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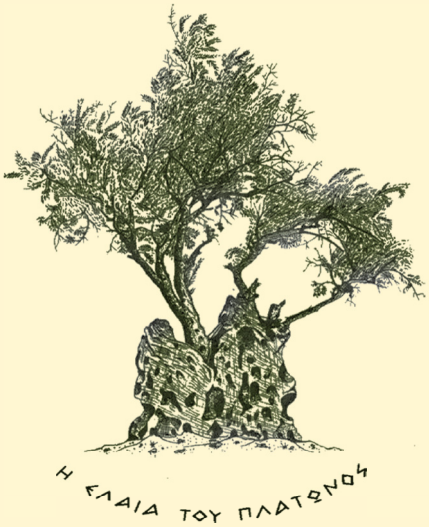
Abstracts

16th Hellenic Phytopathological Congress



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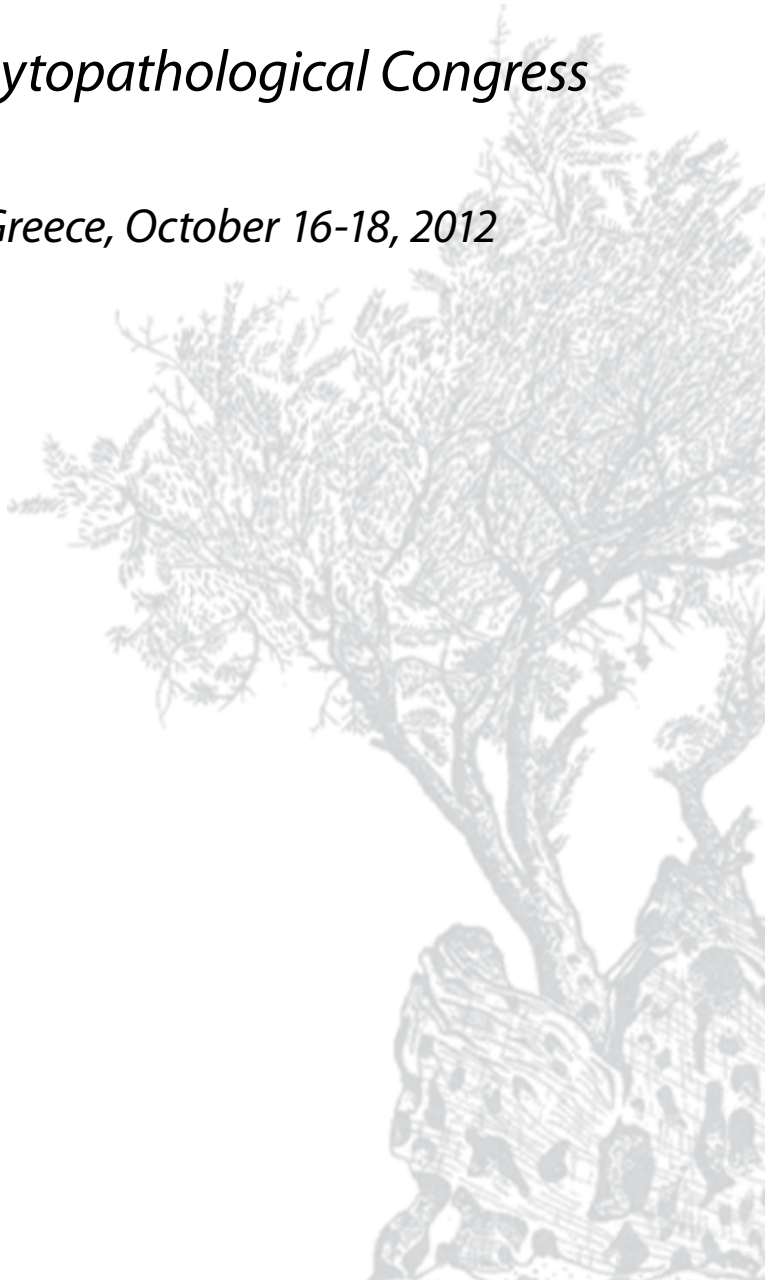
The olive tree of Plato in Athens is the emblem of the Benaki Phytopathological Institute

Abstracts

16th Hellenic Phytopathological Congress

Thessaloniki, Greece, October 16-18, 2012



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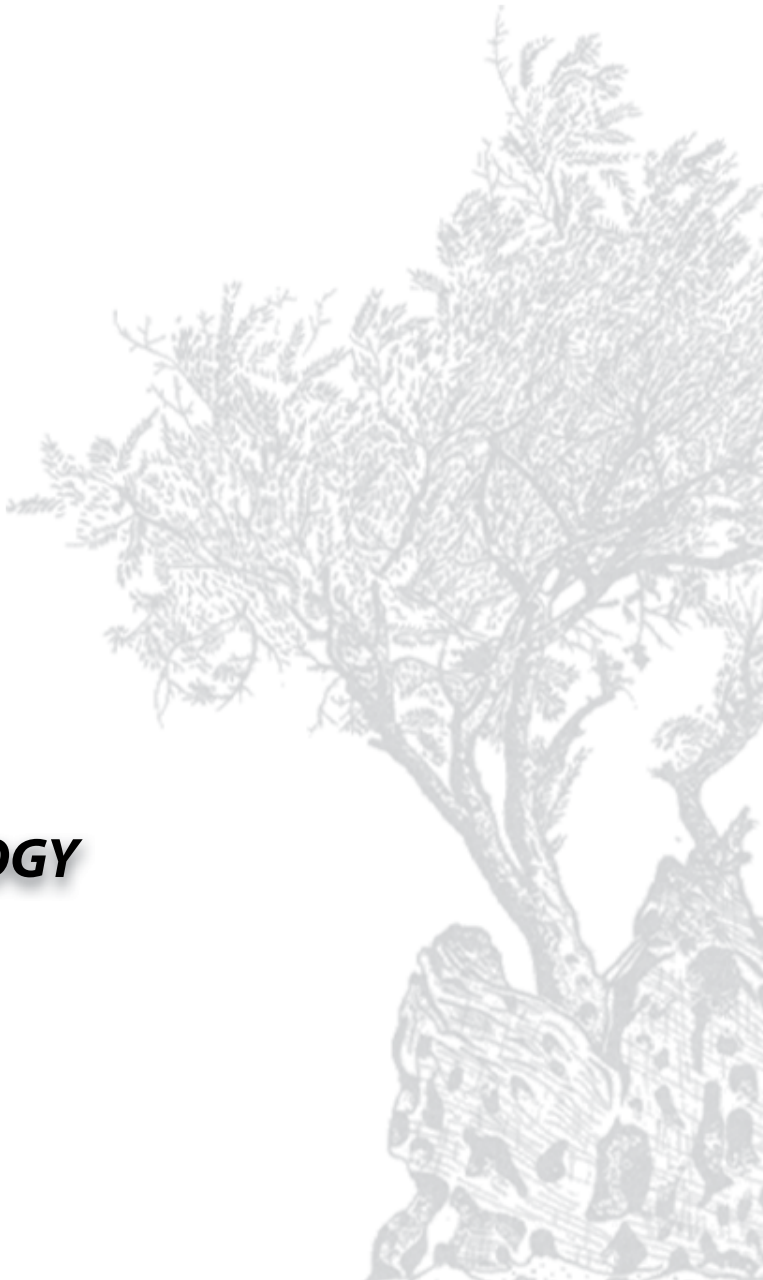
ABSTRACTS

Summaries of invited lectures, oral and poster presentations given at the 16th Hellenic Phytopathological Congress Thessaloniki, Greece, October 16-18, 2012

The 16th National Phytopathological Congress, organized every two years by the Hellenic Phytopathological Society (HPS), was held in Thessaloniki, Greece, on October 16–18, 2012. The meeting was attended by more than 400 participants, and 61 oral presentations, 63 posters and 7 invited lectures were presented dealing with plant diseases caused by fungi, bacteria and viruses, non-parasitic disorders, molecular diagnostics and characterization of pathogens, host-pathogen interaction, and biological, integrated and chemical disease control. In addition, one round table discussion was held on "Production of certified propagative material of fruit trees: international experience and Greek legislation- Problems related to its application". Abstracts of the invited lectures, oral presentations and posters of the congress are presented below.

Mycology



Invited Lectures

Phytophthora ramorum: an emerging pathogen of forest and ornamental plants in Europe and North America

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Phytophthora ramorum is an emerging pathogen with a destructive impact on forest ecosystems of North America and Europe. It was described as a new species in 2001, although has been known since 1993 to infect rhododendron and viburnum plants in nurseries of the Netherlands and Germany. *P. ramorum* is considered to be an exotic pathogen, introduced separately into Europe and North America from an unknown region, speculated to be Asia. Up to the present, three clonal lineages NA1, NA2 and EU1, have been distinguished by the use of molecular markers in North America, while in Europe only the latter (EU1) has been detected. The pathogen has a large host range (more than 140 plant species) and the host-list continues to expand, and includes a significant number of forest trees and shrubs as well as many ornamental plants. Certain hosts have shown a high susceptibility to *P. ramorum*, with lethal stem and branch infections (formation of bleeding cankers). In California and Oregon, the disease, known as "sudden oak death" (SOD), is lethal to certain oak species (*Quercus* spp.) and tanoak (*Notholithocarpus densiflorus*), having a devastating impact on forest ecosystems. Recently (in 2009) the pathogen was found to cause significant mortality in Japanese larch plantations in southern England. The disease can be also lethal to rhododendron and viburnum plants in nurseries and parks. However, in the majority of hosts *P. ramorum* causes less serious diseases, infecting mostly the leaves and the young shoots. In some cases infections are not conspicuous; the plants are considered healthy and are transferred by trade, resulting in disease spread to new areas. *P. ramorum* is a quarantine organism in Europe, North America and other areas of the world. Since 2002 EU regulations have been imposed in order to prevent further spread of *P. ramorum* among ornamental plants in the member countries. Surveys in the EU have detected the pathogen in 20 of the 27 member countries. In Greece, *P. ramorum* was initially detected in the Phthiotida prefecture in 2010, on rhododendron plants imported from Belgium. During 2011-2012 the pathogen was found in three more areas of Greece (Athens, Pelion and Drama), infecting nursery plants of viburnum and camellia produced in the country, as well as rhododendron plants imported from Belgium. The hot and dry climatic conditions in many areas of Greece do not favour the spread of *P. ramorum*; however, there are many suitable habitats for the establishment of the pathogen, including natural oak and beech forests in the highlands as well as maquis scrublands with evergreen oaks and other hosts of *P. ramorum*. There is also the possibility of infection of cultivated plants.

Pomegranate diseases. Remarks on Greek and global problems

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Pomegranate cultivation has rapidly expanded in recent years in Greece and worldwide. This presentation refers to findings concerning diseases of pomegranate in Greece ob-

tained from field observations, laboratory isolations and experimental studies, and from data from international literature on serious diseases.

Our Greek data are focused on fungal, non-parasitic and postharvest diseases with references to their causes, difficulty in diagnosis, their impact and their control. The commonest pathogens causing fungal diseases are *Eutypa / Libertella, Botryosphaeria/ Neofusicoccum,* wound pathogens of non-parasitic origin *(Cytospora / Valsa, Pestalotiopsis, Pestalotia)* and vascular wilts (*Verticillium dahliae* or *Ceratocystis fibriata* or *Ophiostoma stenoceras-sporothrix schenckii* complex). Regarding Greek ophiostomas it was demonstrated that our isolates were not *Ceratocystis fibriata* but of the *Ophiostoma stenoceras-sporothrix schenckii* complex that showed limited pathogenicity, causing mild symptoms on young pomegranate plants.

Regarding post-harvest diseases the fungi *Botrytis cinerea, Alternaria alternata, Aspergillus niger* and *Cytospora* sp. have being frequently observed.

As for non-parasitic diseases, which often lead to secondary attacks by pathogenic fungi, we underline the impact of frost damage and damage by rodents. Also herbicide toxicities and bad cultivation practices are included.

Regarding most recent international data, the fungus *Ceratocystis fibriata* causes severe symptoms and is considered the most serious pathogen of pomegranate in India, China and Iran. The disease appears as yellowing and defoliation in one or more branches in a few days or after 2-3 months to reach full wilting and necrosis. The disease is characterized by grey-brown discoloration of the wood vessels and adjacent tissues.

Another significant bacterial disease of pomegranate is caused by *Xanthomonas axonopodis* pv. *punicae*, which has been found to cause serious damage in India and threatens to spread to other countries.

Finally, viral diseases or virus-like diseases have been rarely reported in pomegranates. The only references relate to a mosaic virus (*Cucumber mosaic virus*) which causes deformation of leaves, yellowing and reduced flowering (1984, in Yugoslavia) and Hop stunt viroid (HSVD) in Turkey (in 2000).

Oral & Poster Presentations

Diplodia corticola: a new pathogen of oak in Greece

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The fungus *Diplodia corticola* (teleomorph: *Botryosphaeria corticola*) was reported for the first time in Greece in 2010, to infect kermes oak (*Quercus coccifera*) in the Messenia prefecture (Peloponnese). In the following years, infections from this pathogen were also observed on holm oak (*Quercus ilex*) and downy oak (*Quercus pubescens*) in the same prefecture. Infections were also noted on kermes oak in the neighbouring Ilia prefecture as well as in the Karditsa prefecture in Thessaly, central Greece. Infected trees showed symptoms of branch and shoot dieback, that were more intense during the summer period. Cankers were evident on infected branches, while abundant pycnidia emerged through the bark. Fungal isolates from infected tissue as well as from pycnidia, on malt extract agar (MEA), were initially white with dense aerial mycelium, becoming dark grey to almost black with age. Conidia formed in culture were similar

in shape and size to those formed in the pycnidia on the cankers; cylindrical with rounded ends, hyaline and unicellular, in some cases two-celled and darker upon maturity, rarely three-celled, (24-34 x 12-17 μm). The identity of *D. corticola* was confirmed by sequencing the ITS1 and ITS2 regions of the rDNA and comparison with known sequences of the fungus. Two isolates of *D. corticola* were used in inoculation tests on branches of kermes and holm oak trees and the fungus was re-isolated from the cankers as well as from pycnidia formed on the inoculated branches.

First report of potato wart disease caused by *Synchytrium endobioticum* (Schilb.) Perc. in Greece: detection, impacts and pathotype identification

I. Vloutoglou[1], G.C.M. van Leeuwen[2], H. Eleftheriadis[3], I. Sarigkoli[3], K.B. Simoglou[3], D. Tsirogiannis[1] and D. Gilpathi[4]

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Potato wart disease, caused by the quarantine fungus *Synchytrium endobioticum,* was detected for the first time in Greece in two potato (*Solanum tuberosum* L.) fields in the Perithori region (Kato Nevrokopi, Regional Unit of Drama) during the 2011 official surveys. The disease exhibited typical wart symptoms on stolons and tubers with some of the latter being largely converted into warts. Pest identification was based on the EPPO diagnostic protocol PM 7/28 (1). Phytosanitary measures are being implemented in the area in compliance with the EU Council Directive 69/464/EEC. The potato crops grown on the infested fields were destroyed, the fields were designated as infested and a safety zone, allowed to be planted only with potato cultivars resistant to the pathotype present, was defined around the infested area. In October 2011, wart material, collected from the two infested fields, was sent to the NPPO of the Netherlands (National Reference Centre, Wageningen), where tests were performed for pathotype identification. The wart material of the two fields was separately composted and the resulting 'compost' (inoculum) was used for Spieckermann tests with the following differential potato cultivars: cv. Markies (susceptible to all pathotypes), Producent, Delcora, Saphir, Miriam and Belita. Based on the resistant reaction of cvs Saphir and Belita and the susceptible reaction of cvs Markies, Producent, Delcora, and Miriam, it was concluded that pathotype 18(T1) is present in both fields. In the present work, the impact of the presence of *S. endobioticum* in the area is also discussed.

Development of a quantitative PCR method to differentiate between viable and nonviable cells of plant pathogenic fungi using propidium monoazide (PMA)

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Department of Agricultural Sciences, Biotechnology and Food Science, Cyprus University of Technology, 3603 Limassol, Cyprus

The quantification of plant pathogens using DNA-based molecular tools can be misleading due to an inability to distinguish signals originating from live and dead cells. However, methods that use DNA-intercalating dyes like propidium monoazide (PMA) have been used to selectively remove cells with compromised cell membranes from the analysis, which can be considered to be dead. These dyes are nearly completely cell membrane-impermeable and therefore can be selectively used to modify only exposed DNA from dead cells while leaving DNA from viable cells intact. Once these dyes enter a cell, they bind to DNA and can be covalently crosslinked to it by light exposure. PCR amplification of such modified DNA is strongly inhibited. In this study we evaluated the suitability of PMA treatment to distinguish between viable and nonviable plant pathogens. A PMA-qPCR combined assay was applied to viable and inactivated fungal pathogens. Cell suspensions were incubated with PMA, and then exposed to light to secure the intercalation of PMA with the DNA of dead cells, and to inactivate any unbound PMA. Treated cells were extracted and the relative ratios of live and dead cells were evaluated by qPCR. After heat treatment and DNA modification with PMA, all fungal species tested showed an approximate 100- to 1000-fold difference in cell viability as estimated by qPCR analysis, which was consistent with estimates of viability based on culturing.

Population genetic structure of *Phytophthora infestans* in Cyprus

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A total of 521 isolates of *P. infestans*, the causal agent of potato late blight, were collected in Cyprus from 2009 to 2011. The scope of the present study was the genotypic characterization of the local population of the microorganism including mating type and DNA fingerprinting patterns using 12 microsatellite markers. During the first two years of the study, when a nationwide collection took place, the presence of both mating types was documented at a ratio of approximately 1:1. In 28.5% of the sampled fields both mating types coexisted, suggesting the potential for sexual reproduction. In addition, 13 genotypes of *P. infestans* were identified, with prevailing types 13_A2, 2_A1 and 23_A1. More specifically, genotype 13_A2, which has been reported in many European countries and which is characterized by high aggressiveness and resistance to the fungicide metalaxyl-M, appeared frequently throughout the sampling period. The 2011 sampling was concentrated in a single potato field and only genotypes of 13_A2 were identified. Overall, the relatively low genetic variability of the Cyprus populations of *P. infestans* indicates the absence of sexual reproduction of the microorganism, despite the existence of both mating types. Monitoring the genetic background of the local population may provide information on the appearance of sexual reproduction of the pathogen, and the potential invasion of new genotypes, primarily from seed exporting countries.

Classification of Cretan *Verticillium dahliae* isolates to races and their virulence characterization in differential hosts

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Race classification and host range pathogenicity determination of 32 *Verticillium dahliae* isolates, originating from 19 plant species from 8 different botanical families, were carried out. The physiological races of isolates were identified using the two differential tomato cultivars Belladona (susceptible to both races 1 and 2 of *V. dahliae*) and Ace 55VF (resistant to race 1, susceptible to race 2 of *V. dahliae*). Among these isolates, 14 were characterized as race 2 (43.8 %), 12 race 1 (37.5 %) and 6 nonpathogenic (18.7 %) on tomato. The host range pathogenicity of isolates was determined using four differential hosts (eggplant, turnip, tomato (*Ve*$^{-}$) and sweet pepper). Among the isolates, 5 were pathogenic to both eggplant and turnip (15.6 %), 21 to eggplant, turnip and tomato (65.6 %), 5 to eggplant, turnip, tomato and sweet pepper (15.6 %) and 1 was pathogenic to eggplant, turnip and sweet pepper (3.2 %). The pathogenicity of isolates on the aforementioned five hosts was investigated on the basis of external symptoms and by calculating the relative areas under disease progress curves (relative AUDPC). Results showed that eggplant was the most susceptible, followed by turnip and tomato cv. Belladona, while sweet pepper and tomato cv. Ace 55VF were less susceptible to the isolates used. The pathogenicity of isolates varied from highly to mildly virulent on eggplant and turnip whilst on Belladona, Ace 55VF and sweet pepper it varied from highly virulent to nonpathogenic. Belladona exhibited a similar level of susceptibility to race 1 and 2 of *V. dahliae*, but was more susceptible than Ace 55VF to race 2. Interestingly, the isolates originating from eggplant were clearly more virulent than those originating from tomato and black nightshade on all solanaceous plants tested.

Phytopathological characterization, morphology, genetics and molecular differentiation combined in an integrated population study of *Verticillium dahliae*

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Understanding the genetic diversity of *V. dahliae* populations and an early recording of their pathogenic profile are essential for disease management. An extended *V. dahliae* population mainly originating from Crete, Greece, was characterized in terms of pathogenicity/virulence, morphology/physiology, vegetative compatibility and mating type. Tomato race 2 has supplanted race 1 and was more virulent on a susceptible tomato cultivar than race 1. Pathotypes of all isolates were determined using four differential hosts (tomato, eggplant, sweet pepper and turnip). All isolates from Crete fell into VCG subgroups 2A, 2B and 4B, while a remarkably high incidence of bridging isolates was recorded. The tomato-sweet pepper pathotype was morphologically distinct from the others, while conidial length and pigment intensity were discriminatory parameters among VCGs 2A, 2B and 4B. The PCR-based molecular marker Tr1/Tr2 was reliable in race prediction among tomato-pathogenic isolates, except for members of VCG 4B, while the application of markers Tm5/Tm7 and 35-1/35-2 was highly successful in distinguishing tomato pathotypes. E10 marker was related to VCG 2B. A SNP in the ITS2 region, and two novel molecular markers, M1 and M2, proved useful for the fast and accurate determination of major VCGs 2A, 2B and 4B, and can be used for high-throughput popu-

lation analyses in future studies. The mating type was unrelated to VCG classification and probably does not control heterokaryon incompatibility in *V. dahliae*.

Interspecific variability and virulence of *Monilinia* spp. isolates in stone fruits

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Brown rot is the one of the most important diseases of stone fruits (*Prunus spp.*) worldwide. The causal pathogens of brown rot include mainly four species of *Monilinia : M. fructigena*, *M. fructicola*, *M. laxa* and *M. polystroma*. Until recently *M. fructigena* and *M. laxa* were thought to be the main agents of brown rot in Europe. However, in recent years, *M. fructicola*, a quarantine pathogen in the European Union, has been reported in many countries of Europe although its presence had not been confirmed in Greece. The first objective of this study was to detect the causal pathogens of brown rot in 4 stone fruits crops (peach, cherry, apricot and plum) in Greece. For this purpose, during 2011 and 2012, 1434 *Monilinia* spp. isolates were collected from two different geographical regions: Central Macedonia and Thessaly. The sampling was conducted during 2 phenological stages: blooming and fruit ripening. All isolates were identified at a species level, by morphological characterization and molecular identification based on the size of the intron of the *cytochrome b* gene of *Monilinia* spp. Two species were detected, *M. laxa* and *M. fructicola,* with frequencies of 59 and 41%, respectively. Specifically, *M. fructicola* was more common on fruits (89%) and *M. laxa* was found in equal frequency in flowers (49.5%) and in fruits (50.5%). The second objective of this study was to compare the aggressiveness of the two species and among isolates of the same species that were collected from the two different stages of infection; flowering and ripening of fruits. Generally, the pathogenicity of *M. fructicola* was found to be significantly higher than that of *M. laxa* on the wounded fruits of cherry and plum, with no significant difference detected on the wounded fruits of peach and apricot. Moreover, no significant variation in levels of aggressiveness among isolates from the two different stages of infection was detected. This represents the first report of *M. fructicola* in Greece. The wide dispersal of this pathogen and its high prevalence necessitates further research on the genetic variability of the fungus, its sensitivity to fungicides, and on the epidemiology of the disease in Greece.

Characterization of *Rhizoctonia solani* from potato in Cyprus

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Eighty isolates of *Rhizoctonia solani* were collected from sclerotia formed on the surface of potato tubers during 2011. Isolates were assigned to the anastomosis group (AG) using molecular primers (ITS-1 and ITS-4) from the ribosomal DNA. Phylogenetic analysis of the obtained PCR products revealed that most of the isolates belonged to AG-3 (92,8%), while the rest belonged to the subgroups AG-4-HG-I (2,8%) and AG-4-HG-II (4,4%), respectively. Optimum growth rates for the AG-4-HG-I and AG-4-HG-II isolates were at 30°C, while for the AG-3 isolates was at 20°C. Sensitivity of the select-

ed isolates to the fungicide pencycuron was tested *in vitro* (EC_{50} values 0.013-0.222 μg/ml). Furthermore, the pathogenicity and aggressiveness of 30 isolates was evaluated *in vitro* on seedlings of barley, lettuce, melon, vetch and wheat. The aforementioned seedlings were chosen based on rotation systems routinely followed by local farmers. The AG-4-HG-II isolates were the most aggressive in all plant species studied, while the AG-3 group showed the lowest levels of aggressiveness. The most susceptible plant was barley for group AG-4-HG-I, melon and lettuce for group AG-4-HG-II and melon for AG-3 isolates. In contrast, the least susceptible were vetch and melon for groups AG-4-HG-I, AG-4-HG-II and AG-3, respectively. The results of the present study could support the development of integrated management programmes for potato infestations by *R. solani* in Cyprus.

Characterization of new genetic regulators of the *Arabidopsis thaliana* innate immune system with homology to programmed cell death genes in mammals

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Programmed cell death (PCD) is a process that normally takes place during development and defence of multicellular organisms. Research in recent years has demonstrated the existence of common biochemical pathways of PCD among plant, animal and microbial cells, and a possible link to serious diseases like cancer and degenerative diseases in humans. The plant hypersensitive response (HR), a form of PCD with many common characteristics with mammalian apoptosis, is associated with the rapid death of host cells triggered during the entrance of the pathogen into plant tissues. Two interesting families of genes likely involved in the activation of the plant defence system are the orthologues of the mammalian *AIF* (Apoptosis Inducing Factor) gene and the DAP (Death Associated Protein) genes that play a crucial role in mammalian PCD. In animals, AIF and DAP genes are associated with diseases related to increased apoptotic events such as infection with HIV, neurodegeneration, and heart attacks. The genetic model *A. thaliana* has five different putative AIF-like proteins and 8 DAP like proteins with regions similar to "Death Domains". We will present the role of *At-AIF* genes in plant-host interactions, in the activation of the plant innate immune system, and in plant resistance or susceptibility to the pathogens *Verticillium dahliae, Hyaloperonospora arabidopsidis, Alternaria brassicicola, Fusarium oxysporum f.sp. raphani*, and *Pseudomonas syringae* pv. *tomato*.

The role of ethylene perception in plant resistance against *Fusarium oxysporum*

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The plant fungal pathogen *Fusarium oxysporum* is the causal agent of root rot or wilt diseases in several plant species and comprises more than 100 *formae specialis*. The aim of the present research was to provide an insight into the host plant-microbe molecular

interactions. For this purpose, the responses of *Arabidopsis thaliana* mutant plants impaired in known pathogen response pathways were used to explore the components of defence against *Fusarium oxysporum* f.sp. *raphani*. It was observed that *etr1-1* plants were the most resistant among the different mutants. Furthermore, the expression of the defence related genes *PR1*, *PR2*, *PR3*, *PR4*, *PR5* and *PDF1.2*. was examined using qPCR. It was revealed that the genes *PR1*, *PR2* and *PR5* were overexpressed in *etr1-1* plants compared to Col-0 plants, indicating their role in plant defence mechanisms.

Investigation of the role of *VdSteA* G protein coupled pheromone receptor in the virulence and biology of the vascular wilt pathogen *Verticillium dahliae*

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V. dahliae is a soil-borne fungus causing wilt diseases in several hosts. The particular biology of this fungus complicates its treatment through conventional methods. Thus, the study of genes implicated in the interactions of *V. dahliae* 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 consisting of seven transmembrane domains. GPCRs are critical factors in regulating the morphogenesis, defence, mating, infection and virulence in various organisms. 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. Seven different groups of GPCRs emerged from the phylogenetic analysis, varying in sensing different environmental signals. *Agrobacterium* mediated disruption of a pheromone GPCR (named as *VdSteA*) in two wild type races, 70V and 25V of *V. dahliae* was performed in order to study the role of this receptor in virulence and morphology. 70V and 25V *ΔVdSteA* mutants displayed a reduction in virulence in eggplant and tomato plants and 70V *ΔVdSteA* mutants exhibited increased microsclerotia formation and conidiation when compared to their corresponding wild types. Both *ΔVdSteA* mutants exhibited higher conidial germination rates compared to wild types.

Study of the role of the secondary metabolite regulatory gene *VdLaeA* in the virulence and biology of the phytopathogenic fungus *Verticillium dahliae*

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Fungal secondary metabolites are compounds with high degree of specialization that fulfil various roles in toxin production, sporulation processes and the biosynthesis of substances of special biotechnological and pharmaceutical interest. Previous studies have shown that the phytopathogenic fungus *V. dahliae* produces phytotoxins and other molecules that induce the process of programmed cell death or other forms of

host resistance. The exact nature, of these compounds in *V. dahliae*, however, remains unknown. In *Aspergillus,* the global regulator of secondary metabolism *laeA* encodes a nuclear protein that is required for the expression of secondary metabolite genes, while its presence is considered indispensable for mycotoxin, antibiotic and mycelial pigment biosynthesis. BLAST analysis of the *V. dahliae* genome with the *laeA* gene of *A. nidulans* led to the discovery of a homologous gene that was named *VdlaeA*. *VdlaeA* was deleted in *V. dahliae* in order to clarify whether products of secondary metabolism play any role in the virulence and physiology of this fungus. Pathogenicity experiments in the greenhouse revealed that the transformed *ΔVdlaeA* strains resulted in significantly reduced disease levels in eggplants, tomatoes and *Arabidopsis thaliana* hosts. *ΔVdlaeA* strains also showed alteration in the rates and morphology of germinating conidia, in mycelial development, and in microsclerotia formation. The study of the regulatory gene *VdlaeA* may contribute to a broader understanding of the molecular mechanisms by which secondary metabolites are produced, and more specifically to the investigation of its role in *V. dahliae* virulence.

Molecular identification of and ochratoxin A production by *Aspergillus* Section *Nigri* isolates from vineyards in Cyprus

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The aim of this study was to investigate the infection of Cyprus vineyards with black aspergilli (A*spergillus* section *Nigri*), the molecular identification and the evaluation of ochratoxin A (OTA) production of the isolated aspergilli. The mycotoxin OTA is considered to be nephrotoxic, teratogenic, immunosuppressive, carcinogenic and neurotoxic. Initially, black aspergilli were isolated from grapes of the varieties 'Marathefiko' and 'Cabernet Sauvignon' during 2010, that originated from four areas of the Limassol district, one of the main wine-producing regions of Cyprus. In total 161 isolates (18%) were selected based on the macroscopic characteristics of black aspergilla. DNA from each isolate was used in PCR reactions with primers that amplified part of the *calmodulin* gene. The PCR products were sequenced and the sequences obtained were compared to those in the NCBI database. It was found that 148 isolates were identified as *A. tubingensis* (92%), 12 as *A. niger* (7.4%) and 1 as *A. carbonarius* (0.6%). Liquid chromatography analysis revealed that 17 of the 161 isolates were toxigenic; 16 of the *A. tubingensis* (1.3 pg/mg-1.96 ng/mg) and the *A. carbonarius* (1.43-1.68 ng/mg). This study confirmed the infection of Cyprus vineyards with black aspergilli and showed *A. tubingensis* to be the predominant species. Moreover, 10.8% of these isolates were toxigenic. The population synthesis seems to be different compared to other Mediterranean countries as only a single isolate was identified as *A. carbonarius*.

Genetic and molecular characterization and evaluation of Greek non-toxigenic isolations of the fungi *Aspergillus* as potential biocontrol agents against aflatoxins

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One of the most efficient strategies to reduce the aflatoxin levels at pre-harvest level in several crops is the application of native biological control agents in the field. One of the mechanisms of this technique is based on the competitive exclusion of the aflatoxigenic fungi *Aspergillus flavus* and *A. parasiticus* by applying non-toxigenic microorganisms such as bacteria, yeasts or non-toxigenic Aspergillus strains. The aim of the present study was the evaluation of 136 non-toxigenic strains of the genus *Aspergillus*, that were isolated from pistachios of the typical Greek variety "Aegina" collected from several orchards in Greece. The major goal is to reduce aflatoxin contamination from the field by selecting and applying the most suitable non-toxigenic strains. For that purpose, the Greek isolates were DNA-characterized and grouped with the method of Simple Sequence Repeats (SSRs) or Microsatellites using a multiple set of primers. Based on SSRs results, the Greek strains were grouped in 20 different vegetative compatibility groups (VCGs), whereas 65% of the total number of isolates grouped to the same VCG. Next, aflatoxin and cyclopionic acid (CPA) gene clusters were investigated for possible indels using a multiplex set of primers with the PCR method. The results showed indels in 7 Aspergillus strains either in aflatoxin or in CPA gene cluster. Also, the efficacy of representative strains from different VCGs in aflatoxin reduction was evaluated with in vitro competition tests in autoclaved maize kernels. Competition tests showed that treatments with 2 non-toxigenic strains from different VCG resulted in 80% aflatoxin reduction compared to control (toxigenic strain alone). The above results demonstrate that some of the isolated Greek non-toxigenic Aspergillus strains can be used as potential biocontrol agents in pre-harvest stages in several aflatoxin susceptible crops in Greece.

Molecular characterization of the *cyp51*, *mdr* and *aflR* genes and the effect of DMI resistance mechanisms on fitness parameters and aflatoxin production in *Aspergillus parasiticus*

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Aspergillus parasiticus mutant strains resistant to DMIs were isolated at a high mutation frequency after UV-mutagenesis and selection on media containing flusilazole. Two different resistant phenotypes, characterized as R_1 and R_2 on the basis of their aflatoxigenic ability, were identified. All R_1 mutant strains produced aflatoxins at concentrations significantly higher (up to 3-fold) than the wild-type parent strain on yeast extract sucrose medium, whereas the majority of the mutant strains (R_2 phenotype) had lost their aflatoxigenic ability. Real-time PCR analysis of the expression levels of the *aflR* gene, a pathway transcriptional regulatory gene in aflatoxin biosynthesis, showed that this gene was not expressed in R_2 mutant strains tested. Study of fitness-determining parameters showed that most flusilazole-resistant mutant strains had mycelial growth rate, sporulation and spore germination rates lower that the sensitive strain. Cross-resistance studies with other fungicides showed that all R_1 mutant strains were also resistant to the DMIs imazalil and tebuconazole, but retained their parental sensitivity to fungicides affecting other metabolic pathways and/or cellular processes. Contrary

to the above, all R_2 mutant strains exhibited a low to moderate multi-drug resistance to DMIs and to several other fungicide classes. Two different homologous genes, *cyp51A* and *cyp51B*, encoding C-14 alpha sterol demethylase (Cyp51) and an *mdr* gene encoding an ATP-binding cassette protein were cloned and characterized. Sequence comparison of *cyp51A* gene revealed an amino acid substitution from glycine (GGG) to *tryptophan* (TGG) at position 54 (G54W) in two out of three of R_1 mutant strains. Analysis of deduced amino acid sequence of *cyp51B* showed that no mutations were associated with DMI resistance. Study of the transcription levels of *cyp51A* showed that this gene was over-expressed in the third aflatoxigenic mutant strain. Neither amino acid substitutions within nor overexpression of the *cyp51A* gene were found in the R_2 mutant strains tested. Real-time PCR analysis showed high levels (up to 25-fold higher) of the *mdr* transcript in all R_2 mutant strains tested. This is the first report describing the existence of two *cyp51* genes and a potential *mdr* gene coding for an ATP binding cassette protein in *A. parasiticus*. These results also indicate that multiple biochemical mechanisms, including target-site modification due to mutation of the *cyp51A* gene, *cyp51A* overexpression, and the function of an ABC transporter protein, are responsible for DMI-resistance in *A. parasiticus*. Our findings suggest that *A. parasiticus* has the genetic and biochemical potential to give rise to highly aflatoxigenic DMI-resistant isolates in the field.

Identification and determination of antibiotic susceptibility of cultured enterobacterial flora from leafy vegetables in Cyprus

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In the last few years, the consumption of raw leafy vegetables has increased due to their well-documented nutritional value. The contamination of these crops by microbes originating from soil, manure and handler microflora is of increasing interest from a public health perspective. In addition to the microbial load, the antibiotic resistance of these microorganisms as a result of antibiotic usage in agriculture and other related fields it is of increasing concern. The aim of this study was to identify and enumerate the enterobacterial microflora on samples of leafy vegetables (lettuce, rucola, spinach, purslane) in Cyprus, and to evaluate the levels of resistance of these microorganisms to the antibiotics ampicillin, cefotaxime, gentamicin and vancomycin. The results showed high levels of total microflora and *Enterobacteriaceae* contamination, within the ranges of 6.31-7.85 and 5.86-6.7 log cfu/g respectively. More than 95% of the total microflora and 87% of *Enterobacteriaceae* identified were resistant to ampicillin. The rates of resistance to cefotaxime (33.41%) and gentamicin (17.35%) for *Enterobacteriaceae* were lower, but for the total microflora resistance to the latter antibiotics was higher (83.33% and 76.58% respectively). Rucola and spinach produced positive results for *Escherichia coli,* with rucola being the only sample found to be contaminated with isolates resistant to cefotaxime (0.43 log cfu/g). The samples also had similar population numbers for yeasts and moulds (4.04-5.67 log cfu/g). The current study shows that leafy vegetables can be a significant source of pathogenic microorganisms, including antibiotic resistant isolates.

Effect of Alternaria leaf-spot on the content of antioxidant compounds in infected kiwi orhards

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Alternaria alternata can infect kiwi leaves causing necrotic lesions and premature defoliation that may lead to complete yield losses. The objective of this study was to evaluate the changes in content of kiwi fruit antioxidant constituents (phenolic compounds and ascorbic acid) after the infection of vines by *Alternaria alternata*. Furthermore the vines were treated with CPPU (forchlorfenuron), a growth regulator agent of the synthetic cytokinins group, which is widely used in kiwi fruit orchards for both, increase of productivity and improvement of product quality. CPPU was applied to both healthy and infected vines in order to study the combined effect of the fungus and of the active ingredient, for chlorfenuron. Total antioxidant capacity was evaluated with FRAP and DPPH techniques, while the chromatographic profile of phenolic compounds and the ascorbic acid concentration were determined by liquid chromatography (HPLC). According to the results the presence of the fungus caused the induction of biosynthesis of phenolic compounds and the reduction of ascorbic acid concentration to untreated with CPPU fruits. In contrast, the application with CPPU caused an increase of ascorbic acid concentration in all kiwi fruits up to 50%. The total antioxidant capacity of fruits was strong correlated to ascorbic acid concentration.

Fungal diseases of sunflower in R.U. Drama in period 2010-2011

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The diseases affecting sunflower in the Regional Unit of Drama throughout the duration of the growing seasons 2010-2011 were recorded. During this two-year period there were incidences of Downy Mildew, Alternaria, Septoria, Powdery Mildew, Sclerotinia, Macrophomina, Phoma and Phomopsis. Systemic mildew, considered the most serious disease of sunflower worldwide, appeared in 2011, with the most highly infected field showing a 15-20% infection rate, while in 2011, a field was observed with a secondary mildew infection. Septoria, considered to be the most important of the foliar diseases (which include Alternaria, Septoria, and Powdery mildew), caused severe infections in 2011, while Alternaria and Powdery Mildew were observed in two separate cases in 2010 and 2011, respectively. Of the fungal diseases that attack the stem and root system (Macrophomina, Phoma, Sclerotinia and Phomopsis), Macrophomina caused the most extensive damage. In dry sandy fields, and in combination with dry and warm conditions, the percentage of infected plants reached in some cases 90%. In 2011 incidences of Phoma, with maximum infection rates of 50%-60% was observed, while incidences of Sclerotinia Wilt (rotting of the stem base) and Phomopsis were observed in individual plants. When sunflowers are grown in the same field for 2 or more consecutive years, the incidence of disease is more common, particularly that caused by Septoria and Phoma.

Inactivation of the *VdVeA* (Velvet A) gene in *Verticillium dahliae* and investigation of its role in the physiology and pathogenicity of the fungus

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Verticillium dahliae is a soilborne plant pathogenic fungus causing the syndrome of wilt diseases and posing a significant threat to several annual and perennial crops worldwide. The inability of common management methods to control *V. dahliae* has led to the investigation of molecular mechanisms that might regulate its virulence. Studies have previously shown that the fungus *V. dahliae* produces phytotoxins and other secondary metabolites that induce cell death or other forms of host defence. It has been found that in several species of *Fusarium* spp. and *Aspergillus* spp. the gene *veA* encodes a protein that can regulate the fungal secondary metabolism, induce the differentiation of fungal development in relation to light, and regulate reproduction and pathogenicity. Along with the VeA, a second protein called LaeA forms a nuclear complex called the Velvet complex, in association with a third protein, VelB. The aim of this study was to elucidate the role of the orthologous gene of *A. nidulans, VdVeA,* in the virulence and morphology of the fungus *V. dahliae*. A gene replacement strategy was applied by incorporating two genomic sequences of about 1000 bp before the start and stop codons respectively, in the binary vector pGKO2. Between these two regions the geneticin cassette was subcloned in order to replace the *VdVeA*gene after transformation. The inactivation construct of *VdVeA* was introduced into various strains of *V. dahliae* using the Ti plasmid of *Agrobacterium tumefaciens* via a double recombination event. The effect of inactivation of the *VdVeA* gene on the physiology and pathogenicity of the fungus was explored in *in vitro* experiments and *in planta* virulence assays in several hosts.

Fast and accurate identification of *Fusarium oxysporum* formae *speciales* complex using High Resolution Melting (HRM) analysis

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The fungus *Fusarium oxysporum* consists by a number of different strains which are grouped together in to groups called formae speciales making F. oxysporum a highly complex species. This complexity is responsible for the timely and difficult discriminate of the different Fusarium formae specials which is performed via biochemical or phenotypic methods. Thus it is of paramount importance to develop novel, rapid, and simple to perform identification methods. Herein, we describe the development of a novel real-time PCR based assay [using universal internal transcribed spacer (ITS) primers] coupled with high-resolution melting (HRM) analysis for the identification and discrimination of *F. oxysporum* formae *speciales* complex. The melting curve analysis of the ITS amplicons succeeded in specifically classify all isolates into seven *F. oxysporum* formae *speciales* and generated seven distinct HRM curve profiles. The smallest DNA sequence difference recognized in this study was one nucleotide. We conclude that based on the mentioned results HRM curve analysis of *Fusarium* ITS sequences is a sim-

ple, quick, and reproducible method which allows both the identification of seven *F. oxysporum* formae *speciales* and at the same time their screening for variants. Our genotyping assay uses the combined information of simultaneously acquired HRM data from an unlabeled probe and the full-length amplicon. Finally, the completion of both reaction and analysis in a closed tube saves time by eliminating the separate steps and reduces the risk of contamination.

Molecular and phytopathological investigation of the role of the global regulator of secondary metabolism *AcLaeA* in the mycotoxigenic fungus *Aspergillus carbonarius*

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The filamentous fungus *Aspergillus carbonarius* is considered one of the main fungi responsible for sour rot in grapes and for the production of the carcinogenic mycotoxin ochratoxin A. Recently, a novel gene named *laeA* that operates as a global regulator of secondary metabolism was discovered in several species of *Aspergillus* and *Fusarium*. Inactivation of the *laeA* gene leads to disruption of mycotoxin biosynthesis. The gene *laeA* encodes a nuclear methyltransferase protein that is required for the expression of secondary metabolite genes, while its presence is considered indispensable for mycotoxin, antibiotic and mycelial pigment biosynthesis. BLAST analysis of the genome of *A. carbonarius* with the *laeA* gene of *A. nidulans* resulted in the identification of an orthologous gene named *AclaeA*. The goal of this study was to investigate the role of the regulatory gene *AclaeA* in the physiology, virulence and ochratoxinA production by *A. carbonarius* in grapes by deleting this gene from the genome of two wild types of the fungus. Using PCR with specific primers, two genome sequences located about 1000 bp before the start and stop codon of *AclaeA,* respectively, were amplified and subcloned into the vector pBluescript. Between these two regions the geneticin cassette was subcloned in order to replace the *AclaeA* gene after transformation. The *AclaeA* deletion construct was transferred to the binary vector pGKO2 and then introduced using the Ti plasmid of *Agrobacterium tumefaciens* via a double recombination event, into two wild type strains of *A. carbonarius*. The evaluation data on the morphological characteristics and virulence experiments in red and white grape varieties of *ΔAclaeA* mutants is presented.

First report of *Diaporthe neotheicola* as pathogen causing shoot blight in kiwifruit

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Kiwifruit is an important crop in Greece. Although it is considered to be a crop with few pests and diseases, new diseases have been identified in the last few years.
In June 2009, shoots of the kiwifruit plants (cvs "Hayward" and "Tsechelidis") were found to be wilted and blighted in many orchards of Episkopi, Naoussa (Imathia Prefecture, Greece). Close examination of these shoots revealed distinct dark cankers. Fungal isolations were made on acidified potato dextrose agar, and the species responsible

was identified based on ITS-5.8S rDNA-ITS2 region and Elongation Factor 1-alpha by the CBS Fungal Biodiversity Centre, Identification Service (Utrecht, Netherlands) as *Diaporthe neotheicola* A.J.L. Phillips & J.M. Santos.
Koch's postulates were fulfilled by the artificial inoculation of 20 segments of 1-year-old woody shoots of the kiwifruit cultivar "Hayward", and 20 fruits. Shoots and fruits inoculated with agar discs without mycelium were used as control. Identical disease symptoms were observed in the inoculated shoots. The pathogen was reisolated from the artificially inoculated shoots and fruits.
In laboratory experiments, the rate of mycelial growth of *D. neotheicola in vitro* was reduced as the temperature was increased from 25 to 30°C, and also when decreased from 20 to 10°C. Growth was totally inhibited at 35 and 2-4°C.
This study is the first report of the occurrence of a shoot blight and canker disease of kiwifruit in Greece caused by the fungus *D. neotheicola*.

Metabolomic analysis of *Verticillium dahliae* races 1 and 2

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The technological advancements in analytical chemistry and computing of the last decade have facilitated study of the metabolomic profile of plants and microorganisms under different treatments. The aim of the present study was to investigate the metabolomic profile of *Verticillium dahliae* races 1 and 2 grown in two different growth media that simulate either xylem or microsclerotia germination conditions. It was observed that the race 2 secreted a higher (by 84%) number of metabolites than race 1 in both media. Furthermore, the number of the secreted metabolites of race 1 and 2 that were common in both media was 20% and 37%, respectively. Among the different metabolites that were identified, were substances with established roles, either as elicitors or effectors, in plant-microbe interactions.

Pathogen identification and incidence of pre- and post-harvest fruit rots of pomegranate in Greece

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In recent years, the cultivation of pomegranate has increased considerably, particularly in northern Greece. Fruit rots, along with other physiological disorders contribute to quantitative yield losses, qualitative deterioration, and restriction of fruit storability. The goal of the present study was to identify and measure the incidence of fungal pathogens causing pre- and post-harvest decay in pomegranate fruit. The sampling of diseased fruit was conducted during 2011 (September to December) in a number of orchards and storage rooms in several regions of N. Greece. Fungal identification at a genus level was based on colony appearance and morphological features of fruiting bodies and spores. In total 5 and 3 pre- and post-harvest pathogens were identified, respectively. It was found that *Aspergillus* spp. (45.9%) and *Penicillium* spp. (40.0%) were the main causal agents of pre-harvest fruit rots. In contrast, post-harvest rots were caused mainly by *Botrytis cinerea* (70.8%) and *Pilidiella granati* (15.4%). *Aspergillus* spp. iso-

lates were identified at a species level as *A. niger* (65.5%) or *A. tubingensis* (34.5%) using the Restriction Fragment Length Polymorphism (RFLP) technique. *Penicillium* spp. isolates were identified at a species level after sequencing of the internal transcribed spacer (ITS) regions of ribosomal DNA (rDNA). The *Penicillium* spp. isolates obtained from fruit with pre-harvest rots were identified as either *P. adametzioides* or *P. brevicompactum* at similar frequencies. *Penicillium* spp. isolates obtained from fruit with post-harvest rot were all identified as *P. adametzioides*. To the best of our knowledge, this is the first report of *P. adametzioides* and *P. brevicompactum* causing pre- or post-harvest fruit rot of pomegranate worldwide. These results, together with those anticipated from new samplings conducted during 2012, will provide significant and useful information for the implementation of successful fruit-rot control measures.

Susceptibility of cultivated solanaceous plants and olive to *Verticillium dahliae* isolated from a new host, the weed *Solanum elaeagnifolium*

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The rapidly expanding weed *Solanum elaeagnifolium* (commonly 'germanos') is considered to constitute one of the most persistent problems in the fields of Greece and southern Europe. The fungus *Verticillium dahliae* is a difficult to control soil-borne fungus with global distribution, that was recently isolated from *S. elaeagnifolium* plants exhibiting wilt symptoms. In the present research the pathogenicity of *V. dahliae,* isolated from germanos was studied on the weed itself, on eggplant cv. Langada, on pepper cv. P13, as well as on olive cv. Chalkidikis, Amfissis and Koroneiki. The results confirmed the pathogenicity of the fungus on the weed, eggplant, pepper, and olive. This is the first report worldwide that the invasive in Europe weed species *S. elaeagnifolium* is a host for *V. dahliae*. There is an urgent need to find successful means to control this weed, as not only can it lead to the exclusion of cultivated species from fields, but it can also increase the levels of fungal inoculum in the soil.

First report of palm rot disease of *Phoenix* spp. caused by *Neodeightonia phoenicum* in Greece

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In July 2007, a severe palm rot disease resembling Diplodia disease was observed on *Phoenix dactylifera* in Heraklion (Crete, Greece). Similar symptoms were later observed on *P. canariensis*. Initial pale elongated spots were gradually converted to dark brown streaks extending along the leaf base and rachis. Decay and premature death of leaves was followed by terminal bud necrosis, and leaf blight and stalk rot were also observed. A filamentous fungus was consistently isolated from leaf base necrotic lesions; according

to morphological (macroscopic and microscopic examination), molecular (sequencing of the ITS1-5.8S-ITS2 region, together with parts of the flanking 18S and 28S rRNA genes, and BLAST search), and phylogenetic (including representatives of relative fungal genera) analyses performed, the pathogen was identified as *Neodeightonia phoenicum* A. J. L. Phillips & Crous (syn. *Diplodia phoenicum*), formerly also known as *Macrophoma phoenicum* and *Strionemadiplodia phoenicum*. The pathogenicity of the fungus to *P. canariensis*, *P. theophrasti* and *Washingtonia filifera* was demonstrated by artificial wound-inoculation experiments. While infections of *N. phoenicum* with *P. dactylifera* are common worldwide, to the best of our knowledge this is the first report of such an infection in Greece. The disease may be favoured by the pruning of older leaves during early Spring and the widespread occurrence of the red palm weevil *Rhynchophorus ferrugineus* in Greece.

First report worldwide of leaf spot disease of *Phoenix theophrasti* caused by *Paraconiothyrium variabile*

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A severe leaf spot disease of *Phoenix theophrasti* was observed in Heraklion (Crete, Greece) in the Spring of 2011. Typical symptoms of the leaves included initially small, round-ovoid brown spots, later changing into streaks (average dimensions 7.3 x 3.3 mm), surrounded by dark brown rings. Finally, the expanding streaks often coalesced to the production of enlarged necrotic lesions resulting in leaf blights. Symptoms were also detected on petioles and leaf bases. Extended spotting and blighting resulted in leaf death and unthrifty appearance of infected trees. A filamentous fungus was consistently isolated from the periphery of the characteristic lesions. According to morphological (microscopic and macroscopic examination), molecular (sequencing of the ITS1-5.8S-ITS2 region, together with parts of the flanking 18S and 28S rRNA genes, and BLAST search), and phylogenetic (including representatives of relative fungal genera) analyses performed, the pathogen was identified as *Paraconiothyrium variabile* Riccioni, Damm, Verkley & Crous. The pathogenicity of the fungus was demonstrated in artificial spraying-inoculation tests on seedlings of *P. theophrasti*. *P. variabile* has so far been isolated from various woody host plants in South Africa, Italy, Turkey, China and New Zealand. This is the first report worldwide of *P. variabile* naturally infecting a palm species.

First report of pink rot of *Phoenix* and *Washingtonia* palm species caused by *Nalanthamala vermoesenii* in Greece

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Pink rot of *Phoenix* and *Washingtonia* species has become common in Heraklion (Crete, Greece) in the last four years. The disease is characterized by leaf spots, chlorosis and necrosis, rachis spots and decay, sheath and trunk rot, decline, and eventually death of infected plants, and a pinkish-orange layer on the surfaces of infected tissues. A filamentous fungus was consistently isolated from the petiole tissues of diseased leaves and from the pinkish-orange characteristic overlay. According to morphological (macroscopic and microscopic examination), molecular (sequencing of the ITS1-5.8S-ITS2 region and part of the 18S rRNA gene, and BLAST search) and phylogenetic (including representatives of relative fungal genera) analyses performed, the pathogen was identified as *Nalanthamala vermoesenii* (Biourge) Schroers (syn. *Penicillium vermoesenii*, *Gliocladium vermoesenii*). Artificial wound-inoculation tests on *P. canariensis*, *P. theophrasti* and *W. filifera* demonstrated the pathogenicity of the fungus to all three palm species, with *W. filifera* being the most susceptible. *N. vermoesenii* has a worldwide distribution and has frequently been documented on several palm species. To the best of our knowledge, this is the first report of a palm disease caused by *N. vermoesenii* in Greece. It may be favoured by the pruning of older leaves during early Spring and the occurrence of the red palm weevil *Rhynchophorus ferrugineus*.

Characterization and distribution of mating type genes in the Dothistroma Needle Blight pathogens

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Dothistroma needle blight, also known as red band needle blight, is one of the most important diseases of *Pinus* spp. The disease is caused by two very similar *Dothistroma* spp., *D. septosporum* and *D. pini*. A total of 23 isolates from the area of Xanthi, N.E. Greece, were assayed in order to identify the occurrence of *Dothistroma* spp. Four different molecular techniques were applied in this study including i) sequencing of the rDNA ITS region ii) a species-specific ITS-RFLP technique iii) mating type primers and iv) a diagnostic microsatellite marker Doth_A. The results of this study showed that all 23 isolates were characterized as *D. septosporum* (Dorog) M. Mopelet, in an agreement with the global distribution of the species. In contrast, none of the isolates was identified as *D. pini*, which had, until recently, been reported only in the USA, Russia and Ukraine. Recently, and for the first time, the occurrence of both species was reported in the same area in France. Subsequently, the two species have been reported to occur in Hungary, at the same location, on the same trees and even infecting the same needles.

Distribution of mating types of *Cryphonectria parasitica* in Greece

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The fungus *Cryphonectria parasitica*, the causal agent of chestnut blight, was observed in Greece for the first time in 1963 (Biris 1964) and particularly in Zagora, Mt. Pelion. Examination of the mating type phenotype of *C. parasitica* isolates can reveal the population structure and the presence, or lack, of sexual reproduction. In 707 virulent and hypovirulent isolates, fungal genomic DNA was extracted after growth on PDA dishes covered with cellophane sheets. Polymerase chain reaction was carried out and the size of the PCR products was determined to be 1,649 kb (MAT1-1) or 594 bp (MAT1-2). The study of the sexual compatibility in our populations showed that the idiomorph MAT1-1 was present in most isolates (92.6%). In contrast, only one isolate (7.35%) was found to represent the MAT1-2 phenotype. This mating type distribution is in agreement with the existing data for the occurrence of only one mating type and the lack of sexual reproduction. The results of our study favour the application of biological control measures against chestnut blight, a technique already in use for management of the disease in Greece.

Necrosis of young almond trees infected by *Botryosphaeria dothidea*

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Several species of fruit trees have been reported to be infected by fungi of the genus *Botryosphaeria*. Amongst these are apple, peach, pistachio, kiwifruit, olive and walnut. Over the period May-June 2011 an unusual outbreak of the fungus was recorded at four new commercial almond (cv. Ferragnes) plantations planted early in the same year in the region of Thessaly, Central Greece (in Tirnavos and New Anchialos). The percentage of dead trees was between 40-70% and the propagative material originated from two different nurseries in Thessaly and North Greece. Infected trees showed initially a yellowing discoloration of the leaves and gradually died during the summer. All the infections were associated with the pruning wound at the point of the union of rootstock/scion. Transverse and longitudinal sections through affected wood revealed a brown discoloration of the wood with a well defined line between the necrotic and healthy tissues. From the rootstock new vigorous shoots had often developed. The species *Botryosphaeria dothidea* (Moug.: Fr.) Ces. & De Not was isolated from all trees examined. The fungus produced hyaline, aseptate, fusiform conidia, 23-29 x 4.5-5 µm in size, belonging to *Neofusicoccum mediterraneum* Crous, M.J. Wingf. & A.J.L. Phillips (anamorph). The pathogenicity of the obtained isolates was proved by inoculating young almond trees cv. Ferragnes grown in the field. All isolates proved to be pathogenic. Re-isolations made from discolored wood yielded the same fungus. This is the first report of infection of almond trees in commercial plantations by the fungus *B. dothidea*, worldwide.

Leaf spots induced in *Ilex aquifolium* by *Alternaria alternata*

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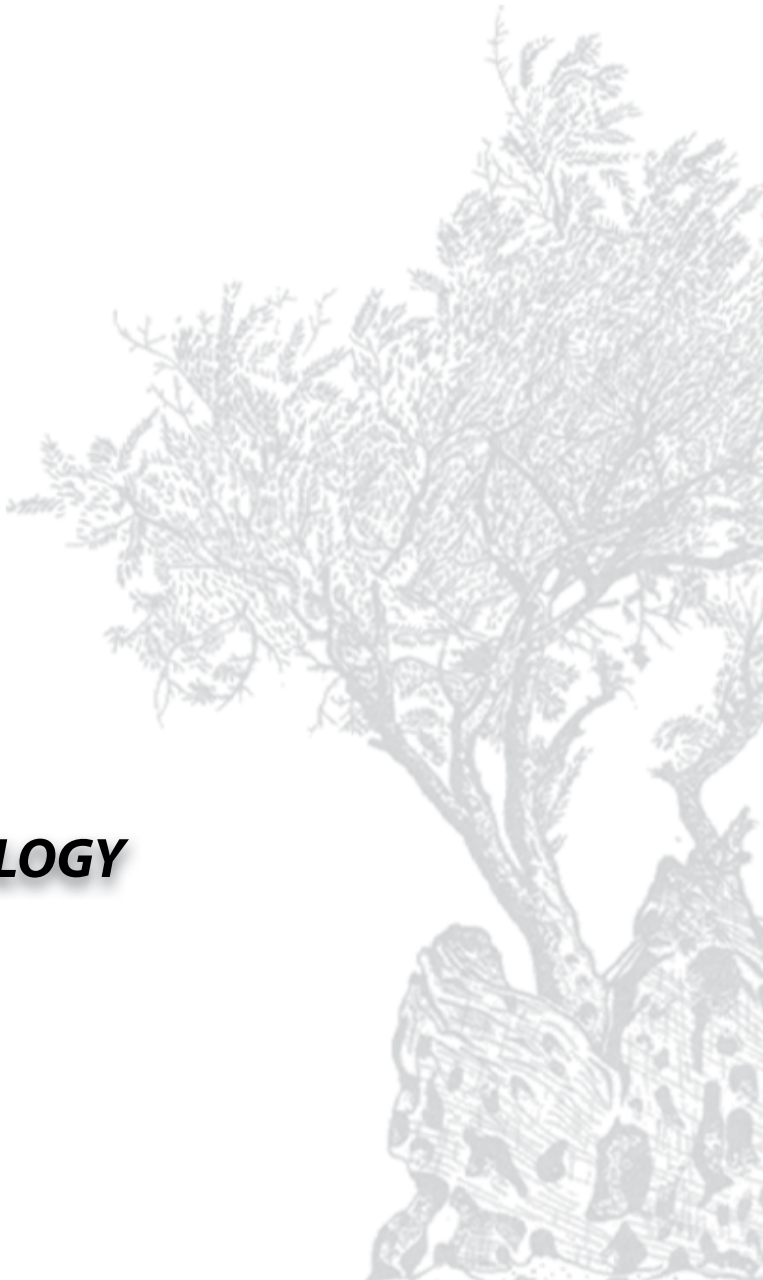
A foliar disease of holly (*Ilex aquifolium* L.) was observed in 2009 in a natural ecosystem in the region of Thessaloniki (Northern Greece, Central Macedonia, Mount Hortiatis). Symptoms consisted of circular, sometimes irregularly shaped, brown necrotic spots with yellow border, 3 to 7 mm in diameter. On some leaves several spots coalesced to form large necrotic areas, covering approximately half of the leaf surface. Older lesions sometimes appeared blackish brown as sporulation occurred on the lesions. The percentage of diseased leaves of the particular population was approximately 30%. Single spore cultures on potato carrot medium (PCA) gave rise to initially white colonies which turned to grayish-black later due to abundant sporulation. Conidiophores were short, septate, branched or unbranched, and green to brown. Mature conidia were produced in long, single or more often in branched chains. The conidia were obpyriform, with a conical or cylindrical beak, ovoid or ellipsoidal measuring, 7.7 – 27.4 x 5.6 -15.0 µm (average 16.3 x 8.8 µm), showing 1 to 5 transverse septa and 0 to 3 longitudinal septa. These data, together with molecular characterization using the specific ribosomal internal transcribed spacer region (rDNA-ITS) confirmed the identity of the fungus as *Alternaria alternata* (Fr.) Keissl. A pathogenicity test was conducted on detached, wounded and unwounded, healthy *I. aquifolium* leaves. Ten days after inoculation, leaf spots similar to the ones observed in the field developed on all the inoculated points (wounded leaves) and on half of the unwounded leaves, while control leaves remained symptomless. *A. alternata* was reisolated from artificially inoculated leaves confirming Koch's postulates. To our knowledge, this is the first report of *A. alternata* causing leaf spot on holly in Greece.



BACTERIOLOGY

Oral & Poster Presentations

Potato blackleg in Greece caused by *Dickeya* sp. biovar 3 (*D. solani*)

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During the years 2007 and 2011, significant bacterial infections were observed in potato crops (*Solanum tuberosum* cv. Spunta) on the island of Crete in Greece. Similar infections were observed at Nevrokopi, Drama, during the spring period of 2012 (cvs. Banba and Safari). Affected plants had blackleg and rotting symptoms at the stem base, brown discolouration of vascular tissues, wilting and soft rot in daughter tubers. Disease incidence varied from 5%-50%. The aim of this study was to identify the pathogen responsible for the blackleg disease isolated from both of the above mentioned regions. Isolations were made from symptomatic tissues onto CVP (crystal violet pectate) and NAG (nutrient agar glucose) media. Single bacterial colonies having characteristic pectinolytic activity (CVP) and/or "fried egg" colonies (NAG) were sub-cultured, purified, and used for further characterisation. Twenty five isolates were characterized as *Dickeya* sp. biovar 3 (syn: *Erwinia chrysanthemi*, *Pectobacterium chrysanthemi*) based on standard biochemical tests. These strains were biochemically identical to the reference strain *Dickeya* sp. IPO2222, while biochemically distinct from the related reference strain *Dickeya dianthicola* BPIC2098, a pathogen of potato. Molecular analysis with a) repPCR (BOX and ERIC fingerprinting), b) specific primers for the PCR amplification of the *pel* (pectate lyase) gene, and c) sequencing of the *dnaX* gene, revealed identical profiles among the isolated strains and the reference strain *Dickeya* sp. IPO2222; which were clearly distinct from the those of related species *D. dianthicola* BPIC2098, *P. carotovorum* TEIC3036, *P. atrosepticum* TEIC3211 and other *Erwinia* spp. Koch's postulates were fulfilled by inoculation onto potato plants and tubers and re-isolation from the sites of observed typical disease symptoms. This is the first report of potato blackleg caused by *Dickeya* sp. biovar 3 (*D. solani*) in a region outside of Crete (Nevrokopi, Drama). As *D. solani*, species include very infectious strains, further spread of the pathogen in potato production in Greece is expected to have a high economic impact.

Molecular characterization of *Pseudomonas viridiflava* strains from several hosts

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The pectinolytic species *Pseudomonas viridiflava* is characterized as a weak opportunistic plant pathogen, with a wide host range, causing foliar and stem necrotic lesions and basal stem and root rots. In Greece this pathogen has been reported as a disease causal agent in a wide range of host plants including celery, blitum, eggplant, acanthus,

tomato, artichoke, rocket, among others. This work reports the biochemical and molecular characterisation of bacterial isolates primarily identified as *Pseudomonas viridiflava,* that have been maintained for the last twenty years in the collection of Bacteriology lab, TEI of Crete. Amongst the 64 isolates, 26 isolates obtained from several hosts were selected and their taxonomy was confirmed based on their phenotypic, biochemical and physiological characteristics, as well as their pathogenicity on different hosts. These results were confirmed by molecular population analyses, where the 18 isolates were tested by rep-PCR fingerprinting (BOX- and ERIC-PCR) as well as by Multilocus Sequence Typing (MLST), utilizing the partial sequences of the genes *gyrB*, *rpoB* and *rpoD*. In conclusion, the biochemical tests and pathogenicity profiling did not reveal any variability among the isolated strains studied. However, the molecular fingerprinting patterns and housekeeping gene sequences clearly demonstrated a significant genomic variability among the 18 isolates tested.

Characterization of isolates of *Pseudomonas corrugata* and *P. mediterranea*, causal agents of «pith necrosis» disease of tomato on Crete

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Pseudomonas corrugate is a plant pathogen that causes pith necrosis in tomato. Infected plants were identified by a general chlorotic appearance, a gradual withering and pith necrosis. Recent findings proposed that *Pseudomonas mediterranea*, which is isolated frequently from cultivated tomato fields of countries in the Mediterranean basin, is a related bacterium acting as a new pathogenic agent causing pith necrosis. This work reports the characterization of bacterial isolates from pith necrosis of diseased tomato and pepper plants on the island of Crete, obtained within the period 1991-2009. Amongst the 72 isolates deposited in the collection of the Bacteriology Lab, TEI of Crete, 36 were chosen based on phenotypic characters such as the absence of fluorescence and a positive oxidase reaction, the ability to elicit the typical hypersensitive reaction on tobacco plants and their pathogenicity on tomato plants. Among these 36 isolates, which were analyzed by classical bacteriological tests, 28 were classified as *P. mediterranea* and the remaining 8 as *P. corrugata*, including the reference strains CFBP5447T and NCPPB2445 respectively. A similar identification was also observed using the antiserum anti-PC14 for *P. corrugata* in immunofluorescence tests. These results were further confirmed by molecular analysis using a) the species specific PCR primer pairs PC1 and PC5, for the identification of *P. mediterranea* and *P. corrugata* respectively, b) by rep-PCR (BOX and ERIC) fingerprinting, and c) by Multilocus Sequence Typing (MLST) utilizing the partial sequences of the genes *gyrB*, *rpoB* and *rpoD*. Phylogenetic analysis of the isolated strains did not reveal significant genetic variability among them. This is the first report of *Pseudomonas mediterranea* as a pathogen of pith necrosis disease of tomato and pepper plants in Greece.

Comparative genomics of multiple strains of *Pseudomonas cannabina* pv. *alisalensis*, a potential model pathogen of both monocots and dicots

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Comparative genomic study of closely related pathogens with different host range provides insights into mechanisms of host-pathogen interaction, differential virulence factors, and pathogen evolution. Moreover, sequencing of various strains of the same pathogen can reveal additional information concerning pathogen diversity and the molecular basis of virulence differences between strains. In the current study we present draft genome sequences and a comparative genomics analysis of four strains of *Pseudomonas cannabina* pv. *alisalensis* (*Pcal*), one of the causative agents of bacterial blight of crucifers, isolated from geographically distant areas of Greece and the USA. Since *Pcal* causes disease in a wide range of plant species and can infect both monocots and dicots, including the model plants *Arabidopsis thaliana* and tomato, draft genome sequences of four *Pcal* strains were obtained to develop hypotheses regarding the molecular basis of virulence and host range determinants of this pathogen. Genomes were also compared to the genome of the recently reclassified strain *P. syringae* pv. *maculicola* ES4326 as identical to *Pcal* ES4326, the model pathogen *P. syringae* pv. *tomato* DC3000 of *Arabidopsis thaliana* and tomato, and *P. syringae* pv. *syringae* B728a bean pathogen. All *Pcal* strains harbour two genomic islands containing genetic elements for type VI secretion systems (T6SSs). Surprisingly, one of the *Pcal* T6SS is phylogenetically closer to *P. aeruginosa* T6SS-I than the *P. syringae* T6SSs. All *Pcal* strains also harbour a *hrp/hrc* gene cluster coding for a type III secretion apparatus (T3SS), which in regard to structure and DNA sequence is most similar to *Psy* B728a, although *Pst* DC3000 is the closer relative, suggesting horizontal gene transfer of the *hrp/hrc* cluster between the strains' ancestors. Although the overall genetic content of each of the four *Pcal* genomes appears to be highly similar, the repertoire of the type III effector proteins (T3EPs) was found to be significantly divergent between the examined strains, reinforcing previous molecular data suggesting the existence of two distinct lineages within this pathovar of *Pseudomonas cannabina*.

Study of the resistance of tomato hybrid CLX3731 transgenic plants overexpressing the *gmgstu4* gene after infection with the bacterium *P. syringae* pv. *tomato*

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In tomato cultivation biotic stresses result in lower production and product quality. The GST isoenzymes participate in the antioxidative defence mechanism of plants contributing to resistance against biotic stress factors. The basic aim of our study was the formation of transgenic lines overexpressing the *gmgstu4* gene using the CLX3731 tomato hybrid and to study their response to bacterial speck disease. For the genetic transformation, cotyledons of 12-15 days old were cocultivated with transformed *Agrobacterium tumefaciens* and subsequently grown on MS_R medium (0.1 mg/L IAA, 0.5 mg/L Z, 250 mg/L Cf και 100 mg/L Km). PCR and RT-PCR were conducted to verify the the presence of the transgene in the lines (B3 και B7), and to study transgene expression, respectively. To examine resistance, *in vivo* transgenic and wild type plants of 4-6 weeks old were infected with the bacterium *P. syringae* pv. *tomato*. The response of the transgenic plants to infection was evaluated 5 days later. The transgenic lines showed 1.5 times fewer infected leaves and 6.4 times fewer necrotic lesions per plant, 1.7 times less electrolyte leakage and 3.7 times more glutathione peroxidase enzymatic activity when compared with the wild type plants. The study of the *gstu4* gene in genetically modified tomato plants may suggest new ways to engineer resistance against biotic stress factors.

First report of a "C*andidatus* phytoplasma solani" related strain infecting two accessions of jimsonweed (*Datura stramonium*) in Greece

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Datura stramonium is a common weed in spring crops in Greece and worldwide, which includes two accessions (*D. stramonium* f. *stramonium*, *D. stramonium* f. *tatula*). During a field trial conducted at the Aristotle University Farm, where the two accessions were evaluated for growth rate and alkaloid content, phytoplasma-like symptoms were observed. Plant samples were taken and tested with a generic nested-PCR targeting the highly conserved 16S rRNA gene of the phytoplasma genome. The ~1200 bp amplicon obtained only from the symptomatic plants of both Datura accessions was sequenced and compared with the NCBI database isolates, using the BLASTn algorithm. Both isolates (acc. no. HE598778 and HE598779 for *D. stramonium* f. *stramonium* and *D. stramonium* f. *tatula*, respectively) exhibited 99% similarity with the "*Ca*. Phytoplasma solani" reference strain (AF248959). Infected plants of *D. stramonium* f. *stramonium* and *D. stramonium* f. *tatula* showed 49-69% and 38% reduction of the above-ground fresh weight, respectively, as compared with the healthy ones. These findings suggest that the phytoplasma is an important pathogen of jimsonweed, which reduces its vigour and makes this weed species a good host-reservoir for this disease. This is, to our knowledge, the first report of a "*Ca*. Phytoplasma solani" related disease in jimsonweed found in Greece.

Pseudomonas cichorii is the causal agent of tomato pith necrosis in Crete

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Recent taxonomic advances based on biochemical as well as genotypic analysis have demonstrated that plant pathogen species *Pseudomonas cichorii* consists of a cluster of closely related genomic groups collectively defined as the *P. cichorii* complex. Prior to this study, three pathovar groups had been described, all sharing common phenotypic and biochemical characters but presenting variability regarding their DNA content. All entities of the complex can cause disease in a variety of hosts, including lettuce, celery and chrysanthemum, among others. In this study, we present the isolation and biochemical and molecular characterisation of *P. cichorii* isolates as the causal agent of pith necrosis in tomato plants. A detailed characterization of the genetic variability among strains belonging to *P. cichorii* was achieved using different molecular typing methods, including rep-PCR (BOX- and ERIC), as well as Multilocus Sequence Typing (MLST), utilizing the partial sequences of the genes *gyrB*, *rpoB* and *rpoD*. Likewise, a number of biochemical tests were also used for the biochemical identification of the tomato *P. cichorii* isolates. To our knowledge, this is the first complete biochemical, molecular and phylogenetic analysis of *P. cichorii* species as the causal agent of tomato pith necrosis. Our results clearly demonstrate the emergence of a new genomic group in the *P. cichorii* complex, consisting of strains that could consistently be separated from other members of the complex. Finally, this is the first record of *P. cichorii* as a pathogen of tomato pith necrosis in Greece.

Infection of watermelon, melon and cucumber plants by a phytopathogenic bacterium of the genus *Acidovorax* in Greece

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During the period of March-May 2012, specimens of young seedlings of watermelon, melon and cucumber from a greenhouse crop in the area of Thiva (Boeotia), exhibiting necrotic, brown, irregular-shaped leaf lesions, of variable size and most often surrounded by a chlorotic halo, were examined in the Laboratory of Bacteriology of the Institute. The lesions were observed on the cotyledons of watermelon, melon and cucumber plants, as well as on a small number of true leaves of the melon plants. The outbreak was reported by the agronomist who supervised the crops to have affected 10 to 100 % of the plants. In all specimens, microscopic examination of affected leaves revealed bacterial streaming from lesion margins. Bacterial isolates obtained from the leaf lesions were consistently identified on the basis of cultural, biochemical and serological assays, as well as a tobacco hypersensitivity test, as a phytopathogenic species of the genus *Acidovorax*. Sequencing of the 16S rDNA region showed significant sequence identity of the isolates to *A. valerianellae*, whereas PCR assays showed them to differ from reference strains of *A. citrulli*. Studies are ongoing to further characterize the isolates at the species level. This is a preliminary report on the presence of a phytopathogenic species of *Acidovorax* causing economically important damage to crops of cucurbits in Greece.

First report of infection of sweet basil plantlets by the phytopathogenic bacterium *Pseudomonas viridiflava* in Greece

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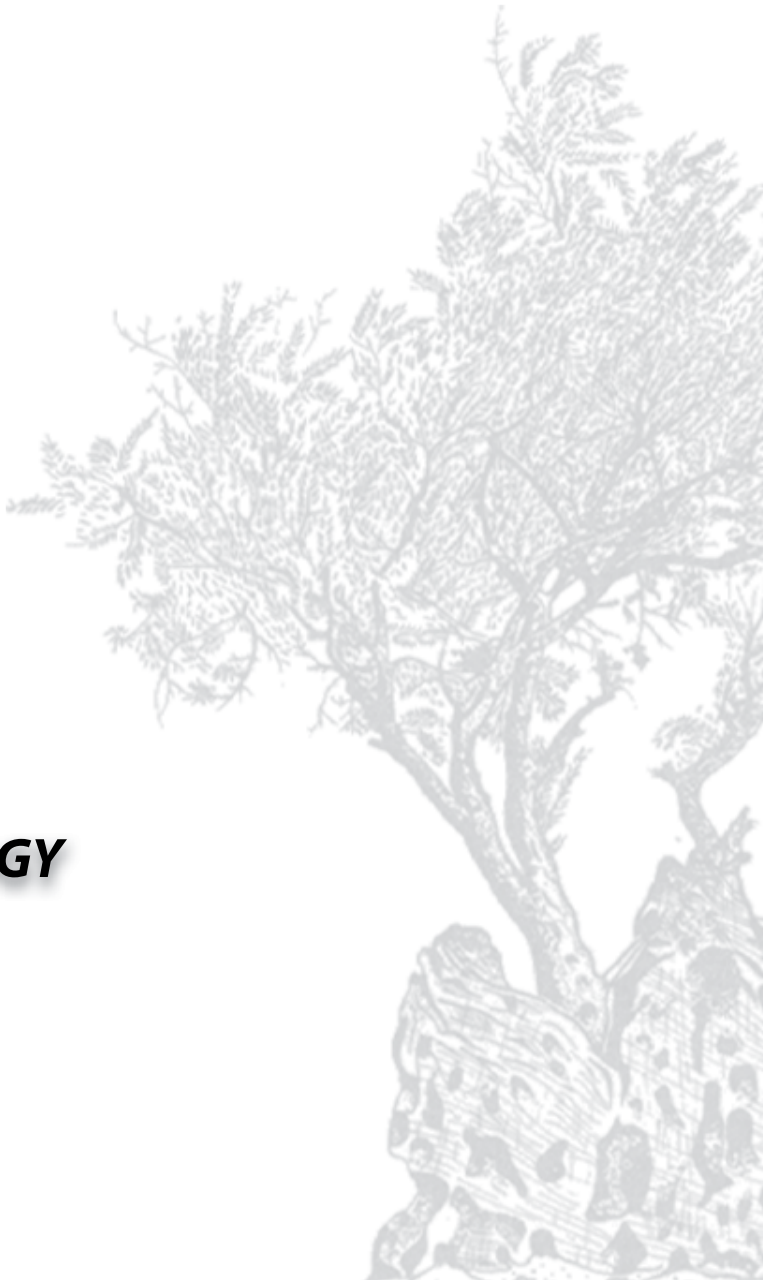
In November 2011, specimens of young plantlets of sweet basil (*Ocimum basilicum* L.) cv. Genovese from a commercial hydroponic culture in the area of Acharnes (Attiki), exhibiting irregular-shaped, necrotic, black lesions of variable size at leaf margins were examined in the Laboratory of Bacteriology of the Institute. This outbreak was reported by the agronomist in charge to have affected about 80 % of the plants grown in a 1000m^2 cultivated area. Symptoms were observed in a relatively small number of fully expanded true leaves per plant. Microscopic examination of sections of affected leaves revealed bacterial streaming from lesion margins. Bacterial isolates obtained from the leaf lesions were consistently identified on the basis of cultural, physiological and biochemical assays as well as a pathogenicity test, as *Pseudomonas viridiflava*. To the best of our knowledge this is the first report of *P. viridiflava* naturally infecting sweet basil in Greece. Further characterization of the isolates and their comparison to *P. viridiflava* reference strains is in progress.



VIROLOGY

Invited Lecture

Deep sequencing and the identification without prior knowledge of phytoviruses: applications to plant disease etiology and to metagenomics

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Similar to viruses infecting other organisms, plant viruses are collectively characterized by an amazing diversity, encoding their genome on various kinds of nucleic acids and sharing as a group no single common gene or determinant. The direct consequence of this situation is that although the detection of a known virus is now generally straightforward, the detection, without prior knowledge, of all viruses present in a plant sample remains a significant challenge. The development of novel sequencing technologies (NGS: next generation sequencing) allows unprecedented sequence data generation at a fraction of the cost of previous technologies and has drastically altered this situation. Indeed, it is now possible to generate vast amounts of sequence information from a plant sample and to then use bioinformatics tools to sift through this data in order to identify any virus that might be present. Various templates have been sequenced in such approaches, including messenger RNAs (mRNAs), small interfering RNAs (siRNAs), double stranded RNAs (dsRNAs) or nucleic acids extracted from semi-purified viral particle preparations. In the case of DNA plant viruses, which to date all have circular genomes, the sequencing of RCA (Rolling Circle Amplification) products has been used.

The first area in which these approaches are likely to have a major impact concerns the field of etiology and the conceptually linked certification and quarantine fields. In each case, the key problem is to be able to quickly identify with great sensitivity any viral agent that may be present in a sample. We have recently used siRNA and dsRNA sequencing in an effort to identify viruses in *Prunus* stone fruit material, illustrating the power and the potential interest of these approaches. In our hands, the best results so far were obtained with dsRNA which can be analyzed in a multiplexed format in order to reduce indexing costs. Although a detailed sensitivity comparison with biological indexing has yet to be performed, the cost of this new technique already compares favourably.

The second area where these strategies are arousing wide interest is the global analysis of viral populations associated with plants in an environment (metagenomics). Such questions were formerly not accessible to experimentation but are now feasible thanks to the democratization of NGS. We are developing this approach in two contrasted environments, the simplified ecosystem of the Kerguelen islands, the second most isolated archipelago on earth, and in a less constrained but anthropized temperate horticultural setting. The first results indicate a very high proportion in dsRNA viruses in the Kerguelen islands and, conversely, an enrichment in pathogenic ssRNA viruses in the agricultural context. They also provide a first glimpse at virus biodiversity in these two highly contrasting situations.

Outside of these first results demonstrating the interest and the potential of these novel approaches, it is clear that new technical and conceptual developments are to be expected in the coming years. On the technological side, NGS is progressing, allowing for ever increasing volumes of sequence at lower cost. Challenges are therefore to be found in the processing of samples (autom-

atization, multiplexing etc.) and in the processing of the vast amount of sequence data generated, were many questions remain. In the conceptual field, these developments are taking us to a new, more integrated vision of viral ecology.

Oral & Poster Presentations

Analysis of siRNAs using a new generation sequencing platform for the detection and characterization of viruses and viroids present in a citrus sample

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The population of virus derived small interfering (si) RNAs (21-24 nucleotides) induced by Dicer processing of dsRNAs formed during RNA virus replication can be sequenced by Ion Torrent next generation sequencing technology. This strategy has been successfully used to analyze a sample derived from a lemon tree of the "Lemonodasos" area in Poros (Trizinia, Prefecture of Piraeus) where *Citrus tristeza virus* (CTV) had been previously detected. Initially, lemon samples were grafted on seedlings of sweet orange cv. Madam Vinous and then siRNAs were isolated. The Ion Torrent platform allowed the determination of 432.632 sequences. The *de novo* bioinformatic analysis confirmed the presence of CTV by detecting several large contigs of this virus species. The isolate present in the sample was reconstructed by mapping the specific siRNAs against several reference isolates, which allowed the determination of almost the full-length 19.251 nt genome of this L192GR-CTV isolate. Only a small gap of 18 nt was identified and was later determined by RT-PCR and direct sequencing. Phylogenetic analysis of the L192GR isolate revealed high molecular homology with the Israeli VT-CTV isolate (GenBank Acc. No EU937519.1) with 98% sequence identity. In addition, the *de novo* analysis of siRNA sequences allowed the reconstruction of the complete full-length genomes of *Citrus exocortis viroid* and *Hop stunt viroid* and the retrieval of partial sequences of *Citrus viroid III* and *Citrus viroid IV*. These results demonstrate the great potential of this next-generation sequencing platform, opening new possibilities for the diagnosis and characterization of citrus viruses and viroids.

Development of a nested RT-PCR for the detection of *Little cherry virus-1* and study of its presence in several host species

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Little cherry virus-1 (LChV-1) and *Little cherry virus-2* (LChV-2), both members of the family *Closteroviridae,* are associated with little cherry disease. In previous surveys, only

LChV-1 was detected in sweet cherry orchards in Greece. Due to the high intraspecies diversity of LChV-1, which can result in unreliable virus detection, we developed a new molecular assay which detects all LChV-1 isolates. For that purpose, several sequences of Greek LChV-1 isolates were obtained with a generic nested RT-PCR, which amplifies the 5΄part of the HSP70h gene (500 bps) of closterovirids. These sequences were aligned with others available in the database and new degenerate primers, amplifying a 200 bps product within the generic nested RT-PCR amplicon, were designed. The new assay exhibited a broader detection range when compared with other available methods and it was also able to detect the virus in sweet cherry trees throughout the year. Surveys conducted with this assay in stone fruit orchards has shown that LChV-1 was present in sweet cherry (70/162), plum (2/82), peach (1/54) and sour cherry (2/5) while it was not detected in almond (0/126) and apricot trees (0/7).

Complete nucleotide sequencing and genome analysis of *Eggplant mottled dwarf virus* (EMDV)

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Eggplant mottled dwarf virus (EMDV) is endemic in the Mediterranean region since the end of 60's. However, it remains one of the less studied members of the genus *Nucleorhabdovirus* in the family *Rhabdoviridae*. The virus has a broad host range including cultivated and ornamental plants. Until recently, only a small part of the glycoprotein gene of EMDV was sequenced. Thus in this study, the full nucleotide genome sequence was determined, which is 13.093 nucleotides (nts) long. The negative sense, single-stranded RNA of the virus contains seven ORFs, which are organized in the order 3'-5' as N-X-P-Y-M-G-L, where N encodes the 52.0 kDa nucleocapsid protein, X an unknown 10.8 kDa protein, P the 32.5 kDa phosphoprotein, Y a putative 31.7 kDa movement protein, M the 27.7 kDa matrix protein, G the 69 kDa glycoprotein and L the 221.7 kDa polymerase. Additionally, the genome also contains 198 and 90 nts long untranslated leader and trailer sequences, respectively. The untraslated regions among the virus genes appear highly conserved and were used as a tool for the determination of the virus genome sequence. Phylogenetic analysis confirmed the classification of EMDV among nucleorhabdoviruses and showed its close evolutionary relationship with *Potato yellow dwarf virus* (PYDV).

Study of the genetic variability of *Eggplant mottled dwarf virus* (EMDV)

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Eggplant mottled dwarf virus (EMDV) has a wide host range including cultivated (*Solanaceae*, *Cucurbitaceae*) and ornamental plants. Although the entire genome of a vi-

rus isolate from eggplant was recently sequenced, studies concerning the genetic variability among isolates from different plant species are still limited. In the present study, a comparative analysis of gene sequences encoding structural and functional proteins between virus isolates from various hosts such as eggplant, caper (*Caparis spinosa)*, honeysuckle (*Lonicera japonica)*, tomato, tobacco, cucumber, rose mallow (*Hibiscus syriacus)* and *Pittosporum tobira* was conducted. More specifically, the comparison was held among sequences of the N, X, Y and G ORFs (849, 294, 633 and 1257 nt, respectively). The analysis revealed high genetic variability in the gene encoding the X protein which was up to 22% at nt level and 23% at amino acid level. N, G and Y genes were the most conserved displaying a maximum variability of 14, 15 and 16% at nt level (1 to 4% at amino acid level). The virus isolates from eggplant, cucumber, tobacco and hibiscus constitute a distinct phylogenetic subgroup which differs from the respective consisting of EMDV isolates from tomato, caper, honeysuckle and *P. tobira*. Certain isolates from caper plants are of particular interest as they have a truncated G ORF, 92 amino acids shorter compared to that of the homologous ORF of the other isolates.

Detection and molecular characterization of viruses belonging to the family *Betaflexiviridae* (CNRMV, CGRMV and CVA) in sweet cherry orchards

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Cherry is susceptible to a number of virus species belonging to the family *Betaflexiviridae*. However, their presence has not been studied extensively in Greece. For that reason surveys were conducted in the spring of 2006, 2007 and 2009 and 166 cherry samples were randomly collected from 12 different areas in Northern Greece. The samples were tested for the presence of viruses of the family *Betaflexiviridae* by using a generic nested RT – PCR that amplifies part of the viral polymerase of betaflexiviruses. Sequence analysis of selected samples showed high similarity with published homologous sequences of *Cherry necrotic rusty mottle virus* (CNRMV), *Cherry green ring mottle virus* (CGRMV) and *Cherry virus A* (CVA). In order to further study their incidence, the generic nested RT-PCR assay was modified to include specific primers for the detection of CNRMV, CGRMV and CVA. The assays performed showed high incidence of CNRMV (64/166), followed by CVA (44/166) and CGRMV (21/166). Sequence analysis of PCR products confirmed the specificity of the methods used and showed high intraspecies variability of the viruses tested. The detection methods developed are currently applied for studying the presence of CNRMV, CGRMV and CVA in other stone fruit trees.

Molecular Characterization and genetic diversity of *Little Cherry Virus-1* (LChV-1) populations

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Little Cherry Virus-1 (LChV1), a member of the family *Closteroviridae,* is one of the two viruses associated with Little Cherry disease (LChD) which causes severe losses in sus-

ceptible varieties by affecting fruit quality. During a survey conducted in sweet cherry orchards for the presence of closteroviruses, one sample in which LChV-1 and -2 could not be detected with specific primer pairs, showed positive reaction in *Closterovirus* infection using a generic nested RT-PCR. Sequencing and comparative analysis of the product amplified with the generic assay revealed the presence of a virus distantly related to the known LChV-1 isolates and therefore it was further characterized. A 7.100 nucleotide part of the virus genome was determined including the Helicase (partial), RNA depended- RNA polymerase (RdRp), the small hydrophobic protein (p4), the HSP70 homolog, the 61kDa protein and the capsid protein (CP). High sequence divergence was found compared to the other known LChV-1 isolates which was ranging from 7-10% in the RdRp and up to 29-35% in aminoacids in the p61 protein. Phylogenetic analysis using part of the HSP70h, RdRp and CP genes of several Greek isolates with others indicated the presence of different evolutionary clades. The effect of the genetic recombination in LChV1 evolution is under investigation.

Epidemiology and genetic characterization of criniviruses associated with tomato yellows disease in Greece

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Tomato infectious chlorosis virus (TICV) and *Tomato chlorosis virus* (ToCV) are transmitted by whiteflies in the semi-persistent manner and are associated with yellows disease in tomato crops in Greece. In 2009-2012, an extensive survey was conducted in order to identify the presence of these viruses in open field and greenhouse tomato crops. Moreover, the presence of TICV and ToCV was also investigated in a number of other vegetable crops that showed mild yellowing symptoms and in weed species (regardless of symptoms); whiteflies were also collected for typing from the affected crops. For the detection of TICV and ToCV a nested multiplex RT-PCR was used, while whitefly identification was done by real time RT-PCR. In total, 1206 tomato, 4 lettuce, 1339 weed samples (42 different species, 17 families) and 1041 adult whiteflies were collected. In tomato crops, results revealed the prevalence of TICV (87%) over ToCV (13%), a fact that signifies the direct correlation of their distribution with the whitefly-vector species that prevails in each geographic area. Weeds seem to play a significant role in the epidemiology of both viruses as 26 species belonging to 15 different families were found to be infected with TICV or/and ToCV. It is worth mentioning that for the first time in Greece lettuce plants showing mild yellowing symptoms were found infected with ToCV. Finally, sequence analysis of the CP and CPm genes from tomato and weed isolates of ToCV and TICV showed that their populations show very low intraspecies genetic diversity.

Tomato yellow leaf curl disease in the eastern Mediterranean basin: virus species, incidence, hosts and transmission properties

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During 2005-2012, an extensive survey was conducted in Cyprus, on Crete, the Dodecanese and the Ionian islands, as well as on mainland Greece, in order to identify the virus species and *Bemisia tabaci* biotypes involved in Tomato yellow leaf curl disease (TYLCD) epidemics. Approximately 8000 symptomatic tomato samples, 4500 weeds and 3000 whitefly samples were collected and analyzed. The host range of TYLCV and TYLCSV isolates was studied using whitefly transmission tests in several plant species. Transmission efficiency of TYLCV and TYLCSV was evaluated using different *B. tabaci* biotypes colonies which harboured different bacterial endosymbionts. Results showed that in Greece, TYLCV was the most prevalent *Begomovirus* species (94.5%), whereas TYLCSV was found in 4.5% of the total samples tested. In Cyprus, TYLCV was the only species found to be associated with TYLCD. Molecular identification of *B. tabaci* biotypes showed that Q was the only biotype found in the mainland of Greece, Peloponnese and on the island of Crete. Both biotypes (B and Q) are involved in TYLCD spread in Cyprus and the Dodecanese islands. Forty nine different weed species belonging to 15 botanical families tested positive to TYLCV under field conditions, suggesting that the host range of the virus is far more extensive that previously documented. Transmission studies showed that TYLCV isolates had a broader host range as well as a higher transmission efficiency than TYLCSV. Finally, TYLCV transmission was somehow correlated with the presence of *Hamiltonella* sp. within the body of the *B. tabaci* insect, as colonies that harboured this bacterium transmitted the virus more efficiently both from and to tomato plants.

Citrus tristeza virus on the island of Crete: a survey and detection protocol applications

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Over a period of two years, more than 5,000 citrus trees were tested for the presence of *Citrus tristeza virus* (CTV) on the island of Crete, resulting in thirty eight positives. Comparisons of the relative transcript levels of CTV p23, coat protein (CP), polymerase (POL) and an intergenic (POL/p33) region using quantitative RT-PCR, revealed consistent differences in abundance for each of these RNAs among flowers, stems, young fruits and leaves of infected orange trees. CTV p23 RNAs accumulated at highest levels, reaching a maximum in the flowers, with lower levels in the leaves, while POL RNAs consistently accumulated at low levels in all tissues tested. A PCR-amplified dig-labelled CTV p23 DNA probe was applied to stem and leaf prints, and to crude and total RNA leaf extracts, using non isotopic hybridization. This technique, when applied to stem or leaf prints, and particularly to total RNA, unequivocally provided strong signals with minimal backgrounds. Moreover, an antiserum with high sensitivity and specificity of CTV detection as shown by DAS and immunoprint ELISA was produced against bacterially-expressed CTV CP. By the former method, stems and flowers contained higher levels of CTV CP when compared to leaf extracts. Taking into account Cretan geog-

raphy and the importance of citrus to the island, systematic surveys for CTV eradication, sustainable control measurements and epidemiological studies need to be undertaken. The observations, materials and methods presented here may assist all three tasks at a local and at a national level.

RNA silencing pathways may have a positive effect on *Potato spindle tuber viroid* infectivity in *Nicotiana benthamiana*

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The role of RNA silencing during viral infection in plants is well studied. However, the role of RNA silencing during viroid infection still remains elusive. *Potato spindle tuber viroid* (PSTVd) is a naked single stranded RNA (ssRNA) plant pathogen, which does not encode for any protein. PSTVd infection leads to the accumulation of abundant viroid small interfering RNAs (vd-siRNAs), the latter being able to trigger the degradation of homologous RNA sequences. Yet, PSTVd genomic RNA itself seems to be resistant to siRNA-directed degradation. Sequencing of PSTVd siRNAs during infection has revealed a hotspot-confined origin, indicating that the rod shape-like structure of the mature PSTVd ssRNA, rather than the double stranded RNA (dsRNA) molecules produced during its replication, may serve as substrate for Dicer-like (DCL) enzymes. In order to examine the role of RNA silencing upon PSTVd infection, we produced *Nicotiana benthamiana* knock down DCL lines (DCLi lines) by RNA silencing and then tested PSTVd infectivity in the DCLi background. Our analyses indicate that PSTVd infectivity is facilitated by DCL1, DCL3 and DCL4, but seems to be unaffected by DCL2. In addition, viroid replication seem also to be influenced in double DCLs knock down plants leading to different theories about their role in its infectivity. Collectively, our results indicate that specific RNA silencing pathways may be advantageous for viroid infectivity.

Heterologous RNA silencing suppressor proteins support *Plum pox virus* infection

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HC-Pro is a multifunctional protein encoded by the genome of potyviruses within the family *Potyviridae,* which acts as a suppressor of RNA silencing (RSS) in plants. Recent studies have shown that the HCPro of *Plum pox virus* (PPV) can successfully be replaced by the P1b protein of *Cucumber vein yellowing ipomovirus*, an RSS coming from a virus of the same family which however doesn't show any sequence similarity with HC-Pro. In this study in order to clarify whether the presence of a specific RSS is necessary for

the establishment of a successful potyvirus infection, we tested the capacity of heterologous RSSs with a different mode of action coming from animal and plant viruses to replace the HC-Pro. For this purpose infectivity studies took place using recombinant infectious clones of PPV which showed that the HC-Pro of the virus can be functionally replaced by some but not all the RSSs studied, including some of animal virus origin. Interestingly, the capacity of a protein to replace HCPro was not strongly associated with its ability to suppress silencing. Overall, the results of this study show that not all RNA silencing suppression strategies are equally suitable for the effective escape of PPV and the establishment of a successful infection.

Studies on the adaptation of *Potato virus Y* to pepper plants

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Potato virus Y (PVY) isolates (genus *Potyvirus*) are classified into four major phylogenetic groups, O, N, C1 and C2. Only isolates belonging to group C1 are able to systemically infect pepper plants. In order to identify the regions of PVY genome that determine the ability of C1 isolates to infect pepper plants, a series of full length infectious virus clones have been constructed comprising of hybrids between a PVY-C1 and a PVY-N or a PVY-C2 isolate, covering in different combinations the entire virus genome. The infectivity experiments on pepper plants using these hybrid viruses have revealed that the major determinants of PVY infectivity on pepper were located within the P3/PIPO and the CI coding regions. In addition, fluorescent microscopy of a PVY-N isolate incorporating the GFP reporter gene showed restriction of the virus to a small number of cells in the inoculated leaves, providing a first insight into the mechanism of resistance.

Further identification of virus-host protein interactions (*Pepino mosaic virus*-tomato) and plant responses to viral infection

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Pepino mosaic virus (PepMV), belonging to the genus *Potexvirus*, was first reported in Peru (1980) in *Solanum muricatum* (pepino). PepMV has spread over the last decade in tomato crops throughout Europe, due to its ready mechanical transfer and seed transmission, and has been included on the alert list of pathogens necessitating control strategies. Four PepMV genotypes exist with sequence divergence that affects host range/symptomatology and most likely also pathogenicity as a result of specific interactions with the hosts. One group of pathogenicity determinants is the result of virus-host protein interactions. We have recently identified two tomato-PepMV protein interactions; Hsc70-PepMV CP, and catalase-PepMV TGBp1 (p25) by yeast two–hybrid interaction, electron microscopy and fluorescence microscopy assays. In the present study, further *in vivo* and *in vitro* confirmation of these interactions is presented. Tomato responses to PepMV infection, as revealed using molecular and biochemical methods are also presented.

In vitro template-dependent synthesis of *Pepino mosaic virus* positive- and negative-strand RNA by its RNA-dependent RNA polymerase

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Pepino mosaic virus (PepMV)-infected tomato plants were used to develop an *in vitro* template-dependent system for the study of viral RNA synthesis. Differential sedimentation and sucrose-gradient purification of PepMV-infected tomato extracts resulted in fractions containing a transcriptionally active membrane-bound RNA-dependent RNA polymerase (RdRp). In the presence of Mg^{2+} ions, ^{32}P-labelled UTP and unlabelled nucleotides, the PepMV RdRp catalysed the conversion of endogenous RNA templates into single- and double-stranded (ds) genomic RNAs and three 3'-co-terminal subgenomic dsRNAs. Hybridisation experiments showed that the genomic ssRNA was labelled only in the plus strand, the genomic dsRNA mainly in the plus strand and the three subgenomic dsRNAs equally in both strands. Following removal of the endogenous templates from the membrane-bound complex, the purified template-dependent RdRp could specifically catalyse transcription of PepMV virion RNA, *in vitro*-synthesized full-length plus-strand RNA and the 3'-termini of both the plus- and minus-strand RNAs. Rabbit polyclonal antibodies against an immunogenic epitope of the PepMV RdRp (anti-RdRp) detected a protein of approximately 164 kDa in the membrane-bound and template-dependent RdRp preparations and specifically inhibited PepMV RNA synthesis when added to the template-dependent *in vitro* transcription system. The 300 nucleotide long 3'-terminal region of the PepMV genome containing a stretch of at least 20 terminal adenosine (A) residues, was an adequate exogenous RNA template for RdRp initiation of the minus-strand synthesis but higher transcription efficiency was observed as the number of A residues increased. This observation might indicate a role for the poly(A)-tail in the formation and stabilisation of secondary structure(s) essential for the initiation of transcription. The template-dependent specific RdRp system described in this article will facilitate identification of RNA elements and host components required for PepMV RNA synthesis.

Incidence of graft-transmissible pathogens in newly established peach orchards

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Production of certified plant propagation material is regulated by the Hellenic Ministry of Rural Development and Food which defines the requirements concerning its import and export. Concerning the stone fruits, certification includes, among others, the absence of *Plum pox virus* (PPV), *Apple chlorotic leaf spot virus* (ACLSV), *Prunus necrotic ringspot virus* (PNRSV), *Prune dwarf virus* (PDV), *Peach latent mosaic viroid* (PLMVd) and the *European stone fruit yellows phytoplasma* (ESFY). In order to determine the presence

of these pathogens in Greece, field samples from new peach orchards of the prefecture of Imathia were collected during the years 2010-2011. Overall, 360 samples of different varieties of nectarine and peach were collected and tested for the presence of these viruses by Polymerase Chain Reaction (PCR) protocols developed for their detection. The results revealed high incidence of the pathogens PLMVd (66.66%) and PNRSV (16.1%), while PPV (0.83%), ACLSV (1.11%), PDV (1.38 %) and ESFY (0.55%) were detected very rarely. According to these results, it is obvious that the implementation of the recent regulation for the production and distribution of certified propagative material should be mandatory.

First report of *Plum pox potyvirus* in almond in Greece in the context of phytosanitary control

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Phytosanitary control is critical for crop health and for transport and certification of plant propagation material, especially in case of quarantine pathogens, such as *Plum pox potyvirus* (PPV). PPV has been naturally found in almond (*Prunus dulcis*, syn. *Prunus amygdalus* Batsch) in Hungary, Romania, Bulgaria, Russia, Turkey and India. In addition, almond resistance to PPV has been well established. In the Control Station for Vegetative Propagation Material of Greek Ministry of Rural Development and Food, phytosanitary control is always practiced by applying DAS-ELISA, an immunochemical method implementing polyclonal antibodies. During the years 2010-2012 PPV was detected in leaf samples from almond trees cvs 'Drepanoto' and 'Retsou', collected at Hemathia and Attica, respectively. The leaves tested were collected from all directions of the canopy and showed no or mild symptoms (mottle, distortion, chlorosis and vein clearing). In order to detect the virus in almond, immunocapture RT-PCR (IC-RT-PCR) was tried in DAS-ELISA- and PPV-positive almond samples, in the presence of proper positive controls. This method is economically efficient for conducting tests, as no separate nucleotide extraction is needed. Application of this method will allow for the rapid detection of the virus in extensive phytosanitary controls in almond, a concealed host of this important pathogen.

Phenotypic and functional analysis of *SERRATE* in *Nicotiana tabacum* plants

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RNA silencing is an evolutionarily conserved mechanism present in most eukaryotes. It is implicated in sequence-specific regulation of gene expression, genome stabilization, as well as in antiviral defense. Small RNAs (sRNAs, 21-24nt-long), including microRNAs (miRNAs) and small–interfering RNAs (siRNAs), are key components of the RNA silencing mechanism. miRNAs are involved in the regulation of developmental processes and

are also central to plant immunity against bacterial, fungal and viral pathogens. In plants, *SERRATE* (SE) encodes a C_2H_2 zinc-finger protein and is considered a post-transcriptional regulator of miRNAs levels, processing *pri-miRNA* precursors through its direct interaction with HYL and DCL1. *SERRATE* mutants in *Arabidopsis thaliana* affect leaf polarity, phase transition, meristem activity, and inflorescense architecture. Here, we present the phenotypic analysis of *N. tabacum SERRATE*-knockdown plants and the response of these plants to viral and viroid infection. *SERRATE* knockdown tobacco plants show abnormal leaf development and phase transition delay from the vegetative to reproductive phase, compared to wild type plants. Our experimental data also suggest that *SERRATE*, and as a consequence miRNAs, participate in plant biotic defence. Further studies are required in order to understand the pathway through which *SERRATE* affects plant defence mechanisms.

First report of *Malva vein clearing virus* (MVCV) and *Bean yellow mosaic virus* (BYMV) in Greece

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Genus *Potyvirus* is one of the largest plant virus genera consisting of 146 members, many of which have been found to infect ornamental plants. During the spring-winter of 2011, ornamentals and weeds exhibiting viral-like symptoms were observed in the central area of Thessaloniki city. Sampling of the aforementioned plants was performed which were in turn subjected to a two step generic RT-PCR capable of detecting members of the P*otyvirus* genus. The presence of potyviruses was detected in two plant species. More specifically, plants of the species *Malva sylvestris* exhibiting symptoms of vein clearing and intraveinal chlorotic patterns and plants from the genus *Cassia* with mosaic inflicted leaves were found to be infected. The generic PCR amplicon was sequenced and the nucleotide sequence was compared with the ones deposited in the NCBI database using the BLASTn algorithm. The Malva isolate was found to be 97 % similar with the *Malva vein clearing virus* and the *Cassia* sp. one was 91 % identical to *Bean yellow mosaic virus*. This is, to our knowledge, the first report of these two viruses in Greece.

First report of a BLRV-like virus in three Fabaceae species

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Bean leafroll virus (BLRV) belongs to genus *Luteovirus* and is one of the most important viruses of Fabaceae, as an early infection causes severe yield losses of the affected plants. During the spring of 2011 typical symptoms of a luteovirus infection were observed in common vetch (*Vicia sativa* L. subsp. *sativa*), bitter vetch (*Vicia ervilia* (L.) Willd.) and alfalfa (*Medicago sativa* L. subsp. *sativa*). Leaf samples were collected from the infected plants, as well as from healthy ones of the same species, which were later on checked for the presence of BLRV with a specific RT-PCR. This method amplifies a 391bp portion

of the viral coat protein (CP), which was obtained from all of the symptomatic samples but not from the healthy controls. Sequencing of the amplicon and comparison via the BLASTn algorithm showed 98-99% similarity of the common vetch (HE601635), bitter vetch (HE601636) and alfalfa (HE601637) isolates with BLRV. An attempt to verify the results with the use of a luteovirus generic detection method did not yield the expected amplicons. The inability to obtain the amplicons from both ORF regions could possibly indicate the existence of variability in the new isolates. This is, to our knowledge, the first report of a BLRV-like virus in these Fabaceae species.

Molecular detection of *Grapevine virus A* (GVA) and *Grapevine rupestris stem pitting associated virus* (GRSPaV) in grapevine cultivars and rootstocks

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*Grapevine virus A (*GVA) and *Grapevine rupestris stem pitting associated virus*, GRSPaV) are members of the genera *Vitivirus* and *Foveavirus* (family *Betaflexiviridae*), respectively. They are both widely distributed worldwide and they are associated with different diseases of the Rugose wood complex. The purpose of this study was to evaluate the presence of GVA and GRSPaV in self-rooted, grafted grapevine cultivars as well as in rootstocks which are cultivated in Greece. In total, we tested 26 samples originating from 20 different rootstocks, 60 samples from 17 self-rooted cultivars and 136 samples from 20 grafted cultivars. Detection of GVA and GRSPaV was done by RT-PCR. In the case of GVA an RT-PCR was developed and applied which uses degenerate primers binding to the capsid protein and shows wide detection range whereas detection of GRSPaV was based on a published method. The results showed that only GRSPaV (7/26) was detected in the rootstocks, whereas only GVA (12/60) was detected in self-rooted cultivars. In grafted cultivars, both viruses were detected with GVA (44/136) being dominant followed by GRSPaV (26/136).

Etiology of yellows disease in cucurbit crops in Greece

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Cucurbit yellows disease (CYD) is a widespread disease in cucurbit crops in Greece. However, its etiology has not been studied sufficiently, despite the fact that it is well known that *Beet pseudo-yellows virus* (BPYV), *Cucurbit yellow stunting disorder virus* (CYSDV) and *Cucurbit aphid-borne yellows virus* (CABYV) are usually implicated. BPYV, CYSDV and CABYV are endemic in Greece. BPYV and CYSDV are transmitted with the whitefly species *Trialeurodes vaporariorum,* and *Bemisia tabaci,* respectively, while CABYV is trans-

mitted persistently by the aphid species *Aphis gossypii* and *Myzus persicae*. During extensive surveys conducted in 2011-2012, leaf samples were collected from cucurbits exhibiting yellowing symptoms in different areas of Greece. From the affected crops, we also collected arable weeds and adult whiteflies. RT-PCR was used for the determination and discrimination of viruses and TaqMan PCR was used for typing of whitefly species. In total, 128 samples from 4 cucurbit crops (cucumber, zucchini, melon, watermelon), 34 samples from 6 weed species and 241 adult whiteflies were collected. Results showed that the etiology of CYD is correlated with the presence of the whitely-vector species that exists in the geographic area. Moreover, CYSDV is the predominant virus (35.64%) in greenhouse crops, followed by CABYV (23.76%) and BPYV (23.76%). In contrast, CABYV prevails (74.1%) in open-field crops, followed by CYSDV (66.6%), whereas BPYV was not detected in open-field cucurbit crops. Infected weeds seem to play an important role in the epidemiology of these viruses, as neither of CYSDV, BPYV or CABYV is seed-transmitted. It is worth mentioning that for the first time, CYSDV and CABYV were detected in watermelon plants that showed mild yellowing symptoms in Greece.

Detection and characterization of *Citrus tristeza virus* (CTV) in Cyprus using molecular techniques

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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 for disease management and characterization, a new diagnostic protocol based on real-time reverse transcription and the polymerase chain reaction (Real-Time TaqMan RT-PCR) was developed and optimized. The protocol is suitable for the generic detection of all virus isolates associated with severe or mild symptoms in the Mediterranean basin. In addition, to discriminate between virus strains identified in Cyprus, six primer pairs were designed suitable for application in conventional or real-time PCR assays. Primer specificity was based on short primer lengths (12-15 nucleotides), and on the incorporation of modified bases known as locked nucleic acids (LNAs), which increase hybridization range and melting temperature. Evaluation of these primers in isolates from Cyprus, Greece, and from other geographical regions showed that they were able to discriminate different CTV strains efficiently and rapidly.

Development of a quantitative one-tube Real Time Reverse Transcription PCR (qRT-PCR) for the detection and quantification of EMDV isolates in different species

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Eggplant mottled dwarf virus (EMDV) is endemic in countries surrounding the Mediterranean basin since 1969. Recently, the entire genome of a Greek isolate originating

from eggplant was sequenced but epidemiological data concerning the presence and dispersal of the virus is still limited. The purpose of the present study was the development of a rapid and sensitive method of reverse transcription PCR (Real Time qRT-PCR) to detect and quantify EMDV in plant tissues and insect vectors. A 210 nucleotides long conserved region of the polymerase (L) gene was used as target of the assay. *In vitro* synthesized EMDV-RNA transcripts of known concentration were used for the evaluation of the system. Moreover, the extraction method of the viral RNA as well as the conditions of qRT-PCR were optimized. The amplification efficiency was 96.9% and the linear range of quantification was from 20 to 2×10^8 RNA transcripts. The above assay was applied to total RNA extracts from tissues of eggplant, honeysuckle (*Lonicera japonica)*, tomato, tobacco, caper (*Caparis spinosa)*, cucumber, *Pittosporum tobira* and hibiscus (*Hibiscus syriacus*) in which the virus was successfully detected. The developed method proved to be a simple and reliable tool for the detection and quantification of the virus in host plants as well as in insect vectors.

Development of a semi-nested RT-PCR for the detection of ApLV and study of its presence in Greece

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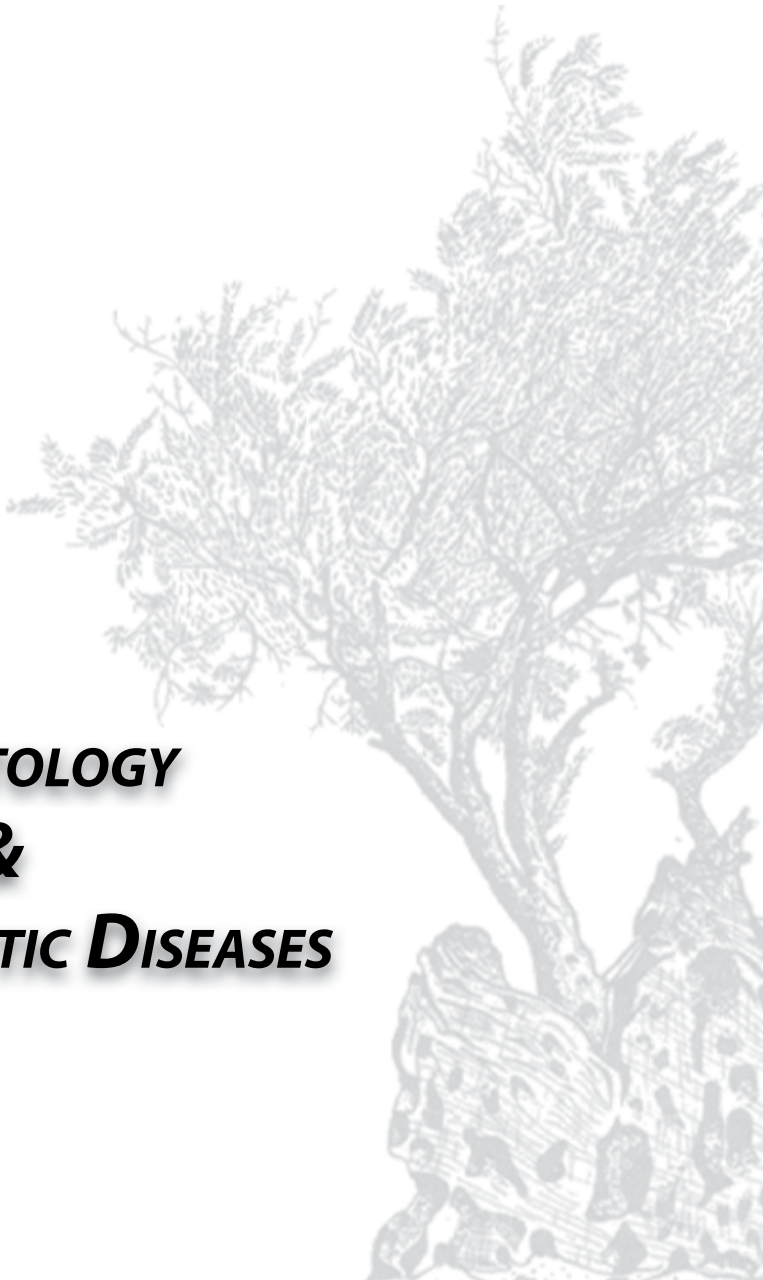
Apricot latent virus (ApLV) is a member of the genus *Foveavirus*, family *Betaflexiviridae*, and infects a wide range of plant species of the family Prunoideae. It is spread in different countries. Although it is usually latent, in some host plants it causes symptoms but without severe yield losses. The available detection methods of ApLV are not reliable as they do not detect all virus isolates. In this study, we developed an improved detection method of ApLV which was also used for testing different plant species of the family Prunoideae for the virus presence. For this purpose, a semi-nested RT-PCR was developed using degenerate primers which bind to conserved sequences of the 5′ untranslated region of the viral genome and the 5′-end of the RNA dependent RNA polymerase (RdRp) gene. The method was successfully evaluated by using four characterized virus isolates (Casserta 12, LA2, SB, A18) and proved to have a wider detection range compared to the available methods as it detects all virus isolates tested during this study. Finally, the developed method was used to test 546 stone fruit samples (almond, apricot, cherry, peach, ornamental plum) from different regions of Greece for the presence of ApLV. The virus was not detected in any of them.



NEMATOLOGY
&
NON PARASITIC DISEASES

Oral & Poster Presentations

Nematodes as soil health bioindicators in an experimental sunflower field

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A 1000m^2 experimental sunflower field, located in Kostakioi, Arta, was divided into 16 plots 40m^2 each, 8 of which had a 5% inclination and 8 of 1%. The soil was tilled, the pilot blocks delineated, the water collection system constructed and sunflower seeds were seeded in 8 of the plots, 4 with 5% inclination and 4 with 1%, in grooves 4-5cm deep, at 70cm distance between the lines and 20cm on the track. The remaining 8 plots were left uncultivated. There were two applications of herbicides, one of oxyfluorfen against broadleaf weeds, and one of quizalofop-p-ethyl against grasses. The herbicides were applied in 8 plots, 4 with sunflower and 4 without cultivation, and in each case, 2 with 5% inclination and 2 with 1%. No herbicides were applied in the other 8 plots, 4 cultivated, 2 with 5% and 2 with 1% inclination, respectively, and 4 uncultivated, 2 with 5% inclination and 2 with 1%. Soil sampling was performed at 0-10cm depth. Nematodes were isolated, the total population per cm3 estimated, species identified based on food preference and / or at family or genus level, where possible, and statistical analysis of data using the program STATISTICA v. 7.0. The experimental field was installed following the principles of a split-split-plot experimental design and statistically significant interaction was observed for each sample ($p<0.05$).

Distinction of live/dead eggs of potato cyst nematodes using propidium monoazide (PMA)

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Potato cyst nematodes (PCN), *Globodera pallida* and *G. rostochiensis* cause severe losses in potato crop. Crop losses are related to nematode population densities in soil, which are therefore used to support decision making for the management of PCN. The standard method to determine the viability of PCN is the microscopic visualization of nematodes stained with Meldola's Blue dye. Meldola's Blue assay, however, is time and labour consuming and frequently leads to overestimation of viable PCN inocula. Furthermore, molecular assays such as Real-Time PCR cannot directly assess the viability of PCN inocula, since DNA of both live and dead cells can be amplified, thus quantifying the total amount of DNA. Recent studies report the use of a DNA-intercalating dye, Propidium Monoazide (PMA), in combination with Real-Time PCR assays for the dis-

tinction of live/dead cells in several microorganisms. The novelty of the method lies in the fact that cell membranes are impermeable to PMA. Thus PMA can be selectively used to intercalate the exposed DNA from dead cells, rendering it unavailable for PCR amplification, and therefore only DNA from viable cells is amplified. In this study, we report a novel method for the enumeration of viable PCN eggs in soil, based on Real-Time PCR in combination with PMA. Quantitative analysis of DNA from viable eggs was performed by using species specific Taqman probes and primers.

"Seed Blight", a non parasitic disease of sunflower

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High temperatures, the sharp rise in temperature and bright sunshine in the process of filling the seed, could affect the seeds of sunflower, causing tanning (burning) accompanied by a complete absence of the endosperm or have rudimentary endosperm. This phenomenon is created when the seed filling stage, in the flowers of the head are directly exposed to solar radiation (at the top of the head). "Burning" of seeds was observed during the 2011 growing season, especially in a particular variety, which lacks a strong inclination of the head. The phenomenon was noticed in late June between stages R6-R7. Optically heads looks healthy, but when we rub the hand over the flowers of the head to reveal the seeds, then there is a spot of seeds that shows in "silver-gray" color. Over the time they dry out and the same happens to the flowers of the affected area of the head as a result making them seem literally "burnt". Affected seeds beneath the dried flowers have a brown tint and are easily rubbed from the head and they are slight because most are hollow or have rudimentary endosperm. In some cases the "burning" of the seeds, proceeds to the top half of the head.

Measurement of ozone concentrations using passive sampling in 13 fruit tree growing areas of the Imathia, Pella and Magnisia prefectures in Greece, during three years

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Ambient ozone (O_3) concentrations were measured using passive samplers and UV or HMOS monitors, in fruit tree growing areas of Imathia (9 sites), Pella (1 site) and Magnisia (3 sites) in Greece, during the summer months of three consecutive years. The O_3 concentrations were also monitored at different positions in an orchard. Results showed that ten-day O_3 concentrations recorded using passive samplers were greater in areas with higher altitude (Rodochori Imathias and Zagora and Chania Magnisias), in comparison with those at lower altitude (Kampohori and Ammos Imathias and Velestino Magnisias). In 2007 the highest O_3 concentrations were recorded, compared with 2005 and 2006. Accumulative AOT(40) values during the day in Naoussa were greater by 1.5 and 3.5 times at 6.000 ppb.h. Diurnal O_3 concentrations in Naoussa and Rodochori followed a campanoid curve with lowest values occuring during the early hours of

the morning, and maximum values during the mid- to late afternoon. In each orchard, the measured O_3 concentrations were greater above canopy compared to within canopy and between rows. Ozone phytotoxic symptoms were observed in the leaves of sweet cherry trees, raspberry, sorbus, clover and rose plants in Imathia.

Biological & Integrated Control

Oral & Poster Presentations

Study the effectiveness of different natural compounds for the protection of pomegranate trees from spring frost

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Spring frosts can cause serious damage to pomegranate orchards, and this was the case in many areas of Northern Greece during 2011. During spring the sensitivity of trees to frost increases and this has been found to be proportional to decreased levels of sugars and the osmotic potential of cell sap. The aim of the present study was to assess the effectiveness of the external application of molasses, glycine-betaine, ethylene glycol, glycerine, sucrose and glucose on conferring frost resistance in pomegranate. Pomegranate trees were sprayed with solutions of the above compounds in the field 8 and 3 days before assessments were made of their frost sensitivity using controlled freezing tests. Frost damage was assessed using the electrolyte leakage test, tetrazolium stain tests in pith and cortex, and visual assessment of injury. The results showed that molasses spray application provided protection as indicated by all test methods used to reveal frost damage. Glucose application protected from low temperatures in all methods apart from the visual rating assessment. Finally, glycine-betaine, ethylene glycol and sucrose application conferred a lesser degree of protection that was only detectable by tetrazolium tests.

Phytochemicals with nematicidal activity in IPM programs for the control of rootknot nematodes *Meloidogyne* spp.

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Amongst all agricultural pests affecting crops, root-knot nematodes (RKN; *Meloidogyne* spp.) represent possibly the most damaging worldwide, with a wide host range, a short generation period, a high reproductive rate, and with the ability to form disease complexes with other soil borne pathogens like fungi. In the past phyto-nematode control has been mainly based on chemical nematicides, but recently many synthetic nematicides have been banned or are under evaluation (Regulation 2009/1107/EU & 2009/128/EU), due to environmental and health concerns. Additionally, the economic cost of research and registration to develop new nematicides is not easily sustained by the industry. There is therefore, a great need for novel nematicides, that are environmentally safer than the synthetic derivatives currently available. An interesting way to search for biorational nematicides is to screen naturally occurring compounds in plants. Plant derived pesticides may find favour in organic food production because many are environmentally friendly, pose less risk to humans and animals, have a selective mode of action, avoid the emergence of resistant races of pest species, and as a result can be safely used in Integrated Pest Management (IPM) programmes. However, botanical nematicides have yet to form the focus of research, unlike insecticides and pesticides. This is a review of our recent studies on *Meloidogyne* spp. control using natural substances of plant origin. We report on the most potent nematicidal botanical extracts, as well as on

the chemical groups of substances exhibiting substantial nematicidal activity. The activity of the extracts was verified against various growth stages of the parasite *in vivo*; while enzyme (AchE) inhibition activities were determined *in vitro*. Fumigant properties were also evident, an effect that is of paramount importance in nematode control as it enhances nematicidal activity in adjacent, untreated soil layers.
Insights into the development of natural nematicidals for use in ecofriendly agriculture are presented.

Effect of several plant extracts and chitosan on *Chrysanthenmum stunt viroid* (CSVd) infections of chrysanthemum plants

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Chrysanthemum stunt viroid (CSVd) constitutes a serious problem for chrysanthemum cultivation worldwide and especially in Japan. Infection is extremely difficult to avoid, as chrysanthemum is propagated vegetatively, CSVd is readily transmissible, and the disease has a long latent period. The present study investigated whether extracts of *Mirabillis japala*, *Pelargonium* sp., *Phytolacca americana*, *Capsicum chinense*, *Oleae europaea*, *Brassica* sp., *Bougainvillea* sp., *Houttuynia* sp., *Dendranthema grandiflorum* Kitam cv. Sei-no-issei, *Allium cepa*, *Allium sativum* plants and Chitosan-100 could reduce CSVd titre in highly infected plants *in vitro,* and/or prevent CSVd titre increase in newly-infected plants *ex vitro*. Ten to 15 plants were used per treatment and the viroid load was estimated weekly using one step Real Time RT-PCR. *C. chinense*, Houttuynia, Bougainvillea and chitosan were the treatments that stood out, resulting in statistically significant (t-test) reductions in CSVd titres, ranging from 35 to 50%. The application of chitosan to *ex vitro* plants prevented CSVd titre increase (50% to 70%) compared to control and seemed to have a positive effect on plant height. Experiments using CSVd RNA treated with plant extracts and chitosan were also performed in order to quantify viroid degradation. Results showed that pepper and chitosan extracts were as effective as sodium hypochlorite treatment. In conclusion, chitosan and extracts of *Capsicum* sp., *Houttuynia* sp. and *Bougainvillea* sp. were found to be promising for use in chrysanthemum stunt viroid disease management.

Impact of *Cucumber mosaic virus* (CMV) infection and benzothiadiazole (BTH) application on the quantitative and qualitative traits of marketable tomato fruits

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This study evaluated the effect of *Cucumber mosaic virus* (CMV) infection and benzothiadiazole (BTH) application (BION 50 WG, 50 mg/L), individually or in combination, on several quantitative and qualitative traits of tomato fruits produced from greenhouse-grown F1 Delos plants during two successive seasons. CMV caused the most severe stunting of tomato plants compared to the other treatments (CMV+BTH, BTH, healthy control plants) and resulted in significant loss of marketable fruits, although the total fruit

number was higher compared to the other treatments. Fruits derived from CMV-infected plants ripened later than those from all other treatments and showed significantly enhanced antioxidant capacity, ascorbate, lycopene and β-carotene contents. Weekly BTH application to healthy plants negatively affected plant growth, fruit size and marketable yield. The nutritional status of tomatoes, as defined by nonstructural carbohydrates, organic acids and antioxidants content, was not significantly affected by the BTH treatment, except for the lycopene and β-carotene contents, which were significantly higher than in control fruits. BTH, applied before and after CMV inoculation, induced systemic acquired resistance (SAR) and clearly suppressed CMV infection, not only reducing the virus infection rate but improving plant growth and fruit size of plants subsequently infected with CMV. Repeated foliar applications of BTH could be used to reduce the damage caused by CMV in serious viral epidemics.

Evaluation of the effectiveness of soil solarization with impermeable plastic films in combination with half the recommended dose of soil fumigants for the control of soil borne pathogens

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The aim of this work was to evaluate the effectiveness of soil solarization with impermeable plastic films (ORGASUN), for 4 and 6 weeks under greenhouse conditions on Crete, in combination with half of the recommended dose of soil disinfectants, against soil borne pathogens. The impermeable plastic film was compared with common polyethylene film. The disinfectants and doses that were used in the experimentation were CONDOR: 20 l/1000m^2. (100%) greenhouse, 10 l/1000m^2. (50%) greenhouse. VAPAM: 55l/1000m^2. (100%) greenhouse, 27 l/1000m^2. (50%) greenhouse. CHLOROPICRINE: Pic (67:33 v/v) l/1000m^2 (100%) greenhouse, l/1000m^2 (50%) greenhouse. The experiments were carried out in tomato greenhouses in the Tympaki region and at the experimental station in Kalessa, Heraklion. The experiment began in July 2011, and the evaluation took place between October 2011 and April 2012 at the end of the cultivation period. The following were studied: the effect of treatments in soil temperature, on the population and in the disease index of knot nematodes (*Meloidogyne* sp). Additionally, the effect of treatments on the population dynamics of *Fusarium oxysporum*, on the severity of the disease caused by *Fusarium oxysporum* f.sp. *radicis lycopersici* and the percentage of diseased plants with symptoms of vessel discoloration and stem rot, was also examined. Moreover, the effect of treatment on the percentage of diseased tomato plants caused by *Pyrenochaeta lycopersici* was also studied. The following conclusions were drawn: the soil temperatures that were recorded during soil solarization in the Tympaki and Kalessa greenhouses were initially suitable to achieve for effective soil solarization. However later, due to the low air temperatures the marginal levels of effectiveness of this method were reduced. The fungal populations were very high in the control greenhouses (disease index 8.46-9.33). Soil solarization in combination with fumigants resulted in a very low disease index. Also an exceptional reduction the in natural soil populations of *Fusarium oxysporum* was noted, with effective control of Fusarium wilt and crown rot caused by *Fusarium oxysporum* f.sp. *radicis lycopersici* and corky root rot caused by *Pyrenochaeta lycopersici* was achieved with the combined use of half doses of fumigants and the impermeable plastic film Orgasun.

It should be noted that from the control greenhouses, high rates of diseased tomato plants infected with *Fusarium oxysporum* f.sp. *radicis lycopersici* with infection rates observed of up to 16% (January 2012) were removed to avoid mass aerial contamination by this pathogen.

Effect of essential oils of five medicinal plants on the mycelial growth and conidia germination of the fungi *Penicillium expansum* and *Aspergillus niger*

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Fruit rots are some of the most important plant diseases worldwide. Because of the policy of the European Union for the production of fruits without pesticide residues, new biological methods to control fruit rot must be investigated. The main aim of this study was to investigate the effects of essential oils extracted from origanum, (*Origanum vulgare* subsp. *hirtum*), rosemary (*Rosmarinus officinalis*), creeping rosemary (*Rosmarinus officinalis Prostratus*), basil, **(***Ocimum basilicum)* and sage *(Salvia officinalis)* on the mycelial growth and conidia germination of the fungi *Penicillium expansum* and *Aspergillus niger*. Mycelial discs of each fungus, 6 mm in diameter, were placed in the middle of petri dishes containing potato dextrose agar supplemented with the essential oils at different concentrations. Results were collected by recording the diameter of the colony 4 days later. In addition, a conidial suspension ($2x10^6$ / ml) of the above fungi was added to petri dishes containing potato dextrose agar supplemented with essential oils at different concentrations. The results were collected by recording the percentage of germinated conidia or germ tube elongation 24 hrs later. The results showed that all the essential oils used in this study inhibited mycelial growth and conidia germination of both fungi. However, the essential oils from origanum and basil were effective at much lower concentrations.

Establishment of the pathogenic fungi *Puccinia punctiformis* and *Colletotrichum gloeosporioides* in the field for the biological control of the weeds *Cirsium arvense* and *Salsola kali*

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Cirsium arvense and *Salsola kali* are indigenous weeds in Greek agriculture, whereas in the USA they are considered as invasive species. In the present study, a methodology for the establishment in the field of their host-specific, native Greek, pathogens *Puccinia punctiformis* and *Colletotrichum gloeosporioides* was developed. The purpose of the research was to study the two pathogenic fungi as potential biocontrol agents to control the weed populations, and to study aspects of the epidemiology of the two diseases. *C. gloeosporioides* was isolated from naturally infected *S. kali* plants in a field in Chalkidiki. The fungus was grown in a large volume substrate containing rice, in order to produce a great number of acervulli. A single treatment with the inoculum, in disease-

free experimental fields in Kozani, resulted in a significant reduction in the *S. kali* weed population, and its almost complete eradication over the following 2-3 years. The distribution of the inoculum was performed in a natural manner, and commenced with the first rains at the end of August. Naturally infected *C. arvense* leaves, bearing the teliosori of *P. punctiformis* were collected from a field in Kozani. Inoculation of young *C. arvense* rosettes, in a disease-free experimental field in Kozani, has shown that successful inoculations take place only during autumn. The percentage of successful inoculations was low, and multiple inoculum applications were necessary during this period. However, the establishment of the pathogen in the field was successful. The progress of the disease over the following years is being studied.

Screening of Greek Trichoderma isolates as potential biocontrol agents of soilborne fungal pathogens

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The general inadequacy of chemical fungicides for the control of *Verticillium dahliae*, *Rhizoctonia solani*, *Pythium ultimum* and *Sclerotinia sclerotiorum* on several plant hosts has led to a survey for biocontrol solutions. Thirty two *Trichoderma* spp. isolates were isolated and identified, on the basis of their morphological characteristics and by molecular analysis of the internal transcribed spacer 1 and 2 regions (ITS1 and ITS2). These isolates were evaluated for their antagonistic activity against the soil borne plant pathogens *in vitro*. Different isolates showed varying degrees of biocontrol activity. Specifically, regarding mycoparasitism tested using a dual culture technique, seven *Trichoderma* sp. isolates, identified as *Trichoderma asperellum* and *Trichoderma harzianum*, showed enhanced mycoparasitic activity on pathogen colonies. Regarding antibiotic activity, two *Trichoderma viride* isolates exhibited increased pathogen suppression due to the production of volatile and non volatile metabolites. The chitinolytic (chitinase and NAGase) activity of the *Trichoderma* species tested varied, as to the carbon source (colloidal chitin, *R. solani* cell wall material) targeted and the location of these enzymes (mycelium, liquid). In detail, *Trichoderma harzianum* B2 and *Trichoderma viride* B4 isolates were characterized as being highly productive of endochitinase, exochitinase and β-N-acetylhexosaminidase. In conclusion, the promising results of this research could form the basis of an extended study, in order to evaluate the efficacy of these biocontrol agents *in planta*.

Evaluation of the biocontrol efficacy of *Paenibacilus alvei* strain K165 against *Fusarium oxysporum* f.sp. *melonis*

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The plant pathogen *Fusarium oxysporum* constitutes one of the most significant threats to farmers' revenue worldwide, having more than a hundred *formae specialis* with differing host specificities. In the present research, the efficacy of the biocontrol agent *Paenibacilus alvei* K165 to control *Fusarium oxysporum* f.sp. *melonis* was studied.

For this purpose, a talc preparation of K165 was either incorporated into the soil (in a proportion of 1%, 5%, 10% and 20%) that was used to grow melon plants, or was used as a seed coating. At the stage of the 2nd leaf the plants were transplanted to soil infested with 10^3 *Fusarium oxysporum* chlamydospores per gram of soil. It was observed that the 10% treatment alone resulted in a statistically significant reduction in disease severity. It is noteworthy that application of the biocontrol agent at 20% resulted in partial reduction in disease severity that was not statistically different from the effective treatment of 10% but at the same time the severity of the disease in this group was not statistically different to that in the non-treated controls. It is tempting to speculate that this difference may be attributable to the phenomenon of quorum sensing as the size of the rhizosphere population of the 20% treatment group was 2-4 fold higher than that of the 10% treatment. On the other hand, the size of the K165 rhizosphere population of the other ineffective treatments (1%, 5% and seed coating) was substantially lower than that seen with the effective treatment of 10%.

Isolation of new biocontrol agents against crown and root rot disease of tomato caused by *Fusarium oxysporum* f. sp. *radicis – lycopersici*

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The need to limit the irrational use of chemical fungicides in plant protection, has led to a tendency to use biological control. In the present research, 383 rhizobacteria, isolated from cultivated plants from different regions of Greece, were studied. *Fusarium oxusporum* f.sp. *radicis – lycopersici* (Forl), which causes crown and root rot disease in tomato was chosen as a target pathogen. The inhibitory activity of the bacteria on the fungus, was tested *in vitro* and *in planta*. For the *in vitro* experiments, a lysis bioassay was performed and the presence of a potential inhibition zone of fungal growth was examined, in multiple and dual cultures of the fungus and the bacteria in Petri dishes. The most effective bacteria in the two previous bioassays were identified by sequencing the 16S *rRNA* gene, or the intergenic spacer region between 16S and 23S *rRNA*. In the *in planta* tests that followed in a gnotobiotic system in pots, and in one indicative field experiment, the disease caused by artificial inoculation of tomato plants with the fungus was significantly reduced by the selected rhizobacteria. Moreover, the ability of these rhizobacteria to promote the growth of tomato was studied in pot experiments. Finally, the presence of antifugal metabolites in the liquid culture supernatants of the biocontrol agents and their organic extracts was investigated. The effect of the metabolites on the growth of the fungus was studied by filter paper, TLC, and microtitre bioassays. The results demonstrated that the strains *Bacillus cereus* S76 and S79, *Serratia rubidaea* S55 and S49, *Serratia marcescens* PiHa5II, S52 and S47 and *Pseudomonas chlororaphis* ToZa7 are effective biocontrol agents that successfully inhibit the growth of Forl, and the disease intensity caused by this fungus in tomato. In addition, the strains *S. marcescens* S47, *B. cereus* S76 and S79 and *P. chlororaphis* ToZa7, showed a statistically significant promotion of tomato plant growth in pots and in the field.

Biological control of the toxigenic fungus *Aspergillus flavus* and aflatoxins produced in shelled pistachio nuts cv. "Eginis"

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One of the most significant threats for food quality and safety are the mycotoxins, particularly toxic and carcinogenic low molecular weight metabolites produced by certain fungal species. Aflatoxins produced by the fungi *Aspergillus flavus* and *A. parasiticus* are highly carcinogenic mycotoxinsthat several times have been detected at high concentrations levels in pistachio nuts in Greece. The aim of this study was to evaluate a collection of 20 yeast isolates and 4 non-toxigenic strains of *A. flavus*, isolated from experimental pistachio orchards located at Fthiotida County for the management of *A. flavus* and aflatoxins. The yeast isolates MR7 (*Candida* sp.) and FR6 (*Aureobasidium pullulans*) were selected as the most effective against the aflatoxigenic pathogen *A. flavus*, strain Δ1.3 AF2 because they led to a 40-50% reduction of *Aspergillus* growth and to a significant reduction in conidiogenesis by approximately 1000 times, in comparison to the control. These 2 yeast strains were further tested on shelled pistachio nuts cultivar "Eginis" for their role in aflatoxin production (assessed by High Performance Liquid Chromatography-HPLC) and led to a significant decrease by 89% (FR6) and 85% (MR7), respectively, in comparison to the control. Evaluation of the non-toxigenic *A. flavus* strains as biological control agents of the disease and mycotoxin in pistachio orchards, AF38, AF51 and AF57 reduced significantly the production of the aflatoxins (assessed by HPLC) AFB1, B2 and G1 of the Δ1.3 AF2 strain by 41%, 48% and 69%, respectively, whereas AF45 strain did not show any effect in comparison to the control. The results of this study contribute to the development of environmentally friendly methods of biological management of mycotoxins in pistachio nuts.

The swarming motility of biocontrol antagonistic Pseudomonas strains possibly inhibits the growth of phytopathogenic fungi

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More than 30 antagonistic culturable bacterial strains were isolated from solarized, tomato rhizosphere soil in greenhouses and characterized for their potential to inhibit the growth of plant pathogenic fungi. In *in vitro* experiments they showed remarkably antagonistic activity against *Fusarium oxysporum, Phytophthora* sp. and *Rhizoctonia solani.* The antagonistic isolates were first characterized using classical microbiological methods and further assessed using 16S rRNA analysis. Most were found to belong to the *Pseudomonas* group (*P. fluorescens, P. poae, P. lurida, P. synxantha. P. moorei* etc). Strains were tested for their swarming motility capacity under different environmental conditions. Two strains, designated as P3 and P23, *Pseudomonas lurida* and *Pseudomonas azotoformans*, respectively, showed hyper-swarming motility and inhibited the growth of phytopathogenic fungi. The results suggest that swarming activity

may contribute to fungal growth inhibition in soil. In this respect it will be of great interest to investigate the behavior of these bacteria under greenhouse conditions and to characterize the bacterial genes involved in fungal growth inhibition.

Selection of non-toxigenic strains of *Aspergillus flavus* from pistachio and cottonseed as biological control agents for aflatoxin management

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The presence of mycotoxins in agricultural products is considered as one of the most serious food safety concerns worldwide. One of the most carcinogenic mycotoxins is aflatoxin produced by the fungi *Aspergillus flavus* and *A. parasiticus*. Aflatoxin has been detected at high concentrations in Greece, including in pistachio. A promising and effective strategy to reduce aflatoxin levels is the application in the field of endemic non-toxigenic *Aspergillus* spp. strains. This technique is based on the gradual substitution and exclusion of *A. flavus* and *A. parasiticus* toxigenic strains by the non-toxigenic strains due to competition. The goal of this study was to create a collection of greek endemic non-toxigenic *Aspergillus* spp. strains. Initially, soil samples were collected from different experimental fields in the Fthiotida region and transferred to the laboratory. The isolation of several strains of *Aspergillus section Flavi* was performed by plating soil-water suspensions on plates of selective medium. Furthermore, several strains of *Aspergillus* section *Flavi* were isolated from pistachio during harvest from experimental fields of Aegina, as well as from pistachio kernels from several other sources in the market. Using thin layer chromatography (TLC) the collection of isolates was evaluated for their ability to produce aflatoxins in order to discover non-aflatoxigenic strains that could be used in biological control experiments. Finally, *in vitro* competition assays were carried out using two wild toxigenic and 12 non-toxigenic strains, in order to study the potential for the reduction of the aflatoxigenic capacity of toxigenic strains by non-toxigenic strains. Most non-toxigenic strains reduced the aflatoxigenic activity of wild type strains, and some strains prevented aflatoxin production completely.

Evaluation of rhizobacterial activity against soil-borne plant pathogenic fungi *in vitro*

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Eight (8) rhizobacterial strains, known for their *in vitro* and *in planta* activity against the plant pathogenic fungus *Fusarium oxysporum* f. sp. *radicis-lycopersici* (Forl), and 3 new strains, were tested *in vitro* for the formation of an inhibition zone in the mycelial growth of 4 soil-borne plant pathogenic fungi. Specifically, the strains *Bacillus cereus* S76 and S79, *Serratia marcescens* PiHa5-II, S47 and S52, *Serratia rubidea* S55 and S49, *Pseudomonas chlororaphis* ToZa7, and the new strains ToZa9-12, ToZa1-5-10, ToZa4-8 of yet unknown genetic identity, were tested against *Verticillium dahliae, Sclerotium rolfsii,* and *Rhizoctonia solani*, with the latter three also being tested against Forl. In addition, S76, PiHa5-II, ToZa7, ToZa9-12 and ToZa4-8 were tested *in vitro* against Forl and *S. rolfsii* for the presence of volatile compounds that inhibit mycelial growth. The most im-

pressive results were obtained by combining all of the (11) rhizobacteria with *S. rolfsii*, or the combination of the 5 strains tested for the effect of volatiles on the same fungus. In almost all treatments mycelial growth was very limited or non-existent, and an inability of sclerotia germination to hyphae was observed. Treatments of *S. rolfsii* with PiHa5-II and S47 were the only exceptions in which the inhibition zone was limited. The effect of volatiles was also apparent, as the strains PiHa5-II, ToZa7, ToZa4-8 and ToZα9-12 prevented mycelial growth and germination of sclerotia of *S. rolfsii* at a distance, whereas S76 showed different results in 3 replications. Finally, combination of *V. dahliae* and S52 gave interesting results with fungal growth being limited, and the formation of microsclerotia inhibited.

Evaluation of Greek Trichoderma isolates effect on plant growth characteristics of aronia (*Aronia melanocarpa*) plants

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Aronia melanocarpa fruit production is a novel crop in Greek agriculture and research is needed in order to define and optimize the cultivation techniques in biological production systems. The present research was focused on the evaluation of three *Trichoderma* sp. isolates, belonging to *Trichoderma harzianum* and *Trichoderma asperellum* species, regarding their effect on plant growth characteristics of aronia plants. All three *Trichoderma* sp. isolates, when applied separately, colonized aronia roots to the same extent, with colonization levels ranging on average from 58% at 2 days after treatment to 94% at 30 d.a.t. However, the combined treatment resulted in asignificant increase in the total number of colonized roots compared with the single treatments, with 80, 87, 94 and 95% root colonization observed at, 2, 7, 15 and 30 d.a.t., respectively. In addition, the combined treatment was superior with regard to all the growth characteristics tested. Data from the single treatments showed that plants treated with *Trichoderma harzianum* isolates B2 or B3 had higher dry root weights and numbers of leaves compared to the untreated rootstocks, while *T. asperellum* B1 treatment was better than the control with regard to dry root and shoot weight. In conclusion, specific biocontrol isolates can form a complementary treatment to assist *Aronia melanocarpa* commercial fruit production in Greece.

Molecular identification of epiphytic yeasts isolated from grapevines and their assessment as biological agents to control *Aspergillus* spp.

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The aim of this study was the isolation and molecular identification of epiphytic yeasts from grapevines and their assessment as biological agents against black aspergilli (*Aspergillus* section *Nigri*). Initially, epiphytic yeasts were isolated from grapes of the varieties 'Maratheftiko' and 'Cabernet Sauvignon' originating from four areas of the Limassol district during 2010. Genomic DNA was isolated from each yeast and was used for the amplification and sequencing of the D2 region of the nuclear large-subunit (LSU) ribo-

somal RNA gene. The sequences were compared to a library (MicroSeq® Fungal Gene library) containing D2 sequence entries from more than 1000 validated species, using MicroSeq® ID Analysis software. The isolates identified included 39 *Aureobasidium pullulans*, 9 *Cryptococcus magnus*, 1 *Pseudozyma onfarclica aphidis*, 1 *Hanseniaspora opuntiae* and 1 *Acremonium glaucum*. After identification, the isolates were screened for *in vitro* antagonism against *Aspergillus tubingensis* by the dual culture technique on PDA plates. The isolates showing antagonism (the presence of an inhibition zone) were also tested in a detached berry test. Berries were immersed in a suspension of the selected yeast isolates and a conidial suspension of *A. tubingensis* was spot inoculated on a wound made on each berry. The inhibition of fungal growth was determined 7 days after *A. tubingensis* inoculation. The isolates with the highest inhibition percentage (93.78% and 90.08%) belonged to the species *Aureobasidium pullulans*.

Biological control of *Aspergillus carbonarius* in pomegranates

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The plant pathogen *Aspergillus carbonarius* causes one of the most significant post harvest diseases in stored fruits leading to rot and contamination with the mycotoxin ochrotoxin A (OTA). The aim of the study was the isolation of epiphytic microorganisms from pomegranates and the *in vitro* testing of these microorganisms for their efficacy against *A. carbonarius*. A number of morphologically distinct microorganisms were isolated and tested against *A. carbonarius* by using a pomegranate bioassay. The pomegranates were immersed in a suspension of the isolated microorganisms and after one day they were infected with the pathogen by making an incision on the surface of the fruits and applying 10 μl of *A. carbonarius* spores at a concentration of 10^5 spores/ml. Two out of the 30 isolated microorganisms significantly reduced *A. carbonarius* rot on the surface of the tested pomegranates; however, only one of these was capable of reducing significantly the total number of pathogen spores produced.

Screening of the biocontrol agents *Paenibacilus alvei* K165 and *Fusarium oxysporum* F2 talc preparations against the plant pathogen fungus *Verticillium dahliae*

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Verticillium dahliae is among the most pathogenic plant microorganisms, mainly due to the lack of effective chemical control strategies. Therefore, it is evident that the development of biocontrol strategies against this pathogen is of vital importance for agriculture. The aim of this study was to develop and evaluate the efficacy of easy to apply formulations of already known biocontrol agents (BCAs) of *V. dahliae*. For this purpose, talc preparations of 2 BCAs, either *Paenibacilus alvei* K165 or *Fusarium oxysporum* F2, were mixed with potting soil at a ratio of 1%, 5%, 10% and 20% and used as substrates for eggplant cultivation, or were used for seed coating. At the stage of the 3rd-4th leaf, the plants were transplanted into soil infested with 20 *V. dahliae* microsclerotia per gram of soil. It was observed that the ratios 1%, 5%, 10% and 20% of both BCAs in soil were

equally effective in controlling Verticillium wilt. However seed coating treatment was ineffective in the case of K165, but F2 treatment partially reduced disease severity. The differences in the efficacy of the different biocontrol preparations to control Verticillium wilt reflected the observed differences in the size of the BCA populations between the treatments of 1%, 5%, 10%, 20% and seed coating.

Spread of hypovirulent strains of the fungus *Cryphonectria parasitica* after biological control of chestnut blight in the Region of Epirus

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Chestnut blight, a bark disease caused by the fungus *Cryphonectria parasitica*, was first reported in Greece, Mount Pelio, in 1963. Until 2002 it was spread all over the mainland country where chestnut is cultivated, while in 2006 it was recorded in the islands of Lesvos and Crete. It is known that the above ascomycete may be infected by dsRNA viruses called *Cryphonectria* hypoviruses (CHVs), a fact that reduces fungal virulence to levels ranging from avirulence to near-virulence. Such infected strains with proved reduction in their virulence termed as hypovirulent (hv) were used for the biological control of the pathogen in the period 2007-2009 in the Region of Epirus (Prefectures of Arta and Ioannina). Two years after the end of artificial inoculations, samples from cankers were randomly selected from three round plots (orchards) in Arta and from six plots in coppice forest in Ioannina. The growth and the characterization of the strains yielded into virulent and hypovirulent were carried out on PDA medium according to morphological features. In Arta 34.9 - 53.1% of the total cankers yielded hv strains, while in Ioannina the percentage fluctuated between 39.2 and 68.5%. Furthermore, 35.9 - 61.85% of the non inoculated cankers sampled yielded hv strains in the Prefecture of Ioannina, while the percentage was 32.3- 48.8% for Arta. The results show that, two years after the end of artificial inoculations, introduced hypovilence was successful in all treated areas as hypovirulent strains of the fungus have settled in orchards as well as in coppice stands and began as well spreading to non inoculated cankers.

Determination of the optimal retention time for sclerotia in soil paste for the isolation of *Sclerotinia sclerotiorum* mycoparasites. A proposed method for the eradication of nematodes and mites.

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In order to determine the optimal time of sclerotia retention in soil paste, seven different retention times, each using four replicate s and 40 sclerotia in total were examined. The sclerotia were placed in the soil paste and incubated at 25^{0}C in the dark with collection and disinfection taking place every five days, beginning at 5 days for the first treatment and ending at 35 for the seventh. For all treatments soil from the same field was used. Subsequently the sclerotia were disinfected and placed for 24 or 48 hours in a environment with 100% relative humidity. After 35, 30, 25, 20, 15, 10 and 5 days in soil, the proportion of sclerotia in a good condition (not dissolved during the process of decontamination) was 0, 10%, 17%, 20%, 80%, 97.5% and 100%. The optimal retention

time for sclerotia within the soil paste was found to be fifteen days. If, by stereoscopic examination nematodes or mites were detected on the sclerotia, the affected plates were then placed in an oven at 80° for four hours. Under these conditions both nematodes and mites were killed while the sclerotia and any mycoparasites present were not adversely affected. Subsequently, the plates were incubated for a further 24-48 hours at 100% humidity until the appearance of either phytopathogenic microbes or mycoparasites was detected.

The presence of mycoparasites of sclerotia of the phytopathogenic ascomycete *Sclerotinia sclerotiorum* in cultivated lands and a possible relationship with organic matter

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More than 80 soil samples from all over Greece, from tree crops, open field vegetables, greenhouse vegetable crops, vine crops and raisins, and gardens, were collected in order to identify sclerotia and to isolate mycoparasites. Cultivation was either by conventional or organic methods. For the detection of sclerotia two metal sieves with holes of 4 and 7 mm, respectively were used. For the isolation of sclerotial mycoparasites using as traps sclerotia and pH, the texture and organic matter were determined in soil samples. Sclerotia were detected in 22.4% of the samples, mainly on vegetables crops, regardless of whether cultivation system was organic or conventional. Hundreds of candidate mycoparasites were isolated and preliminary evaluation showed that the majority had mycoparasitic qualities or abilities. From each soil sample 5 candidate mycoparasites were isolated on average. Most of them belonged to the genera of *Gliocladium*, *Fusarium*, *Trichoderma*, *Coniothyrium* and the class of *Phycomycetes*. Mycoparasite presence was directly proportional to the concentration of organic matter. Also, in organically-cultivated vegetable crops, the presence of parasitized sclerotia was greater. It seems that the parasitism of sclerotia and the overall presence of mycoparasites is higher in soils where the concentration of organic matter is increased. Mycoparasites were also isolated from soil samples where only traces of organic matter were present. The treatment of organic matter should be a top priority for all crops and basic fertilization should be rational and based on analysis of the soil.

Evaluation of surfactants in activation of the innate immune system of the plant *Arabidopsis thaliana*

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Verticillium wilt caused by the soilborne pathogen *Verticillium dahlia* and bacterial speck caused by *Pseudomonas syringae* pv. *tomato* are two very serious diseases of crop plants. Management of these diseases is mainly based on prevention, thus the discovery of alternative means for their control is essential. Induction of systemic acquired resistance (SAR) could be an alternative strategy of these diseases since there are data of successful control of various plant diseases with this method. The aim of this

study was the evaluation of five different stimulants that belong to ammonium bromide surfactants (A, B, C, D, E), for their ability to induce the plant defense mechanisms. Pathogenicity experiments were performed in *Arabidopsis thaliana* plants infected with *V. dahliae* or *P. syringae* pv. *Tomato* and tomato plants infected with *P. syringae* pv. *tomato*. The five surfactants were applied as droplets in various concentrations on leaf surfaces in order to determine the optimum concentration of each agent that can limit the disease spread. It was found that in case of *V. dahliae*, factor D at the concentration of 10 mM, was the most effective compared to other factors decreasing the disease severity by approx. 20%. In the case of *P. syringae* pv. *tomato,* no significant differences were observed in the percentage of the disease for all the treatments with the five factors. Finally, in preliminary experiments investigating the mode of action of the five factors, it was found that factors C and D (at the concentration of 10 mM) had the ability to form a zone of inhibition against the aforementioned two pathogens. Gene expression studies demonstrated that ammonium bromide surfactants A and B have the potential to induce genes that play a key role in plant defense in *A. thaliana* such as the PR-1 and PR-5 Pathogen Related proteins.

Risk management measures to eradicate and prevent the spread of the quarantine potato fungus *Synchytrium endobioticum* (Schilbersky) Percival in Greece

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In August 2011, during official plant health surveys, the quarantine organism *Synchytrium endobioticum* (Council Directive 2000/29/EC) was detected for the first time in Greece, in two potato (*Solanum tuberosum* L.) fields in Perithori, Regional Unit of Drama. Galls of irregular shape and various sizes were observed on the stolons and tubers of infected plants. Pathotype identification (as pathotype 18) was made by the National Reference Centre (The Netherlands) on gall samples originating from the infested fields. The traceability of the propagating material was investigated and a large number of potato fields in the area were inspected and tested, with negative results. The local potato producers were informed about the disease, its impacts and the phytosanitary measures to be implemented, in compliance with the Ministerial Directives 259959/1984 (B' 260) and 456/5861/18-01-2012 (B' 159). The two fields were officially designated as infested, placed under phytosanitary supervision, and the produce was destroyed. A buffer zone was defined around the infested fields, based on the pathogen biology, the disease epidemiology and the area topography. In the present work, the phytosanitary measures implemented in the area with the aim to manage the risk of disease spread (e.g. a ban on grazing animals, prevention of the movement of infested soil, disinfection of agricultural machinery and packing equipment, etc.) and to eradicate the pathogen (e.g. a ban on potato cultivation in the infested fields, cultivation of resistant potato cultivars in the buffer zones, etc.) is presented.

Evaluation of zeolite and Agri-fos 600® in wilt disease management and the bacterium *Pseudomonas syringae* pv. *tomato*

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Plants, during their long evolutionary path have developed several complex mechanisms for resistance to a plethora of invading pathogens (fungi, bacteria and viruses). The phenomenon of induced and systemic acquired resistance is part of the innate immune system of plants and may be potentiated by biochemical or chemical stimulation of latent resistance mechanisms, through the the use of nonpathogenic microorganisms, or chemical compounds. The objective of this study was the evaluation of zeolite and Agri-fos 600® in the control of *Verticillium* wilt disease and bacterial speck caused by *Pseudomonas syringae* pv. *tomato*. Zeolite is a microporous, aluminosilicate mineral with specific physicochemical properties commonly used as a commercial adsorbent and catalyst. Zeolite is commonly used as a soil improvement substance, but its role in disease management has not been studied. Agri-fos 600® is a special formulation consisting of potassium phosphonate anions that, apart from their role as nutrients, have the ability to induce the defence mechanism of plants. Pathogenicity experiments were performed in *Arabidopsis thaliana* infected with *Verticillium dahliae* or *Pseudomonas syringae* pv. *tomato* and in aubergine infected with *Verticillium dahliae*, where zeolite and Agri-fos 600® were applied in the form of root drench at various doses. Virulence assays showed that zeolite and Agri-fos 600®have the ability to reduce the rate of Verticillium wilt in eggplant and in *A. thaliana*.

Evaluation of Renovation Sekamosa to control Verticillium wilt of eggplant

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The ability of the plant extract "Renovation Sekamosa" to control the soilborne pathogen *V. dahliae in vitro* and *in planta* was evaluated. "Renovation Sekamosa" successfully inhibited the growth of *V. dahliae in vitro* with the EC_{50} determined between 0,025-0,05%. "Renovation Sekamosa" suppressed significantly Verticillium wilt symptoms in greenhouse experiments. The lower disease symptoms associated with the use of "Renovation Sekamosa" were associated with decreased fungal biomass in the xylem vessels of the eggplants, whereas plant growth promotion, as indicated by the higher total number of leaves and the final height of plants, was also observed.

Suppressive effects of compost against Verticillium wilt of eggplant on the basis of phenolic composition

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Evaluation of the suppressive effect of six composts (A, B, C, D, E and Z) against verticillium wilt of eggplant was carried out. The suppressive action of the compost, on the basis of its phenolic composition was further investigated. Results showed that composts C, D, E and Z reduced verticillium wilt symptoms, as well as the fungal biomass in the xylem vessels, despite the high inoculum density (45 microsclerotia g^{-1} soil) which was applied. Composts E and Z (originating from plant residues and olive leaves, olive mill extracted press cake, and olive mill waste water, respectively) showed the highest suppressive effects, reducing significantly the disease severity, disease incidence and relative area under disease progress curve (relative AUDPC). The observed decrease in symptom severity was associated with significant reduction of *V. dahliae* biomass in the vascular tissues as well as with a lower total phenol content in plant stems, indicating lower levels of pathogen infection. The total phenol content of the pure composts as well as of mixtures (consisting of 20% compost - 80% substrate) where the plants grew with differing disease severity, was also assessed. Results showed that the total phenol content of composts with significant suppressive effect against *V. dahliae* (C, D, E and Z) was significantly higher that that of the non suppressive composts. In addition, the total phenol content of mixtures (20% compost - 80% substrate) with a significant suppressive effect was about 400% higher than those of the pure substrate and the non suppressive compost A, indicating the importance of these compounds for disease suppression mechanisms.

Comparative study of phytopathogenic fungi growth in two greenhouse tomato farming systems

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Soil microorganisms significantly affect plant growth, but in agricultural ecosystems their growth, diversity and activity depend to a large degree on the management of the soil. The purpose of this study was to investigate the effect of different cultivation techniques on the development of soil microorganisms and phytopathogenic fungi. Soil samples were collected (2009-2010), from three greenhouses (Triphylia area, Kyparissia) sown with different tomato varieties, and exposed to different regimes of application of fertilizer and pesticides. Soil microbial biomass was determined using the Chloroform Fumigation Extraction Method (CFE), the respiratory activity of microorganisms (Rbasal) was examined, and the total number of soil fungi (phytopathogenic or not) was determined *in vitro* by the method of successive dilution and colony-forming units (CFU) assay. The nutrient media used for the isolation of fungi were PDA, oatmeal agar, corn meal agar, strep RBA, *Botrytis* sporulation agar, Czapek (Dox) agar, and *Fusarium* medium. A comparative evaluation of the samples showed that the variation in soil species biodiversity depends on the addition of organic matter and im-

port of nutrients by fertilization. By increasing the content of organic matter and of the macro elements in soil samples, microbial biomass and the total number of different groups of microorganisms was increased. There was also a change with regard to how frequently the groups of fungi appeared in cultivated soils in comparison to non-cultivated soils. Sclerotia of *Botrytis sp, Sclerotium sp* were isolated from the soil of the greenhouse where solarization had taken place.

Sustainable use of chemical fumigants for the control of soil-borne pathogens in the horticultural sector (LIFE 2008 -SustUse Fumigants)

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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:

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

Suppression ability of composts prepared using municipal solid wastes and olive oil byproducts against the tomato pathogen *Fusarium oxysporum* f.sp. *radicis-lycopersici*

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This trial examined the suppressive ability of two different composts on the tomato soil-borne pathogen *Fusarium oxysporum* f.sp. *radicis-lycopersici*. The first compost was prepared using a mixture of agricultural wastes (olive press cake and olive leaves) while the second utilized the organic fraction of municipal solid waste. Both composts were used at a low ratio mixture (5-10% w/w) with sphagnum peat in soilless plant growth media, due to their high electrical conductivity. The results showed great potential for the suppression of the pathogen by both composts. Disease reduction ranged between

10 and 50% compared to the controls, and was dependent on the ratio of the compost to the soilless growth media. The effective control of *Fusarium oxysporum* f.sp. *radicis-lycopersici* could be explained by the suppressive activity of the indigenous compost micloflora rather than the direct effect of the compost on the pathogen. The microorganisms most likely effect pathogen suppression through various means, including competition for nutrients, antibiosis and/or the induction of host resistance.

Chemical Control & Development of Resistance

Invited Lectures

Biological properties of Xemium, the new SDHI from BASF

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Despite of their long history of use, succinate dehydrogenase inhibitors (SDHI) form one of the most interesting classes of agricultural fungicides with a fascinating line of innovations. Being introduced in the late 60s of the last century, SDH inhibitors are among the longest studied group of agricultural fungicides. While the first generation of SDHI fungicides have a narrow spectrum of disease control against basidiomycetes, and are mainly used in seed treatment applications (e.g. carboxin), the disease and crop spectrum has broadened and the intrinsic efficacy levels have increased significantly with the new generations of SDHIs (e.g. boscalid). Xemium, the latest SDHI molecule from BASF is a broad-spectrum fungicide that controls a wide range of economically important diseases from the classes of basidiomycetes, ascomycetes and deuteromycetes in specialty crops including vegetables, stone fruits, and pome and other fruits, berries and grapes and also in arable crops such as cereals, oilseed rape, potatoes and others. After foliar application, the molecule is systemically (acropetally) distributed within the crop and continuously released from depots on the leaf. Xemium provides excellent preventive, curative and long lasting efficacy and contributes strongly to green leaf tissue and high yield. With the investigation of SDHI resistance, a complex picture is forming. Several mutations in the target protein at different positions, conferring resistance to SDH inhibitors were detected. Differences in the impact of such mutations on the efficacy level of single SDHI molecules has lead to sophisticated scientific and technical discussions. The high value of this group of fungicides for farmers worldwide and the complexity of SDHI resistance in agricultural pathogens call for efforts to maintain efficacy of SDHIs. Monitoring assays based on genetic analysis have been developed to improve the sensitivity of monitoring of a number of pathogens targeted by SDHIs.

Dynali 60/30 DC, the combination of difenoconazole with cyflufenamid creates a new benchmarking product for the management of powdery mildew on vineyards

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Dynali 60/30 DC is a dispersible concentrate (DC) containing 60 grams per litre (g/L) difenoconazole and 30 grams per litre (g/L) cyflufenamid for use on grapes. Difenoconazole is a translaminar (weakly xylem-mobile) triazole fungicide with long-lasting preventative and curative broad-spectrum-control, including leaf spot diseases, powdery mildews, rusts and scab of annual and perennial crops. It is active against plant pathogens belonging to the *Deuteromycota*, *Basidiomycota* and *Ascomycota*. Cyflufenamid belongs to a novel and a chemically unique class of fungicides, the amidoximes). Although the biological mode of action of cyflufenamid against pathogens is still un-

known, it has been demonstrated to differ from those of commercial fungicides such as benzimidazole, demethylation inhibitor (DMI) and strobilurin. Cyflufenamid is highly active on a wide range of powdery mildews, with preventative, residual and curative activity. On the plant, it has contact, translaminar and vapour properties. The combination of difenoconazole and cyflufenamid in Dynali 60/30 DC provides a broad spectrum foliar and bunch fungicide to control *Erysiphe necator* (powdery mildew), *Guignardia bidwellii* (black rot) and *Pseudopezicula tracheiphila* (syn. *Pseudopeziza tracheiphila*) (rotbrenner), with excellent crop safety. The curative properties of the triazole difenoconazole are enhanced by the curative activity of cyflufenamid. The curative activity is extremely useful in practice, even in the broadly recommended preventative spray programmes. Under field conditions, especially under very high disease pressure, purely preventative action is frequently not enough to provide reliable control because several stages of the pathogen are simultaneously present. For powdery mildew management it is strongly recommended that a mixture of products is used as well as alternating products with different modes of action, in order to avoid the selection of strains that are resistant to single site active substances. For this reason the availability of new products is essential in order to design efficient and long-lasting effective crop protection programmes.

Oral & Poster Presentations

Study of the inherent risk of resistance to dicarboximides and phenylpyrroles by *Alternaria solani*

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Mutants of *Alternaria solani* moderately (RF: 15, based on EC_{50s}) and highly (RF: 150 to >1000) resistant to phenylpyrroles were isolated at high mutation frequency after UV-mutagenesis and selection on media containing fludioxonil. Cross resistance studies with other fungicides showed that the mutation(s) that conferred resistance to fludioxonil also reduced the sensitivity of mutant strains to the aromatic hydrocarbon (quintozene) and dicarboximide (iprodione and vinclozolin) fungicides. No effect of phenylpyrrole resistance mutation(s) was observed on the fungitoxicity of the triazole flusilazole, the imidazole imazalil, the carboxamide boscalid and the QoIs pyraclostrobin and azoxystrobin. Furthermore, an increased sensitivity (RF: 0.5-0.07) of the mutant strains to the anilinopyrimidine fungicide pyrimethanil was observed in all fludioxonil-resistant strains tested. Interestingly, a significant reduction in the sensitivity of a mutant strain to pyraclostrobin was observed. Study of fitness determining parameters showed that the mutation(s) conferring resistance to phenylpyrroles did not affect, or only slightly affected, the mycelial growth rate, osmosensitivity, pathogenicity and conidial germination in most mutant strains tested. On the contrary, most of the fludioxonil-resistant strains produced fewer conidia than the wild type strain. Almost all fludioxonil-resistant strains retained their resistance levels even after 9 subcultures on fungicide-free medium. The above data indicate that there is a considerable inherent risk of *Alternaria solani* resistance to fludioxonil and that appropriate anti-resistance strategies should be implemented to avoid future control failures.

Characterization and distribution of fungicide resistant phenotypes in *Botrytis cinerea* originating from lettuce crops in Greece

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The minimum inhibitory concentration (MIC) for spore germination and mycelial growth of 447 *B. cinerea* isolates to nine fungicides from different chemical groups was determined *in vitro*, by the point inoculation method. Three phenotypes with multiple resistance to stobilurins (QoI), carboximides (Bos), hydroxyanilides (Hyd), anilinopyrimidines (Ani), phenylpyrroles (Phen), dicarboximides (Dic) and benzimidazoles(Ben),[$QoI^{HR}Bos^{HR}Hyd^{HR}Ani^{HR}Phen^{MR}Dic^{MR}Ben^{HR}$, $QoI^{HR}Bos^{HR}Ani^{HR}Dic^{MR}Ben^{HR}$, $QoI^{HR}Bos^{HR}Dic^{MR}Ben^{HR}$] were detected at frequency 7%, 12% and 6%, respectively. These phenotypes were mainly isolated from a glasshouse lettuce crop located in Thessaly. Isolates with single high resistance to benzimidazoles counted 11% in total, and mainly originated from open field lettuce grown in the Macedonia or Peloponnese regions. Phenotypes with various resistance combinations to 1-5 chemical groups were found less frequently (<5%). No resistant phenotypes to fluazinam (dinitroanilides) and chlorothalonil (phthalonitriles) were detected. *B. cinerea* phenotypes exhibiting multiple resistance to 7 different groups of fungicides, high resistance to fenhexamid (Hyd) and moderate resistance to fludioxonil (Phen), were recorded for the first time in Greece. To reduce grey mould losses the application of anti-resistance strategies is considered necessary.

Molecular characterization, methodology of *sdh*B mutation identification, and cross-resistance in SDHI-resistant *Botrytis cinerea* isolates

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Succinate dehydrogenase inhibitors (SDHIs) have been used against grey mould since the end of the previous decade. However, shortly after their introduction into the spray programmes resistance emerged both in Greece and worldwide. The current study was conducted to: i) investigate the molecular mechanism of resistance in 25 *B. cinerea* isolates showing different levels of resistance to the SDHI fungicide boscalid, ii) develop molecular methods for the rapid identification of resistance mutations and iii) investigate the cross-resistance relationships among members of the SDHI group. Sequence analysis of the *sdh*B, *sdh*C and *sdh*D subunits of the gene revealed five mutations leading to amino acid substitution in the *Sdh*B subunit (P225F, N230I και H272L/R/Y). To facilitate rapid detection of these mutations associated with resistance to boscalid, a primer introduced restriction analysis PCR (PIRA PCR) was developed. The method was successfully applied to the entire resistant subpopulation. To study the cross-resistance relationships, 30 isolates (5 per genotype) were characterized for their sensitivities to 8 SDHI fungicides. The results showed different sensitivities and cross resistance patterns between structurally different SDHIs. P225F mutants were resistant *in vitro* to all SDHIs tested. Similarly, isolates possessing the H272L mutation were highly resistant to boscalid, but showed low to moderate levels of resistance to other SDHIs. The N230I mutants were moderately resistant to boscalid, fluopyram and fluxapyroxad and showed low levels of resistance to isopyrazam, bixafen, fenfuram, benodanil and carboxin. The H272R mutants showed moderate levels of resistance to boscalid

and low resistance levels to isopyrazam, fenfuram and carboxin, but retained the wild-type sensitivity to fluopyram, bixafen, fluxapyroxad and benodanil. Similarly, the H272Y showed moderate levels of resistance to boscalid, and very levels of resistance to isopyrazam, bixafen, fenfuram and carboxin, but showed an increased sensitivity to benodanil and fluopyram (negative cross-resistance). The findings of the current research can be useful both for future sensitivity monitoring programmes and in the implementation of anti-resistance strategies.

Resistance of *Botrytis cinerea* populations from strawberry and greenhouse-grown tomato to botryticides

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Botrytis cinerea is a high risk pathogen for fungicide resistance development and during the past has presented the problem of fungicide resistance development worldwide. The present study was conducted to investigate the fungicide sensitivity profile of pathogen isolates obtained from strawberry (from the Pieria and Ilia regions) and greenhouse-grown tomato (regions of Preveza, Kyparissia, and on Crete). In total 1,160 single spore isolates of the pathogen were tested for sensitivity to the SDHI fungicide boscalid, the QoI pyraclostrobin, the anilinopyrimidine cyprodinil, the hydroxyanilide fenhexamid, the phenylpyrrole fludioxonil, and the benzimidazole carbendazim. The isolates were characterized as either sensitive or resistant to each of the fungicides using a bioassay technique with distinct discriminatory fungicide doses. The isolates were grouped based on their resistance phenotype profile. Results showed that in fungal populations obtained from green-house grown tomatoes on the island of Crete, the predominant phenotype was that of double resistance to benzimidazole and anilinopyrimidine fungicides (48.9% of the population), in the region of Preveza the predominant phenotype was that of single resistance to benzimidazoles (57.4% of the population), while in the Kyparissia region the sensitive isolates dominated the population (57.4%). In contrast, within the populations obtained from strawberry fruits, a high proportion of isolates (57%) exhibited multiple resistance to anilinopyrimidines, benzimidazoles, QoIs and SDHIs. None of the isolates tested was found to be resistant to hydoxyanilide or phenylpyrrole fungicides. The results revealed a widespread prevalence of boscalid resistant phenotypes in the strawberry population ranging from 46.7% to 76.8% in the Pieria and Ilia regions, respectively. The resistance frequency in the tomato population was significantly lower, with boscalid-resistance frequency values of 0.8, 9.8 and 13.2% in the Crete, Messinia and Preveza populations, respectively. In addition, in SDHI-resistant isolates the *sdh*B mutations associated with resistance were identified using a PIRA-PCR technique, which showed that H272R was the predominant mutation in all of the sampled populations.

Fitness and competitive ability of *Botrytis cinerea* strains possessing different mutations in the *sdh*B gene

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Succinate dehydrogenase inhibitors (SDHIs) are a fungicide class with increasing relevance in grey mould control. However, they are also a fungicide class at high risk for resistance development. Recent studies have shown that resistance of *B. cinerea* to SDHIs is associated with mutations in the *sdh* gene. The objective of the current study was to investigate the effect of the H272Y/R/L, N230I and P225F mutations in the respiratory activity, the fitness, and the competitiveness of *B. cinerea* isolates. Fitness parameters measured were: i) mycelial growth and conidial germination *in vitro*, ii) aggressiveness and sporulation capacity *in vivo* and iii) sclerotia production *in vitro* and sclerotia viability under different storage conditions. The competitive ability of the resistant isolates was measured both in the absence and in the presence of boscalid and fluopyram. Measurements of oxygen uptake showed that N230I mutants had the lowest respiratory activity, followed by the H272Y mutants. In contrast no differences in respiratory activity were observed among the H272R/L, P225F mutants and the wild-type isolates. The measurements revealed significant differences in most of the parameters measured. In terms of fitness, the H272R isolates most closely resembled the sensitive isolates. In contrast, the H272Y/L, N230I and P225F isolates showed reduced fitness values when compared to the sensitive isolates. In the competition experiments it was found that, in the absence of fungicide selection pressure, after 4 disease cycles the sensitive isolates dominated the population in all the mixtures tested. In contrast, when the competition experiment was conducted under the selective pressure of boscalid, a gradual decrease in the frequency of sensitive isolates was observed while the frequency of H272L and P225F isolates increased. When the competition experiment was conducted in the presence of fluopyram the sensitive isolates were eliminated, in some cases after a single disease cycle, and the P225F mutants dominated the population. These results indicate that the *sdh*B mutations adversely affect the mutants. The observed dominance of sensitive isolates in the competition experiments conducted in the absence of fungicides suggest that the application of SDHIs in alternation schemes may delay the selection or reduce the frequency of SDHI-resistant mutants.

Phytopathological and molecular characterization of *Penicillium expansum* mutant strains resistant to new succinate dyhydrogenase inhibitors

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Mutants of *Penicillium expansum* highly resistant (RF: >300, based on EC_{50s}) to the new succinate dehydrogenase inhibitors (SDHIs) were isolated at a mutation frequency of 2.3×10^{-6} after UV-mutagenesis and selection on media containing boscalid. Cross resistance studies with other fungicides showed that the mutation(s) for resistance to boscalid also reduced the sensitivity of mutant strains to isopyrazam, fluopyram, thifluzamide and carboxin, but not to fungicides affecting other cellular pathways or processes, such as the triazole flusilazole, the phenylpyrrole fludixonil, the anilinopyrimidine cyprodinil and the benzimidazole benomyl. An increased sensitivity of most boscalid-resistant mutant strains to fluopyram and pyraclostrobin was observed. Studies of fitness-determining parameters showed that the mutation(s) for resistance to boscalid may or may not affect the mycelial growth rate, but had no adverse effect on sporulation, conidial germination or pathogenicity. Analysis of mycelial extracts from the wild types

and mutant strains, using thin layer chromatography (TLC) and high performance liquid chromatography (HPLC), showed that most *P. expansum* mutant strains produced patulin and citrinin *in vitro* at significantly higher concentrations than the wild-type parent strains. Similar results were found in tests on artificially inoculated apples. Gene sequence analysis of the *Sdh*B subunit of complex II, revealed that single point mutations within a highly conserved region of the iron-sulphur protein (Ip) conferred resistance in most cases. Mutations resulted in replacement of histidine with arginine (H272R) or tyrosine (H272Y) at position 272. The H272R mutation had no effect on the strains' sensitivity to fluopyram, while the H272Y mutation resulted in increased sensitivity to fluopyram. However, in one strain no mutations were found in the Ip gene, though the strain showed high resistance to boscalid (RF: 300) and medium-high resistance to fluopyram (RF: 10). This is the first study reporting *P. expansum* strains resistant to SDHIs due to the biochemical mechanism of target-site modification, resulting from amino acid substitutions in the *Sdh*B subunit of complex II. Moreover, the data of the present study indicate for the first time that *P. expansum* have the genetic and biochemical potential for the appearance of SDHI-resistant isolates in the field.

Molecular characterization and PCR-RFLP detection of the E198A benzimidazole resistance mutation in field isolates of *Monilinia laxa* from Greece

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Sensitivity to benzimidazoles in isolates of the brown rot pathogen *Monilinia laxa* collected from stone fruit in central and northern Greece was evaluated and the molecular basis for resistance was investigated. *M. laxa* isolates were classified as benzimidazole sensitive (S) or highly resistant (HR) based on their sensitivity profiles to carbendazim. Thirty seven percent of the isolates belonged to the HR phenotype, carried no apparent fitness penalties and exhibited resistance factor values (based on EC_{50} values) greater than 500. Highly resistant isolates were also less sensitive to the benzimidazoles benomyl and thiophanate-methyl but were more sensitive to the *N*-phenylcarbamate diethofencarb and the benzamide zoxamide, when compared to isolates belonging to the S phenotype. Fungitoxicity tests with fungicides belonging to other chemical classes revealed no cross-resistance relationships between benzimidazoles and the dicarboximide iprodione, the phenylpyrrole fludioxonil, the hydroxyanilide fenhexamid, the carboxamide boscalid, the triazole tebuconazole and the strobilurin-type fungicide pyraclostrobin, indicating that a target site modification is probably responsible for the resistant phenotypes observed. Comparison of the b-tubulin gene DNA sequences between resistant and sensitive isolates revealed a point mutation resulting from the E198A substitution of the corresponding protein in all HR isolates tested. An Eco31I restriction site in the b-tubulin gene, which was destroyed in HR *M. laxa* isolates, allowed the development of a PCR-RFLP diagnostic for the detection of the E198A resistance mutation.

Penthiopyrad (Fontelis® 20SC) a new fungicide for the control of a large number of diseases of fruits and vegetable crops in protected and open field environments

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Penthiopyrad (Fontelis® 20SC) is a novel fungicide belonging to the chemical family of carboxamides (FRAC Group 7 - SDHI, Succinate DeHydrogenase Inhibitors). Penthiopyrad controls a broad spectrum of crop diseases caused by many *Ascomycetes* and *Basidiomycetes*. In Greece, it is currently under registration for the protection of apple and pear against apple and pear scab, powdery mildew and brown spot of pear. It is also under registration for the control of grey mould on field and greenhouse vegetables (tomato, eggplant, pepper, cucumber and zucchini). High efficacy and its unique characteristics, (good translaminar penetration and local systemic movement, very good rainfastness, a favorable toxicological and eco-toxicological profile) make it highly compatible with Integrated Pest Management programmes.

Biological evaluation of INITIUM® against downy mildew diseases. Field trial data on grape, potato and tomato crops

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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 with fungicide classes such as Qo inhibitors, phenylamides and carboxylic acid amides. Initium is highly effective in the inhibition of zoospore formation and release, zoosporangia release, motility and germination. In numerous field trials carried out worldwide, Initium has been shown to be highly selective in a wide range of specialty crops and providest the best performance when applied as a protectant spray before disease is established in the crop. Initium applications should begin before infection of the target diseases, on a preventive scheme, or when ambient conditions are conducive for the disease and the crop is at a sensitive stage.
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, onions, and lettuce).
In Greece, Initium was tested in replicated field trials in ready mix products with either dimethomorph or metiram during the 2006-2012 growing seasons and showed high effectiveness compared to standard fungicides.
The performance features of Initium in terms of biological efficacy, selectivity and environmental safety, will contribute significantly to crop protection, and support integrated production of high quality agricultural products.

Investigation of the effect of pyraclostrobin on grapevine physiology and disease control

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The aim of this research was to investigate the effect of pyraclostrobin on physiological parameters, grape attributes and disease control in grapevine. The experiment was conducted in a commercial vineyard in the Amyntaio area (Greece), planted with cv. Sauvignon blanc onto 1103P. Experimental design included fungicide spray programmes with one (pre-bloom: 1S), two (pre-bloom and berry set: 2S) and three (pre-bloom, berry set and veraison: 3S) applications of pyraclostrobin. Control plants (C) received a standard fungicide spray programme according to the local agricultural practice. During the vegetative period, measurements of downy and powdery mildew severity on grape leaves and bunches were conducted. The incidence of grey mould and black rot infections was measured after harvest. Black Aspergilli were identified molecularly at a species level as either *Aspergillus carbonarious* or *A. niger*. Estimation of physiological parameters included leaf chlorophyll, midday gas exchange parameters and leaf carbon isotope discrimination. Grapes were picked at commercial harvest and grape yield and composition attributes were measured. Vinification of each vineyard block was carried out and experimental wines were analyzed for standard chemical parameters and the precursor of Sauvignon blanc varietal aroma. Measurements of powdery mildew severity showed that disease severity was very low without statistical differences among treatments. Downy mildew and grey mould infections were completely absent during the summer period. A high incidence of *Aspergillus spp*. presence was observed on grape berries, possibly related to the warm and dry conditions that prevailed from the veraison until the harvest period. The lowest incidence of *Aspergillus spp*. was observed in bunches obtained from 3S treatment suggesting that the application of pyraclostrobin at veraison contributed to better fungal control. Pyraclostrobin applications significantly increased the chlorophyll content in Sauvignon blanc leaves, with highest levels in 1S, 2S and 3S compared to C plants. Concerning vine physiological response to pyraclostrobin, results showed lower values of stomatal conductance and assimilation rate in all pyraclostrobin treatments compared to C plants but increased transpiration levels, leading to lower instantaneous water use efficiency in the pyraclostrobin treatments, throughout the study period. However, pyraclostrobin treatments manifested higher photosynthetic nitrogen use efficiency than C plants which could allow for maximum carbon gain at a lower nitrogen levels. No differences were observed among treatments for yield and berry chemical characteristics (with the exception of must ammonia nitrogen, which was higher in the pyraclostrobin treatments). No differences were observed among treatments in the chemical composition of the experimental wines or in the concentration of the precursor of Sauvignon blanc varietal aroma.

Efficacy evaluation of pyraclostrobin against bacterial and viral diseases of tomato and investigation of its mode of action

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Strobilurins have rapidly become an important class of agricultural fungicides. Their activity is due to their ability to inhibit mitochondrial respiration, thus disrupting the energy cycle within the fungus. In addition to serving as potent fungicides, they have been reported to offer protection to plants by increasing their capacity to activate cellular defence responses and promote plant growth (greening effect). The strobilurin class fungicide pyraclostrobin is registered for use in several crops across Europe. In this work, we present the efficacy evaluation of pyraclostrobin against bacterial and viral diseases. Specifically, the incidence and development of bacterial speck disease, caused by *Pseudomonas syringae* pv. *tomato*, in tomato plants treated with pyraclostrobin was assessed in three independent experiments. Moreover, the effect of pyraclostrobin against *Cucumber mosaic virus* (CMV) and *Potato virus Y* (PVY) was assessed in an environmentally controlled greenhouse, as well as in field trials in commercial (toll type) greenhouses. Disease incidence and development was affected by pyraclostrobin application in all experiments conducted so far and further verification of results and analysis is in progress. In a second set of experiments, the elicitation of defence responses by pyraclostrobin, as indicated by a potential increase in the transcription of systemic acquired resistance (SAR)-linked genes, was assessed.

Chemical control of grey mould on lettuce grown in hydroponic systems and fungicide residues at harvest

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For two consecutive years (2010, 2011) the effectiveness of various fungicide spray applications against grey mould on lettuce grown in hydroponics, was evaluated. Two standard applications were made at seedling stage followed up by one or two more sprays after transplanting, at 15-day intervals. The last application was made at least 4 weeks before harvest. Applications at the seedling stage with each of Daconil SC (chlorothalonil 50%) 3ml/lt, Teldor WG (fenhexamid 50%) 1.5g/lt, Signum WG (boscalid 26.7% + pyraclostrobin 6.7%) 1.5 g/lt, Switch WG (fludioxonil 25% + cyprodinil 37.5%) 0.5g/lt and Ortiva Opti SC (azoxystrobin 8% + chlorothalonil 40%) 2.5ml/lt, reduced significantly the disease incidence and severity, compared to untreated controls. The fungicides Switch WG and Signum WG provided the best control, whereas Teldor WG and Daconil SC were the least effective. The level of the disease was further reduced with one or two more fungicide applications after transplanting. To avoid the development of resistance, alternative fungicide applications were found necessary. With the exception of applications of Daconil SC (chlorothalonil 50%) after transplanting, in all other treatments either no fungicide residues or residue levels below acceptable European MRLs were detected at harvest.

Biological activity of the succinate dehydrogenase inhibitor fluopyram against *Botrytis cinerea* and fungal baseline sensitivity

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Succinate dehydrogenase inhibitors (SDHIs) constitute a fungicide class of increasing relevance in crop protection. These fungicides could play a crucial role in the successful management of grey mould disease. In the current study the effect of fluopyram, a novel SDHI fungicide, on several developmental stages of *Botrytis cinerea* was determined *in vitro*, and its protective and curative activity against the pathogen was determined in strawberry fruit. Furthermore, fungal baseline sensitivity was determined in a set of 192 pathogen isolates. Germ tube elongation was found to be the most sensitive growth stage affected by fluopyram, while mycelial growth was found to be the least sensitive growth stage. Fluopyram provided excellent protective activity against *B. cinerea* when applied at 100 μg ml^{-1} 96, 48 or 24h before the artificial inoculation of the strawberry fruit. Similarly, fluopyram showed a high curative activity when it was applied at 100 μg ml^{-1} 24h post-inoculation, but when application was made 48 or 96h post-inoculation disease control efficacy was modest or low. The measurement of baseline sensitivity showed it to be unimodal in all populations tested. The individual EC_{50} values for fluopyram ranged from 0.03 to 0.29 μg ml^{-1}. In addition, no correlation was found between sensitivity to fluopyram and sensitivity to other fungicides including cyprodinil, fenhexamid, fludioxonil, iprodione, boscalid and pyraclostrobin. The obtained biological activity, baseline sensitivity and cross-resistance relationships data suggest that fluopyram could play a key-role in grey mould management in the near future and encourage its introduction into spraying programmes.

Sensitivity of isolates of the fungi *Aspergillus niger* and *Aspergillus carbonarius* in registered fungicides in viticulture

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The filamentous fungi *Aspergillus niger* and *A. carbonarius* cause significant rot problems in grapes at pre-harvest and post-harvest level and produce various carcinogenic mycotoxins (i.e. ochratoxin A) in grape berries and wine. The goal of this study was to evaluate 14 *A. niger* and *A. carbonarius* isolates from the fungal collection of the Laboratory of Plant Pathology in Agricultural University of Athens (isolated from grapes in different regions of Greece) for sensitivity in various fungicides that are included in viticulture plant protection programs. The first experiments were performed with the active ingredients fludioxonil, cyprodinil and azoxystrobin used alone and/or in combination with salicylhydroxamic acid (SHAM-inhibitor of alternative cellular respiration). The applied concentrations of the active ingredients fludioxonil and cyprodinil were 0.01, 0.05, 0.1 and 0.5 ppm for azoxystrobin 0.01, 0.1, 1.0 and 10.0 ppm and 100.0 ppm for SHAM. First, cyprodinil showed higher inhibition rates in all of the tested isolates compared to fludioxonil at all concentration rates. After six days of incubation of the plates with the fungal isolates, the two fungicides showed a statistical difference between them only in the two lower concentrations. The tested *A. niger* and *A. carbonarius* isolates did not demonstrate any statistically significant difference between them regarding their sensitivity to the two active ingredients of fludioxonil and cyprodinil. In azoxystrobin, all isolates showed moderate sensitivity which was increased significantly when SHAM was added in the medium. The exception was the isolation 315 (an *A. carbonarius* strain isolated from the grape variety Corinthiaki Stafida from Trikala in Corinthia) that showed a small inhibition in all four treatments of fludioxonil, cyprodinil, azoxystrobin and azoxystrobin+SHAM. This particular isolate has preserved this resis-

tance even in the treatment of azoxystrobin 10.0 ppm+SHAM 100.0 ppm. The results of this study indicate that the isolates used (except for 315) do not demonstrate high resistance levels to any of the active ingredients tested that are registered for use in viticulture.

Impact of fungicides contained in wastewaters from the fruit packaging industry on soil microbes

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Thiabendazole (TBZ), imazalil (IMZ), and *ortho*-phenyl phenol (OPP) are fungicides used in fruit packaging plants for the control of *Penicilium* sp. and *Geotrichum* sp. infestations during storage. Despite their indoor, post-harvest use high loads of these fungicides are annualy released in the soil environment through direct land spreading of wastewaters from the fruit packaging industry. Taking into consideration the major role of microbes in soil nutritient cycling, a laboratory study was conducted to monitor the effects of these fungicides on soil microbial diversity and function. Fungicides were applied at 50 mg/kg assuming a direct soil disposal scenario and potential effects were recorded on a temporal basis post application. Potential nitrification, β-glucosidase, acid and alkaline phosphatases, and fluorescence diacetate hydrolyzing activity were determined to assess impacts on soil functions. Furthermore, structural changes on basidiomycetes community, being main carbon decomposers, were determined by denaturating gradient gel electrophoresis (DGGE). A general inhibitory effect was observed for potential nitrification in the presence of OPP, in contrast to TBZ which promoted potential nitrification. Enzymatic activities were significantly affected by fungicides, however no clear temporal patterns of fungicide effects were observed. DGGE analysis indicated a rather rich and complex soil basidiomycetes community. Multivariate analysis of the binary data matrix revealed that fungicides significantly altered the structure of basidiomycetes community, however as with enzymatic activities no clear temporal patterns of fungicide effects were evident. To conclude, potential risk may exist to soil microorganisms and their activities in soils treated routinely by wastewaters from the fruit packaging industry, therefore management measures are necessary to control the waste disposal of spent application solution and prevent soil contamination.

In situ effectiveness of different fungicides against selected resistant phenotypes of *Botrytis cinerea* originating from lettuce

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The efficacy of seven fungicides with different modes of action against nine multiple resistant isolates of *B. cinerea,* was tested *in situ*. The fungicides used were: pyraclostrobin (strobilurin), boscalid (carboxamide), fenhexamid (hydroxyanilide), cyprodinil (anilinopyrimidine), fludioxonil (phenylpyrrole), fluazinam (dinitroaniline) and chlorothalonil (phthalonitrile). Young lettuce leaves (c.v. Penelope RZ) were immersed in aqueous fungicide suspensions at the recommended spraying concentrations (a.i.%). When the

leaves dried they were placed in Petri dishes containing sterilized water agar. Then, inoculation was effected place by transferring either an inverted 5 mm mycelial disc, or a drop of a spore suspension ($5x10^4$/ml) onto the adaxial leaf surface (wounded or not). Fungicide efficacy was evaluated by measuring the size of the infection lesion after a 3 (mycelium) or 6 (spores) day incubation, respectively, in a growth chamber at 18°C and 12 h light. The fungicides pyraclostrobin (0.01%) and fenhexamid (0.075%) failed to inhibit the development of Botrytis lesions by the resistant isolates, in all tests. Similar results were also obtained for boscalid (0.04%), cyprodinil (0.018% and 0.038%) and fludioxonil (0.012% and 0.025%) when mycelial discs were used as the inoculum. In contrast, the fungicides fluazinam (0.02%) and chlorothalonil (0.15%) were in all cases effective against all isolates examined. These results show the inability of recently introduced fungicides to inhibit infection by *B. cinerea* resistant phenotypes obtained from lettuce crops.

PROLECTUS™ (Fenpyrazamine): A new fungicide for the control of *Botrytis cinerea* on grape, vegetables, strawberry and *Monilia* spp. on stone fruits

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PROLECTUS™ is the trade name of a fenpyrazamine (S-2188) based product developed by Sumitomo Chemical Co., Ltd., as a fungicide for foliar applications. Fenpyrazamine belongs to the chemical family of Pyrazolinone and is characterized by persistent action and high efficacy against a range of fungi including *Botrytis* sp., *Sclerotinia sclerotiorum* and *Monilia* sp. The product has translaminar motion and is active against the agent of grey mould by inhibiting germ tube elongation, mycelium growth, and spore formation in the lesions, as well as preventing lesion development, by acting on ergosterol biosynthesis. No cross resistance was observed with the dicarboximide, benzimidazole, strobilurin, triazole and pridinamine fungicides. PROLECTUS™ will be released as water dispersible granules containing 50% fenpyrazamine. The very good profile regarding the health of operators, workers and bystanders has lead to a non-classification from a toxicological point of view. Regarding physicochemical properties, the results of residue trials support a 14 to 7 days PHI for grapes (wine or table) and 1 day PHI for vegetables. PROLECTUS™ is also safe for beneficials. PROLECTUS™ at a dose between 0.8 and 1.2 kg per hectare demonstrates a high efficacy against *Botrytis cinerea* on grapevine, vegetable crops and strawberry. Its spectrum of activity also extends to fungi of the genus *Monilinia* and *Sclerotionia*. As the product exhibits excellent selectivity and rainfastness, a long-lasting action, favourable toxicity classification and a short PHI, PROLECTUS™ is therefore an ideal partner for IPM programs, in line with the needs of a modern control strategy.

Effect of pyraclostrobin on the physiology of durum wheat and disease control

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During the growing season 2010-11, a field experiment was established with two durum wheat cultivars (Agapi and Elpida) aiming to investigate the effect of pyraclostrobin on both physiology and disease control in wheat. Experimental design included single applications of pyraclostrobin at two different vegetative stages, double application of pyraclostrobin at both stages, and a triple application, to investigate the effect on *Fusarium* head blight. Disease severity measurements showed that pyraclostrobin application significantly reduced powdery mildew and *Septoria tritici* blotch severity in both cultivars. The frequency of *Fusarium*-infected kernels was relatively high, without significant differences being detected between treatments. Chlorophyll content was highest in the treatment of a single application of pyraclostrobin at the stage BBCH 39, exceeding by 8.69% the respective value of the controls. Treatments did not show any differentiation with regard to SLA. Compared to controls, all treatments had higher ΔT (ΔT: T_{canopy}-T_{air}) values. All treatments outyielded controls (2180 kg/ha) by 8% to 56.2%. Protein content and vitreousness were higher in the treatments with single pyraclostrobin applications either at BBCH 30 or BBCH 39. In conclusion, the present preliminary work indicates possible physiological effects of pyraclostrobin on durum wheat, which also reflected on yield and quality. Most effective was the BC treatment (double spraying at BBCH 30 and 39) but even C treatment (spraying at BBCH 39) could provide an acceptable effectiveness regarding yield and quality traits.

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