to set up an early diagnostic technique to detect and quantify the presence of *B. mediterranea* directly in the host tissues. The ITS1-5,8S-ITS2 regions of rDNA of 21 isolates *B. mediterranea* and related species were sequenced and aligned. Conspicuous inter-specific differences in sequence permitted the design of two primers, named MED1 and MED2, able to selectively amplify *B. mediterranea* rDNA. In repeated experiments these primers always amplified the expected product from *B. mediterranea* DNA, but never amplified other fungal species or host DNA's. By this technique *B. mediterranea* was successfully detected in host tissues following total DNA extraction. Pathogen presence in host tissues was always confirmed by *in vitro* isolation. The correspondence between products obtained from naturally infected host tissues and *B. mediterranea* DNA's was confirmed by Southern hybridisation. These results represent a promising starting point for further studies on aspects of *B. mediterranea* life cycle connected with its latent phase, on its localisation in host tissues and on the shift from endophytic to parasitic habitus.

***PSEUDOMONAS SAVASTANOI* POPULATION VARIABILITY IN A SINGLE OLIVE GROVE. Stefania Tegli*, N. Menditto and G. Surico.** **Istituto di Patologia e Zoologia Forestale e Agraria, Università di Firenze, Piazzale delle Cascine 28, I-50144 Firenze, Italy. Fax:+39.055.354786; e-mail: tegli@ipaf.fi.cnr.it*

Fifty-eight isolates of *Pseudomonas savastanoi* were collected in 1998 from a single olive grove located at Bagno a Ripoli (Florence), each isolate from a different knot. Knots were collected from 6 plants randomly distributed in the grove. For comparison, 25 isolates were obtained from plants in neighbouring groves, located on the four cardinal points of the test grove. All isolates were tested for pathogenicity on olive and oleander. Other characteristics examined were: colony morphology on nutrient-sucrose-agar; production of indolacetic acid (IAA), levan, fluorescent pigments and bacteriocins; plasmid and Rep-PCR profiles. Almost all the 58 isolates induced knots on olive but not on oleander plants. They were levan-positive and fluorescent-negative, unlike the usual *P. savastanoi* phenotype. They produced bacteriocins and IAA (from 3.03 to 26.99 mg l^{-1} of culture filtrate). There was a great variaion in their plasmid profiles, regardless of plant origin. Some plasmids (6, 8, 63 and 75 kb) were however represented in all the isolates. ARDRA (Amplified Ribosomal DNA Restriction Analysis) confirmed that all the isolates belonged to *P. savastanoi*. Rep-PCR experiments showed identical profiles for all the isolates from Bagno a Ripoli and for the olive isolates from international collections as well. Comparison with isolates from the neighbouring olive groves showed that the *P. savastanoi* population examined did not spread over long distances.

EPIDEMIOLOGY OF *PLASMOPARA VITICOLA*. Annamaria Vercesi* and D. Liberati. **Istituto di Patologia, Università di Milano, Via Celoria 2, I-20133 Milano, Italy. Fax: +39.02.70631287.*

Seventeen epidemics of *Plasmopara viticola* on leaves were described by means of the Gompertz model, with a rate of disease increase varying as a function of temperature, rain and relative humidity. Epidemic data were collected in untreated vineyards located in Oltrepò Pavese and in Veneto, from the appearance of the first symptoms until the end of July. The rate of disease increase is modulated by rain: each rain causes the beginning of a new infection cycle whose entity is proportional to the rain square root. The appearance of symptoms is distributed along a Gauss curve, centered around the mean incubation period, calculated according to Goidanich and coworkers. Two parameters, y_0, the initial amount of disease, and pot, the infectiveness of inoculum issued from oospores, were calculated on the basis of the Gompertz curves. Pot greatly influences the epidemic progress, since its value contributes to the definition of the curve width.

SPREADING OF PLUM POX VIRUS STRAIN M IN THE SARCA VALLEY. Maria Elisabetta Vindimian*, M. Da Vià, P. Miorelli, M. Chemolli and F. Dalpiaz. ** Istituto Agrario San Michele all'Adige, Via E. Mach 1, I-38010 San Michele all'Adige (Trento), Italy.*

Plum pox virus (PPV), the causal agent of Sharka disease, was first found in Trentino in 1975 and is now present in all plum growing areas of this province. In 1985 an eradication plan was initiated in the Sarca valley, the most important production area of the typical plum cv. 'Dro' (*Prunus domestica)*, but the virus was not eradicated. In the same area peach trees are rare and seldom grown in specialized orchards. Isolates of PPV fall in four groups, which can be differentiated epidemiologically, molecularly, and serologically, *i.e.*, PPV-D, infecting mainly plum and apricot, PPV-M, infecting mainly peach, PPV-C, recorded from sour and sweet cherry, and PPV-EA, found in Egypt. PPV-D, PPV-M and PPV-C strains are present in Italy, whereas only PPV-D and PPV-M are kown to occur in the Trentino plum growing areas. In this study 109 PPV isolates from plum (104) and peach (5), the latter coming from a single orchard, were tested for strain typing by DASI-ELISA, using the universal monoclonal antibody Mab5B and monoclonal antibodies specific to serotype PPV-M (MabAL). These tests showed that 14 isolates recovered mostly from young plum plants and spreading in the northern part of the valley, belong to strain PPV-M. Only two of the peach isolates were classified as PPV-M. Since PPV-M appears to be as virulent as PPV-D, it is important to continue the disease management plan.

DNA POLYMORPHISM AND PATHOGENICITY OF *FUSARIUM SEMITECTUM* ISOLATED FROM ALFALFA. M. Zaccardelli, M. Carelli, C. Scotti, A. Santori and Luciana Corazza*. **Istituto Sperimentale per la Patologia Vegetale, Mi.P.A., Via C.G. Bertero 22, I-00156 Roma, Italy. E-mail: mc_ispave@www.inea.it.*

Fusarium semitectum is a polyphagous fungus widespread in cultivated and uncultivated soils in tropical and temperate areas. Most frequently, *F. semitectum* is pathogenic in association with other *Fusarium* spp. on seedlings and adult plants of several crops. In July and October 1998, from 4 alfalfa fields in the Po Valley (Italy), some plants, with chlorosis and wilting symptoms were collected, and isolation on PDA and Komada substrates were performed. From monosporic cultures, 25 *F. semitectum*, 4 *F. avenaceum*, 2 *F. equiseti* and 1 *F. proliferatum* were identified on CLA. All these strains and other 18 *Fusarium* spp. strains, were characterized for DNA polymorphism by mini and microsatellite amplification and by AFLP technique. The *F. semitectum* were distributed in 4 of 15 groups with 5'-GAGGGTGGCGGTTCT-3' primer designed on minisatellite sequence of the M13 phage; in 4 of 16 groups with microsatellite primer (GACA)4; in 6 of 15 groups with (GTG)5 primer. With AFLP analysis, most of the *F. semitectum* strains were distributed in two cluster. Preliminary pathogenicity tests performed with *F. semitectum* strains, gave weak disease symptoms. With the *F. proliferatum* strain isolated from alfalfa, severe symptoms of chlorosis and wilting were observed. Probably, the pathogenicity of *F. semitectum* is sinergic with *F. proliferatum* on senescent plants in old alfalfa fields (3-4 years) like those in this study.

**SUSCEPTIBILITY AND RESISTANCE OF ITALIAN ALFALFA ECOTYPES TO *FUSARIUM* SPP. AND *VERTICILLIUM* SPP. Massimo Zaccardelli*, M. Carelli, S. Gnocchi, L. Ferrari, P. Gaudenzi and C. Scotti. ****Istituto Sperimentale per le Colture Foraggere Mi.P.A., Viale Piacenza 29, I-20900 Lodi, Italy. E-mail:iscfbiol@telware.it.*

Fusarium oxysporum f.sp. *medicaginis, Verticillium alboatrum* and *V. dahliae* are the main fungi responsible for alfalfa wilting. Growing resistant varieties of alfalfa is extremely important. In this work, 30 accessions of alfalfa from 10 Italian ecotypes were studied for resistance to *Fusarium* and *Verticillium*. For each accession, 64 plants, grown on an inert substratum in a climatic chamber, were inoculated by immersion of roots in a suspension of *Fusarium* spp. or *Verticillium* spp. (3 x 106 spores ml^{-1}). Control plants were treated with sterile distilled water. After 8 days, an index of infection was attributed to every plant according to the following scale: 1 = no symptoms or minimum chlorosis of basal leaves; 2 = chlorosis of basal and median leaves; 3 = chlorosis of all the leaves (with or without leaf wilt). The survey was performed respectively on 5280 plants for *Fusarium* test and 5040 for *Verticillium* test. The statistical analysis, pointed out a rather continuous variability in response to the inoculum among the ecotypes tested. The ecotypes 'Sicilia', 'Friulana di Premariacco' and 'Campania' showed resistance to *Fusarium*; one accession respectively of 'Polesana', 'Cremonese' and 'Romagnola' were susceptible. The most resistant accessions to *Verticillium* resulted two from 'Romagnola' and one from 'Cremonese' and 'Tipica Basso Friuli' respectively; the most susceptible were 'Leonicena' and one accession of 'Romagnola'. These results suggest that sources of resistance can be found, at different frequency, in all the italian alfalfa ecotypes.

**MOLECULAR CHARACTERIZATION OF *FUSARIUM SOLANI* FROM POTATO. Massimo Zaccardelli*, S. Vitale, L. Luongo and L. Corazza. ****Istituto Sperimentale per le Colture Industriali, Mi.P.A,. Strada Statale no. 18 156, I-84091 Battipaglia (Salerno), Italy. E-mail:iscibattipaglia@sintesi.net.*

Potato dry rot is one of the most widespread storage diseases of the tubers. *Fusarium solani* var. *coeruleum* is the most important causal agent, followed by *F. sambucinum*, *F. avenaceum* and *F. solani*. The distinction between *F. solani* var. *coeruleum* and *F. solani*, is based on morphological and cultural characters often not easily interpreted. In this study, 5 strains of *F. solani* var. *coeruleum* and 11 strains of *F. solani*, isolated from potato and other material, were molecularly characterized. By the minisatellite primer 5'-GAGGGTGGCGGTTCT-3' of the M 13 phage and the microsatellite primers (GACA)4 and (GTG)5, the *F. solani* var. *coeruleum* strains were distributed in two distinguishable groups with respect to the other *F. solani* strains. The same results were obtained by AFLP technique. Only one strain, isolated from chickpea and identified as *F. solani*, was included in one of the two groups of *F. solani* var. *coeruleum*. An incorrect identification of *F. solani* var. *coeruleum* is possible, because some strains potentially lost the characteristic pigmentation. Further investigations on more strains are in progress, to confirm the reliability of the molecular characterization of *F. solani* var. *coeruleum*.

OBSERVATIONS ON A NEW BACTERIAL DISEASE OF *ANTHURIUM* IN ITALY. **Astolfo Zoina*, A. Raio and A. Spasiano.** **Dipartimento di Arboricoltura, Botanica e Patologia Vegetale, Università di Napoli "Federico II", Via Università 100, I-80055 Portici (Napoli), Italy. E-mail: zoina@unina.it*

Xanthomonas axonopodis pv. *dieffenbachiae* (XAD) was isolated from stems and leaves of wilted *Anthurium* plants cultivated in a greenhouse farm in Pompei (Italy). Tropical and Laguna were the most heavily affected cvs (80-100%) among the six *Anthurium* varieties grown on the farm. Seven bacterial isolates were obtained from different diseased plants. They showed very similar biochemical and physiological characteristics and were all identified with the Biolog ML1 System as *X. campestris* pv. *dieffenbachiae* (synonym of XAD). Moreover, all the isolates induced HR in tobacco leaves and when inoculated in *Anthurium* and *Dieffenbachia* plants, they induced bacterial blight disease symptoms. This is the first report of XAD in a European country apart from the Netherlands, the most important producer and distributor of *Anthurium* plant material in Europe. Bacterial infections and disease spread are strongly affected by environmental conditions and cultivation methods. A strict observation of prophylactic measures, the use of indexed plantlets, the stringent control of temperature and humidity in the greenhouses, the careful manipulation of plants during leaf and flower cutting at harvest, the disinfection of tools and operator hands, the cultivation of plants in pots rather than in troughs can be very helpful in reducing the incidence of the disease.