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ABSTRACTS

Abstracts of invited lectures, oral and poster presentations given at the 15th Hellenic Phytopathological Congress, Corfu, Greece, 5–8 October 2010

The 15th National Phytopathological Congress, organized every two years by the Hellenic Phytopathological Society (HPS), was held in Corfu, on October 5–8, 2010. The meeting was attended by more than 450 participants. 5 invited lectures, 53 oral presentations and 54 posters were presented dealing with plant diseases caused by fungi, bacteria, viruses and non-parasitic disorders and with the disease control. In addition, one round-table discussion was held on *"European research projects and phytopathology in practise"*. Abstracts of the invited papers, the oral presentations, and the posters of the congress are presented in this issue.

NEW DISEASES – ETIOLOGY

Oral Presentations

Invasion of the fungus *Ceratocystis platani* in Epirus: A potential threat of an environmental disaster in the natural ecosystems of plane trees. P. TSOPELAS* and N. SOULIOTI. *NAGREF-Institute of Mediterranean Forest Ecosystems, Terma Alkmanos, 11528 Athens, Greece. *E-mail: tsop@fria.gr*

Canker stain disease of plane trees, caused by the fungus *Ceratocystis platani*, is one of the most destructive forest tree diseases worldwide. *C. platani* is considered an indigenous species of North America that was introduced into Europe during WWII. In Greece, the pathogen was detected for the first time in 2003 in Messenia prefecture and gradually invaded the neighbouring prefectures of Ilia and Arcadia, while in 2009 it was found in the Achaia prefecture. In these areas of Peloponnese *C. platani* has already killed thousands of oriental plane (*Platanus orientalis*) trees of all ages and sizes and it is steadily spreading into new areas. In 2010, infection foci were detected for the first time in the region of Epirus, in NW Greece close to the border with Albania. There the disease was found in the area of Tyria in the Ioannina prefecture, close to the newly constructed highway "Egnatia", as well as along the Kalamas River in Thesprotia prefecture. The distance between the two areas is about 30 km. It is possible that more infection foci exist in the Epirus region and they have not been detected yet. From the number of infected trees, it is assumed that the pathogen has been spread into these areas in the last 4–5 years. Most likely, inoculum of *C. platani* was transferred to Epirus from Peloponnese with terracing machinery that was used in the construction of "Egnatia" or in some other construction site. The disease was locally spread in these areas by humans, involving construction works and other activities of municipalities and of other institutions. In both areas that the disease was found terracing machinery has been used. Furthermore, small infection foci initiated by contaminated tools used in pruning operations, especially along local roads, were observed. The main target of control measures should be the prevention of further spread of the disease into new areas through human activities. Herbicides can be used for the devitalisation of infected as well as healthy neighbouring trees in order to create a buffer zone and minimize the risk of transmission through the roots; trenches can also be created around infection foci in order to stop fungal spread by this pathway. If no control measures are taken soon, the pathogen has the potential to spread in natural ecosystems of oriental plane in Epirus and other areas of Greece as well as in neighbouring countries, causing a huge ecological disaster.

Shoot blight caused by *Botryosphaeria* spp. on apple trees in Mt. Pelion in Greece. I.C. RUMBOS. *Plant Pro-*

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tection Institute of Volos, National Agricultural Research Foundation, 380 01 Greece. E-mail: johnrumbos@yahoo.gr

Botryosphaeria canker on apple trees was recorded in Greece for the first time at the beginning of the decade 2000 in orchards of the Mt. Pelion to cause the death of scaffold limbs or even of whole trees. In the spring of 2010 an unusual outbreak of the fungus was recorded at the same apple growing area resulted to the death of young shoots mainly on the cv. Starking Delicious. Symptoms observed were confused by the growers with those caused by the pathogen of fire blight disease *Erwinia amylovora* (Burrill) Winslow. However, they can be easily differentiated by the numerous pycnidia of the fungus formed on the bark which receives a brown discoloration. The infection on the young shoots was usually correlated with pruning wounds. This new and unusual outbreak of the fungus causing extensive dead shoots could be attributed to the prolonged high temperatures occurred during May and June 2010 which favor the development of the fungus. Control strategies include use of water-based latex paints for protecting the pruning wounds, inoculum removal via pruning of infected shoots and use of chemicals applied against the apple scab fungus *Venturia inaequalis* (Cooke) Wint. which are referred to the literature to be effective for the control of *Botryosphaeria* fungus on pistachio trees.

Diversity of phytoplasma populations that cause small fruiting in the orchards of the Pilion mountain. A. RUMBOU[1*], I. ADAMOPOULOS[1], B. SCHNEIDER[2] and M. KUBE[3]. *[1]National Agricultural Research Foundation/ Plant Protection Institute, Fytokou str., 38001, Volos, Greece. [2]Julius Kühn Institute / Institute for Plant Protection in Fruit Crops and Viticulture, Schwabenheimer Str. 101, 69221, Dossenheim, Germany. [3]Max Planck Institute / Institute for Molecular Genetics, Ihnestr. 63-73, 14195, Berlin, Germany. *E-mail: amirumbou@yahoo.gr*

The small fruiting phenomenon in apple trees was observed a decade ago in the orchards of the Pilion Mountain and has already reached an epidemic level. The yield losses range from 20–40% in the lower elevations (≤ 400 m), 40–70% in the main production zone (400–600 m), and in the higher elevations, where apple orchards neighbour beech forest, the losses reach 70–100%. The majority of the symptomatic trees are grafted on seedling rootstock, are aged (35–50 years) and are mainly from cv. Starking Delicious. The phenomenon was related with the presence of *Candidatus* Phytoplasma mali, the causal agent of the "witches' broom" disease, which was detected with the PCR-RFLP diagnostic method in a high number of trees exhibiting small fruiting and in local cv. Firiki branches grafted on symptomatic 'Starking' trees. Phytoplasma strain diversity was investigated with the use of sequence analysis of fragments of the 16S rDNA region from root samples collected from different orchards on the mountain. Except from *Ca.* P. mali, also *Ca.* P. pyri, the causal agent of Pear decline, was identified. Furthermore, sequences that showed up to 90% similarity with phytoplasmas, but were not assigned as a known phytoplasma type, were isolated. These results constitute evidence for phytoplasma strain and species differentiation even in the limited area of one orchard.

Reasons of increase and consequences of Verticillium wilt in intensive olive groves and in promoted alternative cultures in our country. P.P. ANTONIOU*, D.J. TSITSIGIANNIS, S.E. TJAMOS, E.J. PAPLOMATAS and E.C. TJAMOS. *Laboratory of Plant Pathology, Department of Crop Science, Agricultural University of Athens, 75 Iera Odos, 118 55 Athens, Greece. *E-mail: ppantoniou@aua.gr*

Verticillium wilt of olive tree was considered as most serous and destructive disease of cultivation of olive trees in our country in regions where is cultivated the variety Amfissa. The progressive however replacement of Amfissa with the tolerant variety Kalamata and also the extension of sensitive variety Chalkidiki did not resolve the problem of the disease. Indeed the cultivation of Kalamata in fields that at the past had been cultivated with cotton or at the first stages of olive plantation were subcultured with cotton or other sensitive in Verticillium dahliae vegetables, created high inoculum levels so that the olive trees have shown severe symptoms. Regions as Etoloakarnania and Fthiotida present severe symptoms of the disease in Kalamata variety. However the prevalence in traditionally olive-growing regions was found important spread in the regions of Chalkidiki, Magnesia and Kalampaka in Kalamata Chalkidiki and Megaritiki varieties. More recent data report appearance of symptoms in olives in the regions of Western Macedonia and Thrace in the variety Chalkidiki. And here it appears that the source of the inoculum came from cotton fields from the period of extensive culture of cotton in those regions. Recent installations of olive orchards in dense or hyperdense planting schedule both in Etolia and in Ilia regions potentially could create respectively with the mentioned before problems. The plantations of sensitive variety Manzanillo in Ilia in former potato fields probably cause respectively problems. Finally the extension of culture of pomegranate in many regions of Macedonia and Thrace but also the new established alternative cultures of Stevia, wild artichoke and oil seed rape can cause new extensive spread of Verticillium wilt. of problem.

Poster Presentations

First report of *Botrytis cinerea, Penicillium glabrum, Aspergilus niger* var. *tubigensis* and *Pilidiella gra-*

***nati* as pre-and post-harvest fruit rot causal agents on pomegranate (*Punica granatum*) in Greece.** G.A. BARDAS, G.D. TZELEPIS, L. LOTOS and G.S. KARAOGLANIDIS*. *Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, POB 269, 54124, Thessaloniki, Greece. *E-mail: gkarao@agro.auth.gr*

During the last decade and after the recent discoveries of the high antioxidant content of pomegranate fruit and juice, the pomegranate industry is characterized by continuous increase. During September and October 2008, in the region of Larisa, pre- and post-harvest fruit rots were observed on pomegranate (cv. Kapmaditika) causing losses estimated to 10–20%. The identification of the isolated fungal colonies was based on macroscopic observations of fruit rot symptomatology, microscopic observations of fungal colonies' morphological characteristics and sequencing of the ITS region spanning ITS1, 5.8S, and ITS2 of the ribosomal DNA. *Botrytis cinerea* Pers.:Fr., *Penicillium glabrum* (Wehmer) Westling, *Aspergillus niger* var. *tubingensis* (Schober) Mosseray and *Pilidiella granati (Sacc.).* To fulfill Koch's postulates, pathogenicity of the isolated cultures was tested by wound-inoculating pomegranate fruits (cv. Kampaditika). Extensive decay, similar to that observed on diseased fruits in the field, was observed on the inoculated fruit, whereas control fruit showed no decay. Pathogens were re-isolated from the decayed tissues, but not from any of the non-inoculated control fruit. To the best of our knowledge, the above mentioned pathogens have not been reported previously in Greece on pomegranate fruit.

Bacterial rot of the plant *Zamioculcas zamiifolia*. A.P. NIKOU, C.X. ATZILAKIS, A.M. KASSELAKI and D.E. GOUMAS*. *Technological Educational Institute of Crete, School of Agriculture PO Box 1939, 71004 Heraklion, Crete, Greece. *E-mail: dgoumas@staff.teicrete.gr*

Zamioculcas zamiifolia is an ornamental potted plant of the Araceae family that was introduced in the market during the past few years. It is a wild, tropical, monocotyledonous, perennial, plant originating from east Africa countries. Recently, mature plants imported in Greece, showed symptoms resembling bacterial infection in the leaf petioles. Specifically, water soaked lesions appeared on the plant at ground level, as well as rotting, complete degradation and fall of the infected tissues accompanied with a bad odour. Similar symptoms were also observed in the rhizomes of the plants. It was estimated that 5% of the imported plants were infected. Under the optical microscope, bacteria were seen oozing from infected tissues. Bacteria were consistently isolated in pure culture from infected tissues while isolations remained free from other suspected known pathogens of *Zamioculcas zamiifolia* (e.g. *Phytophthora* spp.). Based on morphological, physiological, biochemical, serological profile and pathogenicity tests, the isolated bacteria were identified as member of *Pectobacterium carotovorum* subsp. *carotovorum*. To the best of our knowledge, this is the first report of the pathogen on *Zamioculcas zamiifolia* in Greece and Europe.

Ink disease of chestnut caused by *Phytophthora cryptogea*. C. PERLEROU[1*], G.T. TZIROS[1], A.M. VETTRAINO[2] and S. DIAMANDIS[1]. [1]*National Agricultural Research Foundation, Forest Research Institute, 570 06 Vassilika, Thessaloniki, Greece.* [2]*Dipartimento di Protezione delle Piante, Università degli Studi della Tuscia, Viterbo, Italy. *E-mail: perlerou@fri.gr*

Ink disease is one of the most destructive diseases of sweet chestnut (*Castanea sativa*) in Europe and it is caused mainly by two *Phytophthora* species. *P. cambivora* which has been reported from France, Italy and Greece and *P. cinnamomi* which occurs in England, France, Portugal and Spain. In November 2006, approximately 10% of 5–7 year-old chestnut trees grown in an orchard in the region of Larissa (Central Greece) showed typical symptoms of the disease. Symptoms included decline of the crown, dead leaves and burrs still remaining on the trees and dark necrosis at the collar with flame-shaped edges. A *Phytophthora* species was consistently isolated from collar lesions and soil under symptomatic trees and was identified as *P. cryptogea* Pethybr. & Lafferty based on morphological and cultural characteristics. The fungus was heterothallic (A2 mating type), appeared fluffy on PDA and did not grow at 35°C. Sporangia were oval to obpyriform, nonpapillate, persistent (32.5–57.5 × 25–35 μm), oogonia were plerotic, antheridia were amphigynous, and hyphal swellings produced in abundance. The ITS sequences of five isolates confirmed that they belong to the species *P. cryptogea*. Pathogenicity of three *P. cryptogea* isolates was checked using 3-month-old chestnut seedlings. Five weeks after inoculation all inoculated seedlings showed crown wilting, collar and root rot. Control plants remained healthy. *P. cryptogea* was easily reisolated from collar tissues and from soil of inoculated pots. This is the first report of ink disease of chestnut caused by *P. cryptogea* in our country.

First records of one genus and three species of wood-rotting basidiomycetes of the family Hymenochaetaceae Donk in Greece. E. POLEMIS[1,2], D.M. DIMOU[1], D. TZANOUDAKIS[2] and G.I. ZERVAKIS[1*]. [1]*Agricultural University of Athens, Department of Agricultural Biotechnology, Lab. of General & Agricultural Microbiology, Iera Odos 75, 11855 Athens, Greece.* [2]*University of Patras, Department of Biology, Panepistimioupoli, 26500 Rion, Greece. *E-mail: zervakis@aua.gr*

Members of the family Hymenochaetaceae (Basidiomycota, Agaricomycetes) are generally characterized by the production of brownish basidiomata that darken when moistened in alkali solutions and of clampless generative hyphae; it includes ca. 400 lignicolous species which grow as saprotrophs or biotrophs in a wide range of trees and shrubs. In addition, several species of this family are plant pathogens attacking the tree sapwood leading to the breaking of branches, weakening and fall of trees, and serious loss of timber. In the frame of a recent study on the diversity of basidiomycetes (subphylum Agaricomycotina) in Kiklades, the wood-rotting species *Inonotus cuticularis* (Bull.) P. Karst., *Phellinus erectus* A. David, Dequatre & Fiasson, *P. rosmarinii* Bernicchia and *Phylloporia ribis* (Schumach.) Ryvarden (family Hymenochaetaceae) were recorded for the first time in Greece. The recorded occurrence of *Phylloporia ribis* constitutes the first report of this genus; it was detected on living trees and shrubs of *Crataegus monogyna* and *Spartium junceum* in Andros and Naxos islands respectively. *Phellinus rosmarinii* and *P. erectus* are two related species, which were previously recorded only in west Mediterranean countries on macchia vegetation causing white-rot of the affected plant tissues; they were found on *Pistacia lentiscus* and *Quercus coccifera* in Amorgos and Naxos islands. *Inonotus cuticularis* causes also white-rot on forest plant species, and it was recorded on *Acer sempervirens* living tree trunk in Andros island.

Artificial inoculation and disease development of *Septoria pyricola* on pear fruit. M. CHATZIDIMOPOULOS, E.K. VELLIOS and A.C. PAPPAS*. *University of Thessaly, Department of Agriculture Crop Production and Rural Environment, 384 46 N. Ionia, Volos, Greece. *E-mail: acpappas@uth.gr*

Superficial sterile spots, commonly observed on pear fruits, were either attributed to no typical *Fusicladium* or *Septoria* infections. In spring of 2010, in order to investigate the causal agent of such spots, artificial inoculations with a suspension of 5×10^5 mL^{-1} pycnidiospores of *S. pyricola* were taken place at full bloom, petal fall (fruit set) and young fruit stage, of pears cv. Krystalli. Inoculated organs were covered with moist polyethylene bags for 48h. With the exception of the inoculation at the full bloom stage, superficial slightly sunken brown dotted spots, 2–4mm in diameter, without pycnidia, were formed on inoculated fruit, after 2–3 weeks of incubation. These spots were similar to those observed under natural conditions, in the field. Fruits inoculated with sterile water remained symptomless, under the conditions of the experiment. The fruit infection by *S. pyricola* was confirmed by the isolation of the pathogen on PDA media. For this, newly formed spots were excised, immersed in absolute alcohol, surface sterilized in 0.5% sodium hypochlorite for 3 min, rinsed three times in sterilized water, dried with sterilized filter paper and plated onto the medium. After 15 days of incubation the characteristic pycnidia of the pathogen were formed. Following Koch's postulates we shown out that infections by *S. pyricola* at early stage of fruit growth, can cause the development of superficial sterile spotting on pears.

Invited Lecture

Effector proteins of the tomato pathogen *Cladosporium fulvum* and their functional homologues in related Dothideomycete species. I. STERGIOPOULOS[1,2*], B. ***ÖKMEN***[1], H.A. VAN DEN BURG[1,2], H.G. BEENEN[1], G.H.J. KEMA[3], and P.J.G.M. DE WIT[1,2]. *[1]Laboratory of Phytopathology, Wageningen University & Research Centre, Droevendaalsesteeg 1, 6708PB, Wageningen, The Netherlands. [2]Centre for BioSystems Genomics, P.O. Box 98, 6700 AB Wageningen, The Netherlands. [3]Plant Research International BV, PO Box 16, 6700 AA, Wageningen, The Netherlands. *E-mail: ioannis.stergiopoulos@wur.nl*

Cladosporium fulvum is a biotrophic fungal pathogen of tomato that belongs to the class of Dothideomycetes. During infection, *C. fulvum* secretes effectors that function as virulence factors in the absence of cognate Cf resistance proteins and induce effector-triggered immunity in their presence. Ten effector proteins have been identified from this fungus including avirulence (Avrs: Avr2, Avr4, Avr4E and Avr9) and extracellular proteins (Ecps: Ecp1, Ecp2, Ecp4, Ecp5, Ecp6 and Ecp7). Although demonstrated for only a few, all Avrs and Ecps are assumed to be virulence factors. Recently we have identified for the first time, homologues of the *C. fulvum* Avr4, and Ecp2 effectors in Dothideomycetes, including *Mycosphaerella fijiensis, Mycosphaerella graminicola,* and several *Cercospora* species. We have demonstrated that *M. fijiensis* Avr4 is a functional orthologue of *C. fulvum* Avr4 that binds to chitin and strikingly also triggers a Cf-4-mediated hypersensitive response (HR) in tomato. Three homologues of Ecp2 were identified in *M. fijiensis,* one of which induces an HR in a *Cf-Ecp2* tomato line. Collectively, our data suggest that Avr4 and Ecp2 represent core effectors with conserved domains that are recognized by single cognate Cf proteins. The presence of homologous effectors in fungal pathogens that are collectively recognized by single resistance proteins provides novel strategies for disease resistance breeding, by transferring such resistance proteins into distantly related plant species.

Oral Presentations

The *1-aminocyclopropane-1-carboxylic acid synthase* (*ACS*) gene is involved in virulence of *Verticillium*

dahliae. M.A. TSOLAKIDOU[1], I.S. PANTELIDES[2], S.E. TJAMOS[1], E.J. PAPLOMATAS[1]* and K.F. DOBINSON[3]. *[1]Laboratory of Plant Pathology, Agricultural University of Athens, 75 Iera Odos, 11855 Athens, Greece. [2]Department of Agricultural Sciences, Biotechnology and Food Science, Cyprus University of Technology, CY-3603 Lemesos, Cyprus. [3]Department of Plant Pathology, The Pennsylvania State University, University Park, PA 16802, United States. *E-mail: epaplom@aua.gr*

Several studies have shown that some plant pathogenic fungi are able to produce ethylene. Three biosynthesis pathways leading to ethylene production are known in fungi, suggesting an important role of this compound among those microorganisms. The soil-borne fungi *Verticillium dahliae* is able to produce ethylene; however, it remains unclear whether the fungal ethylene produced *in planta* is required for the development of the pathogen or it acts as a virulence factor. In order to investigate the role of ethylene in *Verticillium dahliae* pathogenicity an *ACS* gene (encodes a key enzyme involved in ethylene biosynthesis) was inactivated in a *Verticillium* isolate through transposon mutagenesis. The inactivation of *ACS* was verified by Real-time PCR gene expression analysis. Pathogenicity experiments showed that the *ΔACS* mutants caused typical symptoms in tomato and eggplants; however, there was a statistically significant reduction in disease severity compared to that of the wild type strain. Overexpression of *ACS* in Δ*ACS* mutant restored the disease severity caused by the pathogen. Quantitative Real-time PCR analysis revealed that the decrease in symptom severity shown in tomato plants inoculated with *ΔACS* mutants was associated with significant reductions in the growth of the pathogen in the vascular tissues of the plants. The results of the present study suggest a role of *ACS* in virulence and vascular colonization of the soilborne fungus *V. dahliae*.

Host proteins interacting with the core replicase protein of *plum pox virus* (PPV) in the yeast two hybrid system. R. SÄGESSER[1], E. VOURVOUHAKI[1, 2], M. TABLER and M. TSAGRIS[1, 2*]. *[1]Institute of Molecular Biology and Biotechnology, Foundation of Research and Technology Hellas, P.O. Box 1389, 71110 Heraklion Greece. [2]Department of Biology, University of Crete, P.O. Box 2208 71409 Heraklio, Greece. *E-mail: tsagris@biology.uoc.gr*

Plum pox virus (PPV) is an important viral pathogen infecting plants of the genus *Prunus*, where it causes considerable yield reduction and reduces the quality of the fruits. PPV infects also a number of annual plants from different genera; one of them is the *Solanaceae Nicotiana benthamiana*. In this work, we have used the two hybrid system in yeast in order to identify host proteins from *Nicotiana benthamiana* which interact with the core replicase protein of this potyvirus, the protein Nib of PPV. The yeast two hybrid system takes advantage of the experimental host *Saccharomyces cerevisiae* (bakers yeast), an important single cell model organism. In the yeast two hybrid system, the modular architecture of transcriptional activators is used in order to measure the interaction of two hybrid proteins, which induce expression of a easily scorable reporter gene. Two hybrid proteins are expressed in yeast, one containing the bait protein (viral Nib in this case) and the DNA binding domain (DB) of a yeast transcriptional activator, and another containing one host protein (pray) and the activation domain (AD) of a yeast transcriptional activator. If the bait protein and the host protein (pray) interact physically in the yeast cell, the two parts DB and AD of the splitted yeast transcriptional activator are brought in close proximity and initiate expression of the scorable reporter gene. We have used a yeast two hybrid expression library of *Nicotiana benthamiana* proteins (a gift of Prof.J.Bol, Leiden University) and screened for interacting proteins with PPV Nib as a bait. For better evaluation of the results and verification of the interactions, we used three different reporter gene systems. Host proteins have been identified interacting with the full length Nib protein. Possible functions of the interacting protein in virus replication will be discussed.

Molecular studies for the identification of protein-protein interactions between *Pepino mosaic virus*-encoded proteins and host (tomato) proteins. M. MATHIOUDAKIS[1,4], R. VEIGA[1], M. GHITA[1], D. TSIKOU[1], V. MEDINA[2], T. CANTO[3], A. MAKRIS[1] and I. LIVIERATOS[1]*. *[1]Mediterranean Agronomic Institute of Chania, Alsylio Agrokepio, Chania 73100, Greece. [2]Departament de Producció Vegetal i Ciència Forestal, Universitat de Lleida, 25198 Lleida, Spain. [3]Centro de Investigaciones Biológicas (CSIC), 28040 Madrid, Spain. [4]Plant Pathology Laboratory, Faculty of Agriculture, Aristotle University of Thessaloniki, P.O. Box 269, Thessaloniki 54124, Greece. *E-mail: livieratos@maich.gr*

Pepino mosaic virus (PepMV) a member of the genus *Potexvirus*, firstly reported in Peru in pepino (1980) and later on in Europe (2000). PepMV is currently subject to Emergency EU legislation in order to prevent the introduction of PepMV into the EU from third countries (South America, Morocco) and also the spread of the virus within the EU with seed. PepMV genome is 6410 nt long, it is capped and adenylated at its 5′- and 3′-termini, respectively. It includes open reading frames that putatively encode for the viral RdRp (164kDa), three proteins (triple gene block proteins [TGB1, TGB2, TGB3; 26, 14, 9 kDa, respectively]), and the capsid protein (CP; 25kDa). We have been investigating PepMV-host system using tomato as a host to reveal protein-protein interactions. Using the yeast two hybrid system, *in vitro* and *in vivo* techniques, specific protein interactions

have been identified possibly shedding additional light into the complex mechanism of potexvirus replication.

The role of the RNA interference in the host-pathogen interaction. A. BOUTLA[1], N. VASSILAKOS[1], **N**. SKANDALIS[1], **E**. DADAMI[2], **O**. KEKTSIDOU[1] **M**. PAPACHRISTOPOULOU[1], K. KALANTIDIS[2] and C. VARVERI[1]*. [1]*Benaki Phytopathological Institute, Laboratory of Virology, 8 S. Delta str., 145 61 Kifissia, Attiki, Greece.* [2]*University of Crete and Institute of Molecular Biology and Biotechnology, Heraklion, Crete, Greece. *E-mail: c.varveri@bpi.gr*

RNA interference (RNAi) is considered to be a defense mechanism against invasive genetic material. In plants, in particular RNAi is the basic defense line against RNA viruses. Our main interest was to define the role of the RNAi in the host pathogen interactions by using technologies taking advantage of the RNAi mechanism itself. Transgenic plants were used in which the essential proteins of the RNAi machinery had been down regulated. Our goal was to examine how these mutants would react in a viral or bacterial infection. The RNAi mutant plants seemed to be more susceptible to viral infections than the wild-type plants. To further investigate the effect of the RNAi on the course of an infection transgenic plants that express viral silencing suppressors, which block different parts of the pathway such as sequestering the siRNAs, were used additionally. The results of the dynamics between the RNAi and the host-pathogen interactions will be discussed.

Phenolic responses of resistant and susceptible olive cultivars induced by defoliating and non defoliating *Verticillium dahliae* pathotypes. E.A. MARKAKIS[1], S.E. TJAMOS[1], P.P. ANTONIOU[1], P.A. ROUSSOS[2], E.J. PAPLOMATAS[1] and E.C. TJAMOS[1*]. [1]*Laboratory of Plant Pathology Agricultural University of Athens, Iera Odos 75, Athens 11855, Greece.* [2]*Laboratory of Pomology, Agricultural University of Athens, Iera Odos 75, Athens 11855, Greece. *E-mail:ect@aua.gr*

Verticillium wilt is the most serious olive disease worldwide. The olive infecting *V. dahliae* pathotypes have been classified as defoliating (D) and nondefoliating (ND), and the disease is mainly controlled in olive orchards by using resistant or tolerant cultivars. Limited information is available about the nature of resistance in most of the olive cultivars. In the present study, the phenolic responses of the susceptible to *V. dahliae* olive cultivar Amfissis and the resistant 'Koroneiki' upon D and ND *V. dahliae* infection were monitored in relation to the fungal DNA levels in the vascular tissues with the purpose to explore the defence mechanisms of olive trees against *V. dahliae*. Quantitative PCR revealed that the decrease in symptom severity shown in 'Koroneiki' trees was associated with significant reduction in the growth of both *V. dahliae* pathotypes in the vascular tissues, compared to 'Amfissis'. In 'Koroneiki' trees, the levels of *o*-diphenols and verbascoside were positively associated with the DNA levels of the D and ND pathotypes. In addition, a positive association was observed between the levels of verbascoside and the fungal DNA level in 'Amfissis' trees; whereas a negative association was revealed between the fungal DNA level and the total phenols and oleuropein content in both cultivars. The levels of verbascoside were clearly higher in 'Koroneiki' trees compared to 'Amfissis' trees, indicating for the first time in the literature the involvement of verbascoside in the defence mechanism of olive trees against *V. dahliae*.

The genetic and molecular role of Apoptosis Inducing Factor (AIF) proteins in activation of *Arabidopsis thaliana* innate immune system. S.D. KOUNTOURI[1], J.D.G. JONES[2] and D.I. TSITSIGIANNIS[1*]. [1]*Agricultural University of Athens, Department of Crop Science, Laboratory of Phytopathology Iera Odos 75, 118 55 Athens, Greece.* [2]*Sainsbury Laboratory, John Innes Centre, Norwich NR4 7UH, United Kingdom. *E-mail: dimtsi@aua.gr*

Plants, like all organisms, have developed an innate immune system that allows them to defeat infection from various pathogens. The first line of defense is to identify the invasive microorganisms and to produce various molecules in order to destroy them. The second line of defense is the programmed death of infected cells, thereby limiting the spread of infection. The programmed cell death (PCD) is a process that normally takes place during development and defense of multicellular organisms and it may be linked to serious diseases like cancer and degenerative diseases. Research in recent years has demonstrated the existence of common biochemical pathways of PCD among plant, animal and microbial cells but, unlike other organisms, this process in plants has not been well characterized. The aim of this study was to investigate a family of genes identified in the genetic model plant *Arabidopsis thaliana*, that have similarities with the mammalian *AIF* gene (Apoptosis Inducing Factor), a phylogenetically old, 57 kDa flavoprotein, which shares similarity to bacterial, fungus and plant oxidoreductases. AIF is associated with diseases related to increased apoptotic events such as infection with HIV, neurodegenerations, heart attacks. In *A. thaliana* five different putative Apoptosis Inducing Factor like proteins were identified and all five genes are expressed in the plant. T-DNA knock-out mutants *At-AIF-2*, *At-AIF-3* and *At-AIF-5* were characterized in Arabidopsis and the mutants were tested for whether they are compromised in HR and disease resistance against several pathogens. Pathogenicity experiments

of *aif* mutant lines with the soilborne pathogen *Verticillium dahliae* showed that *aif3* mutants had higher resistance to infection compared to *aif2* and *aif5* mutants that showed higher rates of disease compared to control plants. Infection experiments with the oomycete *Hyaloperonospora arabidopsidis* showed that *aif3* and *aif5* mutant lines were more susceptible while *aif2* more resistant than the wild type strain. Infection with the bacterium *Pseudomonas syringae* pv. *tomato* DC3000 showed no significant difference between the mutant genotypes. Finally, PCD experiments showed that genes *At-AIF3* and *At-AIF5* play an important role in the development of Hypersensitive Response. The present study showed for the first time that *At-AIF* genes play an important role in the activation of the plant immune system and the resistance or susceptibility to a number of important crop pathogens.

Poster Presentations

Susceptibility of tobacco stem and root tissues to pythium stem rot caused by *Pythium aphanidermatum.* D.F. ANTONOPOULOS* and A.L. MILA. *North Carolina State University, Department of Plant Pathology, Campus Box 7405, Raleigh, North Carolina, USA, 27695. *E-mail: antdim75@yahoo.com*

In North Carolina (NC), pythium stem rot in tobacco fields is caused by *Pythium aphanidermatum*. In the past 10 years, this disease has been reported more frequently than before. During the past 10 years, tobacco flue-cured cultivars carrying the *Php* gene that confirms immunity to race 0 of *Phytophthora parasitica var. nicotianae* (*Ppn*) have also been deployed in NC. It has been questioned whether the introduction of cultivars carrying this resistance gene contributes to the increasing incidence of pythium stem rot. We used a strain of *P. aphanidermatum* expressing b-glucuronidase (GUS) reporter gene to investigate the relative susceptibility of stem and root tissues of four flue-cured tobacco cultivars to pythium stem rot. NC71 and RJR15 were less infected by *P. aphanidermatum* comparing to K326 and K346. Furthermore, incidence was significantly different among different parts of the plant. That is, percentage of infected adventitious roots (first-order roots) was significantly higher than the percentage of true roots. High numbers of adventitious roots emerging at the stem base of tobacco cultivars in combination with the NC warm and cloudy summer conditions that occurred in the recent past years might contribute to pythium stem rot increased incidence in tobacco fields in NC, rather than using resistant cultivars to *Ppn*.

The relationship between aflatoxin B_1 contamination and dried figs' nutrition. V. DEMOPOULOS*, S. SOTIROPOULOS, C. PASCHALIDIS and M. ZOKOS. *TEI of Kalamata, 2410, Kalamata, Greece. *E-mail:vdimo@teikal.gr*

Figs of the "tsapelosika" variety were harvested in two different times at a ten- day interval and dried under the sun according to the cultivation practices of the Messinian region. The concentration of nutrient elements (N, P, K, Ca, Mg, Na, B, Fe, Mn, Zn and Cu) in dried figs and aflatoxin B_1 contamination as a result of natural infection by aflatoxigenic fungi were studied. The occurrence of aflatoxin B_1 was determined by ELISA method and the measurements were calculated according to the recovery which showed a linear increase from 102% for lower concentrations to 227% for the higher ones. The results showed the occurrence of aflatoxin B_1 in all samples tested but ranged at much lower levels than the safety limit of 2 $\mu g\ kg^{-1}$. Statistically no significant differences between the first and second harvest (average 0.62 ± 0.11 and $0.59 \pm 0.07\ \mu g\ kg^{-1}$ respectively) were observed. All dried fig nutrient contents matched the concentration levels of the reference values reported for the "calimyrna" (sarilop) variety. The concentrations of Ca, Mg, K and Cu in dried figs between the first and second harvest were observed to be significantly different. In spite of other published reports, there were no significant correlations between aflatoxin B_1 contamination and any of the nutrient elements studied in dried figs.

The effects of alloplasmatic of the two sorts of rise on the attack of *Fusarium oxysporum* f. sp. *Oryza.* M. PAPADOPOULOU. *Technological Educational Institute of Kalamata, 24100, Kalamata, Greece. E-mail: mashapapoulou@yahoo.gr*

In the laboratory of plant protection of the Institute for Rise on South Kazakstan we separated the use of resistant forms in order to control the Rise root rot disease that was caused by the fungi *Fusarium oxysporum* f. sp. *oryza*, Schlecht, Biali. In more detail, the effect of genes of cytoplasm on control of resistance of rise to the mentioned above pathogens has been studied. In the experiments eight sorts of rise were used and other two alloplasmatic series of theirs with replaced cytoplasm which were created by constant reciprocal crossing of the cytoplasm male –sterile plants, an African sort Indica – Gambiaca (Gam.) and wild sort of rise Oryza sativa f. spantanea (WA). After the plants were inoculated by a conidia suspension (10^6 propagule mL^{-1}) of the pathogen fungi, the durability of the sorts in field and laboratory conditions was tested. The number of the infected plants and the intensity of the infection were measured. The results showed that the genes of the cytoplasm have a peculiar effect on the genes of the core resulting in changing of the disease's intensity. Differences were observed between the sorts that had their own cytoplasm and their alloplasmatic series. In conclusion the replacement of the cytoplasm helps the reduce of the

intensity of the plant's disease and depends upon the degree of the plant's durability and the reactions of the core genes with the non core.

Cloning and functional analysis of the β-1,6-endoglucanase gene in *Verticillium dahlia*. L. EBOIGBE, A. TZIMA and M.A. TYPAS*. *Department of Genetics and Biotechnology, Faculty of Biology, University of Athens, Panepistimiopolis 15701, Athens, Greece. *E-mail:matypas@biol.uoa.gr*

Many fungi produce β-glucanases including the non-cellulolytic β-(1,3) and β-(1,6)- glucanases that degrade β-(1,3)- and β-(1,6)-glucans. In combination with other polymers they provide rigidity and strength to the fungal cell wall, protecting the fungus from adverse host responses and in the initial stages of infection by entrapping hydrolytic enzymes within the cell wall. These enzymes are usually released upon contact with the host plant, thus acting to depolymerize the host cell wall. Nucleotide sequence analysis of the 3′ and 5′ ends of clones from a genomic library of the fungus showed the presence of part of the endo-1,6-B-glucanase gene in a 3.5 kb genomic fragment. Using this clone as a probe and by employing genome walking approaches the 3′ and 5′ of the gene were determined bringing the entire gene (*bgn*1,6) size to approx.1.800 bp. An internal fragment (1.2 k.b) of *bgn1,6* was used to disrupt the wild-type gene of the tomato race2 *V.dahliae* strain 123V and the knock-out mutant strain was tested for pathogenicity on tomato plants and showed a 7.5% reduction in disease symptoms caused on tomato plants as compared to the wild type. Growth on minimal medium supplemented with different carbon sources showed reduced ability of the mutant to breakdown cellulose, whereas growth on glucose, pectin and sucrose was similar to the wild type. Since the cell wall of higher plants is not generally known to contain Beta-1,6-glycosidic linkages, the functional relationship between beta-1,6-endoglucanase and other cell wall degrading enzyme is discussed.

Role in pathogenesis of an endo-β-1,4-xylanase gene from the vascular wilt fungus *Verticillium dahliae*. L. EBOIGBE[1], A. TZIMA[1], E.J. PAPLOMATAS[2] and M.A. TYPAS[1]*. [1]*Department of Genetics & Biotechnology, Faculty of Biology, University of Athens, Panepistimiopolis 15701, Athens, Greece.* [2]*Laboratory of Plant pathology, Agricultural University of Athens, Iera Odos 75, Athens 11855, Greece. *E-mail: matypas@biol.uoa.gr*

Plant pathogenic fungi produce extracellular enzymes which degrade plant cell components in a coordinated action. Functional redundancy is a major drawback in elucidating the role of cell wall degrading enzymes (CWDE). Nevertheless, among different CWDE studied, endo-β-1,4-xylanases have been shown to be important for infection in several plant pathogenic fungi. Endo-β-1,4-xylanases catalyze the endohydrolysis of xylan, the major structural polysaccharide of the plant cell wall. In this study the role of the β-1,4-endoxylanase gene (*xyl*A) in virulence of *V. dahliae* was investigated. Through the analysis of clones from a genomic library of *Verticillium dahliae* strain 76 and shotgun ESTs from xylem sap growing fungus the x*ylA* gene was isolated, its nucleotide sequence was determined and the predicted amino acid sequence showed significant homology with family 11 xylanases. The gene was disrupted by targeted inactivation due to a single crossover event in a *V.dahliae race 2* tomato strain. The knock-out mutant was compared with the wild type strain for disease symptoms on tomato plants, shown to result in a small (7%) reduction in disease severity. Growth of the mutant strain on minimal medium containing cellulose as the sole carbon source was reduced compared to the wild type indicating for a role of x*ylA* in the breakdown of complex components of the cell wall.

Molecular and phytopathological insight of *VdLaeA*, a regulator of secondary metabolism in *Verticillium dahliae*. A.M. GIANNAKOPOULOU and D.I. TSITSIGIANNIS*. [1]*Agricultural University of Athens, Department of Crop Science, Laboratory of Phytopathology Iera Odos 75, 118 55 Athens, Greece. *E-mail: dimtsi@aua.gr*

Secondary metabolites are compounds with high degree of specialization and in fungi play various roles concerning toxin production, sporulation processes and biosynthesis of substances with special biotechnological and pharmaceutical interest. Previous studies have shown that the plant pathogenic fungus *V.dahliae* produces phytotoxins and other molecules that induce the process of cell death or other forms of host's resistance. The exact nature though of these compounds of *V. dahliae* remains unknown. In *Aspergillus nidulans* the gene *laeA* is a global regulator of secondary metabolism and encodes a nuclear protein required for the expression of secondary metabolite genes, while its presence is considered indispensable for mycotoxin, antibiotic and mycelial pigments biosynthesis. In order to elucidate whether products of secondary metabolism play a role in pathogenicity or other physiological aspects of *V. dahliae*, we designed the disruption of *laeA*. BLAST analysis of *V. dahliae* genome using the *laeA* gene of *A. nidulans* led to the discovery of a homologue gene called *VdlaeA*. Primers were designed to multiply two regions of approx. 1000 bp before the initiation and termination codons respectively that were then cloned into the pBluescript vector. Geneticin was also subcloned between the two regions followed by the transfer of the entire construct into the binary vector

pGKO2. The binary vector was introduced in *Agrobacterium tumefaciens* which transferred the full cassette into the fungus *V. dahliae* resulting in disruption of the gene *VdlaeA* through double crossing-over in different races or isolates of *V. dahliae* from different hosts. The role of the gene *VdlaeA* in *V. dahliae* will be characterized from the virulence tests of the mutants in various hosts and through the *in vitro* physiological and morphological experiments of the mutant strains. The study of the regulatory gene *laeA* will contribute to a broader understanding of the molecular mechanisms by which secondary metabolites are produced in *V. dahliae* and more specifically to the confirmation of its role in *V. dahliae* pathogenesis or virulence process.

Insight of the role of *vfb* genes (Vier F-Box Proteine) in the innate immune system of the plant *Arabidopsis thaliana*. C. KOUTELIERI and D.I. TSITSIGIANNIS*. *Agricultural University of Athens, Department of Crop Science, Laboratory of Plant Pathology Iera Odos 75, Athens 11855, Greece. *E-mail: dimtsi@aua.gr*

Previous studies led to identification of two E3 ligases (one F-box and one U-box protein) involved in degradation of cell proteins through the 26S proteasome. Both genes, the F-box and U-box are expressed in the first 30 minutes after the recognition of the protein complex Avr9/Cf9 where the Avr9 is an effector of the fungus *Cladosporium fulvum* and Cf9 is a tomato resistance protein. The two E3 ligases are involved in Hypersensitive Response (HR) and plant sensitivity to different pathogens such as *Tobacco mosaic virus* (TMV), *Pseudomonas syringae* pv. *tabacci* in tobacco and *C. fulvum* and *Pseudomonas syringae* pv. *tomato* in tomato. The model plant *Arabidopsis thaliana* contains 694 F-box and almost 60 U-box proteins in its genome, the role of which have not been well clarified so far. Recent studies led to the characterization of 4 F-box proteins, VFB (Vier F-box PROTEINE) in *Arabidopsis*, belonging to the subfamily C of F-box proteins. This subfamily also includes very well characterized proteins such as a) the Transport Inhibitor Response (TIR 1)/ Auxin Signaling F-box (AFB) and b) the EIN3 Binding F-box that regulates the reactions of plant hormones auxin and ethylene. Inactivation of *vfb* genes resulted in reduced plant growth and less ability to form lateral roots. Part of this study was the characterization of the mutant lines *vfb 1-1,vfb 2-1, vfb 3-1* and *vfb-4* (*vfb-4* gene has been silenced using RNA interference technology) as well as the double, triple and quadruple *vfb* mutant line (*vfb 1-1;vfb 2-1; vfb 3-1; vfb 4*) on the development of HR and the resistance/sensitivity to various pathogens of *Arabidopsis* such as *Hyaloperonospora arabidopsidis*, *Pseudomonas syringae* pv. *tomato* DC 3000 and *Verticillium dahliae*.

Investigation of the role of new genetic regulators of disease resistance in the model plant *Arabidopsis thaliana*. M.C. PANAGIOTOPOULOU[1], H.A. VAN DEN BURG[2,3], J.D.G. JONES[3] and D.I. TSITSIGIANNIS[1]*. *[1]Agricultural University of Athens, Department of Crop Science, Laboratory of Plant Pathology Iera Odos 75, Athens 11855, Greece. [2]Laboratory of Phytopathology, Wageningen University and Research Centre, 6708 PB, Wageningen, The Netherlands. [3]The Sainsbury Laboratory, John Innes Centre, Norwich NR4 7UH, United Kingdom. *E-mail: dimtsi@aua.gr*

The F-box protein ACF1 plays a crucial role in innate immune system of plants by initiating the hypersensitive reaction (HR) and contributing to the sensitivity against the pathogens *Cladosporium fulvum* and *Pseudomonas syringae* pv. *tomato* in tomato, *Tobacco mosaic virus* (TMV) and *Pseudomonas syringae* pv. *tabacci* in tobacco. The characterization of the two *Arabidopsis* ACF1 orthologous genes, *AtSKIP2* and *AtFb116*, has demonstrated that these proteins are also involved in HR triggered by non compatible races of the oomycete *Hyaloperonospora arabidopsidis* and the *Arabidopsis* sensitivity to the soilborne pathogenic fungus *Verticillium dahliae*. Three putative protein-targets of ACF1 identified by the Yeast 2 Hybrid System (Y2H): a Ser/Thr protein kinase (*At-PK*), a bHLH transcripton factor *(At-bHLH*) and a protein with a LIM functional domain (*At-LIM1*) which might be the protein-targets that are degraded by ACF1 or other interacting proteins creating the complex of ACF1 during the host-pathogen interaction. The T-DNA insertion lines of the genes *At-PK*, *At-bHLH* and *At-LIM1* have been characterized in Arabidopsis and the mutant genotypes were checked for the resistance or sensitivity to infection by the following pathogens *H. arabidopsidis, P. syringae* pv. *tomato* DC3000 and *V. dahliae* as well as the development of HR.

Prediction of G Protein coupled receptors (GPCRs) in *Verticillium dahliae* through bioinformatics and phylogenetic analysis. I.A. STRINGLIS, E.J. PAPLOMATAS and D.I. TSITSIGIANNIS*. *Agricultural University of Athens, Department of Crop Science, Laboratory of Plant Pathology Iera Odos 75, Athens 11855, Greece. *E-mail : dimtsi@aua.gr*

Verticillium dahliae is a soil-borne fungus causing wilt diseases in several hosts. This fungus has a particular biology that complicates its treatment through conventional methods. As a result, the study of genes implicated in interactions of the fungus with its hosts is necessary to unravel the pathogenicity or virulence mechanisms and to discover putative novel methods to control the disease. G Protein-Coupled Receptors (GPCRs) represent the largest family of transmembrane receptors. GPCRs consist of seven transmembrane domains and are critical factors in regulating morphogenesis, defense, mating, infection and virulence in various organ-

isms. Protein sequences of characterized GPCRs of the well studied fungi *Aspergillus nidulans* and *Magnaporthe grisea* were used for alignment comparison with the genome of *V. dahliae*, in order to detect potential GPCRs. After performing phylogenetic analysis, the sequences of *V. dahliae* that showed high homology to the GPCRs of *A. nidulans* and *M. grisea* were selected in order to sort out the receptors by their molecular relativity. Six different groups of GPCRs emerged from the phylogenetic analysis, varying in sensing different environmental signals. Further studies for some of these GPCRs will be carried out using genetic engineering and gene disruption approaches in order to better understand their role in pathogenicity/virulence of *V. dahliae.*

MOLECULAR TYPING OF PLANT PATHOGENS

Oral Presentations

Development of new diagnostic tools for gram$^-$ phytopathogenic bacteria based on i*n silico* analysis of protein secretion systems. N. SKANDALIS[1,2]*, P.F. SARRIS[2,3], M. IOANNOU[3], D. KAFETZOPOULOS[3] and N.J. PANOPOULOS[2,3]. [1]*Laboratory of Bacteriology, Department of Phytopathology, Benaki Phytopathological Institute, St. Delta 8, 14561 Kifissia, Attica, Greece.* [2]*Department of Biology, University of Crete, 71409 Heraklion, Greece.* [3]*Institute of Molecular Biology and Biotechnology, FORTH, 71110 Heraklion, Greece. *E-mail: nskandalis@bpi.gr*

Protein export systems of gram-bacteria include the extensively studied type III and the poorly characterized type VI secretion systems (T3SS/T6SS) which both have the potential to translocate effector proteins into eukaryotic host cells. PCR diagnostics based on T3(6) SS gene sequences promise welcome alternatives or supplements to conventional identification but their application is still limited. In this study a systematic *in silico* analysis of putative T6SS core and effector proteins in six *Pseudomonas syringae* pathovars was carried out for the first time. The amino acid sequences of proteins encoded by the three T6SS gene clusters of multihost pathogen *Pseudomonas aeruginosa* were used as initial queries for BLASTP and reverse BLAST searches against the available genome sequences of *P. syringae* strains. To obtain preliminary evidence for transcriptional expression we carried out RT-PCR to detect putative RNA transcripts of a T6SS gene (*icmF)* orthologues. Based on extensive phylogenetic grouping of both T6SS and T3SS core genes, conserved primer binding sites flanking regions of sufficient variability for strain differentiation were identified. A pilot oligoarray microplate was printed with oligos designed to hybridize to probes representing core gene fragments of the secretion apparatus, amplified by means of Cy3-labelled primers of low degeneracy. These microarrays allowed specific detection of economically important bacterial plant pathogens. Our results support the idea that diagnostics based either in sequencing or microarray hybridization of PCR- amplified gene segments coding for virulence–related secretion system components should be feasible.

Molecular identification of *Pseudomonas syringae* pv. *alisalensis* isolates from arugula in Greece. I.B KARRI, P.F. SARRIS and D.E. GOUMAS*. *Technological Educational Institute of Crete, School of Agriculture PO Box 1939, 71004 Heraklion, Crete, Greece. *E-mail: dgoumas@staff.teicrete.gr*

Samples of infected *Eruca sativa* leaves from commercial plants were collected from different areas of Greece between March 2007 and February 2010. Isolations were performed from tissues showing the characteristic symptoms of *Pseudomonas syringae* pv. *alisalensis* infection, i.e. water soaked, angular, necrotic, papery, lesions with or without necrotic halo. Ten isolates were chosen (2 per area) from the total, for further study and comparison with the reference strains CFBP 6866 and CFBP 6870 of *Pseudomonas syringae* pv. *alisalensis* and CFBP 1657 of *Pseudomonas syringae* pv. *maculicola.* In the LOPAT assays, arugula isolates appeared positive in the production of levan and negative in the production of oxidase, the potato soft rot and the breakdown of arginine. Isolates also caused the typical hypersensitive reaction in tobacco leaves. Furthermore isolates in api tests (api 20 NE and 50CH) exhibited the same biochemical profile with the reference strains. Application of molecular indicators rep (BOX-ERIC) on arugula isolates clearly showed that they are different from the reference strains CFBP 1657 of *Pseudomonas syringae* pv. *maculicola* and DC 3000 of *Pseudomonas syringae* pv. *tomato*. They were identical to the reference strains CFBP 6866 & CFBP 6870 of *Pseudomonas syringae* pv. *alisalensis*. Koch's postulates were fulfilled by artificial inoculations of arugula, broccoli, oat and *Bromus diandrus* plants, the latter seemingly being a new monocotyledonous host for the pathogen. The results of this study show that *Eruca sativa* bacterial blight in different areas of Greece is caused by *Pseudomonas syringae* pv. *alisalensis* which was recently proposed to be named *Pseudomonas cannabina* pv. *alisalensis.*

Development of novel molecular tools for the genetic study of phytopathogenic fungus *Verticillium dahliae* populations. I.A. PAPAIOANNOU[1], M. ABDELHALIM[2], A.G. DOULIS[2], E.K. LIGOXYGAKIS[3], D.I. VAKALOUNAKIS[3] and M.A. TYPAS[1*]. [1]*Department of Genetics & Biotechnology, Faculty of Biology, National and Kapodistrian University of Athens, Panepistimiopolis, GR-*

*15701 Athens, Greece. [2]Laboratory of Plant Biotechnology, Institute of Viticulture, Floriculture & Vegetable Crops, NAGREF, GR-71003 Heraklion, Greece. [3]Laboratory of Plant Pathology, Institute of Plant Protection, NAGREF, GR-71003 Heraklion, Greece. *E-mail: matypas@biol.uoa.gr*

The phytopathogenic fungus *Verticillium dahliae* exhibits extraordinary genetic plasticity, capable of colonizing a broad range of hosts in diverse ecological niches. Based on the competence of various isolates to form viable heterokaryons, the species is further divided into groups of compatible strains, referred to as *V*egetative *C*ompatibility *G*roups (VCGs). Their usefulness in population genetics studies as well as their partial correlation with fungal pathogenicity characteristics renders the development of new tools for rapid VCG classification and determination of their relations an urgent task. To this goal, a population of 80 *V. dahliae* isolates from Crete and 20 international reference strains as well as representatives of other related species was employed. In general, no correlation between VCGs of Greek isolates was observed with geographical, phytopathological (tomato/eggplant/pepper pathotype, tomato race 1 or 2), morphological (growth rate, density of aerial hyphae, pigmentation, conidiogenesis, conidial dimensions) or molecular characteristics (fAFLPs with four primer combinations, three widely used PCR-based molecular markers, ITS1-5.8-ITS2 sequencing, search for group-I introns in 18S and 28S loci, mating type determination), thus underlining the need to develop novel, more informative tools. The IGS region of all isolates was determined and sequenced. In addition, we identified four primer combinations for the amplification of unrelated nuclear loci, which were tested with all isolates and found to produce characteristic patterns related to certain VCG subgroups. The combination of the latter approaches: a) provides the scientific community with a reliable alternative for the grouping of isolates in relation to VCGs, b) has high potential for resolving population structure within *V. dahliae* and may contribute to gaining insight into the evolutionary and phylogenetic relations between VCGs, c) questions the generally accepted genetic isolation of some VCG groups.

Frequency and characterization of mating type MAT1-1 and MAT1-2 isolates in *Cercospora beticola*. *A. PAPAVASILEIOU, G.A. BARDAS and G.S. KARAOGLANIDIS*. Laboratory of Plant Pathology, School of Agriculture, Aristotle University of Thessaloniki, P.O. Box 269, GR541 24 Thessaloniki, Greece. *E-mail: gkarao@agro.auth.gr*

Cercospora beticola causes the most important foliar disease of sugar beet known as Cercospora leaf spot (CLS). The fungus causes significant yield losses and attempts to control the disease have been made by using resistant cultivars and fungicide applications. The long-term objective of this research project was to study the effect of resistant sugar beet cultivar deployment on the genetic structure of the fungal populations. Recently indirect evidence was provided for sexual reproduction by identifying the mating-type locus in this fungal species. Each isolate of this fungus possesses a single gene for mating type with one of two alleles, either MAT 1-1 or MAT 1-2. The objective of this specific study was to determine the mating-type distribution of *C. beticola*. We used a PCR assay to determine the mating-type of 344 isolates collected from experimental sugar beet fields cultivated with 4 cultivars of differential resistance to CLS. Two hundred and six isolates were found to be MAT1-1 while the remaining 138 were found to be MAT1-2, giving a ration of 1.5:1. In 3 of the cultivars tested the MAT1-1/MAT1-2 ratio was found to be 1:1 or 1.5:1, while in cultivar Europa the most susceptible cultivar the ratio was 6:1. Furthermore, in 60 randomly selected MAT1-1 and MAT1-2 isolates the sequence of the mating gene was determined to investigate phylogenetic relationships. Moreover, in the same 60 isolates parameters such as the effect of temperature on the mycelial growth rate, the cercosporin production, the aggressiveness and the level of sensitivity to benzimidazole and sterol demethylation inhibitors (DMIs) were measured. Results showed that there were no differences among the MAT1-1 and MAT1-2 isolates in non of the parameters tested. The equal distribution of mating type genes provide further support to the hypopthesis that *C. beticola* may propagate sexually.

Post harvest apple fruit rots: causal agent incidence, variety susceptibility and patulin production. S. KONSTANTINOU[1], G.A. BARDAS[1], E. DOUKAS[2], I. MINAS[3] and G. S. KARAOGLANIDIS[1]*. *[1]Laboratory of Plant Pathology, School of Agriculture, Aristotle University of Thessaloniki, P.O. Box 269, GR541 24 Thessaloniki, Greece. [2]Laboratory of Pesticide Science, Agricultural University of Athens, 75 Iera Odos, 118 55 Athens, Greece. [3]Laboratory of Pomology, School of Agriculture, Aristotle University of Thessaloniki, P.O. Box 269, GR541 24 Thessaloniki, Greece. *E-mail: gkarao@agro.auth.gr*

Postharvest apple fruit decay contributes to high yield quantitative and qualitative losses. The study was conducted to: a) determine the disease causal agent frequency in 4 of the most important apple varieties cultivated in Greece (Red Delicious, Granny Smith, Golden Delicious and Fuji), b) measure the variety susceptibility in the two most commonly isolated pathogens and to correlate the level of susceptibility with several fruit qualitative parameters (fruit firmness, flavonoid, carotenoid and phenol concentration and antioxidant capacity) and c) to measure patulin production in the fruit of the 4 varieties tested. Fruit sampling was carried out

during the 2008–2009 and 2009–2010 storage periods from packinghouses located in the region of Imathia, N. Greece. In total more than 2,000 fruits were sampled for pathogen isolation. Pathogen identification was based on the morphological characteristics of the fungal colonies and fruiting bodies and in the sequencing data of the ITS1 and ITS2 regions of the ribosomic DNA. In total, 10 different fungal species were identified as post-harvest rot agents. Based on the results of the identification *Penicillium expansum*, *Botrytis cinerea* and *Alternaria tenuissima* were the 3 predominant species. Significant differences in the isolation frequency of the causal agent species were observed among the 4 varieties. Variety susceptibility measurements showed that Golden Delicious was the most susceptible variety to *P. expansum* infections, while Granny Smith was the most tolerant. Similarly, Fuji was the most susceptible variety to B. cinerea infections, while Red Delicious and Granny Smith were the most tolerant. Measurements of patulin production on fruit artificially inoculated by *P. expansum* showed that patulin production was higher in the fruit of Red Delicious and Golden Delicious compared to that in the fruit of Granny Smith and Fuji varieties.

Identification and quantification of potato cyst nematodes populations in Cyprus. M. CHRISTOPHOROU[1*], L. PAPAGIANNIS[2], D. TSALTAS[1], G. NEOPHYTOU[3], P. FELLAS[3] and N. IOANNOU[1]. *[1]Department of Agricultural Sciences, Biotechnology and Food Science, Cyprus University of Technology. Archbishop Kyprianos 31, 3603 Limassol, Greece. [2]Agricultural Research Institute. P.O. Box 22016, 1516 Nicosia, Greece. [3]Department of Agriculture. Louki Akrita Av., 1411 Nicosia, Greece. *E-mail: m.christoforou@cut.ac.cy*

Potato cyst nematodes (PCN), *Globodera rostochiensis* and *G. pallida*, cause major economic losses to potato crops in Cyprus. The identification and quantification of the populations of the two species is essential for the development of an integrated disease management system. In an initial study, carried out in the major potato production area of Kokkinochoria, PCN species were identified using a previously described PCR assay. In order to achieve high-throughput discrimination, identification and quantification of the two *Globodera* species a multiplex TaqMan® real-time PCR assay was subsequently developed and evaluated. This method is more accurate than the conventional PCR assay, offering quantitative data concerning inoculums' levels in the tested samples (or the traditional counting approach with the use of a stereoscope). The assay was successfully used to detect and quantify the number of eggs in infested fields. Results confirmed the presence of both species (*G. pallida* and *G. rostochiensis*) on the island. Significant differences were observed between the two species with regard to their geographical distribution and their population densities. The analysis and interpretation of results will enable the development of an integrated disease management program emphasizing to the reduction of PCN populations through the use of resistant varieties and crop rotation.

Mapping of the distribution of potato cyst nematodes in Cyprus using geographical information systems. M. CHRISTOPHOROU[1*], G. IOANNOU[2], D. TSALTAS[1] and N. IOANNOU[1]. *[1]Department of Agricultural Sciences, Biotechnology and Food Science, Cyprus University of Technology. Archbishop Kyprianos 31, 3603 Limassol, Greece. [2]Department of Information Technology Services, Ministry of Finance, Greece. *E-mail: m.christoforou@cut.ac.cy*

Potato cyst nematodes (PCN) *Globodera rostochiensis* and *G. pallida* comprise one of the most important phytopathological problems of potato in Cyprus. To alleviate losses, growers make excessive use of nematicides having a negative impact on both the environment and human health. In the context of a research project funded by the Cyprus Research Promotion Foundation, soil samples were collected from different potato and seed potato growing areas in Cyprus and the two PCN species were identified by molecular and morphometric methods. Biotypes of the two PCN species are also identified through the use of a set of differential hosts. Results from the above studies with their spatial location, were introduced in a Geographical Information System (GIS), which combines satellite images and spatial data thus allowing storage, retrieval, visualization and geographical analysis of biological data concerning the species and biotypes of PCNs. Digital mapping of the species and biotypes enables visualization of the geographical distribution of nematode infestation, assessment of the risk for further disease spread and consequently the forecasting and management of new infestations. This approach provides a useful tool for the development of an integrated management system for PCN and other pests and diseases. Finally, our proposed platform introduces a Decision Support System (DSS) as a tool of precision agriculture that will assist professionals in their decision making process with regard to PCN management.

Cultivated prunus trees infected by European Stone Fruit Yellows phytoplasma in Greece. E.K. VELLIOS[1*], E. KARATSIORI[1], F. LIOLIOPOULOU[2], I. KARAYIANNIS[3], A.C. PAPPAS[1] and P.E. KYRIAKOPOULOU[4]. *[1]University of Thessaly, Department of Agriculture, Crop Production and Rural Environment, Fytokou str., GR38446 Volos, Greece. [2]Aiolou 23, GR382 22 Volos, Greece. [3]Pomology Institute, National Agricultural Research Foundation, P.O. Box 122, R.R. Naoussas 36, GR59200 Naoussa, Greece.*

[4]*D. Areopagitou 15, 151 25 Amarousion, Athens, Greece. *E-mail: evellios@agr.uth.gr*

The European Stone Fruit Yellows is a disease of *Prunus* trees occuring in many European countries. The causal agent of the disease is a phytoplasma belonging to the "Candidatus *Phytoplasma prunorum*" proposed species and causing symptoms, such as, the off-season growth in winter, yellowing and leaf-roll in summer, dieback and more or less rapid decline follow. Twenty-two samples of almonds, 40 samples of plums and 6 samples of apricots were collected with the symptom of off-grown season from several areas of Greece in February 2009. After total DNA extraction from the branch phloem, the samples were examined for the presence of phytoplasma by PCR with the universal primers P1/P7, R16F2n/R16R2, U4/U5 that amplify part of the ribosomal genes 16SrRNA and 23SrRNA and the ITS region between them. 28 samples (25 plums and 3 apricots) gave a positive reaction and the R16F2n/R16R2 products were further submitted to RFLP analysis by using four restriction enzymes (*Taq* I, *Rsa* I, *Mse* I, *Hinf* I). The RFLP profile of the samples confirmed the presence of a phytoplasma belonging to "Candidatus *Phytoplasma prunorum*" (16SrX- B group) (that causes the disease of the European Stone Fruit Yellows) in the phloem of the trees. The sequencing analysis to five PCR P1/P7 products (four plum samples and one of apricot), showed 100% homology with a Spanish isolate (GenBank accession AJ575105) and a German one (GenBank accession AJ542545).

Poster Presentations

Determination of *Phytophthora parasitica* var. *nicotianae* races 0, 1 and 3 with the use of tobacco isolines. D.F. ANTONOPOULOS[1*], R.S. LEWIS[2] and A.L. MILA[1]. [1]*North Carolina State University, Department of Crop Science, Campus Box 7405, Raleigh, North Carolina, USA, 27695.* [2]*North Carolina State University, Department of Plant Pathology, Campus Box 7405, Raleigh, North Carolina, USA, 27695. *E-mail: antdim75@yahoo.com*

Three physiological races (0, 1, and 3) of *Phytophthora parasitica* var. *nicotianae* (*Ppn*) have been reported in North America on tobacco (*Nicotiana tabacum*) based on their ability to infect cultivars and/or breeding lines with different resistance background. Single gene resistance to *Ppn* from *N. longiflora* (*Phl*) or *N. plumbaginifolia* (*Php*) have been incorporated into burley and flue-cured tobacco cultivars, respectively. Single gene resistance provides complete resistance to race 0, but no resistance to race 1 (*Php* and *Phl*) and race 3 (only *Phl*) of *Ppn*. The use of these cultivars for *Ppn* race determination has been criticized, because these cultivars have also unknown level of partial resistance to *Ppn*. In this study, *Ppn* race determination was based on use of tobacco isolines. Nine *Ppn* isolates from North Carolina, previously race characterized with the aforementioned tobacco cultivars as race 0, 1 and 3 (three isolates per race), were used. In addition, three isolines [Hicks, Hicks (*Php*), Hicks (*Phl*)], two breeding lines [NC1071 (*Php*), L8 (*Phl*)] and the flue-cured tobacco cultivar K326 (partial resistance) were used. Plants were evaluated 7, 14, and 21 days after inoculation. All isolates of *Ppn* race 0 infected only K326 and Hicks seedlings in all trials. Race 1 isolates infected all isolines and cultivars in all trials, while race 3 isolates provide ambiguous results. Additional studies should be carried out to determine if race 3 represents a different race.

Genotypic characterization of *Phytophthora infestans* populations in Cyprus using a set of microsatellite markers. L. KANETIS, L. PITTAS, D. TSALTAS* and N. IOANNOU. *Department of Agricultural Sciences, Biotechnology and Food Science, Cyprus University of Technology, CY-3603 Lemesos, Cyprus, Greece. *E-mail: dimitris.tsaltas@cut.ac.cy*

For many years potato late blight (PLB) on worldwide basis was kept to tolerable levels by agronomic practises and fungicide use. However, in the 1980s, the disease resurged causing significant crop losses. This dramatic change was attributed to a major displacement of the original population of *Phytophthora infestans* by a new one that immigrated to Europe and contained both mating types of the pathogen. Since then the European population of *P. infestans* has become highly diverse and there is evidence of sexual reproduction, resulting in more aggressive lineages. The increased diversity in the pathogen population resulted in virulent races that overcame host resistance genes, rendering previously resistant commercial cultivars vulnerable to the pathogen. Currently, the impact of PLB to potato crop in Cyprus during "epidemic" years can be devastating. The aim of the present study was to determine genotypic characteristics for a set of 40 *P. infestans* isolates collected in five districts of Cyprus during 2009. Characterization included mating type and DNA fingerprinting patterns based on simple sequence repeats (SSR). Due to their high variability and dense distribution throughout the genome, SSRs offer a detailed taxonomic resolution for the analysis of individual isolates within local populations. Thus, this work will provide important information regarding the biology and genetic attributes of the *P. infestans* population in Cyprus.

Implementation of Real-time PCR technology for identification of plant pathogens in Cyprus. L.C. PAPAYIANNIS*, T. KAPARI-ISAIA and A. KYRIAKOU. *Agricultural Research Institute, P.O.Box 22016, Nicosia 1516, Cyprus, Greece. *E-mail: l.papayiannis@arinet.ari.gov.cy*

During the past years, the rapid development of biological sciences has enabled the implementation and use of new modern diagnostic techniques for the sensitive and accurate identification of several plant pests and diseases. Within the context of an infrastructure development project entitled "Development of molecular diagnostic tools for identification of plant pathogens" and funded by the Cyprus Research Promotion foundation, the scientific facilities of Plant Pathology laboratories have been modernized and equipped with state of the art molecular biology equipments. The main goal of this project is to provide new detection protocols for the rapid, highthrouput identification of important plant pathogens occurring in Cyprus. The new diagnostic techniques are mainly focused on real-time PCR technology, targeting at plant viruses, viroids, insects and nematodes. Until now, new Taqman® assays have been optimized and used for the discrimination of two virus species involved in Tomato yellow leaf curl disease (*Tomato yellow leaf curl virus*, TYLCV and *Tomato yellow leaf curl Sardinia virus*, TYLCSV), two other viruses related with the etiology of cucurbit yellowing caused by *Cucurbit yellow stunting disorder virus* (CYSDV) and *Beet pseudo-yellows virus* (BPYV) and finally the virus *Cucumber green mottle mosaic virus* (CGMMV). In addition, new diagnostic assays have been incorporated for the rapid differentiation of the tobacco whitefly (*Bemisia tabaci* Gennadius) biotypes and the potato cyst nematodes (*Globoddera pallida* and *G. rostochiensis*). In a direct comparison between developed real-time PCR assays and the traditional conventional PCR and serological techniques, the latter ones have been shown to present higher sensitivity and accuracy for the detection of all studied pathogens.

Potato late blight forecast in Cyprus during the years 2008–2010. L. PITTAS[1], L. KANETIS[1], D. TSALTAS[1*], G. NEOPHYTOU[2], P. FELLAS[2] and N. IOANNOU[1]. *[1]Department of Agricultural Sciences, Biotechnology and Food Science, Cyprus University of Technology, CY-3603, Lemesos, Cyprus, Greece. [2]Department of Agriculture, Ministry of Agriculture Natural Resources, and Environment, CY-1411 Nicosia, Cyprus, Greece. *E-mail: dimitris.tsaltas@cut.ac.cy*

Meteorological data collected from experimental potato plots during three consecutive growing seasons (2008–2010) were analyzed with nine late blight forecasting models (Hyre, Wallin, Fry, Ullrich, Negfry, Smith, Blitecast, Winstel and Forsund). Each forecast was compared with the actual emergence and progress of the disease in the experimental plots. The Fry model was more effective giving the most reliable results in all three years compared with the other models. In plots where spraying program a spraying program was applied according to recommendations by the Fry model, the disease severity did not differ significantly compared to that in plots receiving an empirical program of sprays as applied by the growers. However, the number of sprays was significantly reduced from 10–11 in the conventional program to 8–9 in the program suggested by the Fry model. The meteorological data were recorded at the height of crop canopy (0.5 m) based on the results of respective experiments which showed that such data (rainfall, temperature and relative humidity) are more reliable for disease forecasting than data recorded at the height of 2 m (standard height of agrometeorological stations).

Formation of microsclerotia by the fungus *Alternaria dauci*. G.T. TZIROS[1*] and A.L. LAGOPODI[2]. *[1]NAGREF – Forest Research Institute, Laboratory of Forest Pathology and Mycology, 570 06, Vassilika, Thessaloniki, Greece. [2]Aristotle University of Thessaloniki, Faculty of Agriculture, Laboratory of Plant Pathology, PO Box 269, 541 24, Thessaloniki, Greece. *E-mail: tziros@fri.gr*

Alternaria dauci (Kühn) Groves and Skolko, the cause of leaf blight of carrot, was observed to produce microsclerotia *in vitro*. Four pathogenic isolates of *A. dauci* (CBS 101592, 1A, 1B and 1C), were used in this study. Cultures were made on four different media (potato dextrose agar, PDA), V8 agar, water agar and Czapek-Dox agar) and were incubated at three different temperatures (10, 18 and 28°C). Microsclerotia were formed at 28°C, on PDA, by only one isolate (CBS 10159). In the beginning of their formation they were easily visible, by naked eye, as node-like mycelial aggregations that appeared at the edges of the culture when the mycelium came in contact with the walls of the Petri dish. Microscopic observation of these structures showed extensive branching of hyphae of the aerial mycelium that became darker, grew towards one another and started to intermingle closely. Mature microsclerotia were dark grey to black in colour, they were made up by compact masses of cells and ranged 11.30–89.10 μm in diameter. Compared to classic sclerotia from other fungi, they were less compact woven, were relatively flat and they did not have clearly defined edges, as in this area hyphae were more loosely arranged and were associated with mycelium. As cultures desiccated, microsclerotia were distributed all over the colony. Closely related multi-celled structures were observed in almost all isolates used; however, they were loosely woven and never resulted in the well defined compact masses of cells described above. Exposure of microsclerotia at -20ºC for fifteen days did not affect their ability to reproduce the mycelium. This is the first report of microsclerotia formation in *A. dauci*.

Molecular characterization of *Camarosporium* blight of pistachio. S.C. PALAVOUZIS, E.J. PAPLOMATAS

and D.I. TSITSIGIANNIS*. *Agricultural University of Athens, Department of Crop Science, Laboratory of Phytopathology Iera Odos 75, 118 55 Athens, Greece. *E-mail: epaplom@aua.gr*

Species of the genus *Botryosphaeria* attack many different hosts and cause significant plant diseases including the *Camarosporium* blight or "panicle and shoot blight" of pistachio. The pathogen that causes this disease of pistachio in Greece has been classified to the anamorph *Camarosporium pistachiae* whereas its teleomorph has not yet been discovered on this host. Based on morphological and molecular characteristics of the anamorph, the pathogen of "panicle and shoot blight" of pistachio in California (USA) has been classified taxonomically to *Fusicoccum* sp. and to the teleomorph *Botryosphaeria dothidea.* In order to clarify the current species status of this pathogen in Greece, the ITS region of 10 Greek isolates from five different hosts, having typical *Botryosphaeria* type blight symptoms, was first amplified using the universal primers ITS4 and ITS5 and was further sequenced. The identification at the species level was performed by BLAST analysis. Five of the pistachio isolates were classified at the anamorphic species *Neofusicoccum vitifusiforme*, regardless of the region that were isolated (Attiki, Fthiotida). The other isolates, one from *Sophora* sp., one from olive tree and one from plum tree, were identified as the teleomorph *Botryosphaeria dothidea.* The two isolates from grapevine belonged to the species *Botryosphaeria obtusa* as has also been shown in literature for *Botryosphaeria* pathogens infecting this host. A phylogenetic tree based on these ITS sequences, was constructed using the MEGA v4 software. All the pistachio isolates were grouped into one clade that was separated from the clade including the *Botryosphaeria dothidea* isolates (of all three hosts) with a divergence of 2.4%. The grapevine isolates were grouped into a different clade and diverged by 4.1% from the isolates of the two other clades. Based on these data, we conclude that sequencing of the ITS region is a suitable molecular tool for the classification of the genus *Botryosphaeria* at species level irrespective of the host or the geographic origin of the isolate.

CHEMICAL CONTROL

Lecture of Major Sponsor

INITIUM®: a new innovative fungicide of a new chemical class for the control of late blight and downy mildew diseases. M. MERK[1], K. BOZOGLOU[2]* and R. GOLD[3]. *[1]BASF Italia SRL, Via Marconato 8, 20031 Cesano Maderno, Italia. [2]BASF Hellas Industrial and Commercial SA, Sindos Industrial Area, 57022 Thessaloniki, Greece. [3]BASF SE, Agrocentre, 67117 Limburgerhof, Germany. *E-mail: costas.bozoglou@basf.com*

Initium is a new fungicidal active ingredient developed by BASF. The innovative compound belongs to a new chemical class, the triazolo-pyrimidylamines. Initium is a mitochondrial respiration inhibitor interfering with the complex III (complex bc1) in the electron transport chain of the pathogen, thus ATP synthesis in the fungal cells is inhibited. Research has demonstrated that Initium does not show cross-resistance to fungicide classes like Qo inhibitors, phenylamides and carboxylic acid amides. Initium is highly effective in inhibition of zoospore formation and release, zoosporangia release, motility and germination. At low concentrations Initium leads to bursting of zoospores within a few seconds. Initium is a non-systemic fungicide which remains on the leaf surface where it is absorbed with high affinity in the epicuticular wax layer of the leaf epidermis and thus forms a stable protective film against fungal attack. The vapour phase activity is minimal. In numerous field trials carried out worldwide, Initium has been shown to be highly selective in a wide range of specialty crops and provides best performance when it is applied as a protectant spray before disease is established in the crop. Initium controls all major Oomycete diseases, e.g. downy mildew caused by *Plasmopara viticola* in grapes, late blight caused by *Phytophthora infestans* in potatoes and tomatoes, and a broad range of downy mildews and late blights in vegetables (e.g. cucurbits, Brassicas, onions, and lettuce). Initium has an excellent toxicological and ecotoxicological profile and is highly suitable for use in integrated crop management system.

Oral Presentations

Frequency of transposable elements presence in *Botrytis cinerea* populations from several hosts and fungicide sensitivity profile. S. SAMUEL, T. VELOUKAS and G.S. KARAOGLANIDIS*. *Plant Pathology Laboratory, Faculty of Agriculture, Aristotelian University of Thessaloniki, POB 269, 54124, Thessaloniki, Greece. *E-mail: gkarao@agro.auth.gr*

Botrytis cinerea (teleomorph *Botryotinia fuckeliana*) is a cosmopolitan fungus attacking more than 200 plant species in the temperate zone worldwide. The presence of the transposable elements Boty and Flipper, within fungal genome, is considered to be one of the most important agents of the high genetic variability observed in this fungal species. The current study was conducted aiming primarily to investigate the presence and frequency distribution of the transposable elements Boty and Flipper in populations of the pathogen in Greece. In addition fungicide resistance frequencies in the sampled population were determined. In total, 304 isolates were collected during 2008 and 2009 from grapes, strawberries, tomatoes, cucumbers, kiwifruit and apple fruit. The presence of the two transposable elements was based on PCR detection.Results showed that in all

the sampled hosts occurred in sympatry the 4 possible different genotypes (isolates of transposa type carrying both transposable elements, Boty–type isolates, Flipper-type isolates and vacuma type isolates carrying neither transposable element. Marked differences in genotype frequencies among populations were observed. In tomatoes, cucumbers, grapes and strawberries transposa isolates carrying both elements were the predominant in the populations, while in kiwifruit and apple fruit populations the vacuma isolates were prevailing. Furthermore, in kiwi and apple fruit populations high frequencies of Flipper-type isolates were observed. In an attempt to explain the observed predominance of vacuma isolates in kiwi and apple fruit populations, mycelial growth rate of a set of vacuma isolates was compared to the mycelial growth rate of a set of transposa isolates at 3 different temperatures (0, 10 and 20°C). In addition, the same set of isolates were used to compare virulence of isolates on wound-inoculated kiwifruits incubated at 2 different temperatures (0 and 20°C), in terms of disease incidence and disease severity. The results showed that vacuma and transposa isolates had similar mycelial growth rates at the limiting temperatures of 0 and 10°C, while vacuma isolates grow faster at the optimum temperature of 20°C. Similarly there was no significant difference in virulence on kiwifruit between transposa and vacuma isolates. Measurements of fungicide sensitivity profile of the isolates revealed the widespread existence of resistance to benzimidazole, dicarboximide and anilinopyrimdine fungicides. None of the isolates was resistant to either phenylpyrroles or hydroxyanilides. Interestingly, resistance frequencies were significantly higher in the transposa subpopulation compared to the vacuma subpopulation in all the sampled hosts.

Assessment of G143A mutation and type I cytb intron frequencies in *Botrytis cinerea* populations from several hosts in Greece. S. SAMUEL[1], L.C. PAPAYIANNIS[2], T. VELOUKAS[1] and G.S. KARAOGLANIDIS[1]*. [1]*Plant Pathology Laboratory, Faculty of Agriculture, Aristotelian University of Thessaloniki, POB 269, 54124, Thessaloniki, Greece.* [2]*Agricultural Research Institute, POBox 22016,Nicosia 1516, Cyprus, Greece. *E-mail: gkarao@agro.auth.gr*

Botrytis cinerea (anamorph *Botryotinia fuckeliana*) is the causal agent of gray mold, a disease observed on several crops. The control of the disease is mainly based on the use of fungicides applied against the pathogen. However, development of resistance to antifungal agents is an important shortcoming for the successful chemical control of the disease. Fungicides belonging in the QoI group have been recently registered for use against *B. cinerea* in several crops. In two recent reports resistance of *B. cinerea* to QoI fungicides has been associated to the presence of G143A mutation in the cytb gene, while fungal populations were divided in two groups according to the structure of the cytb gene. The current study was conducted to investigate the frequency of the G143A mutation and the presence of the typeI cytb intron within pathogen populations on several hosts. For this purpose 263 single spore isolates were obtained from grape, strawberry, tomato, squash, kiwis and apples during 2008 and 2009. In the study were also included 43 isolates collected from greenhouse grown tomatoes during 2005, before the introduction of QoIs into the spray programs. Resistance frequency to pyraclostrobin was determined using a single discriminatory concentration of 0.3 μg mL^{-1}, while EC_{50} values were measured in the strawberry isolates using the microtiter method. The whole population tested, was screened for the presence of the G143A mutation and the presence of the typeI cytb intron supposed to prevent the presence of the G143A mutation, using allele-specific primers. A gel-free, real-time TaqMan® PCR assay was developed and applied for the detection of the intron and the mutation presence in all tested samples. Determination of resistance frequency to pyraclostrobin using the discriminatory concentration revealed that QoI-resistant isolates there were only within the strawberry population, while a limited number of 2 isolates were also detected within the tomato population. However, molecular detection of the G143A mutation, showed that there was a wide spread presence of the mutation within all the pathogen populations tested. The frequencies of the mutation ranged within values of 19% in the tomato population to 91% in the strawberry population collected in 2009. Isolates carrying the resistance mutation were found at a frequency of 5% even within the population collected before the introduction of QoIs. Isolates that did not carry the G143A mutation were carrying the typeI cytb intron while at low frequencies were also detected some isolates that did not carry neither the mutation nor the intron. However, the observed G143A mutation frequencies did not necessarily correlate with the level of isolates sensitivity to pyraclostrobin. Measurements of EC_{50} values to pyraclostrobin in the isolates collected from strawberry showed the existence of isolates that were carrying the G143A mutation and their sensitivity was ranged from 0.005 to >50 μg mL^{-1}, while isolates that did not carry the G143A mutation showed EC_{50} values ranging from 0.001 to 0.1 μg mL^{-1}. The results of the study suggest that a high risk for selection of QoI highly-resistant strains exists in crops extensively treated with QoIs. In addition, the widespread presence of fungal strains carrying the typeI cytb intron, do not seem to be a factor contributing to lower risk since under the fungicide selection force they are eliminated, in favor of strains carrying the G143A mutation.

Phytopathological and molecular characterization of *B. cinerea* field isolates resistant to benzamides, ben-

zimidazoles and phenylocarbamates. A.A. MALANDRAKIS*, A.N. MARKOGLOU and B.N. ZIOGAS. *Laboratory of Pesticide Science, Agricultural University of Athens,75 Iera Odos, Votanikos, 188 55 Athens, Greece. *E-mail: tasmal@aua.gr*

Sensitivity profiles of *Botrytis cinerea* field isolates to zoxamide and the molecular basis of the resistance mechanism involved in cross resistance relationships between benzamides, benzimidazoles and N-phebylcarbamates were investigated. *B. cinerea* isolates collected from southern, central and northern Greece where characterized based on their sensitivity to zoxamide, the benzimidazole carbendazim and the N-phenylcarbamate diethofencarb. Isolates exhibiting baseline sensitivity to carbendazim and zoxamide but no sensitivity to diethofencarb were considered wild type (S phenotype) and accounted for 44% of the total strains sampled. Thirty three percent of the isolates had increased sensitivity (HS phenotype) to zoxamide and diethofencarb and were highly resistant to carbendazim compared to S isolates. Eight percent of the sample was highly resistant (HR phenotype) to all anti-tubulin agents studied. The rest of the isolates were moderately resistant to zoxamide (MR phenotype) and equally sensitive to benzimidazoles and N-phenylcarbamates compared to isolates of the S phenotype. Fungitoxicity tests with botrycides belonging to other chemical classes revealed no cross resistance relationships between zoxamide and the phenylpyrrole fludioxonil, the dicarboximide iprodione, the hydroxyanilide fenhexamid, the anilinopyrimidine cyprodinil, the carboxamide boscalid and the strobilurin-type fungicide pyraclostrobin. Study of fitness characteristics did not show any significant difference between zoxamide resistant and sensitive isolates in the respectful parameters tested. PCR-RFLP analysis of a part of the b-tubulin gene sequence detected mutations in position 198 for both HS and HR zoxamide sensitivity phenotypes. DNA sequence analysis of the *B. cinerea* β-tubulin gene revealed two previously described benzimidazole resistance-confering mutations. The first one was the glutamic acid (GAG) to alanine (GCG) change at position 198 (E198A) which was identified in all HS isolates. The second mutation (E198K) was a GAG-to-AAG substitution resulting the replacement of glutamic acid with lysine present in all *B. cinerea* isolates highly resistant to all three anti-tubulin classes of fungicides. A number of mutations in other positions of the β-tubulin gene were detected in the moderately zoxamide-resistance phenotype but their role in resistance -if any- needs to be further investigated.

Botrytis control with chlorothalonil and fungicide residue levels at harvest in hydroponic culture of lettuce. M. CHATZIDIMOPOULOS, F. BALOTI, N.G. TSIROPOULOS and A.C. PAPPAS*. *University of Thessaly, Department of Agriculture Crop Production and Rural Environment, 384 46 N. Ionia, Volos, Greece. *E-mail: acpappas@uth.gr*

Crown rot caused by *Botrytis cinerea* is the main problem on lettuce grown during the winter period in hydroponics. Infection started from the lower senescent leaves and expanded via the pedicels to the neck of the plant. For the control of the disease chlorothalonil (0.1%) was applied at the early stage of plant growth and the fungicide residues were measured at harvest. Three different fungicide application schedules at 10 day intervals, were investigated. (a) two applications at seedling stage, (b) two at seedling stage plus one after transplanting and (c) two at seedling stage plus two after transplanting. Lettuce were harvested 39–55 days after the last fungicide application. For the fungicide residue determination it was used a GC-ECD system after extraction with a mixture (1:1:1) acetone, dichloromethane and petroleum ether. Botrytis infections was less that 3% in all fungicide application programmes with significant difference from the untreated control. The chlorothalonil residues at harvest were: (a) <0.01, (b) 0.49 and (c) 0.66 mg kg^{-1}, respectively. In this experiment it was shown that two chlorothalonil applications at seedling stage protected lettuce plants from crown rot in hydroponic systems, without fungicide residues at harvest.

Estimating rates of fungicide resistance in natural populations of *Penicillium digitatum* using a new air-sampling method. L. KANETIS[1]*, H. FORSTER[2] and J.E. ADASKAVEG[3]. [1]*Department of Agricultural Sciences, Biotechnology and Food Science, Cyprus University of Technology, CY-3603 Lemesos, Cyprus, Greece.* [2]*Department of Plant Pathology, University of California, Davis 95616, USA.* [3]*Department of Plant Pathology and Microbiology, University of California, Riverside 92521, USA. *E-mail: loukas.kanetis@cut.ac.cy*

Fungicide resistance was identified in natural populations of *Penicillium digitatum,* the causal agent of citrus green mold to two new postharvest fungicides before their commercial use in USA. Using a new air-sampling method, in citrus packinghouses, large populations of the pathogen's propagules were exposed to agar plates with a continuous, wide-range fungicide concentration gradient and isolates with reduced sensitivity to fludioxonil or pyrimethanil were obtained. Resistance frequencies to fludioxonil and pyrimethanil ranged from 9.5×10^{-7} to 1.5×10^{-6} and from 7.3×10^{-6} to 6.2×10^{-5}, respectively. Isolates with reduced sensitivity to fludioxonil or pyrimethanil were also obtained in laboratory selection studies. Fludioxonil resistant isolates fell into two categories, based on mycelial growth: moderately resistant isolates with EC_{50} values of 0.1 to 1 μg mL^{-1} and highly

resistant isolates with EC_{50} values >1 μg mL^{-1}. Representative isolates of the two categories varied widely in their virulence and sporulation capacity, as measured by the incidence of decay and degree of sporulation on inoculated fruits, respectively. All isolates resistant to pyrimethanil had EC_{50} values >8 μg mL^{-1} and were mostly similar to the wild-type isolate. This new method enables us to estimate fungicide resistance frequencies and to characterize types of resistance within populations of a fungal species. This information will be used to design resistance management strategies for previous and newly registered postharvest fungicides of citrus.

SDH Inhibitors: mode of action, mode of resistance and resistance management. G. STAMMLER[1], K. BOZOGLOU[2]* and A. GLÄTTLI[3]. [1]*BASF SE, Agricultural Center, Speyerer Strasse 2, 67117 Limburgerhof, Germany.* [2]*BASF Hellas Industrial and Commercial SA, Sindos Industrial Area, 57022 Thessaloniki, Greece.* [3]*BASF SE , Specialty Chemicals Research, Carl-Bosch Strasse, 67056 Ludwigshafen, Germany. *E-mail: costas.bozoglou@basf.com*

The target protein of SDHIs is the succinate dehydrogenase (SDH), which is a major component of two crucial cellular processes, the tricarboxylic cycle and the mitochondrial electron transport chain. SDH consists of four subunits (A, B, C, D), a hydrophilic flavoprotein (A), an iron sulphur protein (B) and two lipophilic transmembrane subunits (C and D) which are necessary to anchor the protein to the mitochondrial membrane. Inhibitors of SDH act via the ubiquinone binding site formed by the subunits B, C, D. A sensitivity monitoring of different target pathogens to SDHIs has been carried out in recent years. While in most target species (e.g. *Mycosphaerella graminicola*, *Pyrenophora teres*, *Rhynchosporium secalis*, *Oculimacula* spp., *Venturia inaequalis*) the sensitivity of all isolates was within the baseline range, cases of resistance were found e.g. in *Botrytis cinerea*, *Corynespora cassiicola* or *Alternaria alternata*. Target gene analysis of such isolates revealed mutations in the SDH-subunits B, C and D. Such data of field isolates and further findings with laboratory mutants carrying additional mutations in the SDH subunits indicate that a number of spatially distinct mutations in the SDH can lead to a loss of sensitivity. Due to the introduction of several new SDHI fungicides in arable crops as well as in fruit, vine, vegetables, ornamentals and turf, appropriate resistance management strategies including extensive monitoring studies are recommended for the target pathogens.

Characterization of fitness parameters and mycotoxins production in field isolates of *Penicillium expansum* resistant to anilinopyrimidine, triazole, phenylpyrrole and dicarboximide fungicides. G.A. BARDAS[1], S. MEGA[2], S. KONSTANTINOU[1], I. KALAMPOKIS[2], E.G. DOUKAS[2], G.S. KARAOGLANIDIS[1]* and A.N. MARKOGLOU. [1]*Plant Pathology Laboratory, Faculty of Agriculture, Aristotelian University of Thessaloniki, POB 269, 54124, Thessaloniki, Greece.* [2]*Pesticide Science Laboratory, Agricultural University of Athens, 75 Iera Odos (Votanikos), 118 55 Athens, Greece. *E-mail: gkarao@agro.auth.gr*

The objectives of this study were to determine the sensitivity of the fungal population in several fungicides applied in apple orchards and to measure fitness components and mycotoxin (patulin and citrinin) production in fungal isolates exhibiting several fungicide-sensitivity profiles. A total of 236 *P. expansum* single-spore isolates were obtained from decayed apple fruits collected from packinghouses and processing industries located in the region of Imathia, N. Greece. Preliminary fungitoxicity tests on the response of the isolates to the dicarboximides (iprodione, Rovral), phenylpyrroles (fludioxonil, Medallion), sterol biosynthesis inhibitors (tebuconazole, Folicur), and anilinopyrimidines (cyprodinil, Chorus) showed several fungicide-resistant phenotypes. Resistance to anilinopyrimidines (Rf: 10–40, based on the EC_{50} values) was widespread accounting for 43% of the population, while only 9% of the population was resistant to tebuconazole (Rf: 2–9, based on EC_{50s}). Furthermore, strains with double resistance to the triazoles and phenylpyrroles (tebuconazole+fludioxonil), to the triazoles and anilinopyrimidines (tebuconazole+cyprodinil), and to the triazoles and dicarboximides (tebuconazole+iprodione) were also determined at low frequency. Interestingly, a small portion of the population (7.5%) showed multiple resistance to tebuconazole, fludioxonil and iprodione. Study of fitness determining parameters showed that the resistance to tebuconazole, fludioxonil and iprodione had a significant adverse effect on mycelial growth rate and virulence. Contrary to the above, these fitness parameters were unaffected in cyprodinil-resistant isolates. Analysis of mycelial extracts and artificially infected apple fruits with sensitive and resistant isolates showed that all isolates tested were mycotoxigenic. Most cyprodinil-resistant isolates produced patulin and citrinin at concentrations significant higher that the sensitive ones. In contrast, a significant reduction in the mycotoxin production was observed in most tebuconazole, fludioxonil, iprodione and multiple resistant strains, obviously due to fitness penalties. The above mentioned data clearly show an increased risk for the predominance of *P. expansum* field strains resistant to anilinopyrimidines with high mycotoxigenic ability, while the risk for resistance to phenylpyrroles, dicarboximides and sterol biosynthesis inhibiting fungicides is rather low.

Molecular characterization of benzimidazole resistance and study of fitness parameters and mycotoxin

production in field isolates of *Penicillium expansum*. S. MEGA[1], S. KONSTANTINOU[2], I. KALAMPOKIS[1], E.G. DOUKAS[1], A.A. MALANDRAKIS[1]*, G.A. BARDAS[2], G.S. KARAOGLANIDIS[2] and A.N. MARKOGLOU. [1]*Pesticide Science Laboratory, Agricultural University of Athens, 75 Iera Odos (Votanikos), 118 55 Athens, Greece.* [2]*Plant Pathology Laboratory, Faculty of Agriculture, Aristotelian University of Thessaloniki, POB 269, 54124, Thessaloniki, Greece. *E-mail: tasmal@aua.gr*

Blue mold, caused by *Penicillium expansum*, is the most important postharvest disease of apples and other stored pome fruits, causing serious problems, not only due to the economic losses resulting from significant yield reduction, but also by compromising food safety worldwide. Today, there is evidence that the extensive use of site-specific fungicides in crop protection may lead to reduced sensitivity of certain mycotoxin-producing fungi, and thus to an increased contamination of agricultural products with mycotoxins. The objectives of this study were to determine the sensitivity of the fungal population in several fungicides applied in apple orchards and to measure fitness components and mycotoxin production in fungal isolates exhibiting several fungicide-sensitivity profiles. A total of 236 *P. expansum* single-spore isolates were obtained from decayed apple fruits collected from packinghouses and processing industries located in the region of Imathia, N. Greece. *In vitro* fungitoxicity tests resulted in the identification of two resistant phenotypes: (a) isolates highly resistant to carbendazim (Rf: >100, based on EC_{50} values), but sensitive to the phenylcarbamate diethofencarb; and (b) strains moderately resistant to carbendazim (Rf: 20–40, based on EC_{50} values) with no increased sensitivity to diethofencarb. Cross resistance studies with other fungicides showed that the mutation(s) for resistance to benzimidazoles do not affect the sensitivity of the isolates to fungicides affecting other cellular pathways or processes, such as the phenylpyrrole fludioxonil, the dicarboximide iprodione, the anilinopyrimidine cyprodinil, the strobilurin-type fungicides azoxystrobin and pyraclostrobin, and the triazoles flusilazole and tebuconazole, indicating that target-site modification is the biochemical mechanism for resistance to the benzimidazoles. Analysis of the sequence of the β-tubulin, the target site of benzimidazoles, in the highly carbendazim-resistant strains revealed an amino acid substitution from glutamic acid (GAG) to alanine (GCG) at the codon 198 (E198A), a mutation previously implicated in benzimidazole resistance. Molecular analysis of the β-tubulin gene in moderately resistant isolates did not reveal any amino acid substitutions. Study of fitness determining parameters showed that the resistance mutation(s) had no apparent effect on mycelial growth rate and pathogenicity in most carbendazim-resistant isolates. Analysis of the mycotoxin production (patulin and citrinine) showed that most highly carbendazim-resistant isolates produced mycotoxins at concentrations significantly higher (4–6 fold) that the sensitive ones on culture medium and on artificially inoculated apple fruits. The above mentioned data indicate, for the first time, the potential risk of increased mycotoxin contamination of apple and other pome fruits and its by-products by the predominance of highly mycotoxigenic isolates of *P. expansum* resistant to the benzimidazoles.

Mating types and fungicide sensitivities of Cyprus isolates of *Phytophthora infestans*. L. PITTAS[1], L. KANETIS[1], D. TSALTAS[1]*, G. NEOPHYTOU[2] and N. IOANNOU[1]. [1]*Department of Agricultural Sciences, Biotechnology and Food Science, Cyprus University of Technology, CY-3603 Lemesos, Cyprus, Greece.* [2]*Department of Agriculture, Ministry of Agriculture, 1411 Nicosia, Cyprus, Greece. *E-mail: dimitris.tsaltas@cut.ac.cy*

In surveys conducted during 2009 and 2010, 265 isolates of *Phytophthora infestans* were collected from all major potato growing areas of Cyprus and analyzed for mating type and sensitivity to metalaxyl *in vitro*. Both the A1 and the A2 mating types were found in both years, with 28.5% of the sampled fields containing both mating types, thus suggesting a potential for oospore formation. The proportion of A1 mating type decreased from 60% in 2009 to 27% in 2010. A similar reduction was observed in the proportion of isolates resistant to metalaxyl, which decreased from 86% in 2009 to 9.5% in 2010, whereas the proportion of isolates found to be moderately resistant to metalaxyl increased from 13.6% in 2009 to 73% in 2010. Fungicide sensitivities were also estimated for 42 *P. infestans* isolates to cymoxanil, propamocarb, and the recently introduced mandipropamid using the agar dilution method. Calculated EC_{50} values for cymoxanil and propamocarb ranged from 0.11 to 0.87 μg mL^{-1} (mean = 0.19 μg mL^{-1}) and from 158.5 to 3,020 μg mL^{-1} (mean = 845.5), respectively. Baseline sensitivities established for mandipropamid ranged from 0.031 to 0.086 μg mL^{-1} (mean = 0.044 μg mL^{-1}). The results reported here provide an initial estimate of the variation in sensitivity to these fungicides in the Cyprus population of *P. infestans* and could be used in order to establish reliable and more effective disease management strategies of potato late blight.

Detection and quantification of cytochrome b A143/S143 alleles conferring Qo resistance in *Cercospora beticola*. A.A.MALANDRAKIS*, A.N. MARKOGLOU, D.C. NIKOU, J.G. VONTAS and B.N. ZIOGAS. *Laboratory of Pesticide Science, Agricultural University of Athens, 75 Iera Odos, Votanikos, 188 55 Athens, Greece. *E-mail: tasmal@aua.gr*

Qo inhibitors play an important role in the effective control of various diseases world-wide. Resistance

development problems, typical in pesticides with site-specific modes of action, have soon emerged resulting from target site modification in many cases of plant pathogens. In most cases a single point polymorphism (SNP) at position 143 of the cytochrome b protein -target of the Qo fungicides- due to the substitution of glycine with alanine (G143A) has been reported to be responsible for high levels of resistance towards strobilurin related fungicides. Recently, strobilurin fungicides azoxystrobin, trifloxystrobin and pyraclostrobin were registered for the control of Cercospora leaf disease in Greece while no reports of resistant field isolates are available. In an attempt to evaluate the inherent resistance development risk, highly resistant laboratory *Cercospora beticola* strains bearing the G143S mutation (substitution of glycine with serine) have been obtained after UV mutagenesis. In order to timely obtain a tool for accurate detection of the resistance mutations an allele specific PCR technique was developed. A real-time PCR assay for the quantitative detection of both G143S and G143A resistance mutations in *C. beticola* was implemented and tested on laboratory and field isolates from sugar beet cultivating regions in Greece. Allele specific primers used for the assay effectively discriminated between S143, A143 and G143 genotypes even in mixtures of 1:10000 resistant:sensitive alleles. All field isolates had no statistically significant proportion of the resistant allele which was in total agreement with the phenotypes revealed by the biotests.

Effect of DMI-resistance on mycotoxin production and fitness parameters of *Fusarium graminearum* (wheat scab). I. KALAMPOKIS, E.G. DOUKAS and A.N. MARKOGLOU. *Pesticide Science Laboratory, Agricultural University of Athens, 75 Iera Odos (Votanikos), 188 55 Athens, Greece. *E-mail: tasmal@aua.gr*

Fusarium graminearum (teleomorph *Gibberella zeae*), the causal agent of fusarium head blight (also known as wheat scab), can cause substantial losses in yield and grain quality, particularly through mycotoxin contamination. The most frequent mycotoxins produced by *F. graminearum* in cereals are the trichothecenes deoxinivalenol (DON), nivalenol (NIV), diacetoxyscirpenol (DAS), T-2 and HT-2 toxins, and the mycotoxin zearalenone (ZEA), which can cause severe toxicities in humans and livestock. The prevention of mycotoxin contamination of cereals is one of the top priorities in human and animal safety. While several pre- or post-harvest techniques have been evaluated for the control of mycotoxigenic fungal species, chemical control seems to be the main measure to reduce the incidence of mycotoxin contamination in most crops. The main objectives of the present study were to evaluate the effectiveness of sterol biosynthesis inhibiting fungicides on growth and mycotoxins production of *F. graminearum*, and further to assess the impact of resistance to sterol biosynthesis inhibitors on the fungal mycotoxigenic ability and ecological fitness characteristics. Laboratory mutant strains of *Fusarium graminearum* resistant to prochloraz (Rf: 10–30, based on EC_{50} values) were isolated at a high mutation frequency (1.8×10^{-5}) after UV-mutagenesis and selection on fungicide-amended medium. Cross resistance studies with other fungicides showed that the mutation(s) for resistance to prochloraz also reduced the sensitivity of mutant strains to other C-14 demethylase inhibiting fungicides (DMIs), such as the triazoles flusilazole, difenoconazole and epoxiconazole and to the imidazole imazalil, but not to fungicides affecting other steps of the sterol biosynthesis (e.g. morpholines and hydroxyanilidines) or other cellular pathways. Study of fitness determining parameters showed that the mutation(s) for resistance to prochloraz may or may not affect the mycelial growth rate, sporulation, conidial germination and pathogenicity on wheat and maize seeds. Analysis of mycotoxins production (DON, ZEA) by the wild-type and mutant strains of *F. graminearum* showed that the resistance to DMIs may or may not affect the *in vitro* mycotoxigenic ability of resistant strains. A correlation between mycotoxins production and phytopathogenic fitness parameters was found in *in vivo* tests with artificially inoculated wheat and maize seeds. The data of the present study indicate, for the first time, the potential risk of increased mycotoxins contamination of cereals after intensive use of DMI fungicides.

Study of the inherent resistance risk to fenhexamid in *Monilia laxa*. N. KOUKIASAS[1], A.A. MALANDRAKIS[1]*, T. VELOUKAS[2] and A.N. MARKOGLOU. *[1]Pesticide Science Laboratory, Agricultural University of Athens, 75 Iera Odos (Votanikos),188 55 Athens, Greece. [2]Plant Pathology Laboratory, Faculty of Agriculture, Aristotelian University of Thessaloniki,POB 269, 54124, Thessaloniki, Greece. *E-mail: tasmal@aua.gr*

Mutant strains of *Monilia laxa* with moderate and high resistance to the hydroxyanilide fenhexamid, a C-4 demethylase inhibitor in the fungal sterol biosynthetic pathway, were isolated at a high mutation frequency (1.5×10^{-5}) after UV mutagenesis and selection on medium containing fenhexamid. Resistance factors, based on EC_{50} values, were 10–30 and 40–80, respectively. Cross resistance studies with other fungicides showed that the mutation(s) for resistance to fenhexamid did not affect the sensitivity of the highly fenhexamid-resistant strains to fungicides affecting other steps in sterol biosynthesis, such as the DMIs (flusilazole, prochloraz), the morpholines (fenpropimorph, tridemorph) and the spiroketalamine spiroxamine, indicating that a target site modification is probably the biochemical mechanism for high level of resistance to fenhexamid.

On the contrary, a reduction in the sensitivity to fenpropimorph, tridemorph and spiroxamine, which inhibit the Δ^{14}-reduction and/or the $\Delta^{8,7}$-isomerization of the sterol biosynthesis, was observed in most moderate fenhexamid-resistant mutants, indicating the existence of a different biochemical mechanism for resistance to fenhexamid. Study of the sensitivity of mutant strains to fungicides affecting other cellular pathways, did not reveal cross-resistance of fenhexamid with the benzimidazole carbendazim, the anilinopyrimidine cyprodinil, the phenylpyrrole fludioxonil, the dicarboximide iprodione, and the strobilurin-type fungicide pyraclostrobin. Study of fitness parameters showed that the mutation(s) for resistance to fenhexamid had no apparent effect on mycelial growth, but did affect one or more of some other determining characteristics, such as sporulation, conidial germination and pathogenicity on apple fruits. Preliminary fungitoxicity tests with field strains of *M. laxa* showed fenhexamid-resistant phenotypes at a significant frequency. The risk of fenhexamid resistance development in *M. laxa* field populations is discussed under the light of these results.

Chemical control of *Camarosporium* blight in pistachio orchards. D.I. TSITSIGIANNIS[1]*, S. PALAVOUZIS[1], E.J. PAPLOMATAS[1], S.E. TJAMOS[1], P.P. ANTONIOU[1], M. DIMAKOPOULOU[1], G. ZAKYNTHINOS[2], T. BARZAKAS[2], G. KARNAVAS[3], T.J. MICHAILIDES[4] and E.C. TJAMOS[1]. [1]*Agricultural University of Athens, Department of Crop Science, Plant Pathology Laboratory, Iera odos 75, 118 55 Athens, Greece.* [2]*Department of Technology of Agricultural Products, School of Agricultural Sciences, Technological Educational Institute of Kalamata, Greece.* [3]*Directorate of Agricultural Development, Perfecture of Fthiotida, Greece.* [4]*Department of Plant Pathology, University of California, Davis, Kearney Agricultural Center, 9240 South Riverbend Ave., Parlier, CA 936484, USA. *E-mail: dimtsi@aua.gr*

Pistachio cultivation is one of the main crops in the area of Makri – Makrakomi (Fthiotida county) and the "Fthiotida pistachio nut" has been qualified as P.D.O. product (Protected Designation of Origin). The disease *Camarosporium* blight in Greece or "panicle and shoot blight of pistachio" caused by the Deuteromyces *Camarosporium pistaciae* (new classification considers it into the genera *Fusicoccum* spp. and *Neofusicoccum spp.*) is the most important phytopathological problem that pistachio orchards are encountered in this area because of the great damage it causes and the fact that there is no satisfactory control method. The teleomorph of the pathogen - *Botryosphaeria dothidea* - has not been found in pistachio in Greece but it has been found in other hosts. The disease is related directly both with quality matters of a P.D.O. product as well as the use of fungicide products, the availability of which as well as the application costs increase the production expenses. The fungus infects severely the leaves, but the main damage is due to panicle infection, which frequently reaches 20–50%. Also, the fungus infects the apical vegetation of the branches resulting in considerable reduction of the production in the following year. The objective of this particular study is the assessment of a program of chemical treatments with four different fungicide products a) Quadris® (azoxystrobin), b) Signum® (pyraclostrobin + boscalid), c) Stroby® (kresoxim-methyl) & d) Switch® (cyprodinil + fludioxonil) in combination with an integrated control program of panicle and shoot blight of pistachio. The chemical products were applied during the blooming period, at the end of the spring and the beginning of the summer 2009. There will be a presentation of the evaluation of the particular fungicides regarding their effectiveness on experimental pistachio orchards in Makri area in the presence of natural inoculum of the pathogen.

Poster Presentations

The Effects of Chemical Control, Cultivar Resistance, and Structure of Cultivar Root System on Black Shank Incidence of Tobacco. D.F. ANTONOPOULOS*, T. MELTON and A.L. MILA. *North Carolina State University, Department of Plant Pathology, Campus Box 7405, Raleigh, North Carolina, USA, 27695, USA.*E-mail: antdim75@yahoo.com*

Black shank, caused by *Phytophthora parasitica* var. *nicotianae* (*Ppn*), is a major disease of tobacco. The rise of race 1 in the late 1990s, after extensive planting of cultivars possessing the *Php* gene, confirming immunity to race 0 of *Ppn*, imposed new challenges to black shank management. The effects of tobacco cultivars and chemical controls with mefenoxam (Ridomil Gold) on black shank incidence were investigated in naturally infested fields. Twenty-five cultivars were tested and the highest resistance against races 0 and 1 of *Ppn* was provided by RJR75 and SP227 based on field and laboratory studies. When race 1 was prevalent, mefenoxam was effective to control black shank. An initial application at an early stage of tobacco growth, such as a few days before or after transplant, was essential to successfully control the disease. In greenhouse experiments, cultivars carrying the *Php* gene produced fewer and shorter adventitious roots than cultivars possessing only partial resistance to all races of *Ppn*. Strategies such as use of mefenoxam, especially at an early stage, when adventitious roots are emerging, and planting a cultivar with high partial resistance or possessing the *Ph* gene when race 1 or race 0, respectively, predominates are critical factors in reducing loss due to *Ppn*.

PREVICUR ENERGY SL® control efficacy trials against *Pythium ultimum* on cucumber plants. T.VELOUKAS[1],

G.A. BARDAS[2*], E. PALEOHORINOS[1], and R. CHATZIGEORGIADIS[1]. [1] *Bayer Hellas AG, Bayer CropScience, Sorou 18-20, 15 125 Marousi, Athens, Greece.* [2]*Laboratory of Plant Pathology, School of Agriculture, Aristotle University of Thessaloniki, P.O. Box 269, 541 24 Thessaloniki, Greece. *E-mail: gbardas@agro.auth.gr*

PREVICUR ENERGY SL® plant protection product is a Soluble Liquid (SL) with the content of the active ingredients: propamocarb 530 g L^{-1} plus fosetyl 310 g L^{-1}. It is authorised against *Pythium* sp.: a) on seedbeds via soil application (tomato, eggplant, cucumber, melon, lettuce, bell peppers), b) in greenhouses via soil application on crops tomato, bell peppers, cucumber, melon, lettuce and against *Bremia lactucae* with foliar sprays on lettuce (field). The present experimental work deals with four greenhouse trials (2005) on four cucumber hybrids (OLYMPOS, KHASSIB, CELEBRITY and RS 26302 F1) technical inoculated with *Pythium ultimum*. Each trial consisted of 5 treatments: inoculated and non-inoculated untreated control plants, two doses of PREVICUR ENERGY SL® and one dose of PREVICUR N®. The fungicides were applied twice in two weeks time intervals, with drip irrigation in soil. Three weeks after inoculation disease severity of positive control plants reached almost 100%. On the other hand, the treatments showed remarkably control efficacy.

Dicarboximides residues in grapes in E.U. A meta-analysis of concentrations (1996–2006). K.B. SIMOGLOU. *Department of Plant Protection and Quality Control. Prefecture Agriculture Directorate of Heraklion. Crete, Greece. E-mail: kbsimoglou@gmail.com*

The increased sensitivity of consumers about pesticide risks has guided the enactment of strict pesticide legislation and food security policy in European Union Member-States. None the less, people concern about their safety as far as pesticides residues are concerned. The objective of the present study was to perform a meta-analysis of dicarboximide fungicides (iprodione, procymidone, vinclozolin) residues in grapes based on the results of the European Pesticide Monitoring Programme (1996–2006). Dicarboximides are used in grapevines for the control of *Botrytis cinerea* and they have been shown to present endocrine disruptor activity. The study was performed using the specialized meta-analysis programme MIX 1.7. Mean residue concentrations were standardized with the inverse variance weight. The random effects model was used because heterogeneity of variances was observed. The half of the analytical level was substituted for non-positive specimens according to the literature. The results of the present study suggest that the mean dicarboximides concentrations that were analysed in 7.391 grapes samples from 1996 to 2006 under the European Pesticide Monitoring Programme were under the MRL that are now in force. With reference to risk assessment the study results suggest that the long-standing dicarboximides residues consumption via nutrition was very limited and lower than 1% of Acceptable Daily Intake.

Ecology, epidemiology and control of the mycotoxigenic fungi *Aspergillus* spp. in pistachio orchards in Fthiotida county. S.P. AGORITSIS[1], G. ZAKYNTHINOS[2], T.H. VARZAKAS[2], S.E. TJAMOS[1], P.P. ANTONIOU[1], M. DIMAKOPOULOU[1], G. KARNAVAS[3], E.I. PAPLOMATAS[1], E.C. TJAMOS[1], T.J. MICHAILIDES[4] and D.I. TSITSIGIANNIS[1]*. [1]*Laboratory of Plant Pathology, Department of Crop Science, Agricultural University of Athens, Greece.* [2]*Department of Technology of Agricultural Products, School of Agricultural Sciences, Technological Educational Institute of Kalamata, Greece.* [3]*Directorate of Agricultural Development, Perfecture of Fthiotida.* [4]*Department of Plant Pathology, University of California, Davis, Kearney Agricultural Center, 9240 South Riverbend Ave., Parlier, CA 93648, USA. *Email: dimtsi@aua.gr*

Mycotoxin contamination of agricultural commodities is considered a serious food safety issue worldwide. One of the most carcinogenic mycotoxins is aflatoxin (AF) produced by *Aspergillus flavus* and *A. parasiticus* that has often detected at high concentration in pistachio nuts. The goal of this study is to evaluate the AF contamination of pistachio nuts in the area of Fthiotida and to propose sustainable management solutions. The principle objectives of this project are to: a) assess the geographical and physiological divergence and distribution among *Aspergillus* spp. in pistachio nuts and orchards, b) assess the dynamics of the population composition of AF producers during the pistachios growing season, c) determine the AF content in nuts and study the epidemiology of AF contamination in correlation with meteorological data, d) evaluate novel biocontrol strategies with the study of antagonistic activity of a collection of yeasts and atoxigenic *Asperigillus* isolates against *A. flavus* and *A. parasiticus* in laboratory and field experiments and e) evaluate the efficacy of several fungicides in laboratory and field experiments against *Aspergillus*. Experiments of the 1st year showed that both *Aspergillus* section *Flavi* and section *Nigri* could be isolated from all parts of healthy and damaged pistachio fruits (hull, shell, nut). A collection of *Aspergillus* strains has been created and is currently analyzed for morphological differentiation, sclerotium size and AF production in order to estimate the diversity of the sample population and to find atoxigenic strains that could be used in biocontrol experiments. HPLC analysis of pistachio nuts from selected orchards in Makri area showed low level of aflatoxin contamination especially under storage conditions of the pistachio nuts.

VIRAL DISEASES

Invited Lecture

Serious virus problems and future risk from insect-borne viruses in relation to their insect vectors. N.I. KATIS. *Aristotle University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, 54 124, Thessaloniki, Greece. E-mail: katis@agro.auth.gr*

The serious epidemics of known insect-borne viruses in new hosts and the increase of their spread rate in crops where they are already present, is mainly related to the presence of efficient insect vectors. In the early 90's in Greece, the introduction of *Frankliniella occidentalis* Pergande (Thysanoptera: Thripidae), a very efficient vector of *Tomato spotted wilt virus* (*Tospovirus*: *Bunyaviridae*), contributed to the expansion of the virus host range and the development of serious epidemics in some vegetable crops where the virus was not previously present. Also, the recent epidemics in tomato of the Begomoviruses (*Geminiviridae*) *Tomato yellow leaf curl virus* and *Tomato yellow leaf curl Sardinia virus* (transmitted by the tobacco whitefly *Bemisia tabaci* Gennadius (Hemiptera: Aleyrodidae)), as well as the incidence of Criniviruses (*Closteroviridae*) such as the *Tomato infectious chlorosis virus* (vectored by *Trialeurodes vaporariorum* Westwood (Hemiptera: Aleyrodidae)) and the *Tomato chlorosis virus* (vectored by *B. tabaci*) the causal agents of the tomato yellowing disease, and the *Cucurbit yellow stunting disorder virus* (vectored by *B. tabaci*) and the *Beet pseudo yellows virus* (vectored by *Trialeurodes vaporariorum)* that cause the cucurbit yellowing disease, are greatly affected by the whitefly species present in a specific area. Finally, the fact that the brown citrus aphid *Toxoptera citricida* Kircaldy (Hemiptera: Aphididae), the most efficient vector of *Citrus tristeza virus* (*Closterovirus*: *Closteroviridae*), is absent from the Mediterranean basin, results to a relatively low virus spread and low transmission rates of highly virulent strains. The eradication of insect-borne viruses already present in an area is a very difficult issue. The avoidance/delay of the introduction of the most efficient insect vectors would result in the avoidance/delay of serious virus epidemics. For this reason, the application of more intensive quarantine measures is a prerequisite for reducing the risk of these epidemics.

Oral Presentations

Immunological and molecular characterisation of *Citrus tristeza virus* lemon isolates. I. MALANDRAKI[1], E. MAROULI[2], V. LIAPPIS[2] and C. VARVERI[1*]. *[1]Benaki Phytopathological Institute, Laboratory of Virology, 8 S. Delta str., 145 61 Kifissia, Greece. [2]Directorate of Agriculture, Prefecture Piraeus, 185 31 Piraeus, Greece.*E-mail: c.varveri@bpi.gr*

Citrus tristeza virus (CTV) was first reported in Greece in 2000 on non certified sweet orange trees grafted on CTV-tolerant Carrizo citrange originating from Spain. Extensive surveys have since shown natural virus transmission in orange trees in Argolis county and in Crete and eradication measures are taken in both cases. Isolates thus far characterized showed high homology with the Spanish mild T385 isolate. In 2009 and 2010, surveys were conducted in the area of Poros (Trizinia, Prefecture of Pireaus), where about 20,000 no longer cultivated lemon trees grow for more than 100 years in an area now consisting virtually a wood protected for its beauty and historical importance. A total of 236 trees were ELISA tested until the summer of 2010 and six were found CTV positive. None of the above mentioned trees showed any symptoms. All positive samples were confirmed with reverse transcription-polymerase chain reaction (RT-PCR), using two sets of primers targeting the p20 and the coat protein (cp) genes. RFLP analysis of positive samples gave identical patterns among them but different from those of standard orange isolates. Partial sequences of the p20 and cp genes of two lemon isolates were further analyzed and high nucleotide identity to the severe VT seedling yellows (SY) strain from Israel (GenBank Accession No. U56902) was obtained. The immunological reaction with the MCA13 monoclonal antibody confirmed the occurrence of a virus strain more virulent than the one thus far recorded in groves. Surveys are being continued intensively in Poros aiming at eradicating this SY CTV strain, not known to occur thus far in the Balkan area of South-Eastern Europe.

Citrus tristeza virus in Cyprus. T. KAPARI, A. KYRIAKOU*, I. GABRIEL, G. SAVVAS, L. PAPAYIANNIS and N. IOANNOU. *Agricultural Research Institute, P.O Box 22016, 1516, Nicosia Cyprus, Greece. *E-mail: kyriakou@arinet.ari.gov.cy*

Citrus tristeza virus was introduced in Cyprus in 1929 from South Africa by infected budwood and was first detected in 1968 when 27 trees of five citrus species were found infected and destroyed. During a virus survey, conducted since 1986, CTV was initially detected in four groves in Ammochostos and Larnaca district. A project for the control of the disease was initiated in 1992 and the basic objectives of the project were: i) the mapping of CTV infection through a systematic survey of citrus, ii) the removal of infected trees or groves where this was feasible against compensation to the growers, and iii) the establishment of a viable citrus certification programme. Samples were tested in the laboratory by enzyme-linked immunosorbent assay (ELISA). From 75 000 trees indexed and obtained from 850 groves with 630,000 trees, 4250 trees were found to be CTV-infected. Disease incidence ranged in the different districts from

2.7% to 18.3%. The highest proportion of infected trees and groves was noted in the districts of Ammochostos and Lemesos.

Detection of Citrus Tristeza Virus in 'calamondin' plants in a nursery unit of Preveza Prefecture and decision of eradication measures.. A. TSAPARAS[1], A. GATSIOS[1], C. VARVERI[2] and P. KOUTRETSIS[3]. *[1]Directory of Agricultural Development of Preveza, 65, Irinis Av., 48100 Preveza, Greece.[2]Benaki Phytopathological Institute, 8, St. Delta st., 14561 Kifisia, Greece.[3]National Station of Multiply Plant Material, 2, Antheon st., 15123 Marousi, Greece. E-mail: u12208@minagric.gr*

During the official surveillance for Citrus Tristeza Virus, which belongs to the list of quarantine pests for our country, a number of certain samplings were carried out, in ornamental citrus plants of a big productive unit consisted of 6 different nurseries in a total area of 20,000 m². The unit (of Israeli interests) begun its activity in 2004 at the area of Nea Sinopi, near Preveza, and it is considered to be a part of another unit, which begun its activity as a cooperative business in Koropi of Attica, almost ten years ago. After the division of the original business, three different productive units had been created; one in Koropi, one in Chalkidiki and the third one was established in this area of Preveza Prefecture. In the nurseries, citrus maternal plants were grown (calamondin=*Citrus mitis* Blanco, *C. Limon*, *C. macrophylla* and *C. volkameriana*) which probably had the same origin (Israel) with the plants of the other two units (in Attica and in Chalkidiki), as well as produced citrus plants in pots, which were mainly sold to Holland and England, and in small quantities in the domestic market. During the spring of 2008 a sampling of about 1,000 maternal plants was carried out and the samples were tested for CTV with ELISA method, by SEAPY (National Control Station for Multiply Plant Material). Although the first results were negative, the sampling was continued during the spring of 2009 (another 1,000 samples), after this procedure and two positive plants were found, from the plants of 'calamondin' variety, which were totally tested (about 800 mother plants). Then the sampling was carried on, in October and November 2009, in a total number of 3,000 produced plants from two different nurseries, which were almost ready for selling. In this process two positive plants were also found. Estimating all this situation, and considering the previous experience in nurseries of Attica and Chalkidiki, where positive samples were also found, and as a consequence, their total production, consisted of 81,560 and 30,000 plants correspondingly, was eradicated and destroyed with fire, the same measures were taken. So, in November 2009 we start the procedure of total eradication and destruction of the plant material of the unit, consisted of about 10,200 maternal plants, and 450,000 produced plants in several stages of growth. The procedure was finished in February 2010. The business had committed serious mistakes: The original plant material was not healthy, and the necessary precautionary measures were not existed, as for example the demarcation of maternal and produced plants in lots, (with the use of insect-proof nets, double insect-proof curtains at the entrance of the nurseries), the isolation (avoidance of free movement of staff, plant material and equipment from one nursery to another) and the inexistence of a well-documented tracing system, and as a result, the total destruction of all Citrus sp. plants was the only choice.

***Pepino mosaic virus*: a new virus disease of tomato crops in Cyprus.** L.C. PAPAYIANNIS[1], C. KOKKINOS[2], T. KAPARI-ISAIA[1*] and A. ALFARO-FERNÁNDEZ[3]. *[1]Agricultural Research Institute, P.O.Box 22016, Nicosia 1516, Cyprus, Greece. [2]Department of Agriculture, Nicosia, Cyprus, Greece.[3]Instituto Agroforestal Mediterráneo. Universidad Politécnica de Valencia, Camino de Vera 14, 46022 Valencia, Spain. *E-mail: theodora@arinet.ari.gov.cy*

Pepino mosaic virus (Genus *Potexvirus*, Family *Flexiviridae*) is a mechanically transmitted viral disease that has emerged as a significant virus problem in greenhouse tomato crops in Europe and all over the world. Previous studies in Cyprus showed that the virus was absent from the island. However, in January 2009, tomato fruits from two major tomato production areas in Cyprus (Parekklisia and Pyrgos, Lemesos District), exhibited symptoms of yellow mosaic and discoloration, similar to those induced by PepMV. During 2009–2010, an extensive survey was conducted in all tomato producing areas of the island in order to identify the incidence and prevalence of PepMV on protected and open field tomato crops. Approximately 3000 samples of tomato plants and weeds were analyzed using serological and molecular methods. A real-time reverse transcription TaqMan® PCR assay was developed and used for the detection of the virus. Results showed that PepMV was rapidly spread on the island, and 384 protected and 122 open field tomato plants were found to be infected. In addition, the virus was detected in 80 weed samples from 20 different species within the families of Amaranthaceae, Chenopodiaceae, Compositae, Convolvulaceae, Plantaginaceae, Malvaceae and Solanaceae. Molecular characterization and phylogenetic analysis of isolates collected from all over the island, showed that they clustered among CH2 strains.

Study of the genetic biodiversity of *Apple chlorotic leaf spot virus* (ACLSV) isolates from species of the *Rosaceae* family. A.T. KATSIANI, V.I. MALIOGKA and N.I. KATIS*. *Aristotle University of Thessaloniki, Faculty*

*of Agriculture, Plant Pathology Laboratory, 54 124, Thessaloniki, Greece. *E-mail: katis@agro.auth.gr*

Apple chlrotic leaf spot virus (ACLSV), type member of the genus *Trichovirus*, has a worldwide distribution and infects several species within the family *Rosaceae*. In the present study the frequency of ACLSV in 723 cultivated, ornamental and wild plants from several regions of the country and the phylogenetic relationships among some virus isolates, were estimated. The serological detection of the virus was performed by DAS-ELISA using two different polyclonal antibodies. For its molecular detection two available RT-PCR methods were evaluated using primers targeting: a) part of the CP gene (380 bps), and b) part of the CP including the 3' untranslated region (677bps). These two methods showed limited detection sensitivity and in order to improve it, a nested PCR combining the primer pairs mentioned above was developed. The phylogenetic analysis were performed using part of the CP gene and the 3' untranslated region (677bps) from Greek isolates determined in the present study and some, already published. The studies indicated that viral isolates from cultivated stone and pome fruits tend to be grouped in distinct host-specific clusters except from some stone fruit isolates which clustered in pome fruit groups and vice versa. No consistent grouping according to the geographic origin of the hosts was observed.

Development of a molecular method for the detection of viruses belonging to the genus *Polerovirus*. L. LOTOS, K. EFTHIMIOU, V.I. MALIOGKA and N.I. KATIS*
*Aristotle University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, 541 24, Thessaloniki, Greece. *E-mail: katis@agro.auth.gr*

Poleroviruses are considered amongst the most harmful pathogens of economically important crops as they often cause significant yield losses. The purpose of this study was to develop a molecular method for the fast and reliable detection of this genus' virus species. In order to achieve that we designed primers which anneal at the genomic region encoding the viral polymerase (RdRp). This region was selected because it exhibits a high degree of conservation within poleroviruses whereas it is significantly differentiated from the other genera of the family *Luteoviridae*. These primers were incorporated in a two stage ramped annealing RT-PCR, with which the amplification of the 593bp expected amplicon was feasible for the total of the isolates tested (23 isolates from 10 different viruses). The developed assay exhibited high detection specificity for the members of the genus *Polerovirus* as no non-specific amplification of viruses from the genetically close genera *Enamovirus*, *Luteovirus* and *Sobemovirus* was observed. Sequencing of the RT-PCR amplicon and phylogenetic analysis can provide us with initial information concerning species-classification of the polerovirus isolates, however this region is not appropriate for revealing the actual evolutionary relationships among different viruses in the genus.

Development of a Real Time PCR for the detection and quantification of several EMDV strains in different hosts. P.G. PAPPI[1], C.I.DOVAS[2], K.E. EFTHIMIOU[1] and N.I. KATIS[1*]. [1]*Aristotle University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, 54 124, Thessaloniki, Greece.* [2]*Aristotle University of Thessaloniki, Faculty of Veterinary Medicine, Laboratory of Microbiology and Infectious Diseases, 54 124, Thessaloniki, Greece. *E-mail: katis@agro.auth.gr*

Eggplant mottled dwarf virus (EMDV) is known to exist in the Mediterranean region since 1969. Worldwide epidemiological data concerning the presence and dispersal of EMDV are rather limited due to the lack of sensitive detection method. The aim of this study was the development of a sensitive and rapid molecular real-time RT-PCR method for the detection and quantitation of the viral load in plant tissues and insects. It included the optimization of the RNA extraction and the reverse transcription step of the viral RNA template. A real-time PCR assay was developed using, as standards, known number of copies of synthetic RNA that encodes part of the viral RdRp gene. The amplification efficiency was 96.9%. The dynamic (linear) detection range was 5×10^8–50 RNA copies. The method was successfully applied for the detection of EMDV in eggplant, tomato, tobacco, caper, cucumber, *Pittosporum tobira* and *Hibiscus rosa-sinensis*. The virus was also detected in adult Cicadelidae insects collected from eggplant crops. The present method is proposed as a useful tool for virus epidemiological investigation in plant hosts and insect vectors.

Identification and epidemiological studies of viruses associated with Tomato yellow leaf curl disease in Greece and Cyprus. L.C. PAPAYIANNIS[1*], J.K. BROWN[2], A. PARASKEVOPOULOS[3] and N.I. KATIS[4]
[1]*Agricultural Research Institute, P.O.Box 22016, Nicosia 1516, Cyprus, Greece.* [2]*Department of Plant Sciences, University of Arizona, Tucson, AZ 85721, USA.* [3]*Directorate of Agriculture, Plant Protection, Kyparissia 24500, Greece.* [4]*Aristotle University of Thessaloniki, Faculty of Agriculture, Plant Pathology lab P.O.Box 269, 54124, Thessaloniki, Greece. *E-mail: lambros@arinet.ari.gov.cy*

Tomato yellow leaf curl disease (TYLCD) is considered to be one of the most important and devastating whitefly-transmitted viral diseases of tomato crops worldwide. In the Eastern Mediterranean region, two *Bego-*

movirus species are involved in the disease epidemics, *Tomato yellow leaf curl virus* (TYLCV) and *Tomato yellow leaf curl Sardinia virus* (TYLCSV). Recent studies have shown that in Greece both species are present, whereas in Cyprus only TYLCV is associated with the disease. During 2006–2010, an extensive survey was conducted in Greece and Cyprus to investigate the epidemiology and characterization of the virus species and whitefly vectors involved in TYLCD. More than 8000 samples of different cultivated plants and weeds, were collected and analyzed together with approximately 2000 adult *B. tabaci* from several districts of Greece and Cyprus. The host range of four TYLCV and one TYLCSV isolate was studied using whitefly transmission tests in several plant species and the back transmission capacity of alternative infected plant hosts to tomato was evaluated. Results showed that in Greece, TYLCV was the most prevalent *Begomovirus* species (92%) whereas TYLCSV was found only in 7% of the samples. Molecular identification of *B. tabaci* biotypes showed that Q was the only biotype found in the mainland of Greece, Pelloponese and the island Crete, and seem to be involved in TYLCD transmission. In Cyprus and Rhodes islands, both B and Q biotypes co-exist. Transmission studies showed that TYLCV isolates have a broader host range than TYLCSV. Back transmission assays from infected weeds and other cultivated plants onto tomato showed that these alternative hosts could serve as important virus reservoirs, contributing significantly to serious outbreaks.

Cellular localization of Peach latent mosaic viroid in peach leaf sections employing a liquid phase in situ RT-PCR technique. I.N. BOUBOURAKAS[1*], A.E. VOLOUDAKIS[2], K. FASSEAS[3], N. RESNICK[4], H. KOLTAI[4] and P.E. KYRIAKOPOULOU[1]. *[1]Agricultural University of Athens, Department of Crop Science, Laboratory of Plant Pathology, Iera Odos 75, 11855 Athens, Greece. [2]Agricultural University of Athens, Department of Crop Science, Laboratory of Plant Breeding and Biometry, Iera Odos 75, 11855 Athens, Greece. [3]Agricultural University of Athens, Department of Agricultural Biotechnology, Laboratory of Electron Microscopy, Iera Odos 75, 11855 Athens, Greece. [4]Institute of Plant Science, Agricultural Research Organization (ARO), Bet Dagan 50250, Israel. *E-mail: ibuburakas@yahoo.com*

In order to prepare for the study of interactions between *Peach latent mosaic viroid* (PLMVd) and its peach host and the verification of the presence of the viroid in cell layers at the very tip of the Shoot Apical Meristem (SAM), a new liquid phase *in situ* RT-PCR technique (IS RT-PCR), based on *Sybr Green*, was developed. The leaves used were from healthy and PLMVd-infected peach plants, including a plant infected by a Peach Calico variant of the viroid. All steps of the assay, except for the signal detection, were carried out in liquid phase in 0.2 mL PCR tubes. The method is based on *Sybr Green* IS RT-PCR amplification of FAA-fixed peach leaf sections pre-treated with pepsin and DNase I. Observation of leaf sections using an epi-fluorescence microscope revealed a bright signal in the peach palisade leaf parenchyma cells with a sub-cellular localization of the PLMVd signals in the chloroplasts, the organelles where it is known that PLMVd replicates and accumulates. The method proved effective for the detection of PLMVd, except for a yellow-green background fluorescent signal in the case of green tissues but not in calico infected albino tissues, presumably due to chlorophyll auto-fluorescence. This is the first report of using liquid phase *in situ* RT-PCR for the cellular localization of a plant pathogen.

The incidence of viruses in lentil crops and breeding perspectives in Greece. E.K. CHATZIVASSILIOU[1]*, A. KARGIOTIDOU[2], I. TOKATLIDIS[2], S.G. KUMARI[3] and K.M. MAKKOUK[3]. *[1]Plant Pathology Laboratory, [2]Laboratory of Plant Breeding and Physiology, Department of Agricultural Development, Democritus University of Thrace, 682 00, N. Orestiada, Greece. [3]Virology Laboratory, ICARDA. P.O. Box 5466, Aleppo, Syria*
**Present address: Agricultural University of Athens, Department of Plant Science, Plant Pathology Laboratory, Iera Odos 75 Votanikos, 11855 Athens, Greece. *E-mail: echatz@aua.gr*

The use of lentil (*Lens culinaris* Medik.) certified seeds is not a common practice when establishing a new crop in Greece, implying high risk for seed transmitted diseases. During April and May of 2007 and 2008 growing seasons, a survey was conducted in six prefectures of Greece (Evros, Fthiotida, Kozani, Larissa, Lefkada, and Viotia) to determine the incidence of seed-borne viruses in lentil crops. A total of 1046 lentil samples exhibiting virus-like symptoms were collected at the flowering-podding growth stage and tested for the presence of viruses using the tissue-blot immunoassay (TBIA) technique with polyclonal antibodies against five viruses. Serological results revealed that *Pea seed-borne mosaic virus* (PSbMV, genus *Potyvirus*, family *Potyviridae*) was the most common seed-borne virus among the tested samples with an overall incidence of 5.5%, followed by *Alfalfa mosaic virus* (AMV, genus *Alfamovirus*, family *Bromoviridae*) (4.0%), *Bean yellow mosaic virus* (BYMV, genus *Potyvirus*, family *Potyviridae*) (1.2%), and *Broad bean stain virus* (BBSV, genus *Comovirus*, family *Comoviridae*) (0.2%). *Cucumber mosaic virus* (CMV, genus *Cucumovirus*, family *Bromoviridae*) was not detected in any of the tested samples. As almost 90% of the collected samples gave a negative reaction against the above mentioned antisera, representative symptomatic plants (360 samples) were further analysed during 2009 and 2010. Two

non seed transmitted viruses prevailed in those samples: *Pea enation mosaic virus-1* (PEMV-1, genus *Enamovirus*, family *Luteoviridae*) (76.4%) was associated with mosaic and mottling symptoms and *Bean leafroll virus* (BLRV, genus *Luteovirus*, family *Luteoviridae*) (50.0%) with stunting, yellowing, and reddening symptoms. In parallel, the honeycomb breeding methodology was established from 2006 to 2009 within a cultivated landrace named "Evros". Selection of individual plants was applied on the seed yield basis, under ultra-low density and for two generations, while virus presence was recorded by Enzyme Linked Immunosorbent Assay (ELISA) tests. Second generation progeny lines were free from PSbMV, present at the rate of 16.0% in the initial germplasm, as well as from BYMV and AMV, identified in the 1st generation progeny lines at the overall rates of 33.0% and 2.0%, respectively. From the breeding perspective in Greece, virus elimination from the cultivated varieties, as well as breeding for tolerance/resistance to widespread viruses is of crucial importance for crop's sustainability, and for conservation of this valuable germplasm in the country.

Poster Presentations

Host range and characterization of *Criniviruses* and whitefly vectors involved in yellowing symptoms of tomato crops in Greece. C.N. DIMITRIOU[1], L.C. PAPAYIANNIS[2], A. GATSIOS[3], C. ORFANIDOU[1], I. FOTIOU[1] and N.I. KATIS[1]*. [1]*Aristotle University of Thessaloniki, Department of Agriculture, Plant Pathology Laboratory, 54 124 Thessaloniki, Greece.* [2]*Agricultural Research Institute, P.O.Box 22016, Nicosia 1516, Cyprus, Greece.* [3]*Directorate of Agricultural Developmentof Preveza, Crop Protection Section, Preveza, Greece. *E-mail: katis@agro.auth.gr*

Tomato chlorosis virus (ToCV) and *Tomato infectious chlorosis virus* (TICV) are two emergent viral diseases of tomato crops in Greece and worldwide. Both viruses cause similar symptoms such as yellowing, interveinal chlorosis and reddish necrotic spots which appear firstly on the older tomato leaves and then expand to the younger ones. In nature, ToCV is transmitted both with the tobacco whitefly (*Bemisia tabaci* Gennadius) and the greenhouse whitefly (*Trialeurodes vaporariorum* Westwood), whereas TICV is only transmitted by *T. vaporariorum*. During 2009–2010 the incidence and distribution of ToCV and TICV, as well as the whitefly-vectors involved in the disease epidemics, were investigated in several plant species from different geographical regions of Greece. Symptomatic tomato samples, asymptomatic plants of other cultivated species, arable weeds and adult whitefly populations were collected from the Prefectures of Thessaloniki, Pella, Trikala, Preveza and Messinia. Plant samples were tested with RT-PCR whereas whitefly identification was performed using molecular probes and real-time PCR tests. In total, 70 tomato, 55 of other cultivated plant species, 298 arable weeds and 850 adult whiteflies were tested. Results showed that TICV was the only crinivirus involved in tomato yellowing epidemics in the sampling areas. For the first time, the virus was detected in several weed species within the families of Amaranthaceae, Compositae, Cruciferae, Malvaceae, Rubiaceae, Scrophulariaceae and Solanaceae and which possibly play an important role in the disease epidemiology. *T. vaporariorum* was found to be the predominant whitefly species as it was detected in 90% of the tested insect population, whereas *B. tabaci* (biotype Q) was only identified in 10%.

Identification of *Pepino mosaic virus* (PepMV) in glasshouse cherry tomato crops in Greece. K.E. EFTHIMIOU[1], A.P. GATSIOS[2], K.C. ARETAKIS[3], L.C. PAPAYIANNIS[4] and N.I. KATIS[1]. [1]*Aristotle University of Thessaloniki, Faculty of Agriculture, Plant Pathology lab P.O.Box 269, 54124, Thessaloniki, Greece.* [2]*Directorate of Agriculture Development of Preveza, Preveza 48100, Greece.* [3]*Asklipiou 15, Preveza 48100, Greece.* [4]*Agricultural Research Institute, P.O.Box 22016, Nicosia 1516, Cyprus, Greece. E-mail: u12208@minagric.gr*

During spring of 2010 mosaic symptoms were observed in cherry tomato crops (hybrid Shiren) in the area of Drymos, Vonitsa, Aitoloakarnania County. No symptoms appeared in tomato fruits of the affected plants. Symptomatic plants were tested for the presence of *Cucumber mosaic virus* (CMV) and *Pepino mosaic virus* (PepMV). CMV identification was based on Enzyme linked-immunosorbent assay (ELISA) with polyclonal antibodies, whereas PepMV identification was based on RT-PCR by using virus specific primers and subsequent sequencing of the obtained 200 bp amplicon. The results showed that some of the symptomatic cherry tomato plants were infected with both CMV and PepMV whereas others were infected only with PepMV. Nucleotide sequence data showed that Greek isolates were identical with US2 strain of the virus which has been reported in USA and Europe. Plant tissue from the plants found to be infected only with PepMV was also used for mechanical inoculation of indicator plants such as *Nicotiana glutinosa* and *N. benthamiana*. Soon after inoculation, test plants were transferred to the glasshouse (temperature 20–24ºC) for symptom development. Two to 3 weeks post-inoculation, *N. benthamiana* plants developed mosaic symptoms, and were found to be PepMV infected by RT-PCR. No symptoms developed in *N. glutinosa* plants which were found PepMV negative when tested by RT-PCR. This is the first report of PepMV in Greece. PepMV is transmitted by tomato seed (although in a very low incidence) and mechanically under field conditions. Therefore, use of virus-free tomato seed is the basic prerequisite for the control of PepMV.

First report of *Cherry green ring mottle virus* (CGRMV) in Greece and development of a new molecular detection method of the virus. A.T. KATSIANI[1], V.I. MALIOGKA[1], C. KTORI[1], E.K. CHATZIVASSILIOU[2] and N.I. KATIS[1]*. [1]*Aristotle University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, 54 124, Thessaloniki, Greece.* [2]*Democritus University of Thrace, Department of Agricultural Development, Plant Pathology Laboratory, 68 200, N. Orestiada, Greece .*E-mail: katis@agro.auth.gr*

Cherry green ring mottle virus (CGRMV, *Flexiviridae*) infects several *Prunus* species in North America and Europe, including sweet and sour cherry, oriental flowering cherry, peach and apricot. Although most of CGRMV infections are symptomless, on sour cherry it causes yellow mottling and ring-like patterns on leaves and misshapen and bitter fruits. During a survey, conducted in spring of 2007 and 2009, on sweet cherry plantations in Northern Greece, for the presence of *Flexiviridae* virus species, CGRMV was identified using a polyvalent nested (PDO) RT-PCR targeting a 362 bp part of the viral RdRp. Initially CGRMV was identified in 2 of the sweet cherry samples tested (Acc. No. FN544780, FN544781). Comparative sequence analysis of both PCR products showed identities to homologous sequences of other published CGRMV isolates, though a high variability was observed. Based on the available RdRp sequences a new degenerate downstream primer was designed and used along with an already published flexi-virus generic upstream one in a nested PCR for the specific detection of CGRMV. The assay was applied for screening 95 cherry trees from different areas of Northern Greece and indicated that CGRMV is rather dispersed (20/95 samples tested) in sweet cherry plantations of the country. The new method exhibited broader detection range when compared to a previously reported PCR assay, which targets the coat protein of the virus. To our knowledge these findings represent the first report of CGRMV in sweet cherries in Greece.

Phytosanitary control of vegetative propagation material samples by the ELISA method in Greece. P. KOUTRETSIS, M. KAPONI*, G. THEODOROPOULOS, I. TSAPARLI and I. AXARLI. *Ministry of Rural Development and Food, Control Station for Vegetative Propagation Material, 151 23 Amaroussion, Athens, Greece. *E-mail: mkaponi@yahoo.gr*

During 2009–2010, a great number of vegetative propagation material samples (seed-potato, grapevine, citrus, pome fruit, stone fruit, squash, strawberry and asparagus) were examined for the presence of viruses, in the context of requisite phytosanitary surveys for the certification and trading of this material in the E.U. The presence of various viruses was tested by ELISA in prebasic, basic and certified propagation material of the species above, mostly traded in the E.U. and secondarily internal. Until now (6/2010), surveys have shown the presence of Potato potyvirus Y (PVY) in 1.274/40.451 seed-potatoes (3,15%), Potato potexvirus X (PVX) in 5/40.451 seed-potatoes (0,01%) and Potato leaf roll luteovirus (PLRV) in 44/40.451 seed-potatoes (0,1%); GFLV (62/8.106 plants, 7,6%), GFkV (82/4.091 mother plants, 2%), GLRaV-1 (61/8.106 plants, 0,7%) and GLRaV-3 in (78/8.106 plants, 0,9%); and Citrus tristeza closterovirus (CTV) in 65/8.242 citrus trees, published and located in productive orchrards (Argolis) and nurseries (Preveza) until eradication. In contrast, the following viruses were not detected; PVA, PVM and PVS in prebasic seed-potato (99 samples); ArMV in 8.106 grapevines and 2 squash rootstocks; PPV, ACLSV, PDV and PNRSV in 677 stone fruit samples; ASGV, ASPV, ApMV and ACLSV in 64 pome fruit samples; SLRSV, ToBRV, RpSPV and ArMV in 2 strawberry crowns; and AV-2 in 8 asparagus crowns. Besides the activation of the phytosanitary legislation, these inspections are important for the prevention of the introduction and spread of quarantine pathogens in Greece, such as CTV.

Detection of *Citrus bark cracking viroid* (CBCVd) on citrus mother trees in Greece. E.M. KOUTSIOUMARI[1], M. AFUNIAN[2], I.N. BOUBOURAKAS[3], P.E. KYRIAKOPOULOU[4], T. AGORASTOU[5], G. MAGRIPIS[5], G. VIDALAKIS[2] and A.E. VOLOUDAKIS[1]*. [1]*Agricultural University of Athens, Department of Crop Science, Laboratory of Plant Breeding and Biometry, 11855 Athens, Greece.* [2]*University of California Riverside, Department of Plant Pathology & Microbiology, Riverside, CA 92521 USA.* [3]*Agricultural University of Athens, Department of Crop Science, Laboratory of Plant Pathology, 11855 Athens, Greece.* [4]*former Professor, Agricultural University of Athens, Department of Crop Science, Laboratory of Plant Pathology, 11855 Athens, Greece.* [5]*Poros Arboricultural Station, 18020, Poros, Troizinia, Greece. *E-mail: avoloud@aua.gr*

Citrus bark cracking viroid (CBCVd, syn. Citrus viroid IV) causes cracking of the bark of the susceptible rootstock *Poncirus trifoliata* and its hybrids, whereas all other citrus species do not show any symptoms. Although citrus viroids are commonly detected in citrus which are not propagated under a certification program, CBCVd was not detected in a recent study performed on citrus mother trees of the Poros Arboricultural Station. In the present study extensive tests were conducted for the presence of CBCVd on citrus trees by: a) biological indexing and bioamplification on indicator-plants (*C. medica*, Etrog citron Arizona 861-S-1) and b) molecular detection by RT-PCR directly from mother trees using two sets of primers (G. Vidalakis lab, Ito *et al*., 2002), followed by sequencing of the PCR products. Using the above methods, a great number of citrus mother trees were found to be infected by CBCVd. These findings

complement the results obtained in the citrus detection project at the greek citrus trees and suggested that RT-PCR method could be applied directly on field samples for the detection of citrus viroids. Following the recent report of the detection of two new viroids, CVd-V and CVd-VI, the necessity for continuous testing for graft-transmitted pathogens and sanitation of the most important citrus cultivars in order to obtain healthy propagating material in Greece, is urgently needed. The tests performed up to now under the citrus sanitation project indicated that trees free of CBCVd have been produced.

Partial characterization of a polerovirus associated with pepper yellows. L. LOTOS, K. EFTHIMIOU, V.I. MALIOGKA and N.I. KATIS*. *Aristotelian University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, 541 24, Thessaloniki, Greece. *E-mail: katis@agro.auth.gr*

In 2008 yellowing symptoms were observed in glasshouse pepper crops in Antalya Turkey and samples were collected in order to identify the causal agent. Previous studies reported the infection of pepper from members of the genus *Polerovirus*, namely BWYV and PLRV. For this reason the samples were initially tested for the presence of poleroviruses using genus-specific primers. Sequencing of the 593bp product amplified with the generic RT-PCR indicated that the pepper virus isolate was differentiated from BWYV and PLRV whereas it was highly similar with TVDV. For further investigation of the taxonomic classification of the isolate (PY), a larger portion of the genome of approximately 1600bp, spanning from the 3' end of the RdRp until the end of the CP, was sequenced. The amino-acid sequences derived from the translation of this portion (MP and CP) were used in phylogenetic analysis applying the maximum likelihood method. Even though the results of the analysis showed a grouping of the PY isolate with the ones belonging to TVDV, the high genetic distances observed between them on both proteins of the CP and MP (11.8% and 16.7%, respectively) indicate that the PY isolate probably belongs to a new species of the genus *Polerovirus* with the putative name *Pepper yellows virus* (PeYV).

The presence of *Peach latent mosaic viroid* (PLMVd) in peach propagative material in Greece. I.N. BOUBOURAKAS*, I. PALYVAKOU and P.E. KYRIAKOPOULOU. *Agricultural University of Athens, Department of Plant Production Science, Plant Pathology Laboratory, 11855 Athens, Greece. *E-mail: ibuburakas@yahoo.com*

In a recent survey, PLMVd was found widely spread in Greek peach orchards, and this is to be mainly attributed to the trade and use of PLMVd-infected peach propagative material. The aim of the present study was to evaluate the status of peach/nectarine germplasm concerning the presence of PLMVd in Greece. During the years 2008–2009, 711 peach/nectarine samples were collected from three nurseries, 353 from one-year seedlings and 358 from 8–10 year mother plants. The presence of PLMVd was ascertained applying RT-PCR and dot-blot RNA hybridization. Of the one-yearold seedlings examined, 51.2 % (181/353) were found infected. None of the 14 varieties examined was found PLMVd-free, while the mostly infected were found the varieties A37 (86%) and Spring Bell (82.5%). The PLMVd-infected mother trees were found very high (82.5%, 305/358). The viroid incidence was greater than 70% in 14 out of 16 peach/nectarine varieties of mother trees tested. The varieties Early Crest, Galtetzi, Honey and Royal Glory were found 100% infected. So, there is an obvious national need for direct action for the production of viroid-free propagative material and for informing the farmers about the need to use healthy such material.

Genetic variability of *Citrus tristeza virus* (CTV) isolates from Cyprus. L.C. PAPAYIANNIS*, A. KYRIAKOU and T. KAPARI-ISAIA. *Agricultural Research Institute, 1516, Nicosia, Cyprus, Greece. *E-mail: lambros@arinet.ari.gov.cy*

Citrus tristeza virus (CTV) was first reported in Cyprus in 1968 and until recently virus detection has been mainly based on Mexican lime (*Citrus aurantifolia*) indexing and ELISA tests. In view of a national project aiming at the disease management, the genetic variability among different CTV isolates was studied using molecular methods. The capsid protein gene (CP) from 31 isolates collected from Cyprus, as well as 4 collected from Greece, was amplified using Reverse Transcription (RT) Polymerase Chain Reaction (PCR). The amplified fragment was analyzed using restriction fragment length polymorphism (RFLP) and single strand conformational polymorphism (SSCP). The nucleotide sequence of the CP gene was determined and phylogenetic analysis was performed. Results showed that 22 symptomless isolates from Cyprus clustered among the mild strains reported from Spain, Portugal and Africa. In addition, five isolates that were responsible for decline of sweet orange, grapefruit and mandarin trees showed high similarity with strains reported from Africa (B249), whereas four other isolates, that caused stem pitting symptoms, clustered with T36, an american severe strain from Florida. All four Greek isolates were identical to strain T385 from Spain.

Serological detection of AMV and CMV in eggplant crops and weeds of Northern Greece with enzyme-linked immunosorbent assay ELISA and molecular characterization of several CMV isolates. P.G. PAPPI,

K.N. MORAKI, P.S. NTASIOU, K.E. EFTHIMIOU and N.I. KATIS*. *Aristotle University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, 54 124, Thessaloniki, Greece. *E-mail: katis@agro.auth.gr*

Cucumber mosaic virus (CMV) and *Alfalfa mosaic virus* (AMV) are among the most important viruses as they have a wide host range, including cultivated and weeds. In the present study, 2052 eggplant samples from Serres, Thessaloniki and Chalkidiki Prefectures and 300 weeds from eggplant cultures from Vassilika (Thessaloniki) were randomly collected in order to evaluate the incidence and the distribution of CMV and AMV by ELISA. CMV was present in 336 eggplant samples and in 5 weeds, belonging to species *Solanum nigrum, Lamium amplexicaule* and *Sinapis arvensis*. The virus was found to be more frequent in the Prefecture of Serres (80%), whereas its incidence was lower in Thessaloniki (9.6%) and in Chalkidiki (3.9%). AMV was detected in 33 eggplant samples, from Thessaloniki (1.9%) and Serres (0.4%). Further molecular characterization of the CMV isolates using RT-PCR, revealed the presence of CMV I, CMV II and the satellite RNA in all the prefectures. The eggplant could be a significant source for CMV epidemics in neighbor susceptible crops.

Survey for the identification of CTV-infected trees and optimization of molecular CTV-detection techniques in Crete. H. AFIFI, D. TSIKOU, A. KARAYANNI and I. LIVIERATOS*. *Mediterranean Agronomic Institute of Chania, Alsylio Agrokepion, Chania Gr-73100, Greece. *E-mail: livieratos@maich.gr*

A survey for the identification of *Citrus tristeza virus* (CTV) in more than 2000 citrus trees in Crete was conducted using a commercial available immuno-print kit. This survey resulted in three tree samples (TI, 342 and 802), all from Western Crete. The nucleotide sequences of the complete coat protein, p23 and HSP70 genes were determined to allow phylogenetic analysis of these CTV isolates. Using these CTV-infected samples, a PCR-amplified CTV dig-labeled probe detected the virus in hybridization experiments using: stem prints, rolling-leaf prints, plant sap and total RNA extracts. Total RNA extracts from CTV-infected plant tissues represented the template with the strongest reaction and minimum background, which often constitutes an issue for both serology and molecular detection techniques. Furthermore, print capture- and immuno capture-PCR protocols were used successfully. Our observations may be of use to extension and phytosanitary services for routine, reliable and low-cost detection of the virus.

Viruses infecting asparagus (*Asparagus officinalis* L.) crops in Greece. E.K. CHATZIVASSILIOU*, E. TSOUPRAS, and F. MIGGOU. *Democritus University of Thrace, Department of Agricultural Development, Plant Pathology Lαboratory, Pantazidou 193, 682 00 N. Orestiada, Greece. *Present address: Agricultural University of Athens, Department of Plant Science, Plant Pathology Laboratory, Iera Odos75 Votanikos, 11855 Athens, Greece. *E-mail: echatz@aua.gr*

Asparagus (*Asparagus officinalis* L.) represents a promising crop in export terms in Greece. Several viral diseases have been reported to infect asparagus worldwide resulting in reduced plant vigor and spears quality, as well as in increased susceptibility to other plant pathogens. *Asparagus virus I* (AV-I, genus *Potyvirus*, family *Potyviridae*) and *Asparagus virus II* (AV-II, genus *Ilarvirus*, family *Bromoviridae*) are considered as the most important ones, suggested to be involved in asparagus decline syndrome. Data on the presence of those viruses in asparagus crops in Greece are lacking. During September 2009, 465 asparagus fern samples were collected from asparagus fields in Evros, Kavala and Pella prefectures, irrespectively of the presence of any symptoms. Additionally, a number of plants with virus-like symptoms were collected separately. Samples were tested with antigen-coated plate (ACP) ELISA tests for the presence AV-II and *Potyvirus*, and with mechanical inoculations onto indicator plants for the presence of *Tobamovirus* and *Potexvirus*. AV-II and *Potyvirus* were recorded in all sampling areas but not the presence of *Tobamovirus* and *Potexvirus*. In 20 asparagus crops sampled from Tychero and N. Vissa areas of Evros prefecture, virus infection reached rates of 40.0% for AV-II and 100.0% for *Potyvirus*. In Chrysoupoli (Kavala) area 14 fields were sampled and AV-II presence was up to 80.0%, while potyviruses were detected in rates from 30.0% to 100.0%. In nine crops from Pella prefecture (Giannitsa and Aridea) infection rates up to 50.0% and 38.9% were recorded for AV-II and *Potyvirus*, respectively. AV-II and potyviruses, either in single or mixed infections, were associated with plants showing necrotic lesions, fern deformities, dwarfing and/or chlorosis. Indicator plants reacted with the development of symptoms representative of AV-I infection, when mechanically inoculated with plants infected with *Potyvirus*. In a number of plants showing virus-like symptoms none of the viruses under study was detected.

Field evaluation of physiological spear rust and fern rust (*Puccinia asparagi* DC) in eight *Asparagus officinalis* L. cultivars. E.K. CHATZIVASSILIOU[1]*, E. KAZAKIS[1], A.S. SIOMOS[2] and Z. ABAS[3]. [1]*Plant Pathology Laboratory,* [3]*Laboratory of Zootechny, Department of Agricultural Development, Democritus University of Thrace, 68200, N. Orestiada, Greece.* [2]*Department of Horticulture, Aristotle University, 54124 Thessaloniki, Greece. *Present address: Agricultural University of Athens, Department of*

*Plant Science, Plant Pathology Laboratory, Iera Odos75 Votanikos, 11855 Athens, Greece. *E-mail: echatz@aua.gr*

This study aimed to evaluate eight asparagus cultivars (Cipres, Darbella, Darlise, Dariana, Darsiane, Grolim, Larac and Steline) for their field response to physiological spear rust as well as to fern rust caused by *Puccinia asparagi* DC, under Greek conditions. Experimental field was established in 2004 in Tychero (Evros prefecture) in a randomized complete block experimental design, replicated three times and evaluation was performed in 2009 growing season. Physiological rust (extended red-brown discoloration and spots) was assessed on harvested spears once a week from 4/4 to 30/5/2009, in the respective daily harvest (in total 9 harvests). The lower number and weight of spears rejected due to physiological rust was recorded for Steline (110 kg ha^{-1}) that was significantly different from that of Grolim and Darianna (500 and 430 kg ha^{-1}, respectively). Most cultivars, except from Grolim and Steline, yielded more spears with physiological rust under conditions of high soil moisture and low temperature. During fern growth, the appearance of orange pustules of urediniospores, and/or black teliospores on stems and needles was used as a diagnostic sign of *P. asparagi* infection. Disease incidence (% infection of the cultivar) was recorded biweekly in three sampling dates from 10/8 to 26/9/2009, and subsequently the area under the disease progress curve (AUDPC) was calculated, while disease severity (the percentage of the main stem covered with urediniospores and/or teliospores) was estimated once at the end of September. Significant differences were recorded among the evaluated cultivars to their susceptibility to fern rust. Steline appeared to be the most susceptible cultivar (70% diseased plants), while Cipres the most tolerant (5%). The most intense symptomatology was recorded on the cultivars Larac, Steline and Dariana (47.3, 43.4 and 40.4% of the stem covered with symptoms, respectively), while Darbella showed only mild symptoms (13.8%). Those evaluation data can be valuable for the choice of the most appropriate cultivar to be cultivated in the area, however our study need to be repeated in the subsequent cropping periods.

BIOLOGICAL AND INTEGRATED MANAGEMENT

Oral Presentations

Sustainable use of chemical fumigants for the control of soil-borne pathogens in the horticultural sector (LIFE 2008 - SustUse Fumigants). D.I. TSITSIGIANNIS, P.P. ANTONIOU, S.E. TJAMOS, S.D. KOUNTOURI, E.J. PAPLOMATAS and E.C. TJAMOS*. *Agricultural University of Athens, Department of Crop Science, Laboratory of Phytopathology Iera Odos 75, 118 55 Athens, Greece. *E-mail: ect@aua.gr*

The project "SustUse Fumigants" is funded by the European Union under the "LIFE + Environment Policy and Governance 2008" and is intended to promote the sustainable use of chemical fumigants and the promotion of non-chemical practices (e.g. soil solarization, resistant rootstocks and biological control agents) for the control of soil-borne pathogens in the horticultural sector, in two agro-ecosystems of Mediterranean agriculture (Greece and Italy) and in a system typical for Central Europe (Poland). The project encourages the judicious use of pesticides by applying principles of Integrated Pest Management (IPM) in order to support the EU policy for successful and sustainable use of pesticides.

The specific objectives of SustUse are the following:

1. To promote the wider adoption of more sustainable crop protection strategies for soil-borne diseases in horticulture
2. To promote the sustainable use of chemical fumigants in horticultural cropping systems
3. To maintain competitiveness of European horticulture in a globalized market, and in particular Italian, Greek and Polish markets.
4. To increase effectiveness of research on sustainable use of pesticides
5. To promote the awareness of growers, fumigators, advisors, policy makers and general public on sustainable crop protection strategies at National and European Level.

Effect of *Trichoderma harzianum* strain T22 on *Pythium ultimum* disease everity during acclimatization phase of GF-677 rootstocks. A. BARDAS[1]*, E. BALLAS[2], K. MAVRODIMOS[2] and C. XILOYIANNIS[3]. *[1]Laboratory of Plant Pathology, School of Agriculture, Aristotle University of Thessaloniki, P.O. Box 269, GR541 24 Thessaloniki, Greece. [2]Fytotechniki, Plant Tissue Culture Company, Filothei, 47042, Arta, Greece. [3]Dipartimento di Scienze dei Sistemi Colturali, Forestali e dell'Ambiente, Universita` degli Studi della Basilicata, Via dell'Ateneo Lucano, 10, 85100 Potenza, Italy. *E-mail: gbardas@agro.auth.gr*

Pythium ultimum mediated damping-off is a limiting factor during acclimatization period of GF-677 rootstocks. The present work deals with biocontrol trials against *P. ultimum* on GF-677 rootstocks, using commercial (TrianumP® and TrianumG®) and pure culture (turf incorporation, root dipping) treatments of *Trichoderma harzianum* strain T22. *T. harzianum* strain T22, an efficient root and rizosphere colonizer, is able to control plant pathogens showing different modes of action. Concerning root and turf colonization experiments, T22 pure cultures when treated by root dipping or by turf incorporation revealed the most promising results compared with commercial treatments. The specific enhanced colonization rates also resulted in greater

values of several plant growth characteristics. Disease control efficacy experiments showed that TrianumG® and pure T22 culture, when incorporated in turf, significantly reduced disease incidence showing 20% and 17% dead plants by the end of the experiments. On the other hand, root dipping treatment, showed greater dfferentiation between commercial and pure T22 culture handlings. More specific GF-677 plants dipped in T22 liquid culture, prior to transplantation, was the most effective method against the plant athogen, showing 9% disease severity. TrianumP® treatment seemed to e ineffective resulting in almost 90% of dead plants. In conclusion, oot dipping of GF-677 using pure culture of *T. harzianum* T22 can be a otential factor in integrated control systems against *P. ultimum* during acclimatization phase.

Observations of the interactions between the phytopathogenic fungus *Fusarium oxysporum* f. sp. *radicis-lycopersici* and the biocontrol agents *Pseudomonas chlororaphis* PCL1391 and *Clonostachys rosea* IK726 on tomato roots using autofluorescent proteins. G.D. TZELEPIS[1,2], N.N. KAMOU[1], E. PANTERIS[3], I.S. PANTELIDES[4], S.E. TJAMOS[4] and A.L. LAGOPODI[1]*
[1]*Plant Pathology Laboratory, School of Agriculture, Aristotle University of Thessaloniki P.O. Box 269, GR541 24 Thessaloniki, Greece.* [2]*Department of Forest Mycology and Pathology, SLU, P.O. BOX 7026, SE-750 07 Uppsala, Sweden.* [3]*Department of Botany, School of Biology, Aristotle University of Thessaloniki P.O. GR541 24 Thessaloniki, Greece.* [4]*Agricultural University of Athens, Plant Pathology Laboratory, Iera odos 75, GR11 855, Athens, Greece. *E-mail: lagopodi@agro.auth.gr*

In a previous work, reduction of crown and root rot severity caused by the fungus *Fusarium oxysporum* f. sp. *radicis lycopersici* (Forl) was reported, after the application of the bacterium *Pseudomonas chlororaphis* PCL1391 and the fungus *Clonostachys rosea* IK726. The phytopathogenic fungus was transformed with the red fluorescent protein gene (DsRed), while *C. rosea* IK726 and *P. chlororaphis* PCL1391 were transformed with the green fluorescent protein (GFP) gene and its analogue yellow fluorescent protein (YFP) respectively. Microscopic observations revealed the ability of the beneficial fungus *C. rosea* IK726 to colonize the root system of tomato. *C. rosea* IK726 covered the surface of the root at places respective to those that the phytopathogenic fungus colonizes. Such places are the root hairs and the junctions of the surface cells, which implies that the disease severity reduction by this specific biocontrol agent is due to antagonism. Although the beneficial fungus was found to grow and sporulate on the root surface, however it was not shown to parasitize within the root cells. Moreover the presence of the colonies of the bacterium *P. chlororaphis* PCL1391 was confirmed at the junctions of the surface cells, at several spots on the root, as well as on the hyphae of the phytopathogenic fungus.

Gaseous ozone treatment of kiwifruit during cold storage induces resistance to stem-end rot caused by the fungal pathogen *Botrytis cinerea*. I.S. MINAS[1], G.S. KARAOGLANIDIS[2]*, G.A. MANGANARIS[3] and M. VASILAKAKIS[1]
[1]*Laboratory of Pomology, School of Agriculture, Aristotle University of Thessaloniki, Thessaloniki, 54124, Thessaloniki, Greece.* [2]*Laboratory of Plant Pathology, School of Agriculture, Aristotle University of Thessaloniki, Thessaloniki, 54124, Thessaloniki, Greece.* [3]*Department of Agricultural Sciences, Biotechnology and Food Science, Cyprus University of Technology, Lemessos, 50329, Cyprus, Greece. *E-mail: gkarao@agro.auth.gr*

Stem-end rot caused by the fungal pathogen *Botrytis cinerea* is the most important postharvest disease of kiwi fruit. Control of the disease is most commonly achieved by pre- and post-harvest fungicide treatments, while cultural methods, like good tree canopy aeration, good orchard hygiene and careful post-harvest fruit handling can merely contribute to successful disease control. However, the worldwide problem of resistance development in fungicides used against the pathogen and the social concerns regarding the problem of pesticide residues on the fruits constitute important limiting factors for the chemical control of the disease, necessitating research for alternative control methods. In the current study the effect of gaseous ozone exposure on the development of gray mold disease caused by *Botrytis cinerea*, on kiwifruit (*Actinidia deliciosa*, cv. 'Hayward') was investigated. Artificially inoculated kiwifruit subjected for 4 months to cold storage (0°C, RH 95%) either in an ozone (O_3) enriched atmosphere (0.3 μL L^{-1}) or in a conventional for kiwifruit cold chamber, where catalytic oxidation of ethylene is applied. Results showed that ozone treatment delayed and simultaneously decreased disease incidence by 56%, while disease severity on infected fruits remained unaffected. Artificially infected fruit formed sclerotia, while no sporulation of the pathogen occurred in the presence of ozone. To elucidate whether the observed disease suppression was mediated by a direct effect of ozone on the fungal pathogen or by the induction of a resistance mechanism in the fruit, two additional sets of experiments were conducted. Kiwifruits were exposed to ozone (0.3 μL L^{-1}) for 0, 2, 8, 24, 72 and 144 h either before or after the artificial inoculation with the pathogen and the effect on the development of the disease in terms of disease incidence and severity was monitored. Results revealed that pre-inoculation exposure of fruit to ozone, at increasing exposure time intervals provided significant disease incidence suppres-

sion while post-inoculation exposure of the fruits did not affect disease incidence and severity. Such results provided direct evidence that ozone treatments induce resistance of kiwifruit against the pathogen. Measurements of fruit quality parameters such as total carotenoids, flavonoids, phenols and antioxidant activity on fruit exposed to ozone for the same intervals showed that there was a strong negative correlation among disease incidence or severity and phenol content. Such results suggest that ozone exposure may induce phytoalexin accumulation, that are mainly compounds of phenolic nature.

Evaluation of surfactants in activation of Systemic Acquired Resistance (SAR) in plants. M. MOSCHOGIANNI, E.C. TJAMOS, P.P. ANTONIOU, J. KUC and D.I. TSITSIGIANNIS*. *Agricultural University of Athens, Department of Crop Science, Laboratory of Phytopathology Iera Odos 75, 118 55 Athens, Greece. *Email: dimtsi@aua.gr*

Plants, during their long evolutionary path, acquired complex mechanisms that confer resistance to a great number of pathogens (fungi, bacteria and viruses). Systemic Acquired Resistance (SAR) is part of the innate immunes system in plants and is expressed as the biochemical stimulation of latent resistance responses, in a way that a plant prior vulnerable to a host-pathogen system becomes resistant. SAR's results for a variety of pathogens are often so effective in the prevention and control of the disease that triggered scientists to try to reproduce the SAR phenomenon in absence of pathogens by using various chemical compounds. In the current study, two cationic surfactants of ammonium bromide were evaluated for their ability to trigger the plant immune system. It is the first time that these surfactants are tested for their capacity to trigger systemic resistance in plants, whereas they were found to play a major role as adjuvants, when tested on other organisms. The two surfactants A and B were applied at different concentrations on plant leaves and evaluated for their role in pathogenicity-virulence of three plant pathogens. The pathogenicity tests were conducted in *Arabidopsis thaliana* plants inoculated with the oomycete *Hyaloperonospora arabidopsidis* or the soilborne pathogen *Verticillium dahliae* and *Nicotiana tabacum* plants inoculated with *Tobacco mosaic virus*. The pathogenicity tests showed that treatment with the factor A at the concentration of 10 mM reduced the sporulation of the oomycte *H. arabidopsidis* by 50% whereas none of the concentrations of the two factors was able to reduce the disease rate caused by *V. dahliae*. Evaluation of the two factors for TMV virulence conducted in tobacco plants showed that factor B at concentrations of 10 and 50 mM was effective in the control of the virus. Further preliminary studies at molecular level, conducted in *A. thaliana*, showed that factors A and B have the capacity to trigger genes that play a major role in plant immunity such as *PR-1* and *PR-5* (Pathogen Related Proteins) providing a first evidence for the putative mechanism of action of these compounds.

Evaluation of surfactants substances in controlling bacterial canker of tomato. E. MORAITIS, A. PANTELIA, C. CHARALAMBOUS, E.C. TJAMOS, D.I. TSITSIGIANNIS, J. KUC and P.P. ANTONIOU*. *Agricultural University of Athens, Department of Crop Science, Laboratory of Phytopathology Iera Odos 75, 118 55 Athens, Greece. *E-mail: ppantonioui@aua.gr*

Bacterial canker of tomato caused by *Clavibacter michiganensis* subsp. *michiganensis* (CMM), constitutes a serious bacterial wilt almost in all regions where tomato is cultivated. Disease control is based mainly in the prevention with reduction and destruction of the pathogen in the seed, rotation and by soil solarization.
Systemic Acquired Resistance (SAR) could constitute another way of controlling the phytopathogenic bacterium, since there are experimental data of successful control of bacterial canker of tomato. The spectrum of effective Systemic Acquired Resistance includes pythopathoginic fungi, bacteria and viruses. The event of SAR can take place also with the use of chemical substances. Based on these data it became effort of evaluation of two cationic surfactants factors of ammonium bromide (A and B) for their ability to induce the defensive mechanism of tomato plants against *Clavibacter michiganensis* subsp. *michiganensis*. These factors have not been studied up to today for their ability to induce systemic acquired resistance in the plants.
The evaluation of these surfactant factors was realized with pathogenicity tests in the system of interaction of host-pathogen tomato – CMM. The application of factors A and B became in different concentrations, so that it is realised the more effective concentration of the two factors in the reduction of the disease symptoms. It was find out that the treatment with factor A in concentration of 10 mM, was more effective compared to the concentrations of 50 mM and 1 mM as well as with the control plants reducing statistically significant bacterial canker at 70%. As for the treatment with factor B in concentration of 10 mM, was more effective compared to the concentrations of 50 mM and 1 mM as well as with the control plants reducing bacterial canker but in lower percentage compared to factor A. The mode of action of these factors will be clarified with further experiments that will follow in molecular level, so that it is show if factors A and B have the ability of induction of genes that plays fundamental role in the defense of plants, such as the PR-1 and PR-5 genes (Pathogen Related proteins).

Poster Presentations

Effects of repeated applications of a garlic essential oil component in combination with entomopathogenic nematodes to suppress *Meloidogyne javanica* on tomato. I. ANASTASIADIS[1], A.C. KIMBARIS[2], M. KORMPI[1], M.G. POLISSIOU[3] and E. KARANASTASI[1]*. [1]*Laboratory of Nematology, Department of Agricultural Entomology & Zoology, Benaki Phytopathological Insitute, 8, St. Delta str., GR-14561, Kifissia, Greece.* [2]*Faculty of Agricultural Development, Democritus University of Thrace, Ath. Pantazidou 193, 62800, Orestiada, Greece.* [3]*Laboratory of Chemistry, Agricultural University of Athens, Iera Odos 75, 11855, Athens, Greece. *E-mail: e.karanastasi@bpi.gr*

The use of entomopathogenic nematodes (EPN) has repeatedly been studied as a possible alternative pathogen and pest – including nematode - control treatment. Also, it is commonly accepted that garlic essential oil and its volatile components that possess fumigant properties against several plant pests and pathogens, are effective against plant parasitic nematodes. The work presented here was a pilot study examining the possibility of a combined action of *Steinernema carpocapsae* and a garlic essential oil component (diallyl disulfide), against the root-knot nematode (RKN) species *M. javanica*. *S. carpocapsae* entomopathogenic nematodes were reared on *Galleria melonella* at 25°C and infective juveniles were recovered using the White traps. The root-knot nematodes were isolated from tomato cultures maintained in a glasshouse at 25ºC. Healthy tomato seedlings were transplanted in 250 cm^3 pots containing sterilised compost, inoculated with 5 nematode eggmasses each and subsequently treated with 7000 live *S. carpocapsae* or 7000 heat-killed (at 70°C for 15 min). Each pot received 20 mL of diallyl disulfide solution in a single or a double application, *i.e.* concurrent with *M. javanica* inoculation, or concurrent and 1 week after *M. javanica* inoculation. The results of this study showed that EPN alone can lower RKN populations in soil; however repeated applications of diallyl disulfide had a stronger impact on RKN, which obscured EPN effects.

The effect of UV-C irradiation on *Botrytis cinerea* postharvest floret specking of cut gerbera flowers. A. DARRAS, V. DEMOPOULOS*, E. KAZANA and C. TINIAKOU. *TEI of Kalamata, Antikalamos, 241 00 Kalamata, Greece. *E-mail: vdimo@teikal.gr*

The direct toxic effect of ultraviolet irradiation (UV-C) on *Botrytis cinerea* growth and its a bility to induce biochemical defense mechanisms in gerbera cvs "intenza" and "ophir" flowers were both studied. Significant reduction of growth rate and even higher reduction of conidia germination were observed when the fungi was exposed to doses of up to 5 $kJ \cdot m^{-2}$ of irradiation. *In vitro* studies showed that "intenza" was approximately two times more sensitive to *B. cinerea* infection compared to "ophir". Florets, artificially inoculated with conidial suspension from irradiated *B. cinerea* culture, showed reduction of the infection on both cultivars as a result of the negative effect of irradiation on the pathogenic strength of the fungi. Flowers' quality was not affected even after exposure of up to 10 $kJ \cdot m^{-2}$ of irradiation. Florets exposed to doses of up to 1 $kJ \cdot m^{-2}$ irradiation and artificially inoculated with conidial suspension of the fungi showed significantly reduced infection on "intenza" and almost complete reduction on "ophir". This supports the hypothesis of biochemical defense mechanisms induced by low doses of UV-C in gerbera flowers and indeed in varying degrees in different cultivars. Irradiated florets, artificially inoculated with conidial suspension from irradiated *B. cinerea* culture, showed even higher reduction of the infection on "intenza" which strongly indicates the accumulating action of UV-C on the complex of host - pathogen.

Effect of several biocontrol agents on plant growth characteristics and *Pythium ultimum – Rhizoctonia solani* disease complex during acclimatization phase of GF-677 rootstocks. G.A. BARDAS[1]*, E. BALLAS[2], K. MAVRODIMOS[2] and N.I. KATIS[1]. [1]*Laboratory of Plant Pathology, School of Agriculture, Aristotle University of Thessaloniki, P.O. Box 269, GR541 24 Thessaloniki, Greece.* [2]*Fytotechniki, Plant Tissue Culture Company, Filothei, 47042, Arta, Greece. *E-mail: gbardas@agro.auth.gr*

A survey during acclimatization phase of GF-677 rootstocks showed the existence of a *Pythium ultimum* and *Rhizoctonia solani* disease complexresulted in severe losses. This work focuses on turf and root colonization ability, plant growth promoting effects and biocontrol trials against *P. ultimum* and *R. solani* disease complex, using three *Trichoderma* species and *Pseudomonas fluorescens* F113. Regarding root and turf colonization, the mixture of *T. harzianum* T22 and *T. asperellum* B1 showed remarkable efficacy. Colonization assays revealed great differentiations between the specific mixture and the combined treatment of *T. harzianum* T22 and *T. viride*. The combination of *T. harzianum* T22 and *P. fluorescens* F113, also, resulted in increased colonization rates. Concerning growth promotion phenomena, the leading effect of *T. harzianum* T22 and *T. asperellum* B1 mixture was obvious compared with all treatments in the presence of the plant pathogens. In the absence of the pathogens, the effect of combined *T. harzianum* T22 and *P. fluorescens* F113 treatment promoted plant growth in a similar way with *T. harzianum* T22 and *T. asperellum* B1 mixture. Finally, disease severity assays showed that only *T. harzianum* T22 and *T. asperellum* B1 mixture can protect GF-677 rootstocks from *P. ultimum* and *R. solani* complex's mediated damping-off.

Evaluation of plant growth-promoting rhizobacteria for growth promotion and biological control of *Fusarium oxysporum* f. sp. *radicis-lycopersici* on tomato. C. MYRESIOTIS[1], G. KARAOGLANIDIS[2]*, Z. VRYZAS[3] and E. PAPADOPOULOU-MOURKIDOU[1]. [1]*Aristotle University of Thessaloniki, Faculty of Agriculture, Pesticide Science Laboratory, P.O.Box 1678, 54124 Thessaloniki, Greece.* [2]*Aristotle University of Thessaloniki, Faculty of Agriculture, Plant Pathology Laboratory, P.O.Box 269, 54124 Thessaloniki, Greece.* [3] *Democritus University of Thrace, Faculty of Agricultural Development, Laboratory of Agricultural Pharmacology and Ecotoxicology, 193 Pantazidou, 682 00 Orestias, Greece. *E-mail: gkarao@agro.auth.gr*

Fusarium oxysporum f. sp. *radicis-lycopersici* (FORL) is the causal agent of tomato crown and root rot (TCRR), one of the most devastating soilborne diseases of tomato worldwide. The relatively poor efficacy of chemical control and the lack of resistance in some commercially important tomato cultivars have focused attention on the feasibility of the biological control of the pathogen using beneficial bacteria. In this study, the plant growth-promoting rhizobacteria (PGPR) commercial products of *Bacillus subtilis*, GB03 (Companion®) and FZB24 (FZB24®), and the PGPR strains *Bacillus amyloliquefaciens* IN937a and *Bacillus pumilus* SE34, applied individually and in all possible combinations of 2-strain mixtures, were evaluated for their capacity to promote growth and control FCRR on tomato under laboratory conditions. Bacterial strains were applied twice, as a soil drench immediately after seeding and 20 days later, and inoculation with the pathogen was performed when the seedlings were 4 weeks old. Disease severity measurements 15 days after challenge inoculation with the pathogen showed that all PGPR treatments resulted in significantly lower disease index compared to the control, with the exception of IN937a. Treatment with IN937a+GB03 achieved the highest biocontrol protection (60%), followed by GB03, FZB24+GB03 and SE34+GB03, which resulted in 47, 40 and 34% protection, respectively, compared to the nonbacterized control. Combined treatments with IN937a+GB03 and IN937a+SE34 demonstrated significantly lower levels of disease than any individual PGPR strain, indicating either additive or synergistic effect on disease reduction achieved by mixing PGPR strains. For growth promotion assays several plant growth characteristics were measured. In general, all PGPR treatments promoted significantly the plant growth parameters compared to the control. Specifically, shoot height, shoot fresh and dry weight were enhanced by all bacterial applications by 18–57%, 22–55% and 23–78%, respectively, compared to the control. The promoting effect was greater when plants were treated with SE34.

Chestnut blight in the Peninsula of Mount Athos – Spread of hypovirulence eight years after application of biological control. C. PERLEROU*, S. DIAMANDIS, A. MITSAKAKI, and Z. NAKOPOULOU. *National Agricultural Research Foundation, Forest Research Institute, 570 06 Vassilika, Thessaloniki, Greece. *E-mail: perlerou@fri.gr*

Chestnut blight caused by the ascomycete *Cryphonectria parasitica* was found in the peninsula of Mount Athos (N. Greece) in 1988. The disease spread rapidly along the peninsula in a 7,000 ha coppice forest. Culture morphology of numerous isolates from different parts of the forest revealed that only the virulent strain was present. No isolate was infected with the CHV1 hypovirus the agent that cause hypovirulence. A well organized project was implemented in the period 1998–2000 when approximately 90 infected trees/ha were inoculated with compatible hypovirulent paste in a more or less uniform network. A local virulent strain converted by a hypovirulent one from Mount Pelion was used as inoculum. In 2008 a survey was carried out to evaluate the spread of hypovirulence in the area. Sampling of cankered and not inoculated trees was conducted in three plots, 100 m^2 each, in coppice stands aged 4, 9 and 14 years old. All *C. parasitica* isolates recovered were assessed for culture morphology on PDA at 25°C, 16 h photoperiod for 10 days. All the isolates with hypovirulent characteristics such as white not or reduced sporulating mycelium, were tested for the presence of viral cytoplasmic dsRNA by cellulose chromatography and electrophoresis through 0.8 agarose gel. From a total of 298 chestnut sprouts that were examined in the three plots 80 of them had one or more cankers. Forty six out of 80 isolates obtained were hypovirulent. Hypovirulence occurred from 38.5% to 73.3% in the three plots. No tree mortality was encountered inside the sampled plots. The results show that eight years after application of biological control, hypovirulence has been established and spread in adequate level to reduce significantly the disease. The presence of only one vc type of the fungus may have a positive effect on the spread of hypovirulnce in the area since the cytoplasmic CHV1 is transferred horizontally (and not vertically) by hyphal anastomosis between vegetative compatible strains.

Antifungal activity of natural extracts of olive tissues, grapes and their by-products. N. SKANDALIS[1,2]*, T. MAVRAKIS[3,4], C. OUSTAMANOLAKIS[2], A. BOTTIN[5], N. MAGAN[4], A.L. SKALTSOUNIS[6], N.J. PANOPOULOS[2,7], F. VERVERIDIS[3]. [1]*Laboratory of Bacteriology, Department of Phytopathology, Benaki Phytopathological Institute, St. Delta 8, 14561 Kifissia, Attica, Greece.* [2]*Department of Biology, University of Crete, 71409 Heraklion, Greece.* [3]*Plant Biochemistry & Biotechnology Laboratory, Department of Plant Science, Technological and Educational Institute of Crete,P.O. Box 1939, Heraklion, Greece.* [4]*Applied Mycology Group, Cranfield University, Silsoe, Bedford MK43*

*0AL, UK. [5]UMR 5546 CNRS-Université Paul-Sabatier, Castanet-Tolosan, France. [6]Department of Pharmacy, University of Athens, 15771 Athens, Greece. [7]Institute of Molecular Biology and Biotechnology, FORTH, 71110, Heraklion, Greece. *E-mail: n.skandalis@bpi.gr*

A specific group of the olive (*Olea europae*) phenolic profile, called secoiridooids, including oleuropein, are natural antioxidants and have antimicrobial activity, and consequently a health-beneficial role. Similar properties are attributed to grape pomace, a by-product of wine making which also is rich in polyphenols compounds, especially stilbenoids and flavonoids. We have examined the *in vitro* and *in vivo* antimicrobial activity of: oleuropein isolated from olive tissue as well as olive mill waste water extract (OMWWE) and grape pomace exctract (GPE) extracts which are rich in polyphenols such as hydroxytyrosol and resveratrol, respectively. All three treatments were found to restrain growth of a series of important fungal and bacterial pathogens, including *Botrytis cinerea, Fusarium oxysporum, Colletotrichum higginsianum,* the oomycete *Phytophthora parasitica* var. *nicotianae*. and *Xanthomonas campestris* pv. *vesicatoria*, *Pseudomonas savastanoi*, respectively. Moreover, extracts restrained conidial germination of *C. higginsianum* and *B. cinerea* in solid media at a concentration 0.5% and/or lower, GPE being the most effective. *In planta* application of extracts redeced bacterial infection by direct antimicrobial activity and – in some cases - by induction of plant resistance mechanisms in pepper, tomato and olives grwon under greenhouse conditions. They also had a similar effect to the endophytic growth of *P. parasitica* in tobacco. Postharvest treatment of table grapes with GPE restrained brown mold (*B. cinerea)* incidence and development. Our results suggest possible uses of these extracts as alternative phytoprotectants at least on a small scale, along with alternative methods for agricultural by-product management.

Isolation from the rhizosphere of a new biocontrol agent against the fungus *Fusarium oxysporum* f.sp. *radicis-lycopersic.* G.D. TZELEPIS[1,2], L.C. LOTOS[1], G. GIOKAS[1] and A.L. LAGOPODI[1]*. [1]*Plant Pathology Laboratory, School of Agriculture, Aristotle University of Thessaloniki P.O. Box 269, GR541 24 Thessaloniki, Greece. [2]Department of Forest Mycology and Pathology, SLU, P.O. BOX 7026, SE-750 07 Uppsala, Sweden. *E-mail: lagopodi@agro.auth.gr*

The aim of this study was to evaluate several bacterial isolates as potential biocontrol agents against *Fusarium oxysporum* f.sp. *radicis-lycopersici* (Forl) causing foot and root rot in tomato. Among a collection of different bacterial isolates, which came from pepper, tomato and potato plants, from different region in Greece (Crete, Rhodes, Zakynthos, Lakonia and Halkidiki) one isolate, designated as ChEm5, isolated from the rhizosphere of a pepper plant grown in Chalkidiki, was shown to be effective against Forl, inhibiting its mycelial growth in dual cultures. The effectiveness of ChEm5 was tested *in planta*, in gnotobiotic experiments as well as in pot experiments. The results of the experiments showed that ChEm5 was able to reduce the disease severity significantly. Identification of ChEm5 was done, amplifying and sequencing the 16S – 23S intergenic space of rDNA using PCR strategies. The sequence analysis was compared with published data (NCBI, GenBank), and ChEm5 was found 99% identical with the bacterium *Serratia marcescens*. The phylogenetic analysis put ChEm5 in the same group with the other *S. marcescens* strains. *S. marcescens* is a well known bacterium with many strains that are human pathogens, others that are plant pathogens and others that work as biocontrol agents.

***Leptosphaerulina australis*, a novel antagonist of powdery mildews**. E.T. TOPALIDOU* and M.W. SHAW. *School of Biological Sciences, The University of Reading, Whiteknights,Reading RG6 6AS, UK. *E-mail: etopalidou@googlemail.com*

Parasitism of oak (Quercus robur) by *Erysiphe alphitoides* (oak powdery mildew) was extremely common in nature. Micro-organisms of at least five morphological groups were observed (*Acremonium* sp., *Ampelomyces* sp., *Trichoderma* sp., *Leptosphaerulina australis* and *Tilletiopsis* sp.). Nearly 90% of mildew colonies were associated with *L. australis*, which is not normally an antagonist of powdery mildews. The association between the two fungi was determined by quantitative studies and proved that *L. australis* was capable to reduce disease severity even under development conditions favourable for the disease. The host range and individual attributes of *L. australis* were investigated in order to study its auto-ecology and infer its potential to be used as an antagonist of powdery mildews. *L. australis* was not host specific since it parasitized also *Podosphaera aphanis* (strawberry powdery mildew) and *Podosphaera xanthii* (=*Sphaerotheca fuliginea*) (cucumber powdery mildew). Although *L. australis* pseudothecia were extensively produced on artificial media (Malt Extract Agar or on oat kernels) and in nature, an asexual stage was never observed. Under very high relative humidity (97%) the temporal pattern of ascospore release was extended, but ascospore release was substantial even under very low (54%) and moderate (85%) RH levels. *L. australis* has some potential as bio-control agent under field and protected environments, although more research is needed.

Integrated pest management of *Verticillium dahliae* in the olive culture, in the prefecture of Halkidiki,

Makedonia, Greece. Z.D. ZARTALOUDIS*, P. IOANNIDIS, and S. MOKIOU. *NAGREF- Plant Protection Institute of Thessaloniki, PC 570 01, P.O.Box: 60324 Thermi, Thessaloniki, Greece. *E-mail: zoan@hal.forthnet.gr*

Verticillium dahliae, has a wide host range. Over 300 woody and herbaceous plant species are known to be susceptible to this fungal pathogen, and among these the olive tree. The disease has been reported as olive tree pathogen, in the countries of Mediterranean basin but also in California (USA). The management of this disease is based on the prevention and the combination of farming practices that aims in the reject of import and restriction of quantity and dissemination of the pathogen in the field. Experiments of control and management of Verticillium wilt, became in the region of Kalyves, Polygyros, Halkidiki. Was evaluated the method of soil solarization with use of a ground plastic leaf: PA 1455 Orgasun, as well as the action of various beneficial micro-organisms as *Glomus coronatum* GO 01, *G. Caledomium* GM 24, *G. Coronatum* GU 53, *Bacillus subtilis* BA 41, *Pseudomonas* spp. SN 02, *P. borealis* PA 37, *P.* spp. PM 46, *Trichoderma harzianum* TH 02, as well as natural product Harpin EA. The above agents treated in the soil with irrigation in 2 seasons (at the beginning of autumn and spring) in trees where it had preceded soil solarization and in trees where it did not precede soil solarization. The results showed that can be limited considerably the symptoms of the disease, particularly if has been preceded soil solarization.