

## ***ABSTRACTS***

### **Summaries of invited lectures, oral and poster presentations given at the 14<sup>th</sup> Hellenic Phytopathological Congress, Dalamanara Argolidas, Greece, 7-10 October 2008**

The 14<sup>th</sup> National Phytopathological Congress, organized every two years by the Hellenic Phytopathological Society (HPS), was held in Dalamanara Argolidas, on October 7-10, 2008. The meeting was attended by more than 450 participants. 5 invited lectures, 50 oral presentations and 71 posters were presented dealing with plant diseases caused by fungi, bacteria, viruses and non-parasitic disorders and with the disease control. In addition, one round-table discussion was held on “*Consequences of the new European legislation concerning the placing of plant protection products*”. Abstracts of the invited papers, the oral presentations, and the posters of the congress are presented in this issue.

## Keynote Lecture

### Plant disease diagnosis: from the classical approach to the molecular dimension

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The fast advancement of molecular biology promised to offer modern powerful tools to biological sciences. In plant pathology research, the potential of applying radical leading edge technologies for the molecular detection and quantification of plant pathogens and in particular of those that are by nature difficult to work with such as soilborne pathogens that live in a complex environment (soil) or non culturable on artificial media organisms (obligate parasites). The continuously increasing need for fast and accurate identification of microbes in various aspects of plant disease studies (epidemiology, spread, population dynamics) aiming to develop more effective control strategies, lead to the wide use of molecular techniques in phytopathological research. Classical approaches that had been widely employed for decades (e.g. selective media) for the detection and identification of pathogens, due to several drawbacks like the inconsistent efficiency of various methods, the prevalence of saprophytes over pathogens in culture media due to better antagonism, the confusion in diagnosis of species sharing common morphological characters and finally the bias of the researcher to a certain methodology, are only some of the inevitable pieces of the diagnosis puzzle of plant pathogens. At the same time, the trend for globalization of agriculture created higher demands in the trade of propagation material after the essential abolishment of geographic borders. The phytosanitary controls and the regulations for quarantine pathogens became stricter while the standards for production and transfer of healthy propagation material increased. The parallel development of computer science in conjunction with the achievements of molecular biology offered the possibility for digital documentation of biological parameters (bioinformatics). This way, the acquisition and deposition of DNA sequences in databases offer the information that lead to the discovery of new and even unknown species of microorganisms. All these data, combined with the classical approach of studying plant pathogens in the field, assisted in opening new eras in the knowledge of pathogens or of microbes in general. However, even if all the aforementioned factors are major components to solve the equation of plant disease diagnosis, the knowledge and expertise of the plant pathologist would always be the exponent parameter. All these powerful molecular tools are useless items in the hands of the user that is deprived of basic knowledge of plant diseases and lacks immediate contact and experience with reality of phytopathology. Nowadays, although we live in the era of molecular revolution, the classical knowledge of plant pathology is the foundation for plant disease diagnosis.

## NEW DISEASES – ETIOLOGY

### Oral Presentations

#### **Molecular characterization of two new grapevine virus species belonging to a distinct lineage within the genus *Ampelovirus***

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Two putatively new virus species (GLRaV-Pr, GLRaV-De) related to grapevine leafroll disease were isolated from greek varieties. These viruses along with GLRaV-4,-5,-6 and -9 constitute a distinct phylogenetic cluster within the genus *Ampelovirus*. In this study, the full and partial nucleotide sequences of GLRaV-Pr and -De were determined respectively, using viral dsRNA extracts. The genome of GLRaV-Pr is 13,696 nt long, one of the smallest in the *Closteroviridae*, and contains 7 open reading frames which potentially encode a 253 kDa polyprotein, a 58.2 kDa RNA-dependent RNA polymerase, a 5.2 kDa hydrophobic protein, a 58.5 kDa heat shock 70 protein homologue, a 60 kDa protein, a 30 kDa coat protein (CP) and a CP minor of 23 kDa. A 4319 nt region was also determined for GLRaV-De corresponding to the HSP70h, p60, CP and Cpm (partial) genes. GLRaV-Pr and -De differ by more than 10% in amino acids from the known closely related *Ampelovirus* species, thus their future assignment as tentative species can be supported. Comparative sequence analysis of GLRaV-4, -5, -6, -9, -Pr and -De showed high uniformity on their genome organization which in combination with the inferred topologies indicate that these virus species follow a distinct evolutionary course and they should constitute a separate genus.

## ***Iris yellow spot virus, an emerging pathogen in Allium sp. crops in Greece***

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*Iris yellow spot virus* (IYSV) (genus *Tospovirus*, family Bunyaviridae) is a destructive pathogen affecting *Allium* species, worldwide. The virus causes a variety of symptoms ranging from chlorotic or necrotic lesions to extended necrosis on onion (*A. cepa* L.) leaves. In Greece, similar symptoms were often encountered in the past however they were generally attributed to fungal infections. During the spring of 2008, 530, 439 and 217 leaf samples were collected from onion, leek (*A. porum* L.) and garlic (*A. sativum* L.) crops, respectively, from different areas of the country. The plants sampled were infested with *Thrips tabaci* Lindeman, the main vector of the virus. Samples were analysed by DAS-ELISA using polyclonal antibodies against the N protein of IYSV as well as by mechanical inoculation onto *Nicotiana benthamiana* L. IYSV was detected in almost all surveyed areas, in 9.4-61.7%, in 0-23.7% and in 0-14.8% of the onion, leek and garlic samples, respectively. A positive ELISA leek sample was subsequently analyzed in a reverse-transcription-polymerase chain reaction (RT-PCR) using N-gene primers of IYSV and the produced amplicon (approximately 800 bp) was subsequently cloned and sequenced. Nucleotide sequence comparisons with the GeneBank IYSV gene showed 99% homology with a Dutch isolate of the virus (GenBank Accession No AF001387). IYSV seems to be very well established in Greece comprising a serious threat for *Allium* sp. crops.

## ***Hop stunt viroid (HSVd), a new pathogen of stone and pome fruit trees in Greece***

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During the last two years, sampling was conducted in pome and stone fruit tree orchards in different regions of Greece, in order to determine possible infection of these orchards with *Pospiviroidae* viroids. Approximately 500 field samples were tested for infection with *Hop stunt viroid* (HSVd), using molecular hybridization and RT-PCR assays. A total of 167 samples were positive for HSVd infection: 14 almond, 102 apricot, 10 plum, 8 sweet cherry, 1 bullace plum (*Prunus insititia*), 22 peach, 6 wild almond (*Prunus* ??) and 1 wild prune (*Prunus spinosa*) stone fruit species, as well as 1 apple and 2 wild apple (*Malus sylvestris*) pome fruit species, from the Argolis, Arcadia, Achaia, Eleia, Emathia, Corinthia and Pella prefectures. Nucleotide sequence analyses of 4 RT-PCR products, 2 from sweet cherry and 2 from wild apple viroid-infected trees, substantiated infection with HSVd. This viroid has not been reported previously to naturally infect apple, wild apple or bullace plum, whereas in sweet cherry it was reported this year in Turkey using only RT-PCR assay, without nucleotide sequencing. In Greece, HSVd was previously reported only in apricot, by molecular hybridization, and in citrus, by both molecular hybridization and RT-PCR. Examination of more samples, including other tree species and other Greek regions, as well as cloning and nucleotide sequencing of HSVd-positive RT-PCR samples, is under way, for obtaining Greek HSVd clones and complete nucleotide sequences, aiming at phylogenetic analyses of the viroid isolates.

**Bacterial blight on arugula caused by *Pseudomonas syringae* pv. *alisalensis* in Greece**

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Arugula (*Eruca sativa*) of the Cruciferae is an annual leafy plant that is eaten as fresh vegetable in salads. In spring 2007, leaf samples from several parts of Greece showed disease symptoms that were not recorded for any of the known diseases of arugula. Initial isolations indicated a bacterial pathogen as the causal agent. Symptoms appeared exclusively on the leaf surface. Specifically, small, water-soaked lesions 2-5 mm with or without a pale chlorotic ring, restricted between the secondary leaf veins, appeared on the adaxial and abaxial sides of the leaf. As disease developed, lesions retained angular edges, turned grey-brown and became necrotic and often with a papery appearance. They coalesced resulting in complete necrosis of the leaf. All isolations made from the infected leaves, showed the fluorescent *Pseudomonas* bacteria. In the LOPAT assays, isolations exhibited the phenotype [+ - - +] of the Ia group that includes *Pseudomonas syringae* pathovars. Based on the morphological, biochemical and physiological phenotype and on the pathogenicity of the isolates from infected arugula plants, we conclude that the causal agent is probably *Pseudomonas syringae* pv. *alisalensis*. Our results may be confirmed by the molecular identification of the pathogen. This is the first report of the bacterium in Europe.

## ***Phytophthora drechsleri* and *Phytophthora cryptogea* complex on cinerarias**

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At Marathon area (Southeastern Greece) and in Corfu Island (Northwestern Greece), a severe disease of cinerarias [*Senecio cruentus* (Masson ex L'Her.) DC.], causing a considerable loss of plants, was observed. Symptoms of the disease, identical in both locations, were root and stem rotting while the aerial parts of the plants showed extensive wilting. The older leaves wilted first, retaining the green color followed by a sudden wilting of the rest of the leaves that resulted in the death of the plant. The central axis of the diseased plants remained upright; irrigation failed to restore the plant vigour. In all cases, isolates of the genus *Phytophthora* were isolated from diseased plants; sporangia were non-papillate, oval to obpyriform, persistent on the stalk, proliferating internally. Marathon isolates grew well at 35 °C while for Corfu isolates the maximum temperature for growth was 34 °C. The morphological and physiological features of Marathon isolates are characteristic of *Phytophthora drechsleri* Tucker species. The Corfu isolate, since did not grow at 35<sup>0</sup> C, was differentiated from those originated from Marathon area, and identified as *Phytophthora cryptogea* Pethybridge and Lafferty. Symptoms observed in the field were reproduced via pathogenicity tests under greenhouse conditions. In addition to morphological-physiological characters, the ITS (internal transcribed spacer) variation of the rDNA (ribosomal DNA) gene was studied using the ITS6 and ITS4 universal primers in standard PCR reactions. Sequence comparison of the amplified ITS fragments could not assign isolates clearly to one or the other of the two species; this is also reported in the literature.

## FUNGAL DISEASES - MYCOLOGY

### Invited Lecture

#### **Mycotoxins: a significant factor for the production of safe and high quality food**

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Mycotoxins are toxic, secondary metabolites of low molecular weight produced by naturally occurring fungi. The determination of which of the many known mycotoxins are significant can be based upon their frequency of occurrence and/or the severity of the disease they cause, especially if they are known to be carcinogenic. Among the mycotoxins fitting into this major group are aflatoxins, produced by *Aspergillus flavus*, *A. parasiticus*, and other closely related fungi; fumonisins, produced by *Fusarium verticillioides*; and deoxynivalenol (DON or vomitoxin), produced by *F. graminearum*. Other fungal toxins include cyclopiazonic acid, zearalenone, patulin, ochratoxin, T-2 toxin, and certain ergot alkaloids produced by *Claviceps purpurea*. Aflatoxins are proven carcinogens, immunotoxins, and cause growth retardation in animals. Fusarium toxins, specifically fumonisins, are reported carcinogens and trichothecenes are reported immunotoxins. In the past, mycotoxin contamination of food was considered as a storage phenomenon whereby grains becoming moldy during storage allowed for the production of these secondary metabolites proven to be toxic when consumed by humans and animals. Subsequently, mycotoxins of several kinds were found to be formed during development of crop plants in the field. Many countries throughout the world have implemented a tolerance level for the presence of different mycotoxins in food for human and animal consumption. While economically strong countries have well-developed infrastructures for monitoring of internal food quality standards, people in developing countries are not protected by food quality monitoring and enforcement of safe standards within their countries. In less developed countries percentages from 22% fumonisin to 56% aflatoxin incidence have been reported. In developing countries, many individuals are chronically exposed to high levels of mycotoxin in their diet. No economically feasible processing procedures are currently available to remove toxins from foods that are already contaminated.

Mycotoxin losses and costs of mycotoxin management are overlapping areas of concern. Mycotoxin losses result from a) lowered animal production and any human toxicity attributable to the presence of the toxin, b) the presence of the toxin in the affected commodity which lowers its market value, and c) secondary effects on agriculture production and agricultural communities. Costs of mycotoxin management include testing and research necessary to try to prevent the toxins from appearing in food and feed products of affected commodities and research production practices.



Despite the extensive medical literature about the toxic effects of mycotoxins on humans and animals as well as the knowledge about the chemistry and modes of action of mycotoxins and their cost to the world, reliable solutions are still few and not applicable. As plant pathologists, this problem is still on our plate after almost 30 years of research. Economically effective solutions are those that are agriculturally and technologically based that exclude the fungi from the host and/or block the production of mycotoxins in the host substrates. Research is needed on 1) inexpensive and appropriate sampling and testing protocols for mycotoxins even at low concentrations, 2) identification and application of appropriate technologies for obtaining low grain moisture at harvest and maintaining low grain moisture during storage are needed, 3) chemical and biological control of mycotoxigenic fungi, 4) developing crop plant cultivars that are resistant (or at least not susceptible) in the field to infection by mycotoxin-producing fungi or the production of mycotoxins. Furthermore, with the complete genomes of several mycotoxigenic fungi, researchers worldwide are working at a rapid pace to identify mycotoxin biosynthetic and regulatory genes in order to find novel and long-lasting solutions in reduction or even elimination of mycotoxin production.

## Oral presentations

### Evaluation of forecasting models for potato late blight in Cyprus

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Late blight (*Phytophthora infestans*) is the most important potato disease in Cyprus. Under favourable weather conditions the disease can assume epidemic proportions causing heavy losses. Control of the disease relies on preventive sprays based on empirical data, the growth stage of the crop and the general weather conditions. This approach involves the risk of false prognosis, leading to ineffective control and excessive fungicide use. During the 2007-2008 growing season a preliminary study was carried out with nine forecast models which were compared with the conventional method applied by growers. An agrometeorological station was installed in an experimental field in the Kokkinochoria area for monitoring rainfall, temperature, relative humidity and leaf wetness. Analysis of the meteorological data was performed with the use of the CASTOR 2.0 software. The potato field was divided into two plots, one for the application of the conventional program and the other for the evaluation of the forecasting models without any fungicide applications. During the course of the study weekly disease surveys were carried out in the experimental and neighboring fields for disease detection and evaluation. Although no late blight symptoms were detected, the use of the conventional program resulted in the application of 6-7 sprays. All forecasting models suggested fewer or same number of sprays compared to the conventional system. Only one model (Wallin) predicted the absence of late blight infection. Prospects for employing the forecast schemes will be discussed.

## Diseases of greenhouse tomato fine roots in Greece

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Lack of vigour, stunting, wilting and reduced productivity indicating poor root performance often occur in greenhouse grown tomatoes in Greece. In order to determine the frequency, extend and aetiology of fine and medium size root losses, a survey was conducted in the main tomato growing areas. Because dead rootlets decompose and disappear quickly in natural soil obscuring the extent of root losses, a floatation - small mesh sieving method was used to separate fine roots from soil. Methods of *in situ* observations of roots using minirhizotron and related devices gave more accurate estimations for the fine root damage and turnover. The loss estimates were high in some instances reaching more than 50% of total fine roots and often were associated with the pathogens *Pyrenochaeta lycopersici*, *Colletotrichum coccodes* and *Pythium* spp., especially when the soil had a solanaceous crop history. There were also single cases of massive fine root attack by *Rhizoctonia solani* and *Phytophthora* sp. Attempts to reproduce root symptoms in seedlings grown in soils naturally or artificially infested with the above pathogens were not always successful indicating the involvement of other also factors. Adverse soil conditions such as unfavorable soil structure, low temperature, flooding and salinity were also associated with increased root loss and colonization by non-aggressive fungi or weak pathogens such as *Fusarium* spp., *Olpidium* sp., *Acremonium* spp., *Microascus* sp., *Alternaria* spp., *Penicillium* spp., *Aspergillus* spp., *Geotrichum* sp. and *Phialophora* spp.

**Characterization of *Rhizoctonia solani* isolates from cotton seedlings using conventional and molecular techniques**

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*Rhizoctonia solani* causes pre- and post-emergence damping-off as well as root and hypocotyl rot in cotton seedlings. The aim of this study was to characterize strains of *R. solani* isolated from diseased cotton plants in Greece. For this purpose 79 pathogen isolates were obtained during the spring of 2007, from diseased cotton seedlings from fields located in the main cotton growing areas of Greece. Among the 79 isolates examined, 13 were binucleate and 66 were multinucleate. The multinucleate isolates were characterized using several conventional characteristics such as mycelial growth rate, sclerotia production, virulence and hyphal anastomosis reactions using tester isolates of known AG groups. In addition, molecular characterization was carried out, on 33 of 66 multinucleate isolates, using the specific ribosomal internal transcribed spacer region (rDNA-ITS). Molecular analysis classified the majority of the isolates within the anastomosis group AG-4 (18 isolates to the subgroup HG-I, 1 isolate to the subgroup HG-II and 7 isolates to the subgroup HG-III) while 5 isolates belonged to AG-7, one isolate belonged to AG-2-1 and one isolate to AG-3. These data were in agreement with the data derived from the anastomosis reactions. Moreover, sequence analysis of the polymerase chain reaction (PCR)-amplified internal transcribed spacer (ITS) rDNA region was used for phylogenetic analysis.

**Infection of *Sequoiadendron giganteum* in Greece by the fungus *Neofusicoccum parvum***

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In the spring of 2006, symptoms of branch die-back were observed on 15-20 years old trees, 4-5 m tall, of *Sequoiadendron giganteum* (Lindl.) Buchholz in two localities in the area of Megalopolis, Peloponnese. Cankers exuding resin were observed on branches and the main stems of the trees. A fungus typical of the Botryosphaeriaceae in morphology was consistently isolated from the bark and the wood near the canker margins. The same fungus was also isolated from branches of a diseased tree of *S. giganteum* in the city of Lamia in central Greece (about 400 km from Megalopolis). Cultures on malt extract agar were initially white gradually becoming grey to dark grey with abundant aerial mycelium and a radial growth of 35-40 mm in 3 days at the optimum temperature of 27°C. Pycnidia were observed at the bases of dead needles in the cankered area of the branches and they were also formed in culture. Conidia were one-celled, hyaline, fusiform to ellipsoid, (15)18-20 µm X (6)7-9 µm. These data, together with phylogenetic analysis of ITS rDNA sequences, confirmed the identity of the fungus as *Neofusicoccum parvum* (Pennycook & Samuels) Crous, Slippers & A.J.L. Phillips (syn.: *Botryosphaeria parva*, Pennycook & Samuels). Pathogenicity tests were performed in the area of Megalopolis, by inoculating the lower branches of *S. giganteum* trees. Characteristic cankers, 4-12 cm in length, were formed on the inoculated branches 8 weeks after inoculation (summer 2006), while the terminal portions of some branches, proximal to the inoculation point, were killed. *N. parvum* was consistently re-isolated from the cankered areas of the branches, up to 6 cm from the inoculation point. These data provide strong evidence to suggest that the die-back disease of *S. giganteum* is caused by *N. parvum*.

**The effect of water stress on the susceptibility of cypress plants to the fungal pathogens *Seiridium cardinale*, *Diplodia cupressi* and *Pestalotiopsis funerea***

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The fungal pathogens *Seiridium cardinale* (W.W. Wagener) B. Sutton & I.A.S. Gibson, *Diplodia cupressi* A.J.L. Phillips & A. Alves and *Pestalotiopsis funerea* (Desm.) Steyaert were used in an inoculation trial with plants of *Cupressus sempervirens* L., in order to investigate the effect of water stress on fungal growth in the plant tissues and the development of cankers. Three-year-old cypress seedlings were planted in 6 L plastic pots, divided in three groups and randomly arranged in a greenhouse. Each group was inoculated with a single isolate from each fungus. After inoculation, half of the plants were grown in water stress conditions and received 150 ml of water per week, while the rest of the plants were watered twice a week with 250 ml of water each time. The water potential of plants ( $\psi$ ) in both groups was measured regularly using a pressure chamber and ranged from -3.17 MPa to -6.5 MPa in the water stressed plants, while in the plants with regular watering it ranged from -1.3 to -2.5 MPa. The plants were harvested 5 months after inoculation. Water stress favored the development of the fungus *D. cupressi*; the mean canker length ( $58.88 \pm 10.51$  mm) of the water stressed plants was significantly longer from that of the unstressed plants ( $19.82 \pm 2.03$  mm). On the other hand, the fungus *S. cardinale* created significantly longer cankers ( $39.82 \pm 1.80$  mm) on the plants grown under normal water conditions compared to the water stressed plants ( $26.84 \pm 2.41$  mm). Finally, no significant difference was observed in the mean canker length of *P. funerea* between water stressed plants ( $11.51 \pm 2.07$  mm) and those grown under normal water conditions ( $13.03 \pm 1.63$  mm). All three fungi were consistently isolated from the cankered areas of the stem.

**Molecular diversity and assessment of biological characteristics of greek  
*Colletotrichum lindemuthianum* isolates**

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The present study focuses on the characterization of *Colletotrichum lindemuthianum* isolates collected in Greece, by means of temperature effect on their biological characteristics (mycelial growth, sporulation and spore germinability) and by molecular diversity revealed by RAM and ERIC – BOX PCR analysis. Temperature effect on the assessed biological characteristics resulted in a similar classification according to isolates origin and virulence patterns. *C. lindemuthianum* isolates originated from areas of Nevrokopi and Vrodou showed better adaptation at the lower temperatures exposed (12° and 18°C) compared to isolates originated from Municipality of Hrisoupolis, which showed better adaptation at the highest temperature tested (24°C). Molecular diversity was detected using RAM and ERIC – BOX PCR primers. Both methods revealed, in a similar way ( $r = 0.58$ ,  $p = 0.05$ ), two main clusters of isolates, in agreement with previous findings using RAPD and RFLP analysis. The majority of the tested isolates were grouped in the same main cluster (29 out of 35 greek isolates for both methods), underlying high level of genotypic similarities between greek populations of *C. lindemuthianum*. This study is an extension of previous research providing further information on population diversity of *C. lindemuthianum*, which can be useful in developing more efficient control strategies of bean anthracnose disease.

## **Hypogeous fungi and prospects of truffle cultivation in Greece**

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Twenty four species of mycorrhizal fungi which produce hypogeous carpophores have been recently recorded in Greece. Among them *Tuber melanosporum*, *T. aestivum*, *T. uncinatum*, *T. brumale* and *T. borchii* are known worldwide for their gastronomically precious truffles. The recording and also the commercial picking of wild truffles, the recent production of truffles from established truffle plantations but also historic documents which reveal the presence of truffles in Greece since antiquity lead to the conclusion that truffle cultivation is possible in Greece. Given that truffle plantations need well draining, sloppy land and poor, alkaline soil, truffle cultivation could exploit a significant area of mountainous and hilly fields. If two more features are added, one that truffle cultivation is by nature organic and second that it may generate a high income, then truffle cultivation may be an attractive alternative for Greece.



## Interactions between *Erysiphe alphitoides* (oak powdery mildew) and fungi naturally associated with it

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Powdery mildew (*Erysiphe alphitoides*) on oak (*Quercus robur*) was common on trees of different height-classes but was more common on trees of 3-9 m. *E. alphitoides* was extremely commonly intimately associated with a variety of other mycoparasitic fungi in nature. Co-existence of five genera, namely *Acremonium*, *Trichoderma-Cladosporium*, *Leptosphaerulina*, *Ampelomyces-Phoma*, *Tilletiospsis*, with *E. alphitoides* was studied through stratified sample surveys for 2.5 years. Nearly 90% of mildew colonies were associated with *Leptosphaerulina* sp., which is not normally an antagonist of powdery mildew. Certain antagonist-combinations were observed more commonly than others in non-random combinations. For example *Leptosphaerulina* sp. relationship with *Acremonium* sp. appears to be synergistic, and *Leptosphaerulina* sp. had also a number of strong relationships with the other antagonists. The time of specimen collection (summer or autumn) has a significant effect on antagonist populations. Populations of *Trichoderma-Cladosporium* sp. were higher in autumn but of *Acremonium* sp. in summer; this could be due to different life-cycles or due to associations, relationships and interactions between the antagonists or between each antagonist and its host. Mechanisms leading to these associations and their implications will be discussed. The variety and high population densities of *E. alphitoides* antagonists during this study suggest that antagonists are an important factor in determining the final density of *E. alphitoides* and the damage caused by the pathogen.

## Poster Presentations

### Discrimination of the plant pathogen *Verticillium dahliae* races 1 and 2 performing <sup>1</sup>H NMR fingerprinting

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*Verticillium dahliae* Kleb. is a soil-borne plant pathogen that causes a vascular wilt and heavy losses to a large number of crops. Until now, two *V. dahliae* races have been identified differing in their pathogenicity; race 2 which is pathogenic to all tomato varieties and race 1 for which resistance gene in tomato plants has been reported. Until today, *Verticillium* races discrimination has been based on pathogenicity tests and molecular techniques. Their rapid and reliable discrimination is of great importance for crop protection. Since metabolic fingerprinting applying <sup>1</sup>H NMR spectroscopy has given good results in discrimination and identification of metabolic differences in biological systems, a metabonomic approach for the rapid and reliable discrimination of *Verticillium* races was developed. The identity of the isolates used in the present study was identified by a pathogenicity test prior to metabonomic analysis. Analyses results applying principal components analysis (PCA) and partial least squares-discriminant analysis (PLS-DA) revealed metabolic differences between *V. dahliae* races 1 and 2 that can be used for their rapid and reliable classification.

## Characterization of *Verticillium dahliae* isolates by fluorescent Amplified Fragment Length Polymorphisms (fAFLPs)

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*Verticillium dahliae* is a polyphagous plant fungus of high economic significance. A degree of genetic diversity exists among *V. dahliae* isolates within and between vegetative compatibility groups (VCGs) that bears phytopathological significance and is worth investigating employing genetic tools of higher resolution than VCGs. Due to their capacity to generate a large number of genome-wide genetic markers amplified fragment length polymorphisms (AFLPs) have been used in genetic, population and evolutionary studies of different fungal species. In the present study AFLPs was used to study genetic variation among 101 isolates of *V. dahliae* from different VCGs (VCGs 4B, 4AB and VCGs 2A, 2B, 2AB), pathotype (tomato vs. eggplant), races (1 vs. 2), geographic origin and hosts. A dendrogram based on the AFLPs data revealed four main clusters (A, B, C, D), in partial concordance to VCGs subgroups. Analysis of molecular variance (AMOVA) demonstrated that isolates within a VCGs subgroup are molecularly similar, to the extent that clustering of isolates correlated with VCGs subgroups regardless of geographic origin and the host source. VCGs differed in molecular variability; the most variable ones being VCG4B and VCG4AB. Tomato isolates were grouped in four clusters clearly distinct from isolates derived from other plant hosts. Conversely, no significant molecular distinction was uncovered either between tomato and eggplant pathotypes or between race 1 and 2.

**Pathogenic ability estimation of the races 1 and 2 of the fungus *Verticillium dahliae* by using EGFP biomarker**

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The virulence of races 1 and 2 of the soilborne fungus *Verticillium dahliae* transformed with the *egfp* gene ( $Vd_{EGFP}$ ) was studied on roots of the tomato varieties Ace 55VF (resistant to race 1, susceptible to race 2) and Planet 96FI (susceptible to both races). Tomato plants were inoculated into a greenhouse with conidia suspension of  $Vd_{EGFP}$  and root samples were collected regularly for estimating the relative fluorescence via digital fluorometer of the extracted EGFP. The disease was started since 3 dpi (days post inoculation), max relative fluorescence value was observed on 5 dpi (Planet 96FI) or 7 dpi (Ace 55VF), whilst to the following dpi the relative fluorescence from tomato root samples of the macroscopically asymptomatic until 14 dpi plants was decreased. However, the relative fluorescence to the resistant variety Ace 55VF against race 1 remained to low levels. Similar results were also derived by the relative pathogenicity test. Using the innovative EGFP technique, wilt pathogen biomass was evaluated easy and fast into plant tissues.

**Characterization of *Rhizoctonia solani* isolates from tobacco seedlings using conventional and molecular techniques**

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*Rhizoctonia solani* is a soil borne pathogen that causes economically important diseases in many crop species. It is known to cause damping-off as well as crown rot in tobacco seedlings. The aim of this study was to characterize isolates of *R. solani* associated with tobacco in Greece. For this purpose 89 pathogen isolates were obtained during the spring of 2007, from diseased tobacco seedling plants from nurseries located in the main tobacco-growing areas in Northern Greece. The isolates were characterized using several conventional characteristics such as number of nuclei, mycelial growth rate, sclerotia production, virulence and hyphal anastomosis reactions using tester isolates of known AG groups. Among the 89 isolates tested, 87 were multinucleate and only 2 were binucleate. Molecular characterization was carried out in 39 out of 87 multinucleate isolates, using sequence analysis of the genomic regions encoding the internal transcribed spacers ITS1 and ITS4 for AG determination. Molecular analysis classified 27 of the isolates within the anastomosis group AG-2-1, 11 isolates belonged to AG-4 (6 isolates to the subgroup HG-I and 5 isolates to the subgroup HG-III) and a single isolate belonged to AG-5. Moreover, sequence analysis of the polymerase chain reaction (PCR)-amplified internal transcribed spacer (ITS) rDNA region was used for phylogenetic analysis. This is the first study of the relative AG composition of *R. solani* populations causing disease in tobacco seedlings in Greece.

**Biodegradation of a mixture of the herbicides linuron and metribuzin by the white rot fungus *Trametes versicolor***

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The white rot fungi are a physiological rather than taxonomic grouping, comprising those fungi that are capable of extensively degrading lignin. Due to this ability many studies were carried out on degradation of different toxic pollutants. In this study, the ability of the white rot fungus *Trametes versicolor* to degrade a mixture of the herbicides linuron and metribuzin, in different concentrations and water stress conditions, was investigated. We used a nutritionally poor soil extract broth: alone (-0.7MPa) and amended with KCl or glycerol to increase water stress (-2.8MPa). Every week and for six weeks herbicides remaining were determined by HPLC. Results showed that linuron was completely degraded within 5 weeks regardless of treatment, while metribuzin was degraded by 50%, only in the soil extract broth amended with glycerol.

## Pathogens causing preharvest fruit rots on peaches in Imathia prefecture

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Peach is one of the most important crops of fruit trees in Greece. The counties of Imathia and Pella in northern Greece are considered the largest producer in canning peaches for domestic and foreign markets, although large quantities of fresh market peach and other stone fruits are also produced. Because of its low elevation and its proximity to the Aegean sea, this area is subjected to high humidity that favors infection of stone fruit by fungi causing preharvest and postharvest decays. The main aims of this investigation were to determine the main pathogens causing fruit rots of mature peaches in Northern Greece, the major peach producing area of Greece. Fungi of genus *Monilinia* were responsible for about 70% and 78% of rotted peaches in 2005 and 2006, respectively. Fungi of the genus *Fusicoccum* caused damages in a percentage of about 30% in the area Mesi Verias – Ammos Verias – Meliki Imathias. In contrast, this pathogen was responsible for less than 3% of rotted peaches in other investigated areas. Damage up to 5% was also caused from the fungi of genus *Phomopsis*. Other pathogens isolated from rotted peaches at a percentage lower than 5% were *Alternaria alternata*, *Aspergillus niger*, *Aspergillus flavus*, *Botrytis cinerea*, *Sclerotinia sclerotiorum*, *Fusarium* spp., *Colletotrichum gloeosporioides*, *Rhizopus stolonifer* and *Gilbertella persicaria*. Until now, to manage preharvest fruit rots, spraying was applied almost exclusively to control fungi of the genus *Monilinia*. This programme should include sprays against *Phomopsis*. Moreover, for the area Mesi Veria - Ammos Veria - Melikis Imathia, spraying should be applied to control fungi of the genus *Fusicoccum*.

## Management of latent infections in peaches caused by fungi of genus *Monilinia*

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Brown rot, caused by the fungi of genus *Monilinia*, is worldwide one of the major diseases of the peaches, especially in humid areas. Problems from this disease occur mainly in the flowering stage, the stage of the fruit ripening and over the fruit maintenance. Although this pathogen does not cause serious damages in the period from the fall of the petals to the stage of the fruit ripening, relative publications have demonstrated potential latent infections which indeed seem to play an important role in the development of the disease. The purpose of this work was to investigate the effect of spray applications in reducing the percentage of latent infection of the peaches. The experiments were conducted in the experimental orchard of the Pomology Institute Naoussa (NAGREF). Spraying applications were conducted in nectarine trees (cvs Fantasia, Venus and Tasty Free) on 16 May and 2 June on different trees. The fungicide used was thiophanate methyl. For the recording of latent infections, the "ONFIT" method was used. Data were collected by recording the percentage of fruits infected from *Monilinia*. Identification of pathogen was based on morphological characteristics. For each treatment, there were 6 trees (replications), while 50 fruits were collected from each tree. Unsprayed trees were used as control. In addition, the percentage of rotted fruits in each treatment was recorded in the stage of ripening fruit. The results showed that the percentage of latent infection was significantly lower in the sprayed trees in comparison to untreated control in all three cultivars used. Trees sprayed on 2 June showed significant lower percentage of latent infection than those sprayed on 15 May. Similarly, the sprayed trees showed significant lower percentage of fruit rots in comparison to untreated control with those sprayed on 2 June having the lowest one. The results of this work showed that a spray of nectarines in early June can significantly reduce the percentage of the fruit rots in peaches caused by the fungi of the genus *Monilinia*.



## **Monitoring and assessing turf canopy health using color and near-infrared digital imagery**

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The possibility of using ground based color and infrared digital imagery for the estimation of turf canopy health and early detection of plant stress and disease was investigated. Different stress agents were applied in 3 m<sup>2</sup> plots of a *Festuca arundinacea* lawn and stress severity was estimated by use of arbitrary visual rating and estimates from digital image analysis data. The Hue, Saturation and Intensity (HSI) color space was successfully implemented to develop a simple model for the estimation of turf health and quality based on color variations of the turf canopy. The near-infrared reflectance data and the use of polarizing filter gave no additional information. Results obtained with image analysis correlated significantly with those of visual ratings and were more consistent and accurate in estimating the daily stress progress in plots of induced drought, shading, increased UV-B radiation, N-deficiency, and herbicide toxicity. High accuracy, repeatability and rapidity obtained when photography and image analysis were performed under a standard operational procedure. The camera was calibrated and adjusted for brightness and white balance of the incident ambient light before shooting. A neutral grey or color palette of known reflectance on the lawn was used as reference for fine white balance and light intensity calibration during image processing. The method is practical, accurate and rapid, and can be used for turf surveillance in spotting of early stages of adverse conditions and disease development.

**Effect of heavy metals Zn, Pb, Cd in combination with nitrogen fertilization on soil microflora and phytopathogenic fungi in a lettuce pot experiment**

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Environmental pollution is leading to changes on soil physico-chemical properties and has a negative impact on microbial processes which consequently alter the existing microbial community. With the change in soil microbial activity both the competitive activity of soil microflora and the properties of soil-borne pathogenic fungi are altered. The aim of this work was to investigate the impact of various concentrations of the heavy metals Zn, Pb, Cd in combination with applied nitrogen fertilization on microflora biodiversity and activity of soil where lettuce plants were grown in pots under greenhouse conditions. As the basic measurement unit of the active soil microbial population, the method of measurement of soil microbial biomass and of soil evolution ( $R_{\text{Basal}}$ ) before and after the addition of active material ( $R_{\text{SIR}}$ ) was chosen. The quantitative and qualitative assessment of different groups of microorganisms and of soil phytopathogenic fungi in soils was performed using standard dilution spread plate method and MPN method. In total, eight different medium plates were used. A significant interaction between Nitrogen and Zn, Pb, Cd heavy metals on the number, activity and biodiversity of different groups of soil microbial populations was evident.

## **First report of chestnut blight in the islands of Lesvos and Crete**

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Chestnut blight, a bark disease caused by the fungus *Cryphonectria parasitica*, was first recorded in Greece in 1963 in Zagora, Mount Pelio by D. Biris. In the next three decades it was spread all over the mainland country where chestnut is cultivated. Until recently, no sign of disease was detected in the chestnut orchards of Crete and Lesvos probably because of the use of the indigenous planting material. The two islands were declared as a Protective Zone for chestnut blight in 2002. In 2006 we detected disease symptoms in an orchard in Chania, Crete and in Agiassos, Lesvos. Bark samples were collected from both areas. All the isolates determined for their vegetative compatibility type (vc type). Two vc types were identified, EU-1 and EU-12 with the majority of isolates belonging to the vc type EU-1. In mainland Greece the most widespread type is EU-12 while EU-1 has been reported only in Evoia and Pieria counties. None of the isolates was found to be infected by the *Cryphonectria hypovirus* (CHV1) which causes hypovirulence. The two new areas have been already included in the next biological control project which will be financed by the IV EU Supporting Programme 2007-2013.

## Molecular polymorphism between populations of *Pseudoperonospora cubensis* from Greece and the Czech Republic and their phytopathological and phylogenetic implications

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Molecular genetic polymorphisms within *Pseudoperonospora cubensis* (Berk. and Curt.) Rostov. (Chromista, Peronosporales) isolates of different geographic origins were investigated to establish phylogenetic relationships. Thirty isolates originating from Greece (Crete; 15), Czech Republic (13), the Netherlands and France (one isolate from each country) were analysed with two distinct molecular techniques (AFLP and *ITS rDNA* sequence analysis). All isolates were obtained from cucumber (*Cucumis sativus* L.) plants showing typical downy mildew symptoms. Sequence analysis of the *ITS* region of rDNA is a well established taxonomic tool within the genus *Pseudoperonospora*. However, AFLP typing has not been used previously for molecular polymorphism analysis in this species. AFLP fingerprinting produced ample polymorphisms and grouped isolates along two separate clusters; one included the Czech (Central Europe) and West European (the Netherlands, France) isolates, while the other only the isolates of Greek origin. Within each group there was some variation which could be accounted for by geographic origin and pathogenicity. *ITS rDNA* analysis showed no variability among isolates in *ITS-1*; however, all *ITS-2 rDNA* sequences of Greek and Czech isolates clustered together with isolates from Austria (NCBI access) forming a large cluster together with *P. humuli* indicating their close taxonomic relationship. Application of classical phytopathological and molecular approaches in the research of genetic structure and dynamics of *P. cubensis* populations is discussed.

**Evaluation of the reaction of five Greek olive cultivars to the plant pathogens *Spilocaea oleagina*, *Mycocentrospora cladosporioides* and *Pseudomonas savastanoi* pv. *savastanoi***

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Olive scab (*Spilocaea oleagina*), cercosporiosis (*Mycocentrospora cladosporioides*) and olive knot (*Pseudomonas savastanoi* pv. *savastanoi*) are among the most frequently observed diseases in olive orchards in Greece, especially in regions with high humidity. The aim of the present work was to evaluate the reaction of the most widely-grown Greek olive cultivars Kalamon, Amfissis, Koroneiki, Adramitini and Manaki to artificial infections by representative strains of the three pathogens (as this was shown by the molecular diversity analyses). For this purpose, fifteen trees of each cultivar were artificially inoculated either by spraying their leaves with a fungal suspension or injuring their twigs and pouring a dense bacterial suspension. One month later, it was shown that cv. Kalamon is the most tolerant cultivar to both fungal pathogens. On the other hand, the cv. Amfissis is the most susceptible cultivar exhibiting a high percentage of defoliation. Furthermore, cv. Amfissis is also the most susceptible cultivar to *P. savastanoi* pv. *savastanoi*, while cv. Koroneiki is the most tolerant, under our experimental conditions.

## Infections in olive orchards of Messinia prefecture by the fungus *Omphalotus olearius*

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In two localities of Messinia prefecture, Peloponnese, symptoms of root rot have been observed in recent years on olive trees uprooted by the wind as well as on trees uprooted for land use change of the orchards. No symptoms were observed on the crown of the trees although a part of the root system was rotten and in some cases the decay was extended into the lower part of the trunk. The wood of the roots showed symptoms of white rot being spongy and friable, white-brown in colour. The fungus *Omphalotus olearius* (DC.: Fries) Singer was consistently isolated from the rotten wood of the roots. The mycelium of the fungus in malt extract agar was yellowish in colour and heterokaryotic, as concluded from the presence of clamp connections. The identity of *O. olearius* was confirmed in mating tests using homokaryotic single-spore isolates from basidiocarps of the fungus. *O. olearius* had been reported in Greece as the cause of “Gelatina” disease of olive roots by Kougeas in the 1960’s, however, there are no detail reports in the international literature on this fungus as a plant pathogen. In 2004, inoculations were performed on the stems of 5-year-old olive trees of Koroneiki variety, using a heterokaryotic isolate of *O. olearius*. No symptoms were observed on the tree crown three years after inoculation, but the fungus had grown along the stems of the trees causing discoloration of the wood at 7-25 cm in length and in some cases small cankers had formed 3-4 cm in length. The fungus was consistently isolated from the discolored wood. *O. olearius* does not appear to have the ability to cause tree mortality, however, the extensive root rotting affects tree physiology and productivity and in some cases predisposes the trees to wind fall.

## BACTERIAL DISEASES

### Poster Presentations

#### ***Pseudomonas viridiflava*: Causal agent of a bacterial disease of *Syngonium podophyllum***

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*Syngonium podophyllum* is an ornamental plant that develops well indoors and outdoors. During the spring of 2007, potted *Syngonium* plants, in one of the TEI–Crete greenhouses, showed severe leaf spotting that gradually resulted in complete foliage necrosis. Disease incidence started with the appearance of water-soaked lesions between the secondary leaf veins. Gradually lesions turned grey to brown-black and became necrotic. Usually, appearance of symptoms starts from the edge of the leaf spreading towards the centre of the leaf surface. Under conditions of high relative humidity, foliage necrosis proceeds fast. Although destruction of the whole plant is rare, loss of its qualitative characteristics is significant. The bacterium *Pseudomonas viridiflava* (Burkholder) Dowson was identified as the causal agent of the disease based on the morphological, biochemical and physiological phenotype and on the pathogenicity of the strains isolated from infected plants.

**Evaluation of resistance of cultivated walnut varieties, selections and crosses to *Xanthomonas arboricola* pv. *juglandis* in Greece**

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Thirty three walnut varieties (16 lateral and 17 terminal fruit fullness varieties), 12 selections and 13 walnut crosses (*Juglans regia* L.) were evaluated for their resistance to *Xanthomonas arboricola* pv. *juglandis* by artificial inoculations *in vitro*. A mixture of four *X. arboricola* pv. *juglandis* strains isolated from walnut natural infected nuts in Greece was used. The inoculum had a concentration of 1-2 x 10<sup>7</sup> cfu/ml and it was injected to nuts with a syringe. As control nuts were injected with sterile distilled water. The inoculated nuts were kept at 25 °C for two weeks in plastic chambers with the appropriate moisture. The experiments were conducted the second week of June 2007 and 2008. The inoculated nuts had a diameter of 2-4 cm. Disease incidence of nuts was assessed with a six level scale of damage: 0 = healthy, 5 = heavy infected (large necrotic areas around the injection spot). The nuts injected with the bacteria, in all treatments showed typical bacterial blight symptoms compared to control. The bacterium was always reisolated on selective medium (brilliant cresyl blue-starch medium). The least susceptible cultivars were Amigo and Iliana and the selection EK-1.



**Application of phenotypic and molecular typing techniques for determining variability of greek pectolytic isolates of *Pectobacterium carotovorum* subsp. *carotovorum* and *Dickeya* spp.**

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Soft rot bacteria infect a wide range of crops world-wide, including potato, cabbage, cucumber, tomato and ornamental plants. The strains associated with soft rot of potato have been studied extensively because of their economic importance. On the basis of results from the examination of samples of potato plants and tubers showing soft rot symptoms, *Pectobacterium carotovorum* subsp. *carotovorum* (Pcc) (συν. *Erwinia carotovora* subsp. *carotovora*) and *Dickeya* spp. (συν. *Erwinia chrysanthemi*) seems to be the main pathogenic agents associated with potato disease, whereas *Pectobacterium atrosepticum* (συν. *Erwinia carotovora* subsp. *atroseptica*) is less frequent in Greece. To investigate phenotypic and genetic diversity of greek populations of Pcc and *Dickeya* spp., 25 strains for each bacterial species, isolated from potato and other host plants from different geographical areas, were used. The variability within the isolates was studied in regard to morphological characteristics of the colonies on three nutrient media, enzymatic activity, metabolic profile and physiological traits. Pathogenicity assays both on potato stems and tubers are under way. PCR techniques are employed for detection of *pel* and *hrp* genes, while genetic fingerprinting techniques BOX-PCR, REP-PCR, ERIC-PCR and ITS-PCR combined with RFLP provide a comprehensive view of the genetic variability. Understanding the diversity within and relationships among pathogenic taxa is an important prerequisite to improve detection systems, control measures and strategies of breeding for resistance to soft rot bacteria.

**First report of natural infection of watermelon plants and fruits by the phytopathogenic bacterium *Acidovorax avenae* subsp. *citrulli* in Greece.**

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Samples of watermelon fruits, hybrid F1 Obla, sent to the Laboratory in July 2005 from the district of Chrysoupoli (Kavala) and samples of watermelon fruits, hybrid F1 Obla as well, sent to the Laboratory in September 2006 from the district of Vagia (Thibes), showed very small, irregularly shaped, water-soaked lesions and brown cracks of the fruit surface. The percentage of infected fruits was high in the first district and 30% in the second one, according to the sample information sheets. Fruit sections revealed brown discoloration of the rind, water-soaked area underneath the lesions and complete watery rot of the flesh of the fruit. From the lesions and cracks, pure cultures of a bacterium were recovered and identified as *Acidovorax avenae* subsp. *citrulli* (Aac) based on cultural, physiological, biochemical and pathogenicity assays. Pathogenicity of these isolates was confirmed on watermelon, melon, cucumber and pumpkin seedlings. Subsequently, in May 2008, a sample of young grafted watermelon plants, hybrid F1 Byblos, from a nursery at the district of Varda (Ilia) was sent to the Laboratory showing symptoms of brown, almost angular necrotic spots and larger necrotic areas on their leaves. According to the sample information sheet, 50% of the plants in the nursery were infected and exhibited severe symptoms. Streaming of bacterial cells from the edges of cut lesions was microscopically observed. The bacteria recovered from the lesions were identified as Aac, based on cultural, physiological, biochemical, serological, molecular and pathogenicity assays on fruits and seedlings of various cucurbits. This is the first report of natural infection of watermelon plants and fruits by the phytopathogenic bacterium *Acidovorax avenae* subsp. *citrulli* in Greece.

## VIRAL DISEASES

### Invited Lecture

#### Emerging virus diseases of vegetable and ornamental crops in Mediterranean countries

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This short review addresses some of the new “emerging” virus diseases economically relevant for vegetable and ornamental crops in the Mediterranean countries. Viruses like *Tomato yellow leaf curl sardinia virus* (TYLCSV) and *Tomato yellow leaf curl virus* (TYLCV), genus *Begomovirus*, were reported to cause heavy infections and substantial crop losses to tomato and other vegetable crops growing mainly in greenhouses (Apulia, Sardinia and Sicily 2001-2003). Similar situations were observed last years in Spain and Greece (2001-2006), with strong and destructive infections in different places of both countries. TYLCSV and TYLCV are transmitted in nature by the whitefly *Bemisia tabaci* (biotypes A and B) in a persistent manner. Two more viruses, i.e. *Tomato chlorosis virus* (ToCV) and *Tomato infectious chlorosis virus* (TICV), genus *Crinivirus*, affected severely tomatoes in Italy especially in southern areas. Explosive epidemics have taken place since 2001 in Portugal, Greece and Spain causing crop losses higher than 30%. *Tomato infectious chlorosis virus* (TICV), infections have recently been observed in lettuce and escarole in southern Italy. ToCV is transmitted in nature by *Bemisia tabaci*, *Trialeurodes abutilonea* and *T.vaporariorum*, and TICV by *T. vaporariorum*, both in a semi-persistent manner. Infections to cucurbits by two additional criniviruses, *Cucumber yellow stunting disorder virus* (CYSDV) and *Beet pseudo-yellow virus* (BPYV), were reported from different Greek provinces. Both viruses are transmitted by *T. vaporariorum* and *B. tabaci* in a semi-persistent manner. Tospoviruses (TSWV and INSV) are pathogenic in a high number of vegetable, ornamental and wild plant species, spread in nature by thrips (*Frankliniella occidentalis* and *Thrips tabaci*) and are endemic in many countries. Both tospoviruses along with TYSCSV and TYLCV, occur often in epidemic form and represent the most destructive pathogens for tomato, pepper, eggplant, lettuce, chicory and ornamentals like gerbera, lisianthus, anemone, *Ranunculus sp.* etc. grown in the field and under plastic cover. In the last two years new virus were found to infect some hosts (some wilds) in different countries. A *flexiviriade* was isolated and studied from *Phlomis fruticosa* in Greece. Olive latent virus (OLV-2) was isolated from wild castor bean (*Ricinus communis*) in Greece and South Italy. A rhabdovirus (EBDV) that induced yello-vein symptoms in *Hibiscus rosa-sinensis* was studied in South Italy. In addition, a tombusvirus infecting different cultivars of *limonium sp.*, an ornamental plant, has been studied just recently.

## Oral Presentations

### Methods of *in vitro* microindexing of viroids in citrus

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The detection of citrus exocortis and related viroids relies mainly on biological indexing on the indicator plant Etrog citron in the glasshouse in conjunction with the RT-PCR test. Biological indexing is a time consuming and expensive method. A new laboratory technique was explored as an alternative procedure to biological indexing of exocortis and related viroids. This laboratory technique is a combination of the shoot-tip grafting technique *in vitro* and the biological indexing method on indicator plants. Three *in vitro* laboratory methods were used and compared with the classical biological method. With the classical *in vivo* method, diagnosis is based on the expression of symptoms on indicators 11-14 weeks after inoculation. With the first *in vitro* method, "Microindexing *in vitro* of citron seedlings by graft inoculation", diagnosis was possible twelve days after inoculation. With the second method, "Microindexing *in vitro* of citron cuttings by graft inoculation", twenty days after inoculation were needed for appearance of diagnostic symptoms and with the third method, "Microindexing *in vitro* of citron cuttings by injection inoculation", forty days after inoculation were required for viroid diagnosis. Inoculated Etrog citron plantlets grown *in vitro* and tested by RT-PCR showed the same viroid content present in the source plants. The *in vitro* microindexing methods for viroids were quicker than the classical *in vivo* method and could replace it. Of the three *in vitro* viroid indexing methods, microindexing on cuttings *in vitro* by graft inoculation was easier and more reliable than microindexing either on seedlings or on cuttings by injection.

## Seed transmission of *Pepino mosaic virus* in tomato

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*Pepino mosaic virus* (PepMV, genus *Potexvirus*), member of EPPO's alert list, is a quarantine virus in Greece and a potential important danger for the country's tomato industry. Within the frame of a European research project (PEPEIRA, STREP FP6) aiming at providing a robust and scientifically sound assessment of the risk that PepMV poses in Europe, an extensive trial on virus seed transmission was carried out to confirm earlier results on limited seed numbers. More than 100,000 seeds were harvested from tomato plants mixed infected with the two common virus strains. This work was performed in the premises of the Belgian participant and the resulted seeds were distributed to the 10 laboratories participating in this study. Seedlings were grown in plots of 10, since the virus is mechanically easily transmitted by contact, and 5 weeks after being germinated each plot of 10 was ELISA tested as a composite sample. Twenty-three out of in total 8,778 plots tested (0.026%) were positive, thus verifying that seed transmission of PepMV can occur, even though at a low level. Differences were observed in the obtained results according to the time interval between virus infection in the mother plants and seed harvest. Seeds harvested 8 weeks after PepMV inoculation resulted in a seed transmission rate of 0.005%, while seeds harvested 15 weeks after PepMV inoculation resulted in a significantly higher transmission rate of 0.057%. The conclusive overall low level of seed transmission (0.026%), obtained under 'worst case scenario' conditions, will be considered in the drafting of the Pest Risk Assessment (PRA) that will be one of the outcomes of the PEPEIRA project.

## **Epidemiology and characterization of Begomoviruses and *Bemisia tabaci* biotypes in Greece and Cyprus**

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In view of a bilateral project between Greece and Cyprus (2006-2008), the epidemiology and molecular characterization of begomoviruses involved in the etiology of tomato yellow leaf curl disease (TYLCD) and molecular identification of biotypes of *Bemisia tabaci* Gen. (Hemiptera: Aleyrodidae) was studied. A total of 5000 samples of tomato, bean and pepper plants and approximately 1000 adult *B. tabaci* were collected from 9 prefectures of Greece and 5 Districts of Cyprus. Results showed that TYLCV is widespread on tomato crops and arable weeds in the mainland of Greece, Crete, Rhodes Island and Cyprus. TYLCSV was only found on Peloponnese and Crete in a very low incidence. TYLCV is also reported for the first time in Greece (Peloponnese) and Cyprus, to cause leaf crumble symptoms on bean plants. Molecular identification of *B. tabaci* biotypes showed that Q is the only biotype found in the mainland of Greece and Crete, whereas biotype B is reported for the first time on Rhodes Island. In the Cypriot *B. tabaci* populations both B and Q biotypes co-exist with biotype B being more widespread in the island.

## Molecular studies on plant virus members of the genus Crinivirus

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The complete nucleotide sequence of the bipartite genome of a Greek isolate (Gr-535) of *Tomato chlorosis virus* (ToCV) was determined. Phylogenetic analysis and pairwise nucleotide and amino acid sequence comparisons showed that the Greek isolate clusters together with the American ToCV isolate. Nevertheless, the Greek and Spanish isolates share several common deletions and extra stretches of nucleotides in the untranslated regions of their genomes when compared to the American isolate suggesting genetic recombination. Prediction of putative structures of the 3'-terminus of ToCV RNA 1, showed the presence of four stem loops and a pseudo-knot, while the putative structure of the 3'-terminus of ToCV RNA 2 varied between the three sequenced isolates. Diagnostic dot-blot hybridization and reverse transcription–polymerase chain reaction (RT-PCR) assays indicate that ToCV can easily be detected in 20 ng of total RNA extracts from infected plants. Dot-blot hybridization can also be performed for virus diagnosis using infected crude plant extracts. Transgenic *Nicotiana benthamiana* plants were infiltrated using *Agrobacterium tumefaciens* to express locally GFP alone or in combination with either ToCV p22, *Cucurbit yellow stunting disorder virus* (CYSDV) p22 or CYSDV p25. These experiments indicated that ToCV p22 and CYSDV p25 represent suppressors of gene silencing.

**Stability of resistance of tobacco transgenic plants  
against Tobacco rattle virus (TRV)**

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Tobacco transgenic plants harboring portion of the replicase gene of TRV were produced and exhibited resistance against the two most genetically distinct isolates of the virus after either mechanical virus inoculation or challenge by viruliferous vector nematodes. Resistance characteristics were in accordance with operation of resistance through RNA silencing. Here, the possible influence of the plant developmental stage, of low temperature and of plant infection by heterologous viruses on the stability of resistance and transgene expression, was studied. None of the factors examined influenced transgenic resistance systemically. The possibility of reduced efficiency of transgenic plants resistant to viruses in field conditions is further discussed.



## The presence of *Peach latent mosaic viroid* (PLMVd) in Greece

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*Peach latent mosaic viroid* (PLMVd) is the causal agent of an economically important disease of peach, responsible for reduction of fruit quality and tree vigor. In Greece, PLMVd has been reported for the first time in 2001, in pear and wild pear samples, and later (2004) in apricot. On the other hand, in Greece, there is no information about the presence of this viroid in its main host, peach and its germplasm. In this study the presence of PLMVd in peach, and other *Prunus* species, in pome fruit species and also in peach germplasm (nursery stock), was examined. Leaf samples were collected from the following regions: Aridaia (Pella), Ano Lehonia (Magnesia), Klenia and Agios Vasilios (Argolida) and Naousa, Institute of Deciduous Trees (Imathia). The presence of the viroids was ascertained applying the methods of RT-PCR, slot-blot hybridization and sequencing. Also, for first time internationally, the method of Reverse Transcription Loop-Mediated Isothermal Amplification, (RT-LAMP) was used for the detection of PLMVd. Peach (48/53), plum (11/24), apricot (4/15) and cherry (2/15) samples were found infected with PLMVd. The viroid was also detected in pear, wild pear and quince samples. It was interesting that more than the 50% of peach germplasm examined was found infected.

## **Sensitive and rapid detection of Peach latent mosaic viroid (PLMVd) by Reverse Transcription Loop-Mediated Isothermal Amplification (RT-LAMP) method**

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A reverse transcription loop-mediated isothermal amplification method (RT-LAMP) for the detection of *Peach latent mosaic viroid* (PLMVd) was developed. LAMP is a novel nucleic acid amplification method quite simple, performed under isothermal conditions (60-65°C) with high specificity, efficiency and rapidity. It is characterized by the use of four different primers: F3 (forward outer primer), B3 (backward outer primer), FIP (forward inner primer), BIP (backward inner primer) specifically designed to recognize 6 distinct regions on the target sequence. Four primer sets (OLD, OLD1, NEW and FUCUTA'S) were designed originally. Based on initial experiments the set OLD1 was selected for further evaluation. Simple and accelerated RT-LAMP were performed using degenerate and no degenerate F-Loop and B-Loop primers. Degenerate primers were selected, and after their best concentration had been determined (0,8 µM), the reaction performed in different temperatures (60-67.5 °C) using Betaine concentrations 1x, 1/2x and 1/4 x. Optimal conditions were found 62.5 °C and 1/2 x Betaine. Under these conditions, using tRNA as template, PLMVd could be detected within only 32 min, compared to 180 min of RT-PCR, using the Real Time Turbimeter (LA200, Teramecs) which measures the turbidity caused by the production of insoluble magnesium pyrophosphate. In addition, the RT-LAMP method was found to be more sensitive than the RT-PCR. PLMVd was detected in Greek samples of peach, plum apricot, pear, wild pear and quince samples.

## Survey of Stonefruits for Virus Diseases in Cyprus

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In the context of the programme “Viruses, sanitation and molecular characterization of stonefruits in Cyprus”, which is funded by the Cyprus Research Promotion Foundation, a survey was conducted for virus diseases which affect peaches, nectarines, plums and apricots on the island. During spring and summer of 2007 and 2008 samples were collected from 20-30% of the trees of each orchard in the main stonefruit growing areas of Cyprus. Every sample consisted of shoots and leaves from the four sides of each tree and was tested by enzyme-linked immunosorbent assay (ELISA) using polyclonal antibodies for *Plum pox virus* (PPV), *Prunus necrotic ringspot virus* (PNRSV), *Prune dwarf virus* (PDV), *Apple chlorotic leafspot virus* (ACLSV) and *Apple mosaic virus* (ApMV). Results from 2667 samples showed that the most widespread virus disease on stonefruits is PDV with an incidence of 36.7%, while PNRSV and PPV follow with an incidence of 10.5%, and 9.6%, respectively. Apple chlorotic leafspot virus (ACLSV) and ApMV are less widespread with an incidence of 3.3% and 0.9%, respectively.

## Poster Presentations

### Cloning and characterization of a mild isolate of *Plum pox virus* (PPV)

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A mild not aphid-transmissible isolate of *Plum pox virus* (PPV) was obtained after low temperature treatment of the isolate PPV-D-GR in *Nicotiana benthamiana* and was designated as PPV-B2. Moreover, PPV-B2 multiplied poorly and caused no symptoms in *N. clevelandii*. A full length clone of the PPV-B2 genome of 9786 nucleotides was obtained after amplification using RT-Long Template PCR. Subsequently, PPV-B2 and PPV-D-GR were fully sequenced from both ends and aligned. The sequence analysis has revealed 14 substitutions between PPV-B2 and PPV-D-GR dispersed two in P1, two in HC-Pro, two in P3, one in CI, two in 6K2, four in NIa and one in NIb protein. The possible role of the substitutions found between PPV-B2 and PPV-D-GR in the diverse symptomatology, aphid transmissibility and host adaptability of PPV-B2 is investigated.

## **Production of virus-free citrus varieties in Greece - Preliminary experimental results**

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Citiculture, a very important industry for Greece, is subjected to serious graft-transmitted diseases (of virus and viroid origin), mainly due to the lack of a respective certification program for citrus propagative material and the use of scions from mother trees of doubtful health. For this reason, a “clean stock” program was recently initiated for selected citrus varieties cultivated in Greece, using the *in vitro* micrografting technique. The initial material derived from the citrus collection of Poros Arboricultural Station and the Ioannis Stamatakos nurseries in Sparta area, from trees of grape-fruit (Shambar, Star Ruby), lemon (Adamopoulou, Vakalou, Zambetaki, Interdonato, Nouvel Athos, Verna), mandarin (Clementine of Poros, Chiotiko, Clasuelina, Encore, Marisol, Page, Tardivo di Ciaculli, Clementine SRA-63, Nova) and orange (Valencia of Poros, Late Navel of Argos, Moro, Navelate, Salustiana, Valencia Olinda, Navelina, Newhall, Washington navel). Young seedlings of citranges and citrumelo were used as micrografting rootstocks. Up to now, at the Agricultural University of Athens 150 micrografting events (19 varieties) have given 22 plants at the tube stage, whereas at the Agricultural Research Institute in Cyprus four (4) of their micrografted plants have already been potted. For biological indexing, complementing the laboratory detection methods (ELISA and RT-PCR), the newly developed *in vitro* indexing method will be employed, requiring shorter time for detection as opposed to the classical indexing. As a result, citrus selected varieties free of their original infection are expected to be obtained faster.

**Incidence of *Prunus necrotic ringspot virus* (PNRSV) and *Prune dwarf virus* (PDV) in almonds of Cyprus**

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Almonds, which are being grown for centuries in Cyprus, cover an area of 4979 hectares and are cultivated on lowlands, coastal areas and the southwestern part of the Lefcosia plain. In the context of the programme “Viruses, sanitation and molecular characterization of stonefruits in Cyprus”, which is funded by the Research Promotion Foundation of Cyprus, a survey was performed for virus diseases which affect almonds on the island. Samples were collected from about 20% of the trees of each almond orchard in the main almond growing areas of Cyprus and were tested by enzyme-linked immunosorbent assay (ELISA) for *Prunus necrotic ring spot virus* (PNRSV), *Prune dwarf virus* (PDV) and *Plum pox virus* (PPV). Results from 624 samples showed that PNRSV and PDV are widespread with an incidence of 54.32% and 26.76%, respectively, whereas all samples were negative to PPV.

## **Study of the effectiveness of acibenzolar-S-methyl against *Cucumber mosaic virus* in tomato**

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*Cucumber mosaic virus* is one of the most destructive aphid borne viruses in tomato and its control is only based on preventive measures of questionable effectiveness. It has been shown that inducers of systemic acquired resistance (SAR) like acibenzolar-S-methyl (ASM) influence plant metabolism increasing resistance of plants also against viruses. At the present study the effect of ASM (formulation BION WG 50, 50 mg L<sup>-1</sup>) against mechanically or aphid transmitted CMV in tomato plants was investigated in greenhouse experiments. Potted tomato plants were sprayed with ASM either once or at weekly intervals and a week after the first ASM application CMV transmission was performed. Three days later, regular monitoring of plants for virus infection by ELISA testing began. Three trials with mechanical inoculation of CMV were carried out and it was shown that a week after virus inoculation the disease incidence in the ASM treated plants was 50-70% lower than in the control plants. When ASM was applied at weekly intervals the difference between treated and control plants reached 85%, 22 days after virus transmission. Two trials with aphid transmission of CMV were also performed and when ASM was applied weekly, a significant 30% difference between treated and untreated plants was obtained 10 days after aphid transmission. The above results confirm that ASM is capable of inducing systemic acquired resistance in tomato plants against CMV, even under the virus natural mode of transmission by aphids.

## Sanitation of stone fruits in Cyprus

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The main aim of the Programme “Virus diseases, Sanitation and Molecular Characterisation of Stone Fruits in Cyprus”, which is funded by the Cyprus Research Promotion Foundation, is the establishment of a Prebasic plantation where all virus-free stone fruit material will be maintained. Local stone fruit propagating material from three Mother Plantations of the Department of Agriculture was collected and was grafted onto healthy seedlings in an insect proof screen house of the Agricultural Research Institute. Fifty mother trees were established, including peach varieties (Royal gold, Spring crest, Red June, Arm King, Suellen crepel, Fail lane, Florida King, Flavour top, Royal April, H. Harley, Bullard’s, Fantasy), cherry varieties (Griotte DV Nord, Utah Gial, B. Lapine, B. Reversion) plum varieties (Santa Rosa, Stanley, President, Red Beauty, Black Amber, Tende) και apricot varieties (Palen, Dixie red, Brecaucia Licia, Μπεμπέκο, Monique). All mother trees were tested for viruses and viroids and results showed that six peach varieties (Spring crest, Arm King, Fail lane, Florida King, H. Harley), three cherry varieties (Griotte DV Nord, Utah Gial, B. Reverchon), a plum variety (Tende), και an apricot variety (Palen) were infected. Meristem culture *in vitro* is performed for elimination of viroids and viruses from the selected stone fruit isolates. In addition, meristem culture *in vitro* is used for sanitation of the popular almond variety Retsou and the local plum variety “formoza”.



**Detection of *Hop stunt viroid*, *Pear blister canker viroid* and *Apple scar skin viroid* in infected stone and pome fruit trees in Greece by molecular hybridization**

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Molecular hybridization was used for the detection of *Hop stunt viroid* (HSVd), *Pear blister canker viroid* (PBCVd) and *Apple scar skin viroid* (ASSVd) in infected pome and stone fruit trees from various parts of Greece. It was done on plant tissue imprints (imprint hybridization), such as cross sections of stems, pedicles, seeds and fruit skin, as well as on dot blots (dot blot hybridization) of total nucleic acid preparations, crude sap and RT-PCR products blotted on positively charged nylon membranes. A modified protocol of an imprint-hybridization method for citrus viroids in infected indicator plants was used (Palacio-Bielsa *et al.*, 1999). Digoxigenin (DIG)-labelled cDNA or cRNA probe of each viroid was used for hybridization. Probes were made from the RT-PCR product of their respective Greek viroids. From testing 765 pome and stone fruit samples, 76/175 were positive for PBCVd (43%), 58/251 for ASSVd (23%) and 167/334 for HSVd (50%). This technique was accurate and sensitive with samples from the greenhouse and the field, the latter with possible lower viroid titer. In addition, this method can be used in the phytosanitary control on large numbers of samples in the context of the certification of plant propagation material in Greece.

**Molecular detection and diversity of *Apple chlorotic leaf spot virus* (ACLSV) isolates originating from wild, ornamental and cultivated hosts species of the family *Rosaceae***

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*Apple chlorotic leaf spot virus* (ACLSV) is a typical member of genus *Trichovirus*, (*Flexiviridae*) and infects *Rosaceae* species. In this study 232 samples of ornamental, wild and cultivated plants were collected from several geographical regions of Greece and tested for the presence of ACLSV. Virus detection was based on nested RT-PCR, using primers targeting the coat protein (CP) gene and amplifying a 380 bp product. ACLSV was detected in 77 samples. The highest virus incidence was found in almond (88.9%), followed by peach (63.6%), ornamental plum (57.14%), apple (50%), blackthorn (50%), ornamental quince (42.10%), plum (36%), cherry (32.4%), pear (28.6%), wild cherry (27.3%), almond-leafed pear (21.4%), hawthorn (25%), wild plum (11.1%) and roses (6.2%). The virus was not detected in any of the *Sorbus* sp. samples tested. Molecular variability was also studied by determining the nucleotide sequence of several ACLSV isolates with primers that amplify part of the CP gene and the 3' untranslated region (UTR). Comparative analysis of the sequences obtained in this study with already published ones showed high molecular variability (76%), which was mainly located in the 3' UTR.

**Application of RNA silencing technology for the generation of transgenic plants resistant to Plum Pox Virus (PPV): evaluation of resistance in T1 and T2 generations**

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PPV is an insect-borne pathogen that affects *Prunus* species, reducing marketability and crop yields. RNA silencing technology has been applied in the herbaceous model species *Nicotiana benthamiana* and successive generations of transgenic lines were evaluated by observation of symptoms and ELISA assays. Transgenic lines were produced using two different plasmids constructions, as reported earlier in this congress. One plasmid harbours a synthetic hairpin gene containing a ca. 500 bp region of the PPV gene for the RNA dependent RNA polymerase (Nib) in antisense orientation, a DNA spacer, and a ca 1500 bases spanning the Nib gene in sense orientation. This cDNA, placed under the control of 35S CaMV promoter of the binary vector ART7/ART27, produces in transgenic plants an asymmetric double-stranded RNA of PPV (called "hairpin PPV plasmid"). The same region of ca 1500 bases was cloned in the ART7/ART27 vector in sense orientation (called "sense PPV plasmid"). Both plasmids are capable of production of PPV specific siRNAs, which may trigger resistance to the incoming virus. Several independent transformants lines derived from both plasmids were isolated and tested for resistance to PPV at the T0, T1 and T2 generation. Transgenic lines were challenged with PPV (two different strains) by mechanical inoculation and infection was assessed by symptom development and ELISA assays. The results of these studies will be presented.

## **Molecular variability of *Apple stem pitting virus* (ASPV) isolates from ornamental, wild and cultivated host species**

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ASPV is a pome fruit-infecting virus with a world-wide distribution. It induces several economically important diseases such as quince fruit deformation, epinasty of Spy227, pear stony pit and pear vein yellow. During a survey in Greece, ASPV was detected in *Cydonia japonica* (ornamental Japanese quince), *Pyrus caleriana* (ornamental pear) and *Pyrus amygdaliformis*. Moreover, ASPV has been recently reported from grapevine, indicating that its host range may be wider than previously considered. To study the variability and phylogenetic relationships among ASPV isolates from different hosts, a small region of the RdRp gene of 12 Greek and Italian isolates was amplified with PCR. Comparative analysis of the sequences obtained showed a high level of variability with an average nucleotide divergence between isolates of 21.3% (maximum divergence 28%). A phylogenetic analysis performed using the deduced amino acid sequences confirmed the close relationship of *Apricot latent virus* (ALV) cluster with that of ASPV. No clear clustering of the ASPV isolates could be identified based either on country or host of origin using the obtained sequence information. This first report of partial sequences of ASPV isolates from ornamental and wild host species highlights, similarly to several other members of the family *Flexiviridae*, a previously unsuspected high level of ASPV variability. Further sequencing of longer genomic fragments should allow a better understanding of the factors that may shape the population structure of ASPV.

## **Elimination of a new ampelovirus (GLRaV-Pr) and *Rupestris Stem Pitting associated Virus 1* (RSPaV-1) from two grapevine varieties**

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A new virus species (GLRaV-Pr) associated to grapevine leafroll disease which is classified in a distinct phylogenetic group of the genus *Ampelovirus* (*Closteroviridae*) was isolated and characterized from greek cultivars. Elimination studies of GLRaV-Pr were carried out in two wine cultivars, Mantilari and Prevezaniko, also infected with RSPaV-1 (*Flexiviridae*). Both viruses were detected by nested RT-PCR assays. In our study, virus elimination was achieved by combining *in vitro* thermotherapy and meristem (<0.2 mm) or shoot tip culture (0.5 mm). The survival rate of meristems was very low (2.5% for Mantilari, 0% for Prevezaniko), while the few regenerated plantlets were only GLRaV-Pr free. From the 74.54% of Mantilari explants that survived thermotherapy, 95.12% and 46.34% of the cultured shoot tips were GLRaV-Pr and RSPaV-1-free, respectively. Both viruses were eliminated from the 43.90% of the plantlets. In the case of Prevezaniko, 82.86% of the plantlets survived thermotherapy. Of those 86.20% and 93.10% were GLRaV-Pr and RSPaV-1 free, respectively while in 51.70% both viruses were eradicated. The results confirmed that virus elimination in grapevine is easier to occur on species of the *Closteroviridae* family than on RSPaV-1. Finally, it seems that eradication of GLRaV-Pr and RSPaV-1 is feasible even with larger plant tissue parts if combined with thermotherapy *in vitro*.

## **First Report of *Cucumber green mottle mosaic virus* (CGMMV) in watermelon crops in Cyprus**

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In Cyprus, watermelon (*Citrullus lanatus*) cultivation is of great economic importance. They are mainly produced in the coastal regions of Ammochostos, Larnaca, Lemesos and Paphos districts. In 2006, a severe disease affecting grafted watermelon plants appeared in several fields located on the eastern part of the island. Dark-brown necrotic lesions were observed on the pedicel surface of the fruit of affected plants. Mature fruits showed interior water soaked yellow discoloration of the peripheral region under the skin and maturation incongruity of the red flesh. No leaf symptoms were observed. These symptoms were similar to those caused by *Cucumber green mottle mosaic virus* (CGMMV). A total of 620 watermelon and 1500 arable weed plant samples consisted of a young, fully developed leaf were collected from 23 fields in 7 locations. Also, 850 seeds from commercially available rootstocks were tested. Samples were tested using DAS-ELISA and RT-PCR for the presence of CGMMV. A small number of samples were mechanically inoculated onto watermelon and indicator plants. Both tests confirmed the presence of CGMMV in all watermelon symptomatic plants and in the rootstock seeds tested. The virus was also detected in many weed species belonging to the families of Amaranthaceae, Cucurbitaceae, Malvaceae, Chenopodiaceae, Solanaceae, Boraginaceae, Portulacaceae and Polygonaceae. This is the first report of a CGMMV outbreak in Cyprus.

**A Real Time RT TaqMan<sup>®</sup> PCR assay for the detection of *Cucurbit yellow stunting disorder virus* (CYSDV)**

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During the last decade, *Cucurbit yellow stunting disorder virus* (CYSDV) (*Crinivirus: Closteroviridae*), native to the Middle East, has become a major constraint to cucurbit production, worldwide. Infected plants exhibit bright yellow, interveinal chlorosis on older leaves, yellow splotches on developing leaves, and overall stunting and reduced fruit quality. The host range of the virus includes cucumber, melons, squashes, and watermelon. The virus is transmitted by *Bemisia tabaci* (Hemiptera: Aleyrodidae) in a semi-persistent manner. The 2007 introduction of exotic CYSDV into Arizona and California, USA and in Sonora, Mexico, mandates a regional management approach based on improved understanding of the epidemiology, including vector dynamics and virus-vector biology, and the identification of natural reservoirs. To aid in research toward effective disease management, a TaqMan<sup>®</sup> real time fluorescent, one-step reverse transcription (RT), polymerase chain reaction (PCR) assay has been developed and optimized. The assay is >100-fold more sensitive than conventional RT-PCR, and involves template preparation that does not require RNA purification. The assay can be accomplished either by first spotting the sap extract on a positively charged nylon membrane and elution, or by the direct addition of crude plant extract, into the real time reaction cocktail. Preliminary results indicate that this real time method is capable of detecting CYSDV in cucurbit samples from the USA, Mexico, and the Mediterranean Basin.

## **Molecular characterization of *Plum pox virus* (PPV) in Cyprus**

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*Plum pox virus* (PPV) is considered to be among the most important virus diseases infecting stonefruits worldwide. In view of an ongoing project on the Virus Diseases, Sanitation and Molecular Characterisation of Stone Fruits in Cyprus, which is funded by the Cyprus Research Promotion Foundation, a study was conducted in order to identify the prevalence, distribution and molecular characterization of PPV isolates in Cyprus. 115 samples from PPV infected peach (*Prunus persica*), apricot (*Prunus armeniaca*) and plum (*Prunus domestica*) trees were tested using molecular methods and specific primers for PPV strains M, D, and EA. The above samples were also tested by Real time PCR and melting curve analysis was performed in the amplified product in order to investigate the existence of different strains. Results of conventional and real-time PCR were consistent and showed that strain PPV-M is widely distributed in Cyprus, whereas PPV-D is reported for the first time in 17 infected peach trees.



## **Production of healthy grapevine propagation material from local cultivars in New Achialos, Magnesia, Greece**

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The lack of certified grapevine propagation material of Greek cultivars combined with the appearance of new pathogenic fungi which infect the propagative material in the nursery make it necessary to develop quick procedures for the production of healthy material of local cultivars. In the grapevine growing area of N. Achialos, Magnesia, Central Greece, a study was carried out during the period 2006-2008 aimed at the production of healthy material of the cultivars Roditis, Sabbatiano and Muscat of Alexandria. Grape stocks were selected from over 15 years old vineyards during summertime on the basis of macroscopic criteria. Virus detection was made by ELISA and comprised the following five viruses: *grapevine fan leaf virus* (GFLV), *grapevine leafroll associated virus 1 and 3* (GLRaV-1 and GLRaV-3), *grapevine virus A* (GVA) and *grapevine fleck virus* (GFkV). In total, 997 vines from 21 vineyards were checked and 865 (86,8%) were found infected by one or more viruses. The most widespread was GLRaV-3 (49,1%), followed by GFkV (17,1%), GLRaV-1 (5,6%), GFLV (3,7%) and GVA (0,7%). The most infected variety was Roditis (95,4%), followed by Sabbatiano (67,3%) and Muscat of Alexandria (51%). The canes of the 132 vines, where the five viruses were absent, will be used for the establishment of new vineyards (category standard). Healthy genotypes of the examined varieties were cultivated in aseptic media and preserved in the Genetic Bank of Greek Grape Varieties in Tissue Culture which exists in the Plant Protection Institute of Volos.

## Elimination of GLRaV-1 and RSPaV-1 from *Vitis vinifera* L. cv. Agiorgitiko

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Agiorgitiko is an important wine-producing Greek grapevine variety. Field monitoring, using ELISA and RT-PCR, revealed that it is heavily infected with both *Grapevine leafroll associated virus 1* (GLRaV-1) and *Rupestris stem pitting associated virus 1* (RSPaV-1). Both viruses reduce the productivity considerably and their elimination from the cultivar seems to be very important. In the present study an attempt was undertaken to produce virus-free propagation material of Agiorgitiko through meristem and shoot tip culture, in combination with thermotherapy *in vitro*. Detection of both viruses was based on nested RT-PCR assays. Meristem culture combined with thermotherapy resulted in successful elimination of both viruses (62%), though sanitation rate for GLRaV-1 was higher (91.2%) than for RSPaV-1 (67.6%). Shoot tip culture coupled with thermotherapy promoted the regeneration of virus-free plantlets, however, elimination rate was lower than meristem culture (73.8% for GLRaV-1 and 50.8% for RSPaV-1). Nevertheless, the survival rate was significantly higher for the shoot tips (80.3%) than in meristems (55.7%). In general the results confirmed the easier elimination of closteroviruses in grapevine compared to RSPaV-1 while the use of shoot tips facilitates the massive production of virus-free plantlets.

## Characterization of a new potyvirus from verbena

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Verbena is a vegetatively propagated ornamental plant known to be susceptible to several viruses. A ‘Taylortown Red’ plant from Michigan exhibited mottling symptoms on young leaves that became necrotic as leaves matured, indicating a possible virus infection. After testing for several known viruses of verbena it was suspected that the ‘Taylortown Red’ plant was infected with one or more novel viruses. Electron microscopy of partially purified preparations from the verbena plant showed the presence of elongated and spherical particles, providing the first evidence of infection with multiple viruses. Double-stranded RNA analysis revealed the presence of several bands ranging from approximately one to nine kilobases in size. After shotgun cloning and partial sequencing three viruses were shown to be present in this ‘Taylortown Red’ verbena plant: a como-, a carla- and a potyvirus. In this communication we present the complete nucleotide sequence of the novel potyvirus, named hereafter Verbena virus Y (VVY), a partial experimental host range, aphid transmission and development of detection methods for the virus.

**Characterization of an infectious clone of *Dulcamara mottle virus* and chimeric clones with *Turnip yellow mosaic virus***

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*Dulcamara mottle virus* (DuMV) is a member of the genus *Tymovirus*, family *Tymoviridae* and was first discovered more than 40 years ago in *Solanum dulcamara* from Great Britain. Although DuMV has been considered a member of the genus *Tymovirus*, previous efforts to sequence the genome indicated that the virus has an adenosine tail instead of the typical *Tymovirus* tRNA-like structure at 3' end of the genome. We sequenced the virus and concluded that DuMV has an internal adenosine track and the acceptor stem of the tRNA structure though it lacks the features that allow for the amino acid charging. We developed an infectious clone of DuMV and showed that the virus replicates successfully with the unusual 3' end. We then created chimeras between DuMV and *Turnip yellow mosaic virus* (TYMV) exchanging the 3' untranslated region (UTR) of the genome of DuMV with that of TYMV and vice versa. One of the chimeras was able to replicate efficiently in the inoculated leaf but had restricted systemic movement. These results indicate that *Tymovirus* polymerases can recognize the DuMV heterogeneous 3' UTR and showed that genome recombination is a potential mechanism for generation of new viruses in the genus *Tymovirus*.

**Association of *Potato virus Y* and *Potato leafroll virus* with native flora of Northern Greece**

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The aim of the study was to record the incidence of *Potato virus Y* (PVY, genus *Potyvirus*, family Potyviridae) and of *Potato leafroll virus* (PLRV, genus *Polerovirus*, family Luteoviridae) in native flora of Northern Greece. A total number of 905 random samples as belonging to 59 different plant species from 30 families were collected from Kilkis and Xanthi prefectures, where the viruses under study are endemic. Samples were tested in ELISA tests using monoclonal and polyclonal antibodies for the detection of PVY and PLRV, respectively. PVY was detected in plants of 29 species within 20 families, while PLRV was detected in 19 species of 12 families. Among those, 14 and 17 botanical species are possibly new hosts of PVY and PLRV, respectively. Most of the identified hosts were found within the Asteraceae (Compositae) family; eight species for PVY and six for PLRV. Higher incidence of PVY was detected among plants of *Conium maculatum* and *Erodium ciconium* and of PLRV among plants of *Convolvulus arvensis* and *Rumex crispus*. Mixed infections were detected in ten species and they were most common in *Stellaria media* plants. In laboratory studies, PVY was very efficiently transmitted by *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) when it was acquired from *Solanum nigrum* and transmitted either to tobacco (*Nicotiana tabacum* L.) cv. Basmás (45%), or to *S. nigrum* (75%) plants.

**The effect of essential oils isolated from plants of the Lamiaceae family in the transmission of *Potato virus Y* by *Myzus persicae***

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The effect of essential oils isolated from Greek plants of basil (*Ocimum basilicum*), mint (*Mentha piperita*) and pennyroyal (*M. pulegium*) (family Lamiaceae) in the transmission of *Potato virus Y* (PVY, genus *Potyvirus*, family Potyviridae) by *Myzus persicae* (Sulzer) (Hemiptera: Aphididae) was studied in laboratory tests. Essential oils were isolated by hydrodistillation and their effect was recorded in the acquisition as well as in the inoculation of PVY, at three concentrations (0.01, 0.1 and 0.5%). *Nicotiana tabacum* L. cv. Basmas plants and a necrotic isolate of PVY were used. Wingless *M. persicae* individuals acquired the virus for 3 min on infected plants, after a starving period of 3 h. In the experiments performed, PVY transmission ranged from 60% to 72% in the control treatment. When the essential oils were applied at the different concentrations on the acquisition plants, PVY transmission was 45-77.5% for basil, 52.5-57.5% for mint and 62.5-75% for pennyroyal. Transmission rates of 32.5-37.5%, of 47.5-60.5% and of 47.5-56.4% were recorded when the oil of basil, mint and pennyroyal were applied on the inoculation plants, respectively. The main decrease in PVY transmission was recorded when the basil oil was applied at the concentration of 0.5%. Pennyroyal essential oil application had no significant effect in PVY transmission. GC/MS analysis of essential oils showed that the most abundant components were linalool (45.8%) and methyl chavicol (16.5%) for basil, menthone (39.0%) and menthol (25.9) for mint, and pulegone (66.4%) and isomenthone (16.8%) for pennyroyal.

## **TRISTEZA: THE MAJOR THREAT FOR THE CITRUS TREES**

### **Invited Lecture**

#### **Current status of Citrus Tristeza Virus (CTV) in Italy**

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Following the Ministerial Decree for the Mandatory control of CTV in Italy, tristeza outbreaks first appeared in 2002 in the Southern Italian regions of Sicily and Apulia. The CTV findings were (i) in the Sicilian provinces of Siracusa and Catania on 'Fortune' mandarin, 'Navelina' and 'Tarocco' sweet oranges; (ii) in the Apulian province of Taranto on 'Navelina' orange and 'Common clementine'. Later other CTV foci were reported in the Sicilian provinces of Palermo and Messina. In 2006 CTV was also reported in Calabria region after testing 100 suspected trees of 'Satsuma' and 'Fortune' mandarins. All infected trees were grafted onto sour orange rootstocks. Mild or severe declining trees as well asymptomatic infected trees were detected in all CTV foci. With the only exception of 'Tarocco' orange, which is a local Sicilian variety, most of the infected trees were presumably originated by infected propagating material supplied by extra-regional nurseries. In Sicily, about 30.000 trees were initially ELISA-tested and 50% were found CTV-infected. After these findings, the virus monitoring was only carried out in the apparently not infected areas, because the eradication programme could not be sustainable. Differently in Apulia, about 80,000 trees were tested by DTBIA and showed different rates of CTV infections in young and aged citrus groves of clementines and sweet oranges. Few infected trees were detected in the nurseries located in the CTV foci. In Apulia region all infected groves and nurseries were partially or entirely eradicated with a decrease of the infection rate. All CTV isolates in the 3 regions were considered mild on the basis of serological and molecular assays. Most of the tested sources showed clear cut tristeza symptoms on Mexican lime by biological indexing. After the first findings, the Italian Ministry of Agriculture demanded to a group of experts the revision of the Ministerial Decree for the mandatory control of CTV in Italy. The revised Decree has not been issued yet. In the meantime, each region decided to adopt different control measures, but only the Apulian Region enforced the existing Decree by issuing a Regional Decree. The Apulian Decree introduced the use of the hierarchic sampling scheme of Hughes and Gottwald (2000), the DTBIA assay instead of the ELISA test and the use of insect proof screenhouses for citrus nursery productions in the CTV foci areas.

## Detection of *Citrus tristeza virus* in calamondin pot plants in a greenhouse in Eastern Attica and measures for eradication

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During the required phytosanitary surveys for transmission of calamondin pot plants (*Citrus madurensis* syn. *Citrus mitis* on *Citrus volcameriana* rootstock) from a large greenhouse cultivation of an enterprise in Koropi in Eastern Attica to EU, in 2007, were tested for *Citrus tristeza virus*: 368 specimens of *C. volcameriana* mother rootstocks by ELISA and found not to be CTV-infected and 540 calamondin pot plants on *Citrus volcameriana* rootstock, where CTV was detected in 11 of them by immunoprinting which was also confirmed by ELISA. In 2008, testing again the *C. volcameriana* mother rootstocks that were not CTV-infected the previous year, CTV was detected in 4 of them, which provides evidence that, above graft-transmission, an aphid-transmission had taken place. For elimination and eradication of CTV, all citrus plants, totaled 81.560 plants, were destroyed by fire, since there were conditions of free communication and direct relationship among all the plants, rootstocks and grafted plants of different age, infected and non-infected with CTV plants. Extensive surveys were taken place in a zone 1.5 km from the greenhouses with the calamondin plants, where CTV was not detected. Citrus plants without phytosanitary plant passport were forbidden to be transmitted and administrative and punitive sanctions were established against both the transmitter-possessor and the producer of citrus propagation material.



## **Citrus Tristeza Virus in Greece. Is the “game” finally over?**

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According to the recent legislation regarding the Plant Health, Regulation (EC) No of 690/2008 of 4th July 2008, which is in effect from the 23/7/2008, the countries of Greece, Malta, France (Corsica) and Portugal (except the island of Madeira) are recognized “*as protected areas that are exposed in particular plant health dangers in the European Union*”. In regard to Greece, the data of the last few years have changed dramatically, after the finding that citrus tristeza virus (CTV) has been detected in various regions of country that cultivate citrus trees. Analytically, the first inspections started in 1994 with extensive sampling according to the decision of the Ministry of Agriculture in Greece, while in June 2000, the first positive sample was found in the region of Katsikania in Argos in an orange tree (variety Lane late) which belonged in a lot of about 50 trees of Standard category (C.A.C) that “entered” illegally in the country from Spain in 1994. From this lot only 18 trees were survived, that had been planted in various regions of Argolida region and 7 of them were found to be positive for the CTV virus. In the spring of 2001, 1038 certified trees of a new clone of Clementine (Clemenpons) were imported from Spain, and 7 of them were found to be infected with the CTV virus. In Argolida, in a total of 49597 examined samples (the inspections were carried out in spring and in the fall), 101 samples were found to be CTV positive using grafting or insect transmission methods. To date, 824 trees have been destroyed because they were found positive or suspect of carrying the virus and among these, in three cases, the entire orchards of a total number of 150, 160 and 454 trees respectively were completely destroyed. In Crete, and particularly in the Prefecture of Chania, with the same manner as in Argolida in 1994, about 20 trees of the variety Lane late were imported from the same Spanish source, from which 2 trees survived that were also infected with the CTV virus. From this small lot, many samples were taken repetitively for grafting citrus scions in several regions of the Prefecture of Chania, as well as in other areas such as in the region of Sisses of the Prefecture of Irakleio. However, despite the extensive initial uproots (>4000 trees), according to recent inspections (Spring 2008) the destructive virus has been detected in several other regions of Crete and the situation appear to be uncontrolled in this island. In the region of Arta in 2005, in inspections and examination of several samples by the professor of University of Bari, Italy Dr. C. Vovla, several positive samples were found in trees of the orange variety W. Navel. The virus has also been located in other regions of Greece and specifically in Skala of Lakonia in 2007, in an orchard that had been planted with the new clone of tangerine tree of Clementine (Clemenpons) that was imported by a producer of that region from Spain in 2002. Laboratory examination of samples of the total of the 930 trees, 200 proved to be positive for the CTV virus. The total numbers of trees in this orchard was uprooted, however according to testimonies of farmers of that region, plant material had been collected for grafting from these trees. In spring 2007, in greenhouses of Eastern Attica that produce the ornamental citrus Calamodin, thousands of trees were found to be infected with CTV in a large area and more than 60000 trees were uprooted and destroyed with fire. In Chalkidiki, in similar

greenhouses of the ornamental citrus Calamodin, a positive sample was also found. The inspections are still in progress while the production has been committed. According therefore with the situation that is presented here in regard to the presence of CTV in various regions of Greece that cultivate citrus fruits, the question of how much more time the country will be under the status of a protected area can not be predicted. How much serious will be the disease caused by CTV in the Mediterranean basin after the spread of the major CTV aphid vector *Toxoptera citricida* (Kircadly) from the regions of Northern Portugal and Northern Spain can not also really be predicted.

## NON-PARASITIC DISEASES

### Oral Presentations

#### **Correlations among concentrations of mineral nutrients in olive leaves**

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In commercial olive plantations, leaves were collected from one year old shoots from March – September. In these leaves, the concentrations of N, S, P, K, Ca, Mg, NO<sub>3</sub><sup>-</sup>, Fe, Al, Mn, Zn, Cu, B, and Mo were determined. The statistical analyses showed that some correlations among the nutrients were significant (P<0.05).

## **Effect of the variety and the growth substrate in the intensity of tip-burn in spring lettuce**

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In order to study the effect of the variety and the growth substrate in the intensity of the physiological disorder 'tip-burn' in lettuce, romaine lettuce plants of the spring varieties Gramsi and Merlin were grown in a glasshouse experiment, in hydroponics, in two solid growth substrates (perlite and rockwool), as well as in a water culture (floating). Twenty days from the transplanting, after the appearance of the symptoms, the fresh and dry weight of the aboveground plant part and the root, the total number of leaves, the number of leaves with symptoms and the length of the stem were recorded. The ratio root to aboveground plant part, the aboveground plant part water content, as well as the concentration of several nutrient elements were determined. The main effect of the growth substrate on the plant growth was that the fresh and dry weight of the aboveground plant part in floating was significantly higher compared to the relevant ones in perlite and rockwool. However, the plants in floating presented significantly higher number of leaves with symptoms and significantly lower Ca and Mg concentration; in contrast, K, P, Mn, Zn and B concentration were found to be significantly higher. The main effect of the variety on growth and nutrient element concentration was that Merlin presented significantly higher fresh and dry weight of the aboveground part compared to Gramsi, lower ratio root to aboveground part, higher water content and a higher number of leaves with symptoms, but lower K, Ca, Mg concentration.

## Poster Presentations

### **Iron deficiency decreases root and leaf ferric reduction activity in grapevine rootstocks irrespective of their tolerance to iron chlorosis.**

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Ferric reduction (FR) activity of roots and leaves was determined in grapevine rootstocks Riparia Gloire (*V. riparia*) και 140 Ruggeri (*V. berlandieri* x *V. rupestris*), susceptible and tolerant to iron chlorosis respectively, under Fe deficient and sufficient conditions. Micropropagated plants were subjected to iron treatments in hydroponic culture for root FR activity determination. Leaf FR activity was measured in mesophyll protoplasts from in vitro growing plants. Iron deficient plants had a significantly lower level of root FR activity compared with Fe sufficient plants, in both rootstocks. Iron supply to Fe deficient plants caused a rapid increase of root FR activity. Mesophyll protoplasts FR activity in Fe deficient plants appeared to be decreased in comparison with that in protoplasts from Fe sufficient plants in both rootstocks. Reduction activity of mesophyll protoplasts from Fe sufficient plants was higher in 140 Ruggeri in comparison with that in Riparia Gloire rootstock. The results suggest that Fe induces FR activity in roots and leaves of grapevine rootstocks irrespective of their tolerance to iron chlorosis.

**Use of the 'quick test' Merck quant method for nitrogen nutrition diagnosis of lettuce**

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The 'quick test' Merck quant method for nitrogen nutrition diagnosis of lettuce was applied in three field nitrogen fertilization experiments. The higher lettuce yield was observed when the mean nitrate nitrogen concentration in soil was  $> 25$  ppm. The relation between the declines in nitrate nitrogen concentration in sap with the increase in shoot fresh weight was found to be curvi-linear. The sap nitrate nitrogen concentration was not related to the concentration of nitrate nitrogen in soil. Based on these results an on farm 'quick test' method for nitrogen nutrition of lettuce is proposed.

## NEMATODE DISEASES

### Poster Presentation

#### Identification of potato cyst nematodes in Cyprus using RT-PCR

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Potato cyst nematodes (PCN) of the genus *Globodera* cause major economic damage to potato crops in Cyprus. The present study aims at the integrated management of PCN through the utilisation of varieties resistant to particular species/biotypes. In a preliminary study carried out at Kokkinochoria the most important potato production area in Cyprus PCN species were identified using Real Time PCR. This method is faster and more reliable than the traditional morphometric approach. Soil samples were collected from fields previously found to be infested with inoculum level exceeding 20 cysts/100g of soil. The results obtained with RT-PCR confirmed the presence of two PCN species in Cyprus, *G. pallida* and *G. rostochiensis* with the first being more frequently detected than the second. At the time of sampling the coordinates of fields surveyed were recorded with the aid of GPS equipment and used to map the distribution of the two species. The use of electronic Geographical Information System (GIS) will assist in the understanding of PCN distribution and in the optimisation of disease control using resistant cultivars.

## HOST-PLANT INTERACTIONS

### Oral Presentations

#### **High protection of tobacco plants against *Cucumber mosaic virus* (CMV) by exogenously applied dsRNA of the silencing suppressor *2b* gene.**

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The control of *Cucumber mosaic virus* (CMV) is of great importance, due to economic crop losses that this virus causes in agriculture. Given the fact that only few sources of natural resistance for this pathogen are known, current research is focused on exploiting means of pathogen-derived resistance. Utilization of transgenic plants expressing viral genes, as well as cross protection using attenuated strains of CMV have been shown to be effective approaches; however, these methods are not permitted, as yet, to be incorporated in the agricultural practice in European Union. Based on our previous experimental data showing that exogenous application of dsRNA of the capsid protein of CMV (dsRNA-CP) induces resistance of tobacco against CMV, in this work the effectiveness of dsRNA of the silencing suppressor *2b* (dsRNA-2b) was investigated. No visual disease symptoms were observed in 35 to 55% tobacco plants inoculated mechanically with a mixture of the highly virulent CMV-G strain and dsRNA-2b produced *in vitro*. Protection against CMV reached 75% with application of dsRNA-2b produced *in vivo* in bacterial cells, and 85% when dsRNA-2b was combined with dsRNA-CP, both produced *in vivo* and applied exogenously. The method is compatible with high-throughput technological means for plant handling.



## Gene expression in *Arabidopsis* following induction of resistance to *Verticillium dahliae*

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In previous studies it has been described the ability of the strain *Paenibacillus alvei* K165 to protect various crops against the soilborne pathogen *Verticillium dahliae*. Furthermore it has been reported the efficacy of strain K165 to induce systemic resistance (ISR) in *Arabidopsis thaliana* plants against *V. dahliae* in a salicylic acid dependent pathway. The aim of the present study was to provide more insight in the interaction of *A.thaliana* mutant plants in the salicylic acid pathway with K165 and *V. dahliae*. For this purpose, we transformed a *V. dahliae* isolate with the *dsRed* gene to quantify its presence in the *NahG* (transgenic line degrading salicylic acid (SA)), *sid2* (SA-induction deficient), *eds5/sid1* (enhanced disease susceptibility), and wt plants. It was shown that K165 reduced pathogen colonization in wt and *NahG* plants, in contrast to the non K165 induced *eds5/sid1* and *sid2* plants. Furthermore, it was demonstrated, by using the Real Time PCR technology, the activation of the *PRI*, -2 and -5 genes upon induction by the K165 strain in the wt and *NahG* plants in contrast to the non K165 induced *eds5/sid1* plants.

**Changes in ethylene perception of *Arabidopsis* plants lead to differential responses during infection by *Verticillium dahliae***

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Preliminary studies on the susceptibility of several *Arabidopsis* mutants to the soil-borne pathogen *Verticillium dahliae* showed that the *etr1-1* (ethylene insensitive) mutants had enhanced disease tolerance compared to the control (Col-0 ecotype) or other mutant lines tested. In order to investigate the possible role of ethylene in plant defence mechanisms, *Arabidopsis* plants mutated in the ethylene perception pathway (*ein2*, *ein3*, *ein4* and *ein5*) were used along with the *etr1-1* plants in pathogenicity tests with *V. dahliae*. It was revealed that the *etr1-1* plants showed less disease severity compared to the other ethylene mutants. Furthermore, fungal biomass quantified by Real-time PCR (RT-PCR) was significantly less in the *etr1-1* plants than that of all the other mutants. To obtain a further insight in the defence mechanisms that could be involved in this phenomenon, the expression of *PR1*, *PR2*, *PR5*, *PDF1.2*, *WRKY 18*, *22*, *33*, *40*, *53* and *60*, *ERF1*, *ERF2*, *AtMYC2*, *rd22*, *VSP2*, *ABII*, *KINI*, *SDR1*, *GSL5*, *GSTF8* and *UGT73B1* genes that have been associated with defence mechanisms in plants against pathogens, was studied using RT-PCR. The expression of genes *GSTF8*, *SDR1* and *KINI* was elevated in the *etr1-1* plants, suggesting a possible role in the defence of plants against *V. dahliae*.

**Quantitative determination of a defoliating and non defoliating pathotype of *Verticillium dahliae* in susceptible and tolerant Greek olive cultivars**

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*Verticillium dahliae* is the most serious olive disease in the Mediterranean countries and worldwide. The olive infecting pathotypes of *V. dahliae* have been classified as defoliating (D) and nondefoliating (ND) according to their ability to defoliate the tree. *Verticillium* wilt is mainly controlled in olive orchards by using resistant cultivars. However, limited information is available about the level and source of resistance in most of the olive cultivars and there are no published data using microsclerotia, the resting structure of *V. dahliae*, as the infective inoculum. In the present study, we quantified the biomass of a D and ND *V. dahliae* strain in the susceptible cv. Amfissis and the tolerant cvs Kalamon and Koroneiki, by using the Real Time PCR technology. The viability of the pathogen in the plant tissues was confirmed by isolating the fungus on PDA plates, while symptom assessment proved the correlation between the amount of *V. dahliae* in plant tissues and cultivar's susceptibility. It was demonstrated that the biomass of the D and ND strain was significantly higher in cv. Amfissis than in cvs Kalamon and Koroneiki. In addition, the biomass of the D strain was higher than ND in cv. Amfissis. It was also observed that the amount of the pathogen in roots is lower than in stems and shoots and declines over time.

**Study of molecular and phytopathological aspects of pathogenicity genes in  
*Verticillium dahliae***

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Studies in several plant pathogenic fungi have shown that G protein  $\beta$  subunit (*Gb*) and Protein kinase A (*PKA*) genes are implicated in signal transduction, while the sucrose non fermenting gene (*SNF*) regulates the induction of cell wall degrading enzymes. In order to investigate the role of these genes in *V. dahliae*, they were amplified by PCR and inactivated in a race 1 tomato *Verticillium* isolate through gene replacement. In virulence assays that were performed, the  $\Delta PKA$  mutants caused typical symptoms in tomato and eggplants, however, there was a statistically significant reduction in the percent of disease compared to that of the wild type strain. Eggplants inoculated with mutants  $\Delta SF$  and  $\Delta Gb$  started to show symptoms about one month after inoculation, while plants inoculated with wild type strains had reached a high percentage of disease. The mild chlorosis symptoms that appeared in plants inoculated with the above mutants never became necrotic. Concerning the influence in physiological characteristics, the  $\Delta PKA$  and  $\Delta Gb$  mutants exhibited only a small difference in mycelial growth compared to the wild type strain, while increased germination frequency was observed in the  $\Delta Gb$  mutants. The above results contribute to the understanding of molecular mechanisms underpinning *Verticillium dahliae* pathogenicity.

## **Proteomics approaches to identify molecular networks of the plant immune system**

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Aim of this study was the biochemical investigation of protein targets and protein complexes that are involved in plant defense mechanisms after the recognition of avirulence proteins (secreted by pathogens) by the plant resistance proteins. Previous studies led to the identification of 2 E3 ligases, the F-box protein ACF1 and the U-box protein CMPG1, that are required to activate the plant defense mechanism triggered by a variety of effectors or elicitors that are produced by different pathogens (Avr9, Avr4, AvrPto, Inf1, P50 helicase of Tobacco Mosaic Virus). E3 ligases are involved in the degradation of proteins in eukaryotic cells via the 26S proteolytic machinery that constitutes the major multi-component complex required for the degradation of proteins in cell. The role of E3 ligases in this complex is the recognition and destruction of target proteins that act as activators or repressors of signalling pathways in cells. Therefore, the discovery of the target proteins of ACF1 and CMPG1 in plants can lead to further clarification of the major pathways followed after the recognition in plant cells of microbial molecules produced by pathogenic plants. The ultimate goal is the discovery of new molecules that could contribute to new and more effective methods to control plant diseases. Modern biochemical and proteomics technologies (epitope tagging, affinity purification, co-immunoprecipitation, LTQ-Mass Spectrometry and HMS-IPC: High-throughput mass spectrometric identification of protein complexes) were applied for the detection of proteins that might interact with the E3 ligases ACF1 and CMPG1.

## Poster Presentations

### Identification of new genetic regulators of plant hypersensitive response based on homology studies with the mammalian programmed cell death - apoptosis

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Aim of this study is the identification of new genetic regulators of the plant hypersensitive response (HR), a form of programmed cell death with many common characteristics with the mammalian apoptosis. HR is associated with the rapid death of host cells triggered during the entrance of the pathogen in plant tissues. When the pathogen elicits a host HR, it fails to multiply to high population levels and causes no disease symptoms. Many recent studies indicate the existence of common biochemical pathways of programmed cell death/apoptosis between the plant, mammalian and microbial cells. Our approach was the detailed bioinformatics analysis of the genome of *Arabidopsis* for the identification of mammalian orthologous genes known to be involved in programmed cell death. A series of new genes that are likely involved in HR and disease resistance in *Arabidopsis thaliana* to different pathogens were identified. In the present study, we investigated the role of mammalian AIF orthologous genes (Apoptosis Inducing Factor) known to play essential role in apoptosis. The mammalian AIF protein is a phylogenetically old, 57 kDa flavoprotein, which shares similarity to bacterial, fungus and plant oxidoreductases. *A. thaliana* contains 5 different putative homologous AIF genes that were named At-AIF-1, At-AIF-2, At-AIF-3, At-AIF-4 and At-AIF-5. T-DNA knock-out mutants At-AIF-2, At-AIF-3 and At-AIF-5 were characterized in *Arabidopsis* and the mutants were tested for whether they are compromised in HR and disease resistance against *Hyaloperonospora arabidopsis*, *Pseudomonas syringae* pv. *tomato* DC3000 and *Verticillium dahliae*. Characterisation of these lines and the results of the virulence and HR assays will be presented.

## **The discovery of the protein complex of the F-box protein ACF1 and study of its role in plant disease resistance**

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The F-box protein ACF1 is expressed in plant cells in the first 30 minutes after the recognition of the *Cladosporium fulvum* avirulence protein Avr9 by the tomato resistance protein Cf9. ACF1 is required for the activation of hypersensitive response (HR) and disease resistance against *Tobacco Mosaic Virus* (TMV) in tobacco, *Cladosporium fulvum* and *Pseudomonas syringae* pv. *tomato* in tomato and *Pseudomonas syringae* pv. *tabacci* in tobacco. Inactivation of the F-box of protein in tobacco using gene silencing methodologies leads to a significant reduction in the development of HR and a significant increase in plant sensitivity to TMV. Characterization of the *Arabidopsis* ACF1 homologues, AtSKIP2 and AtFbl16 led to the conclusion that these proteins are also involved in activation of HR triggered by incompatible races of *Hyaloperonospora arabidopsis*. Next goal was to identify the protein targets that are responsible for the degradation of ACF1 or other interacting proteins that form the ACF1 complex during the host-pathogen interactions. Using the yeast-two-hybrid assay of NtACF1 with two *A. thaliana* libraries, 4 putative interacting protein targets were identified: one Ser/Thr protein kinase, one Myb transcription factor, one bHLH transcription factor and one LIM-domain containing protein. T-DNA knock-out mutations of these genes were characterized in *Arabidopsis* and the mutants were studied for the susceptibility and/or resistance to different pathogens i.e. *Hyaloperonospora arabidopsis*, *Pseudomonas syringae* pv. *tomato* DC3000 and *Verticillium dahliae* as well as the development of HR. Characterisation of these lines and the results of the virulence and HR assays will be presented.

**Function alterations of the ethylene receptors *Never ripe* and *LeETR4* affect the susceptibility of tomato plants to the fungal pathogen *Verticillium dahliae***

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Previous experimental evidence derived from pathogenicity tests of *Verticillium dahliae* on ethylene insensitive *Arabidopsis thaliana* plants (*etr*) showed increased tolerance of the *etr* mutants compared to the wild type control (Col-0). To investigate the role of the *LeETR4* gene, which encodes an ethylene receptor in tomato, a *Tobacco rattle virus* (TRV) based VIGS (virus induced gene silencing) system was employed. A mixture of *Agrobacterium tumefaciens* cultures (carrying TRV RNA1, RNA2 containing an *LeETR4* gene fragment) was vacuum infiltrated into tomato leaves of cv. Ailsa Craig. The efficiency of the VIGS system was confirmed by silencing the *LePDS* gene (responsible for carotenoid biosynthesis) resulting in a photo-bleaching phenotype. Plants infiltrated with a mixture of *A. tumefaciens* with TRV RNA1 and RNA2 were used as control. The *LeETR4* expression level was assessed by Real-time PCR (RT-PCR). Silenced (*TRV:LeETR4*) and mutant *Never ripe* (*Nr* – ethylene receptors deactivated) plants were inoculated with *V. dahliae* ( $10^7$  spores/ml) and disease symptoms were scored for 30 days. Both *TRV:LeETR4* and *Nr* plants showed significantly less symptoms compared to the control plants. In addition, quantification of *V. dahliae* by RT-PCR revealed that the fungal biomass in the *Nr* and *TRV:LeETR4* plants was significantly less than that in the control plants. These findings suggest a key role of ethylene perception in the interaction of tomato with *V. dahliae*.



**Pathogen-derived effectors as functional markers for genomic analysis and disease resistance breeding in plants**

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“Effector” proteins produced by plant pathogens (bacteria, viruses and fungus) are recognized by matching plant resistance (R) proteins or their “guards” and trigger defense-associated responses such as the hypersensitive response (HR). Failure to recognize pathogen-produced effectors results in disease susceptibility. We are exploring the concepts of using effectors in functional genomic analysis, as novel breeding tools in conventional breeding for disease resistance, and as a tool in phylogenetic analysis in species of agronomic and agro-ecological interest. Preliminary results on screening of parental lines and progeny from crosses between selected tobacco cultivars with bacterial and viral effectors will be discussed.

## Evolution of the Oomycete Chitin Synthase genes

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The Oomycetes, a group of diploid eukaryotes which includes the most important plant pathogens, were classified for many years within the Kingdom *Fungi* because of their similar ecological and morphological traits, as well as due to the common infection strategies. A feature usually mentioned when distinguishing oomycetes from true fungi is the presence of cellulose and the absence of chitin in their cell wall. However chitin was unambiguously detected by biophysical analyses in some oomycete species of the *Leptomitales* and *Saprolegniales* orders, the latter group including *Aphanomyces euteiches*, the cause of seedling and root rot diseases on many legumes. Two putative chitin synthase (CHS) genes were identified by data mining of an *A. euteiches* EST collection and Southern blot analysis. The full length of CHS1 (bp) and CHS2 (bp) genes was sequenced by 5'-RACE PCR using cDNA as a template (after their induction with nikkomycin Z-treated mycelium at early stages of inoculation). Moreover, a putative CHS sequence was identified in *Phytophthora parasitica* (syn *nicotianae*) var. *nicotianae*, the cause of root rot disease in numerous plant species. By RT-PCR with degenerated primers designed according to the conserved CHS sequences of *Phytophthora* species we amplified the central and more conserved region of the gene. The full length clones sequences (bp) were obtained again by applying 3' and 5'-RACE PCR using nikkomycin-induced mRNA cDNA as a template and showed high homology to the *P. infestans* CHS1. We compared all the generated CHS sequences to an assembled Oomycete and fungal CHS database. Phylogeny analysis indicated that oomycete CHS diversification into two major divisions occurred before the divergence of the major oomycete lineages and that oomycete CHS cluster closer to the fungal division 1 CHS than to any other eukaryotic species.

## **Functional analysis of type III effectors proteins from *Bradyrhizobium japonicum***

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Type III secretion systems (T3SSs) from bacteria deliver effector proteins (type III effectors) into eukaryotic cells to manipulate the host metabolism, e.g. to suppress defense responses. Type III effectors are virulence factors that play a key role in pathogenesis of human, animals and plants. On the other hand, eukaryotic host cells developed strategies to perceive type III effectors in order to “sense” the invading bacterium. In recent years, genomic studies have revealed the presence of T3SSs in rhizobia, in certain endosymbionts and in some non-pathogenic plant-associated bacteria. Aim of the present study is the functional analysis of candidate type III effectors from *Bradyrhizobium japonicum* USDA110. Initially, we isolated two genes encoding proteins with weak similarity to AvrPphB protein of the bean pathogen *Pseudomonas syringae* pv. *phaseolicola*. The candidate effector proteins were studied for their ability to be translocated via a heterologous type III secretion system as well as to induce the hypersensitive reaction in plants. Mutagenesis analysis of the genes under study determined protein regions essential for the protein delivery through the type III secretion system in plants and also for their ability to trigger the hypersensitive reaction.

## CHEMICAL CONTROL

### Lecture of Major Sponsor

#### **Biological and physico-chemical properties of mandipropamid, a new fungicide for the control of Oomycete pathogens**

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Mandipropamid is a new fungicide developed by Syngenta for the control of Oomycete pathogens. The effect of mandipropamid in the infection cycle of important target pathogens was examined by light microscopy. These tests show that the compound is highly effective on cystospore and sporangial direct germination. It inhibits mycelial growth and haustoria formation and reduces sporulation. Uptake and translocation of mandipropamid were investigated by chemical analysis and bioassays. Following foliar application, a significant proportion of the applied active ingredient binds immediately to the wax layer of plant surfaces. The adsorption to the wax layer protects the active ingredient from being washed off by rain as soon as the spray deposit has dried. From the surface deposit and the material adsorbed to the epicuticular wax small amounts of active ingredient migrate progressively into the plant tissue. Due to the high intrinsic activity the amount taken up into the plant tissue is sufficient to provide good translaminar activity and curative disease control during the incubation period. These biological and physico-chemical properties explain the consistently excellent disease control observed under field conditions, when mandipropamid is applied as preventive treatment.

## Oral Presentations

### **Fluopicolide: a new fungicide mode of action from Bayer Cropscience for more efficient Oomycete disease control in high value crops**

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Fluopicolide is a novel fungicide from the new chemical class acylpicolides, discovered and developed by Bayer CropScience. Fluopicolide is highly active on oomycete organisms, causal agents of a number of economically important diseases such as *Phytophthora* and *Plasmopara* species in various crops like potatoes, vines, vegetables and ornamentals. *In vitro* and *in vivo* studies conducted on *Phytophthora infestans* demonstrated that fluopicolide is active at several stages of its lifecycle on both direct germination and liberation of zoospores. The strong and extremely quick effect on zoospores at very low concentrations of fluopicolide is already visible under the microscope. Zoospores lose their mobility immediately after contact with fluopicolide and within less than five minutes these zoospores swell and burst. Studies performed on *P. infestans* and *Plasmopara viticola* showed that fluopicolide affects the release and motility of zoospores and the germination of cysts, as well as mycelial growth and sporulation. The biochemical mode of action of fluopicolide is novel and unique and it has been demonstrated that there is no cross resistance with other downy mildew fungicides since it controls oomycete strains resistant to phenylamides, strobilurins, and carboxylic acid amides (CAA). Fluopicolide induces a fast redistribution of spectrin-like protein(s) from the membrane to the cytoplasm in both hyphae and zoospores. This effect is extremely fast in accordance with the symptoms induced on zoospores. Cytoskeletal proteins such as spectrin provide a structural stability to cells as they form a network sustaining the plasma membrane. Fluopicolide destabilize this network, leading to cell disorganization and cell death. None of the tested established oomycete compounds showed any similar effect on spectrin like proteins. Fluopicolide alone and in combination showed consistently high efficacy levels under various climatical conditions. The immediate reaction on zoospores combined with an outstanding long-lasting effect delivers strong preventive protection from the start right through to the end of the application interval. This is visible in field trials carried out since 1998. Fluopicolide is delivering excellent efficacy on leaves and stems as well as protection on berries and tubers. Fluopicolide-based products provide a range of significant benefits to the farmer. They are perceived as products of choice by demonstrating a good profile for Integrated Crop Protection (ICM) programmes to fulfil requirements of the foodchain. Fluopicolide is being developed world wide in combinations, for active anti-resistance management such as mixtures with propamocarb-HCl for use in potatoes and vegetables (under the trade name Infinito®) and with fosetyl-Al for use in vines (to be marketed as Profiler®). Since 2006 fluopicolide based products have been successfully introduced and will be launched globally in more than 60 countries.

## **The legal framework and procedures relating to the control of plant protection products in the market**

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Plant protection products (ppps) are active substances and preparations intended among other purposes to protect plants or plant products against all harmful organisms or prevent the action of such organisms. The controls during all stages from the manufacturing of ppps, guaranteed composition, labelling, sale and application considerably improve the protection of users of plant protection products, the protection of consumers of plants and plant products and the protection of the environment. Ministry of Rural Development and Food and Agricultural Services of Prefectures are conducting systemic controls in all stages and severe sanctions are imposed in case of infringements. Results of sanctions imposed during 2003-2007 show the intensiveness and the efficacy of the controls resulting to the safety of the domestic plant products. In addition these results are used for programming future controls aiming to the quality of the plant products.

**Phytopathogenic and mycotoxigenic characterization of laboratory mutant strains of *Aspergillus carbonarius* and *Penicillium expansum* resistant to phenylpyrrole fungicides**

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Mutants of *Aspergillus carbonarius* and *Penicillium expansum* highly resistant (Rf: 100-130) 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) for resistance to fludioxonil also reduced the sensitivity of mutant strains to the aromatic hydrocarbon (tecnazene, tolclofos-methyl) and dicarboximide (iprodione, vinclozolin) fungicides. No effect of phenylpyrroles resistance mutation(s) on fungitoxicity of triazoles, anilinopyrimidines, phenylpyridinamines and to the non-site specific inhibitor chlorothalonil was observed. However, an increased sensitivity (Rf: 0.06-0.1) of fludioxonil-resistant strains to the strobilurin-type fungicides azoxystrobin and pyraclostrobin was observed in all mutant strains tested. Tests for the evaluation of osmosensitivity of isolates showed that most fludioxonil-resistant isolates were more sensitive to high osmotic pressure than the wild-type parent strains. Study of other fitness determining parameters showed that the mutation(s) for resistance to phenylpyrroles may or may not affect the mycelial growth rate, sporulation, conidial germination and pathogenicity. However, in a few mutant strains these fitness parameters were unaffected or only slightly affected. 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 *A. carbonarius* and all *P. expansum* mutant strains produced mycotoxins (ochratoxins and patulin, respectively) at significantly lower concentrations than the wild-type parent strains. However, in few *A. carbonarius* mutant strains the ochratoxin (OTA and OTB) production was much higher (up to 4-fold) than the wild-type strain. All *P. expansum* mutants produced citrinin at concentrations much higher than the wild-type strain. Interestingly, in all mutant strains tested the mycotoxigenic ability was further increased when they were grown on fludioxonil-amended medium. Similar results were also found in tests with artificially inoculated grapes and apples. The data of the present study indicate, for the first time, the potential risk of increased mycotoxin contamination of grapes and apples after intensive use of dicarboximides and/or phenylpyrroles.

## **Fitness of anilinopyrimidine-resistant strains of *Botrytis cinerea***

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The objective of this study was to investigate the fitness of anilinopyrimidine-resistant isolates of *Botrytis cinerea* collected from vegetable crops in Greece during 2005. Fitness parameters measured were mycelial growth, spore production *in vitro* and *in vivo*, virulence, percentage spore germination and competitive ability of the resistant isolates in four pairs with sensitive isolates. The measurements of the fitness components in individual isolates showed high variability within both sensitivity groups in all, except virulence, fitness components tested. As a group, resistant isolates showed significantly lower ( $P < 0.05$ ) mycelial growth and virulence than the sensitive isolates. In addition the resistant isolates showed higher ( $P < 0.05$ ) spore production *in vivo* but there was no difference ( $P > 0.05$ ) between the two sensitivity groups in spore production *in vitro* and in the percentage of spore germination. However, the correlation to test if there is any relationship between the values of each fitness component and the level of cyprodinil sensitivity of each isolate was not significant ( $P > 0.05$ ) for all fitness components, except spore production *in vivo*. This absence of significant correlation coefficient values suggests that development of resistance to anilinopyrimidine fungicides did not affect the fitness of the resistant isolates. Competition of the resistant versus sensitive isolates was isolate-dependent, since in two of the isolate pairs the resistance frequency decreased significantly after five culture or disease cycles while in the remaining two pairs resistance frequency increased significantly after five disease cycles or remained stable for one pair after five culture cycles on artificial nutrient media.



**Fungicide sensitivity of *Septoria pyricola* and chemical control of the disease in pear cv. Krystalli**

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Thirty six *S. pyricola* single spore isolates, collected the period 2003-2007 from 10 pear orchards, were tested on PDA media enriched with fungicides, by the use of point inoculation method. Twenty eight isolates were found highly resistant (MIC >100mg/L), 3 moderately resistant (MIC 10mg/L) and 5 sensitive (MIC 0.1mg/L) to benzimidazoles (carbendazim). The MIC to DMI fungicides (bitertanol, flusilazole, myclobutanil) was almost equally distributed within the limits 0.001 to 1.0 mg/L. MIC to strobilurins (azoxystrobin, kresoxim-methyl, pyraclostrobin, trifloxystrobin) and to boscalid (carboximides), was ranged from 0.005 to 0.1 mg/L, for each fungicide. However, two isolates showed up to 1000-fold less temporal insensitivity to some strobilurins. This insensitivity was lost after consecutive subculturing on fungicide free media. On artificially inoculated pears with a mixture of spores (1:1) of one sensitive and one benzimidazole resistant isolate, a single application with flusilazole or azoxystrobin inhibited the infection and disease development, when they applied at pink bud stage and at the recommended by their manufacturers' dose.

**Molecular and biochemical study of resistance to zoxamide and other oomycete fungicides in *Phytophthora infestans***

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Mutants of *Phytophthora infestans* with high resistance to the benzamide fungicide zoxamide were isolated from a wild-type strain after UV-mutagenesis and selection on medium containing zoxamide. Cross-resistance studies showed that the mutation(s) for resistance to zoxamide also greatly reduced the sensitivity of mutant strains to other oomycete fungicides from different chemical groups. Three possible mechanisms of fungicide resistance were studied to elucidate the resistance of *P. infestans* mutant strains: (a) The mechanism of target site modification with isolation and sequencing of  $\beta$ -tubulin gene from the wild type and mutant strains. (b) The mechanism of increased secretion with inhibitors of energy production, with the study of intracellular fungicide concentration by LC-MS chromatography and with molecular analysis of a part of ABC-transporters gene. (c) The mechanism of detoxification with the use of synergists. The sequency of  $\beta$ -tubulin gene, the site of action of benzamide zoxamide and benzimidazoles, showed common mutations in all resistant strains studied at the position 200 from methionine to phenylalanine and in other sites close to the positions of 200, 198 and 167, which are known mutation sites for benzimidazole resistance in other fungal species. From the above data, it seems that the target site modification explains the resistance of *P. infestans* to zoxamide. However, the resistance to other oomycete fungicides is not explained by a modification of  $\beta$ -tubulin gene. Study of the other mentioned above mechanisms did not provide positive results and more efforts are needed for that. An over expression of an ABC transporter gene using real time PCR, possibly, will give an explanation of multi drug resistance phenomenon in *P. infestans*.

## **Effect of triazole resistance mutations on the fumonicin production by *Fusarium moniliforme***

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Mutants of *Fusarium moniliforme* (teleomorph *Giberella fujikuroi*) resistant to the triazole fungicides (Rf: 20-60) were isolated at high mutation frequency ( $1.8 \times 10^{-5}$ ) after UV-mutagenesis and selection on media containing epoxiconazole. Cross resistance studies with other fungicides showed that the mutation(s) for resistance to epoxiconazole also reduced the sensitivity of mutant strains to other demethylase inhibiting fungicides (DMIs) as flusilazole, difenoconazole, propiconazole, flutriafol and imazalil. No effect of epoxiconazole-resistant mutation(s) on fungitoxicity of fungicides which affect other cellular pathways or other steps of the sterol biosynthesis, was observed. Study of saprophytic fitness determining parameters showed that the mutation(s) for resistance to epoxiconazole did not significantly affect the mycelial growth rate, sporulation and conidial germination. Pathogenicity tests on maize seedlings under greenhouse conditions showed that most mutant strains presented infection ability similar to the wild-type strain. Liquid chromatographic-mass spectrometric (LC-ESI/MS) analysis of mycelial extracts from the wild-type and mutant strains that were grown on PDA medium showed that all epoxiconazole-resistant isolates produced fumonicins (FB<sub>1</sub>, FB<sub>2</sub>) at similar or even higher (up to 6-fold) concentrations than the wild-type parent strain. In addition, in most of these mutant strains the mycotoxigenic ability was further increased (2 to 4-fold higher) when the mutants were grown on epoxiconazole-amended medium. Similar results were also found in tests with artificially inoculated corn seeds. The data of the present study indicate, for the first time, the potential risk of increased fumonicin contamination of cereals after intensive use of triazole fungicides.

## **Proquinazid (Talendo<sup>®</sup> 20EC), a new fungicide against powdery mildew on grapes**

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Proquinazid (Talendo<sup>®</sup> 20EC) was discovered by DuPont in 1993 and it belongs to the new quinazolinone chemical class. Although proquinazid's specific target site is not yet known, it seems to interfere with signal transduction between the pathogen and its host, necessary for successful infection. Proquinazid (Talendo<sup>®</sup> 20EC) inhibits spore germination of *Uncinula neactor*, while it can prevent appresorial formulation even in lower concentrations. Because of its novel mode of action, it is able to control powdery mildew strains resistant to other fungicide classes like triazoles and strobilurins. Translaminar penetration and local systemic movement of proquinazid (Talendo<sup>®</sup> 20EC) fully protects treated leaves and bunches, while local vapor activity shields treated and untreated tissues, penetrates into bunches and protects new growth. Field trials' results indicate that proquinazid (Talendo<sup>®</sup> 20EC) is very effective against powdery mildew on grapes even under very high disease pressure situations. On leaves, proquinazid performed equally to the chemical standards. On bunches, the performance of proquinazid was equal or even superior to that of the chemical references.

## **Risk analysis of cases of non compliances with plant protection products legislation requirements**

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Infringements related to plant protection products legal requirements during 2003-2007 and specifically infringements related to guaranteed composition, labeling, sale and use of plant protection products put on the market involve risks and hazards which in the majority of the cases examined is not significant. Conclusions raised by the risk analysis of the non compliances are useful for programming controls aiming to the safety of the user of plant protection products, the consumers of plants and plant products and the environment. Risk factors as type, incidence and consequence of the infringement are used for conducting the risk analysis.

## **Evaluating the contribution of agricultural vocational training courses to the proper use of crop protection agents in tobacco cultivations**

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The aim of the present study was to evaluate the contribution of agricultural vocational training courses to the adoption of good agricultural practices by the tobacco growers, taking into consideration specific factual data which concern the use of Crop Protection Agents (CPA's). During the first semester of 2007, Komotini "Dimitra" Training Center carried out six 60-hour training courses concerning the "Integrated Tobacco Crop Management" in 5 different locations (training locations, TL) of the mountainous regions of the Prefecture of Rodopi. The courses were voluntarily attended by 136 tobacco growers, most of them  $\geq 25$  year old males. The implementation of crop protection standards and the compliance of the tobacco growers with their obligations in relation to the proper use of CPA's in their crops were among the subjects which were underlined during the courses. Tobacco samplings originated from the Prefecture of Rodopi were carried out by SEKE Xanthi throughout September-October 2007 and the concentrations of specific CPA's were evaluated (chlorothalonil, cypermethrin, methamidophos), also being compared with the respective results in samples obtained during the aforementioned period of 2006. The concentrations of CPA's in all samples taken from TL were ranged below MRL's. Furthermore, a reduction of the residues in TL samples (80% to 98%) was recorded when compared with the results evaluated in 2006. Comparing to the 2007 average results for the Prefecture of Rodopi, a reduction of 26% (cypermethrin) to 85% (chlorothalonil) was noticed as regards the residues of CPA's in the samples obtained from the final product of the trained tobacco growers.

## Poster Presentations

### Evaluation of the fungicide VOLARE from Bayer Cropscience, against oomycetes, on the crops of potatoes, tomatoes and cucumbers in Greece

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The new specific oomycetes fungicide Volare 687,5 SC ( 62,5 gai/L Fluopicolide + 625 gai/L propamocarb hydrochloride), due to the Fluopicolide it contains, is the first representative of a new chemical class, the acyl picolides. Details of the mode of action of Fluopicolide are still under evaluation, but it is already evident that Fluopicolide interferes with spectrin like proteins, which are part of the cytoskeleton. Volare 687,5 SC provides excellent efficacy against a range of economically significant phytopathogens of the oomycetes class responsible for a number of important diseases in crops such Potatoes, Tomatoes, Cucumbers, Vines and other vegetables. During the years 2002-2007 the following field trials took place in Greece: 6 field trials against *Phytophthora infestans* on Potatoes, 5 field trials against *Phytophthora infestans* on Tomatoes and 3 greenhouse trials against *Pseudoperonospora cubensis* on Cucumbers. One type of formulation of the product was used EXP 11120A SC (62,5 gai/L Fluopicolide + 625 gai/L Propamocarb hydrochloride), in which Fluopicolide is in mixture with Propamocarb hydrochloride. Volare 687,5 SC was applied foliar in a protective application program within an interval of 7-10 days. The relevant EPPO guidelines were followed in order to lay out, apply the treatments and assess the field trials. From the trials in Greece came out that Volare 687,5 SC, at the dose rates of 1,4 L/HA and 1,6 L/HA , in spray application interval of 7-8 days, can provide excellent control against *Phytophthora infestans*. The dose rate of 1,6 L/HA is recommended under very favorable for the disease conditions. Concerning the trials against *Pseudoperonospora cubensis*, the infection was at high pressure level and the results showed that Volare 687,5 SC at the dose of 1,4 – 1,6 L/HA, in an application interval of 7-10 days can efficiently control *Pseudoperonospora cubensis*.

**Evaluation of the efficacy of new fungicides against powdery mildew (*Leveillula taurica*) on pepper crop**

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During the period summer/autumn 2006, the efficacy of 6 new fungicides against the disease development on three pepper cultivars (Kerato, Gemisti, Florinis type), in an artificially inoculated experimental crop in Velestino area, was evaluated. Commencing August 9<sup>th</sup>, pepper plants were inoculated on 7 occasions at 3-6 day intervals with a spore suspension (20.000 – 70.000 spores/ml). Fungicides were applied twice, 30 day apart and at the recommended doses, commencing the appearance of first disease symptoms (25 days after the 1<sup>st</sup> artificial inoculation). The fungicides were evaluated for their effectiveness against both the amount of the disease (frequency, intensity) and the yield (weight, number of developed fruit). Compared to untreated controls, all fungicide applications significantly reduced the disease on the three pepper cultivars. Quinoxifen gave the best control of the disease followed up by azoxystrobin, boscalid+pyraclostrobin, penconazole spiroxamine and flusilazole. Applications with penconazole and the mixture boscalid+pyraclostrobin, increased significantly the yield whereas, a tendency of reducing the weight and the number of fruits, was noticed in applications with the fungicides quinoxifen and flusilazole.



## **Effect of boron on controlling the development of fruit rots in peaches**

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With recent public concerns regarding pesticide residues on fruit, there is a need for alternative disease management practices that will reduce risk to consumers. This study investigated the effect of different boron products for controlling the development of *Monilinia* sp. and *Rhizopus* sp. on peaches. Symptomless mature peaches were collected at harvest time and disinfested by dipping them in 10% vol/vol domestic bleach solution (4.85% NaOCl) for 15 min. The peaches which would be inoculated with *Monilinia* sp. were then immersed in solutions of Power-B or Borax and those which would be inoculated with *Rhizopus* sp in Clawbor. The percentage of total surface area infected was determined. The treatments were arranged in a randomized design and there were 5 replications of 20 fruit for each treatment. The control treatment included non-inoculated fruits and fruit immersed in sterile distilled water. The results showed that boron products reduced significantly the percentage of fruit rot caused from *Monilinia* sp. or *Rhizopus* sp. In addition, the percentage of boron in flesh of peaches was increased. It is most probable that the appropriate use of Boron could increase the resistance of peaches to fruit rots. Applying postharvet dipping of peaches in boron solution could be an effective method to reduce, but not to eliminate, the losses from *Monilinia* and *Rhizopus* infections. Therefore, this method should be used together with sanitary and chemical fungicides with low residues.

## **Effect of copper on the aflatoxin contamination in dried figs**

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Figs of the ‘tsapelosika’ variety, while still on the trees, were inoculated with a strain of *Aspergillus flavus*. The infection took place by spraying the figs with a spore suspension of the fungi in two different developmental stages: ‘green with eye open’ and ‘yellow’. In some treatments, prior to the infection, a commercial product of oxine-copper was applied. The dried figs were harvested in three different times at seven days interval over three weeks and analyzed for aflatoxin B1 contamination by ELISA method. The results showed that, in the case of the ‘green figs with open eye’ infection, the third harvest had almost five times the aflatoxin of the first one. This can be explained with the acceptance that aflatoxin is mostly produced on mature figs and affected by the incubation time. In the case of ‘yellow figs’ infection, the amount of aflatoxin produced was generally lower (with the exception of the first harvest) and remained almost stable through the three harvests. This could be attributed to the high temperatures (>40°C), observed during the ‘yellow’ infection period which probably affect the pathogenicity of the fungi. In all cases, the aflatoxin production was increased in the presence of copper ions. This phenomenon could be attributed to the antibacterial action of copper which affect the antagonistic epiphytic microflora, leading to increased infection of the figs by the fungi.

**Developments regarding setting of pesticide Maximum Residue Levels according to Regulation (EC) No. 396/2005**

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Directives 76/895/EEC, 86/362/EEC, 86/363/EEC and 90/642/EEC for setting pesticide Maximum Residue Levels (MRLs) on fruits, vegetables, cereals, products of animal origin and other products of plant origin have been amended several times. For reasons of clarity and simplicity, the above mentioned directives, are repealed and replaced by a single act. This act is Regulation (EC) No 396/2005 of the European Parliament and of the Council, of 23 February 2005, on maximum residue levels of pesticides in or on food and feed of plant and animal origin and amending Council Directive 91/414/EEC. As a Regulation, 396/2005 does not require transposition into national law in the Member States and its precise requirements are to be applied at the same time and in the same manner throughout the Community. This way, MRL setting, based on Good Agricultural Practice, ensures the effective functioning of the internal market and trade with third countries in relation to fresh, processed and/or composite plant and animal products intended for human consumption or animal feed in which pesticide residues may be present, whilst providing the basis for securing a high level of protection for human and animal health and the interests of consumers. The regulation is consisted of ten chapters and several annexes and will fully enter into force at September 2008. Where a Member State envisages granting an authorization for the use of a plant protection product in accordance with Directive 91/414/EEC, that Member State shall consider whether, as a result of such use, an existing MRL set out in Annex II or III to this Regulation needs to be modified, whether it is necessary to set a new MRL, or whether the active substance should be included in Annex IV of Regulation (EC) No 396/2005.

## **Multi annual national control programs on pesticide residues**

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According to articles 41-44 of Regulation (EC) No 882/2004 of the European Parliament and of the Council, of 29 April 2004, on official controls performed to ensure the verification of compliance with feed and food law, animal health and animal welfare rules (OJ L191/28.05.2004), Member States shall organize a system of official controls on food and feed based on multi annual national control programs (MANCPs), which cover all stages of production, processing and distribution. MANCPs take into account guidelines defined by the European Commission, which lead to a solid, overall and complete approach based on risk analysis and the detection of the most informative and representative stages. The guidelines for MANCPs' planning are described on Decision 2007/363/EC of the Commission. Part of MANCPs is the official control of pesticide residues on food and feed. The basic principles, priorities and key points of planning coordination and application of the official control of pesticide residues on food and feed will be presented.

**Chemical control of leaf spot diseases and fungicide residues on celery plants grown in pots under cover**

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In the spring of 2006, the efficacy of 4 fungicides against leaf spot diseases caused by *Cercospora apii* and *Septoria apiicola* on celery grown in a polyethylene tunnel, was studied. Potted plants replicated 3 times (3 plants/treatment) in randomized blocks were used. Fungicides applied on three occasions at 15 day intervals and at the recommended dose by the manufacturers. Immediately after the first and the second fungicide application plants were artificially inoculated with a spore suspension (10–50.000 spores/ml) of each pathogen. Following inoculation the plants were covered for 36 h with a moist polyethylene film and then maintained in greenhouse conditions until evaluation. Commencing the symptom appearance on the untreated controls, disease intensity was evaluated on three occasions at weekly intervals on a scale 1-5. All fungicide applications reduced significantly the amount of the disease, caused either by *C. apii* or *S. apiicola*. The best control provided by chlorothalonil followed up by azoxystrobin, flusilazole and the mixture of boscalid+pyraclostrobin. In case of chlorothalonil and azoxystrobin, fungicide residues were determined in leaf samples collected 15 days after each application, by the use of GC with a ECD detector. Chlorothalonil residues 11.75, 37.86 and 76.08 mg/Kg and azoxystrobin 6.90, 15.10 and 11.50 mg/Kg were detected in these samples following the 1st, 2nd and 3rd fungicide application, respectively.

**Control of *Botrytis cinerea* benzimidazole- and anilinopyrimidine-resistant strains with boscalid and pyraclostrobin**

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Emergence of resistance fungicides represents one of the most important limitation factors of the sustained control of gray mold caused by *B. cinerea* on vegetable crops. The introduction into the spray programs applied against gray mold, of new fungicide compounds, with good efficacy against the disease is a prerequisite for successful disease control and resistance management to fungicides already in use. In the current study was tested the control efficacy of two novel fungicide compounds, boscalid and pyraclostrobin, against *Botrytis cinerea* isolates resistant- and sensitive to benzimidazole and anilinopyrimidine fungicides. Control efficacy of boscalid and pyraclostrobin was measured using six *B. cinerea* isolates, two benzimidazole-resistant (BENR) isolates, two anilinopyrimidine-resistant (ANIR) and two of wild-type sensitivity (WTBC). Both pyraclostrobin and boscalid provided satisfactory control of all the six isolates used in the study, when applied at 25  $\mu\text{g ml}^{-1}$  (control efficacy 69-94%) and very high levels of control when applied at 50 and 100  $\mu\text{g ml}^{-1}$  (control efficacy 90 – 99%). Control was independent of the isolate sensitivity to carbendazim and cyprodinil. In contrast, carbendazim applied at 100  $\mu\text{g ml}^{-1}$  failed to control sufficiently the benzimidazole-resistant isolates (control efficacy 25-27%), while cyprodinil applied either at 50  $\mu\text{g ml}^{-1}$  or 100  $\mu\text{g ml}^{-1}$  failed to provide satisfactory control of the anilinopyrimidine-resistant isolates of the pathogen (control efficacy 25-60%). Such results suggest that boscalid and pyraclostrobin could play a key-role in gray mold management and in the management of resistance developed to other fungicide classes.

**Baseline sensitivity of *Botrytis cinerea* isolates from vegetable crops to pyraclostrobin and boscalid**

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Fifty five isolates of *Botrytis cinerea* collected from vegetable crops were used to determine the pathogen` baseline sensitivity to two new fungicides boscalid that inhibits the enzyme succinate dehydrogenase in the electron transport chain, and pyraclostrobin that blocks electron transport between cytochrome *b* and cytochrome *c<sub>1</sub>*. Measurement of sensitivity to boscalid was based both on the inhibition of mycelial growth and spore germination while measurement of sensitivity to pyraclostrobin was based only on the inhibition of spore germination. For both fungicides the sensitivity distribution was a unimodal curve with a mean EC<sub>50</sub> value of 0.033 µg ml<sup>-1</sup> for pyraclostrobin and 2.09 and 2.14 µg ml<sup>-1</sup> for boscalid based on the inhibition of mycelial growth and spore germination, respectively. No cross-sensitivity relationship was observed between the two fungicides (r = 0.09). In addition, no cross-resistance relationship was observed between these two fungicides with other botryticides; cyprodinil, pyrimethanil, fenhexamid, fludioxonil and iprodione.

## **Influence of fungicides on the growth of the fungus *Clonostachys rosea* IK726**

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The fungus *Clonostachys rosea* IK726 is effective not only against many serious soilborne pathogens like *Fusarium oxysporum* f.sp *culmorum*, *Bipolaris sorokiniana*, etc. but also against foliar pathogens like *Botrytis cinerea*, *Alternaria dauci* etc. The aim of this study was to investigate the influence of some fungicides on the mycelial growth of this fungus *in vitro* and to calculate EC<sub>50</sub>. Totaly 13 fungicides were checked: Fosetyl – Al, Pyraclostrobin, Captan, copper-hydroxide, Propineb, Thiophanate-methyl, Mancozeb, Carbendazim and Boscalid that are applied as foliar sprays, and Thiram, Hymexazole, Previcur and Iprodione that are applied by soil irrigation. According to the results, Carbendazim and Pyraclostrobin were the most active of all with EC<sub>50</sub> = 2 µg ml<sup>-1</sup> and 5 µg ml<sup>-1</sup> respectively, followed by Thiram and Copper–hydroxide with EC<sub>50</sub> = 27 µg ml<sup>-1</sup> and 29.7 µg ml<sup>-1</sup> respectively. Regarding Iprodione, Thiophanate-methyl, Captan, Mancozeb, Hymexazole and Propineb the EC<sub>50</sub> was 217.5 µg ml<sup>-1</sup>, 240 µg ml<sup>-1</sup>, 250 µg ml<sup>-1</sup>, 320 µg ml<sup>-1</sup>, 360 µg ml<sup>-1</sup> και 1300 µg ml<sup>-1</sup> respectively. Finally, Boscalid and Previcur did not affect the mycelial growth of the fungus at doses of 500 µg ml<sup>-1</sup> and 432 µg ml<sup>-1</sup> respectively. The results demonstrate that some fungicides like Boscalid, Previcur, Propineb, Hymexazole etc could be combined with *C. rosea* IK 726 in integrated pest manangement systems.



## **A novel approach for real time forecasting and mapping of potato late blight in Cyprus**

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Potato production is one of the most important sectors of the agricultural economy in Cyprus. Late blight (*Phytophthora infestans*) is the most destructive disease of potato, causing severe losses especially in humid years. To prevent yield losses potato producers apply fungicides based on empirical and calendar information. This approach is of moderate effectiveness and frequently leads to excessive pesticide use, with negative impact to the environment and human health. In the context of a 3-year research project aiming to introduce to Cyprus a late blight forecasting scheme, during 2007-2008 a network of agrometeorological stations was established in the potato growing area of Kokkinochoria. These stations broadcast wirelessly the micro-environmental conditions, and forecasting models are run to suggest respective spraying schemes. The forecasting schemes are compared to the conventional control practice with a view to develop a prognosis system that would achieve a significant reduction of fungicide application combined with satisfactory control of late blight. The data from the weather stations and the results of the forecast schemes will be incorporated on a web-based Geographic Information System (GIS) platform that will eventually be freely accessible to agronomists and growers. This platform is expected to enhance the dissemination of information while providing new possibilities to researchers for the development of more detailed prognosis models, adjusted to local conditions.

## **Safe Use Initiative for the use of plant protection products: Farmers' education and communication**

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In the light of the pilot project regarding the Safe Use of plant protection products, which commenced at Ierapetra in 2005, the Hellenic Crop Protection Association (HCPA) prepared a variety of educative material (brochure and DVD) which are used for the education of farmers through the official Organization of Agricultural Vocational Education Training and Employment (OGEEKA – Dimitra), in an attempt to inform and sensitize in issues of environmental protection, but also in the use of personal protective equipment. Moreover, for the demonstration of the proper application of plant protection products and appropriate choice, use and storage of personal protective equipment, HCPA administered the creation of three (3) demo-farms in Crete (Messara, Ierapetra, Chania), where practical trainings take place. The program is expanding to the rest of Greece, starting from crops in Central and Western Greece, in olives in Viotia and grapes in Corinth. As far as communication is concerned, radio and TV spots, posters and newsletters were prepared. For easy access communication to the public, the site [www.safeuse.gr](http://www.safeuse.gr) was prepared. Furthermore, the project was presented to newspapers, conferences, exhibitions, farmers' unions, but also to official bodies, such as the Ministry of Rural Developments & Food, educational institutions and universities.

## **Safe Use Initiative for the use of plant protection products - Experimental part**

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The project regarding the Safe Use of plant protection products is conducted by the Hellenic Crop Protection Association (HCPA) in collaboration with the Laboratory of Toxicological Control of Pesticides of the Benaki Phytopathological Institute, while it is being financed by the European Crop Protection Association (ECPA). HCPA performed comfort tests using four (4) types of cotton coveralls. Two of them were used in field tests for the determination of the exposure of users during the application of plant protection products, while, afterwards, they were tested against their permeability from the Laboratory of Toxicological Control of Pesticides of the Benaki Phytopathological Institute. Results showed that the Resist Spills ® coverall had the lowest permeability. Due to that, the Resist Spills ® coverall was sent to Germany and after it successfully passed all the tests, it was certified according to the German model DIN 32781. The coverall was named ΑΙΓΙΣ (AIGIS) and it is already being sold in agricultural stores. In Crete, at the same time, relative tests took place with modern spraying equipment (Fumicar) from the Gandhi Agricultural Institute, results of which showed decrease of exposure of user in comparison to the classic spray gun. Fumicar is used by producers in areas of Crete. The variant of this spraying equipment, the Novi-F, was also used in relative field tests in Crete. The analysis of samples was conducted by the Laboratory of Toxicological Control of Pesticides of the Benaki Phytopathological Institute.

**Effectiveness of alternative treatments on *Botrytis cinerea* control in pepper cv. Florinis**

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The effectiveness of vapor heat treatments and acetic acid fumigation in the control of gray mould caused by *Botrytis cinerea* on inoculated pepper cv. Florinis was studied. All treatments applied after inoculation completely inhibited the infection compared with controls. The most promising treatments were those of 48°C for 15 min and the same treatment in combination with 4 mg/L AA fumigation. These reduced the infection levels by 100% on the 12<sup>th</sup> day, compared with controls, without affecting fruit quality. Heat damage was observed on fruit heated at 50°C for 15 min, with the damage appearing as a slight peel pitting on the fruit surface. It is concluded that vapor heat treatment and acetic acid fumigation at relatively low concentrations are an effective physical treatment for controlling *B. cinerea* on stored sweet red pepper, with real commercial potential.

## BIOLOGICAL AND INTEGRATIVE CONTROL

### Oral Presentations

#### **Current applications of soil solarization for the control of soilborne pathogens in plastic houses or in open field cultivations**

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Watermelon plants grafted on rootstocks resistant to *Fusarium oxysporum* f.sp. *niveum* are able to avoid Fusarium wilt. However, this protection is not extended to *Verticillium dahliae* infections. Indeed watermelon cultivations established in fields previously cultivated with potato or cotton (both susceptible to Verticillium wilt) cause widespread infections of watermelons regardless of the use of *Fusarium* resistant rootstocks. In July 2007, strip soil solarization was applied in 10 hectares sandy loam field previously cultivated with potatoes. A strip of 2 m wide was covered mechanically by impermeable transparent plastic films (actual width 2.80 m) while an intermediate zone of 1.50 m wide was left uncovered. Mechanical application of the plastic film was carried out by a specifically regulated tractor in dry soil while irrigation tubes 1 m apart were also inserted during covering to be used to extensively water the soil. Two months after the application of soil solarization the plastic film was teared in the middle to allow rain to water the field during winter. In February 2008 the soil was cultivated with a ripper without soil mixing while the plantation was established in March 2008 and transparent plastic tunnels covered the plants. Enumeration of *Fusarium oxysporum* propagules in solarized or unsolarized control strips revealed that soil solarization for two months almost destroyed all *Fusarium oxysporum* propagules. Regarding symptom development in unsolarized strips up to 50-60 % of the plants developed brown vascular discoloration indicating vascular wilt diseases. Laboratory examinations and pathogen isolation revealed that almost 80% of symptomatic plants were infected by *Verticillium dahliae*. On the contrary neither vascular wilt symptoms nor *Verticillium dahliae* was isolated from watermelon plants grown in solarized strips proving the effectiveness of the root stocks against Fusarium wilt. Preliminary calculations of the effect of solarization on the number of produced watermelon fruits per plant with commercial value showed that the difference between treated and untreated plots was not significant (the corresponding figures were 2.5 to 3.2). However, the mean weight of watermelon fruits from the untreated was per 8-10 Kg compared to 14-16 Kg from the solarized plots. This significant difference in the total production and the size of fruits justified the low cost extensive machine application of soil solarization in the Amaliada region of Helia county in Peloponnesus Greece (Cost of application 750 Euro per ha). Various examples of successful soil solarization application refer to globe artichoke, lettuce, tomato and cucumber against several soilborne pathogens. In Iria region, soil solarization has been applied to control *Verticillium dahliae* and *Sclerotinia minor* for the last 25 years as well as in the region of Lechaina (Helia) and Lapa (Achaia) for the control of *Sclerotinia minor* and other soilborne pathogens of lettuce. Soil solarization is also applied in

Preveza county for the last 30 years for the control of *Pyrenochaeta lycopersici*, *F. oxysporum* f.sp. *cucumerinum* and *Clavibacter michiganensis* subsp. *michiganensis*. Solarization is a useful alternative of methyl bromide for the control of soilborne pathogens in Greece.

## **Current status and future prospects for integrated management of olive diseases in the Mediterranean basin**

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*Verticillium* wilt has a significant negative impact on olive cultivation all over the world. The situation has become more complex by the appearance of a defoliating strain of *Verticillium dahliae* in the USA and recently in the Mediterranean region. Current screening of olive germplasm for selecting resistant cultivars or rootstocks appears to be promising. Soil solarization or chamber solarization has been suggested; available fungicides are unable to control the pathogen. *Spilocaea oleagina* is effectively controlled by copper compounds, while strobilurin-based fungicides could also be efficient. Trials with copper oxychlorides in Italy indicated that four treatments could control *Pseudocercospora cladosporioides*. *Clitocybe olearia* and *Armillaria mellea* are causing root rot and wood decay in old olive orchards, but *Fomitiporia mediterranea* is an emerging threat. Severity of olive knot disease is directly related to susceptibility to frost, hailstorm and harvesting injuries. Phytoplasmas are ubiquitous, but a clear correlation between a given syndrome and the presence of one or more phytoplasmas did not emerge. Olive trees host up to 13 different viruses without significant impact so far. Molecular hybridization tests on dsRNA-positive samples in Apulia, revealed the presence of three nepoviruses, *Arabis mosaic virus*, *Cherry leaf roll virus* and *Strawberry latent ring spot virus*, plus *Olive leaf yellowing-associated virus* and *Olive latent virus-1*. Aspects related to integrated management of the diseases and problems related to dispersal of pathogens by exporting olive plant material in southern hemisphere countries will be discussed.

**Interactions between the biocontrol agents *Clonostachys rosea* IK 726 and *Pseudomonas chlororaphis* PCL 1391 against tomato foot and root rot caused by *Fusarium oxysporum* f.sp. *radicis* – *lycopersici*  
Influence of fungicides on the growth of the fungus *Clonostachys rosea* IK726**

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The aim of this study was to investigate the interactions between the fungus *Clonostachys rosea* IK 726 and the bacterium *Pseudomonas chlororaphis* PCL 1391 against the phytopathogenic fungus *Fusarium oxysporum* f.sp. *radicis* – *lycopersici* (*F.o.r.l.*) that causes tomato foot and root rot. Results of *in vitro* experiments, in dual cultures with *P. chlororaphis* PCL 1391 and *F.o.r.l.* and with *P. chlororaphis* PCL 1391 and *C. rosea* IK 726 showed production of an inhibition zone at the fungal colonies in the vicinity of the bacterium. Isolation of the antibiotic phenazine-1-carboxamide, which is produced by this bacterium, by Thin Layer Chromatography, followed by a bioassay demonstrated that this antibiotic inhibits the growth of both fungi. However, results of *in planta* experiments in a gnotobiotic system showed that, there was a synergistic effect between the biocontrol agents against the pathogen since their combined application reduced the disease index significantly, compared to the positive control and the plants in which these biological agents were applied separately. Results of *in planta* experiments in pots have shown that the combined application of the biological agents reduced significantly the disease index, in comparison to positive control, but not in comparison to plants on which these biological agents were applied separately. A further microscopy study will unravel more aspects regarding the interaction between the three microorganisms in the tomato rhizosphere.



**Phyllosphere grapevine yeast *Aureobasidium pullulans* Y1 reduces *Aspergillus carbonarius* (sour rot) incidence in wine-producing vineyards in Greece**

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Ochratoxin A (OTA) has been reported to be a dangerous nephrotoxic and carcinogenic mycotoxin, produced by various *Aspergillus* and *Penicillium* spp. In the vineyards, the main ochratoxicogenic producing species is *Aspergillus carbonarius*. The main objectives of this work were to (i) isolate yeasts from the phyllosphere of vine leaves or canes (ii) evaluate the activity of isolates against *A. carbonarius*, (iii) evaluate the ability of most efficacious isolate to reduce sour rot in wine-producing vineyards, (iv) determine the species of *Aspergillus* in wine-producing vineyards and (v) determine the OTA levels in juice from macerated grapes (must). Yeasts were isolated from the phyllosphere of vine leaves or canes and evaluated in a detached berry assay for their ability to suppress *A. carbonarius* growth. Seventeen of the 21 yeast isolates significantly reduced *A. carbonarius* growth, compared to untreated controls. The most effective yeast isolate *Aureobasidium pullulans*, isolate Y-1, was field tested on two varieties of red grape, Grenache Rouge and Agiorgitiko located on the Island of Rhodes and in Corinthos County. It was demonstrated that *A. pullulans* Y-1 was as effective as the commercial fungicide fludioxonil + cyprodinil, in reducing sour rot infection, *A. carbonarius* presence on berries at harvest and ochratoxin A contamination in must.

**Antifungal activity of ethanolic *Glycyrrhiza glabra* L. extracts against infection of tomato plants by *Phytophthora infestans***

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Ethanolic extracts from plant tissues of 25 different species were tested against late blight on detached tomato leaves in Petri dishes. Results designated high anti late blight properties of *Glycyrrhiza glabra* leaves extract (2.5% w/v), whereas the rhizomes extract was ineffective. The disease inhibition levels were 100%. The EC<sub>50</sub> and EC<sub>99</sub> values of the extracts were 0.325% and 0.995 % (w/v), respectively. Trials conducted to test the influence of the duration and extraction pattern on the extract anti late blight effect, showed that the use of ethanol in a soxhlet apparatus for 5 hours, gave the highest disease control (100%). Among different solvents used for the extraction (1% w/v) in a sohxlet apparatus, ethanol gave the best effect. Further trials showed that the extract had no curative effect against the disease. The best effect was achieved by extract application at the day of inoculation (100% inhibition). Trials on potted tomato plants confirmed the anti late blight properties of ethanolic *G. glabra* extracts (1% w/v). The disease reduction level was equal to that of the fungicide Fosethyl-Al for a time period of 20 days after the inoculation. Three applications of the extract, every 14 and 20 days, reduced late blight severity for 42 days; the reduction level was similar with that achieved by the applications of the fungicide.

## Application of bioactive plant substances from olive tissues and grapes in non-chemical disease control

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A specific group of the olive (*Olea europae*) phenolic profile, called secoroids, including oleuropein, are natural antioxidants and have antimicrobial activity, and consequently a health-beneficial role. Grape pomace, a by-product of wine making represents as much as 20% of total grape weight and is rich in polyphenols compounds, especially stilbenoids, flavonoids and other polyphenolic substances, which have also been shown to exert beneficial effects for human health and plant defense against invading pathogens. We have examined the *in vitro* and *in vivo* antimicrobial activity of: oleuropein isolated from olive tissue, an olive mill waste water extract rich in polyphenols such as hydroxytyrosol, as well as a grape extract also rich in polyphenols such as resveratrol. In particular, all three compounds were found to restrain *in vitro* growth of a series of important bacterial and fungal pathogens, including *Xanthomonas campestris* pv. *vesicatoria* (*Xcv*), *Pseudomonas syringae* pv. *tomato* and *Botrytis cinerea*, *Fusarium oxysporum*, *Colletotrichum higginsianum*, *Alternaria alternata* respectively. Moreover, these natural extracts were shown to inhibit or restrain spore germination of fungi both in solid media and plant tissues. They also had similar effects to both epiphytic and endoparasitic mycelial growth in different pathogen-host interactions, such as the *Phytophthora parasitica* (syn. *nicotianae*) var. *nicotianae* hyphae infected tobacco leaves. In addition, oleuropein significantly reduced disease incidence in pepper plants artificially inoculated with *Xcv*.

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**Characterization of antimicrobial metabolites produced by the bacterium  
*Pseudomonas fluorescens* X**

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*Pseudomonas fluorescens* X is an efficient biological antagonist of soil borne phytopathogenic fungi that cause damping-off of seeds and seedlings. In order to investigate the mechanism of its biological activity, mutants deficient in the antifungal activity *in vitro* and *in vivo* were generated by mutagenesis using the transposon Tn5. Fungitoxicity tests of bacterial extracts from solid cultures of the wild type and mutant strains showed significant reduction of mycelia growth of *Pythium ultimum* only in the case of the wild type. These results indicate that the possible mechanism of biological activity of *P. fluorescens* is due to the production of antimicrobial metabolites. A method using semi-preparative high performance liquid chromatography (HPLC), was developed for the isolation of these substances. *In vitro* bioassays were followed to test the isolated compounds for antifungal activity. Direct application of each of these purified metabolites with *Pythium ultimum*, resulted in growth suppression of fungi. Structure elucidation using atmospheric pressure chemical ionization-mass spectrometric detector (APCI-MSD) suggested that they are probably antibiotics due to their low molecular weight. Chemical structure is currently being analyzed by FT-IR.

## Study on the nematicidal efficacy of garlic essential oil and two of its components

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Garlic essential oil, diallyl sulphide 98% and diallyl disulphide 70%, were tested with respect to their nematicidal properties, initially against the plant parasitic nematode species *Xiphinema* sp., *Helicotylenchus* sp., *Tylenchorhynchus* sp., *Tylenchus* sp., *Heterodera* sp., and against non parasitic nematodes and secondly against *X. index*, *X. italiae* and *Tylenchulus semipenetrans*, which are known parasites of important cultures. Garlic essential oil was isolated by hydrodistillation from garlic cloves and its chemical composition was determined by GC/MS analysis resulting methyl allyl trisulfide as the primary volatile (19.8%) followed by diallyl trisulfide (16.3%), 2-vinyl-[4H]-1, 3-dithiin (13.9%), 3-vinyl-[4H]-1,2-dithiin (11.1%), dimethyl trisulfide (10.1%), diallyl disulfide (7.2%) and methyl allyl disulfide (4.7%). The nematodes were extracted from soil samples using a variation of the Baermann funnel method. Efficacy was estimated by counting the number of living individuals after 4 hours in the first set of experiments, and at 0, 1, 2, 3, 4, 5, 6 and 24 hour intervals in the second set. The essential oil was very effective at all instances, even at the concentration of 0.1 μl/ml, and its efficacy was possibly affected by the species and the size of the nematode. Diallyl disulphide was also effective, though apparently higher concentrations and/or longer durations than with the essential oil are required. However, both compounds appear to have rather nematostatic than nematicidal properties.

**G21-3 (*Gliocladium* spp) and F12-9 (*Trichoderma* spp), two new aggressive mycoparasites of sclerotia of the phytopathogenic Ascomycete *Sclerotinia sclerotiorum***

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One hundred ninety nine candidate mycoparasites from soils of organic crops in Southwestern Greece were isolated by using the method of trapping. After preliminary evaluation the best eighteen were selected and subjected to further evaluation. The mycoparasites were applied in the form of hyphae and spore suspension and were tested in water agar, sterile and non-sterile soil. Finally, they were tested in pot experiments in greenhouse. A sequence of events during mycoparasitism process of three mycoparasites of different origin and aggressiveness (among them G21-3 και F12-9) was studied through an optical microscope. Additionally, both germination and entry course of mycoparasites on host's surface were observed through an electronic scanning microscope. Mycoparasites produced good results for the control of sclerotia of *Sclerotinia sclerotiorum*, while the G21-3 (*Gliocladium* spp) proves to be an exceptional mycoparasite and a competent antagonist as well followed by the F12-9 (*Trichoderma* spp). G21-3 destroyed completely the sclerotia within 15 days even earlier in all experiments. The development of sclerotia parasitism shows many common features in all three mycoparasites indicating very likely both common course and mechanisms. The ideal period to isolate aggressive mycoparasites of sclerotia of *Sclerotinia sclerotiorum* from soil paste is 15 days.

## Poster Presentations

### Effect of the antagonist *Paenibacillus alvei* to the fungus *Verticillium dahliae* growth in eggplant root tips via EGFP technology

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Studying the effect of potential biological agents against the disease that is caused by the fungus *Verticillium dahliae*, it might be the alternative way of controlling it. Investigation of the *in vivo* interaction between the plant growth-promoting rhizobacterium *Paenibacillus alvei* strain K165 and the soilborne fungus *Verticillium dahliae* (VdEGFP) transformed with the *egfp* (enhanced green fluorescent protein, EGFP) gene was observed by a fluorescent microscope. Estimation of the corresponding relative fluorescence by using digital fluorometer showed that K165 restricted statistically significant the colonization level and ramification process of VdEGFP into eggplant root tips, variety Black Beauty. In addition, disease severity was reduced about 50% to the eggplants that K165 had been applied to their root systems, in comparison to control-plants. In the present study the effect of the rhizobacterium K165 in root tips of a host, the most susceptible plant tissue for the pathogen entrance, to the VdEGFP biomass growth was evaluated according to EGFP biomarker. Transforming every antagonistic agent with the *gfp* gene could also contribute to study in detail the potent mechanisms of biocontrolling soilborne pathogens.

**Biological control of tobacco pathogens *Rhizoctonia solani*, *Sclerotinia sclerotiorum* and *Pythium* sp. in the greenhouse, by using saponins, *Trichoderma viridea* και *Streptomyces griseoviridis***

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Pythium damping off, sclerotinia stem rot and collar rot are the most devastating diseases of tobacco seedlings production in greenhouses, in USA. The effectiveness of saponins to control Pythium damping off of tobacco was investigated in greenhouse experiments and compared to the effectiveness of the fungicide Terramaster at the recommended rate. Also the effectiveness of two biofungicides, i.e. *Trichoderma viridea*, and *Streptomyces griseoviridis*, to control sclerotinia stem rot and collar rot was investigated and compared to the control provided by the fungicide Rovral.



**Genetic characterization of the antagonism against phytopathogenic fungi and oomycetes in the bacterium *Pseudomonas fluorescens* X**

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*Pseudomonas fluorescens* X has the ability to suppress seed and seedling damping off in sugar beet and cucumber. Screening a library of 12000 mutants, generated by Tn5 insertion, resulted in isolating nine insertion mutants that were defective in suppression of *Pythium* damping off on cucumber plants. Wild-type genes inactivated by Tn5 insertion were isolated after plasmid rescue. Six different genes were found to affect biocontrol of *Pythium ultimum* *in vitro* and *in vivo*. Three (*pqqE*, *pqqD*, *pqqF*) encode for proteins involved in the biosynthesis of pyrroloquinoline quinone (PQQ), one encodes for glucose dehydrogenase (*gcd*), one shows extensive similarities to cupin protein and one encodes for a protein of unknown function. Only genes *pqqE* and *pqqD* seem to reside in the same cluster while all the other are localized in separate clusters. Mutants carrying insertions in *pqq* genes as well as those carrying insertions in *gcd* did not lack glucose dehydrogenase activity, and could utilize all carbon sources comparing to wild type. This indicates differential role of these genes, other than carbon metabolism. Wild type phenotype was restored in all mutants by complementation with recombinant plasmids carrying PCR-amplified wild type genes in pBBR1MCS5, a broad-host range expression vector. These results provide a basis for understanding the mechanism that *P. fluorescens* X uses in order to suppress *Pythium ultimum*.

**Effect of edible coatings on the pepper fruit rot caused by *Botrytis cinerea* and *Alternaria alternata***

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Food coatings of natural (plant or animal) origin are a new approach in the post-harvest management of fresh horticultural products. Individual packaging made of these membranes is aimed, amongst other things, at reducing the incidence of infections from pathogens. In the present study several hydrocolloid coatings, such as chitosan and pullulan, were tested, as well as two waxy substances (beeswax, propolis) and one commercial product – Pro-Long. The study focused on the effect of edible coatings on reducing rot on injured or intact peppers which had been inoculated with two fungi: *Botrytis cinerea* and *Alternaria alternata*. The study showed that the benefits of the application of edible coatings were visible on uninjured peppers. The application of beeswax, chitosan and propolis on uninjured fruits inoculated with *B. cinerea* reduced the rate incidence by 87, 75 and 52%, respectively, in comparison with the control, and the diameter of rot by 88, 80 and 54%, respectively. In the case of peppers covered with propolis and chitosan, the rate incidence of rot from *A. alternata* was reduced by 86 and 79%, respectively, compared to the control, and the diameter of the rot was reduced by 95 and 76% respectively, for the same coatings.

## Potential use of two botanical fungicides against grey mould and powdery mildew in vegetables

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Currently, the EU supports the use of natural products of plant origin as botanical pesticides. In the present study, a microencapsulated mixture of eugenol, thymol and geraniol essential oils (ETG, 4 trials), and a mixture of canola oil derivative and thymus oil (CT, 2 trials) were tested against grey mould (*Botrytis cinerea*) and powdery mildew (*Podosphaera xanthii* syn. *Sphaerotheca fuliginea*) in greenhouse-grown tomato and potted zucchini plants, respectively. Preventive spray applications of EGT to control grey mould on tomatoes had an overall efficacy of 30%, under high disease severity (74% infected leaf area in control plants, 102 d after transplanting). However, when EGT was applied curatively (13.9% infected leaf area), the disease severity on leaves and fruit did not differ significantly ( $P=0.05$ ) from that on the control plants. EGT did not affect the yields of the 1<sup>st</sup> and 2<sup>nd</sup> truss or the concentration of antioxidants (methods TEAC & FRAP) in fruit harvested from disease-free plants. *B. cinerea* mycelial growth was inhibited by 95%, *in vitro* at a concentration of 0.14  $\mu\text{g ml}^{-1}$  EGT. The mixture had no effect against powdery mildew in zucchini plants under conditions of high disease severity (60% infected leaf area in control plants). CT reduced the severity of powdery mildew in zucchini plants by *ca* 60%, when applied (i) prior to disease onset, and (ii) after the appearance of the first disease symptoms (1.5% infected leaf area). In both trials, powdery mildew severity (% infected leaf area) on control plants was >50%, 60 d after transplanting.

**Biological control of three *Colletotrichum lindemuthianum* races using *Pseudomonas chlororaphis* PCL1391 and *Pseudomonas fluorescens* WCS365**

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*Colletotrichum lindemuthianum* is the causal agent of anthracnose one of the most important diseases of bean worldwide. *Pseudomonas chlororaphis* PCL1391 and *P. fluorescens* WCS365, were tested *in planta* against three *C. lindemuthianum* races. *Pseudomonas chlororaphis* PCL 1391 in the absence as well as in the presence of the pathogen promoted several plant growth characteristics. The promoting effect was greater regarding certain growth characteristics when this strain was tested in combination with *P. fluorescens* WCS365. Treatment with *P. chlororaphis* PCL1391 resulted in best biocontrol of anthracnose. The combined bacterial treatment did not differ from the treatment with *P. chlororaphis* PCL1391 alone. Colonization experiments showed that *P. chlororaphis* PCL1391 and *P. fluorescens* WCS365 are excellent colonizers of bean roots. However, their combined treatment resulted in increased concentration of the total bacterial population on the root tips and reduction of the *P. fluorescens* WCS365 population. *In vitro* experiments showed that *Pseudomonas chlororaphis* PCL1391 reduced pathogen's growth, sporulation and conidial germinability. Similar results were obtained when both bacteria were used in combination. In contrast, *P. fluorescens* WCS365 did not affect any of these characteristics. It was assumed that phenazine–1–carboxamide produced by *P. chlororaphis* PCL1391 was the crucial factor for the *in vitro* activity of this strain. This hypothesis was confirmed by absence of fungal growth over phenazine–1–carboxamide on a TLC plate seeded with *C. lindemuthianum* spores.

**Effect of two foliar elicitors Milsana<sup>®</sup> and Chitoplant<sup>®</sup> on powdery mildew development, plant growth and quality characteristics of tomatoes**

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In the frame of the EU funded research project “Truefood” the effect of two foliar elicitors (inducers of resistance) namely Milsana<sup>®</sup> (formulated plant extract of the knotweed *Reynoutria sachalinensis*) and Chitoplant<sup>®</sup> (a formulation of chitosan) was examined on: a) powdery mildew development in greenhouse grown tomatoes (cv Belladonna), b) plant growth (height, number of open and total flowers per floescence, chlorophyll and nitrogen content of leaves), c) yield (weight of fruits) and d) quality of harvested fruits. Two greenhouse experiments were conducted in parallel in 2007 in two different locations in Greece (March to July). Milsana<sup>®</sup> and Chitoplant<sup>®</sup> were tested at three rates (2, 3, 4 ml L<sup>-1</sup> and 0.4, 0.5, 0.6 g L<sup>-1</sup>, respectively). Sulfur 80 WP (reference fungicide allowed in organic farming), water and the elicitors were applied onto plants as foliar sprays, at 7 day intervals. Plant height, flower production, chlorophyll content and nitrogen concentration in the dry material of leaves and yield did not statistically differ among treatments both in the absence or in the presence of the disease. A slight difference in the production rate of total flowers per plant in Milsana<sup>®</sup> and Chitoplant<sup>®</sup> was not reflected to final yields (1 trial). In almost all treated plots it was shown that the two foliar elicitors delayed disease development compared to the control. In addition both elicitors were equally effective to sulfur in terms of disease reduction. Quality analysis of fruits is currently in progress.

**Biological control of Verticillium wilt of olive trees by using the biocontrol agent  
*Paenibacillus alvei* K-165**

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In the present work it was investigated the ability of the biocontrol agent K165 to protect olive trees against *V. dahliae*. For this purpose young olive trees of the susceptible to *V. dahliae* cv. Amfissis and the tolerant cv. Kalamon were planted in an artificial infested field. Sixteen months after transplanting, (i) Verticillium wilt symptoms were recorded (ii) the biomass of *V. dahliae* in the xylem vessels of the trees was quantified by using the real time PCR technology and (iii) the viability of the pathogen in the plant tissues was confirmed by isolating the fungus on PDA plates. It was shown that in the K165 treated cv. Amfissis trees the disease percentage and the biomass of the fungus in the plant tissues was lower than in the control trees. On the other hand, the application of K165 in the resistant to Verticillium wilt cv. Kalamon did not result in less symptom development and fungal biomass compared to the control trees.

**Study of the interaction of *Verticillium dahliae* with the non-pathogenic isolate *Fusarium oxysporum* F2**

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Verticillium wilt is a devastating disease of a wide range of herbaceous and woody plant hosts, incited by the soilborne fungus *Verticillium dahliae*. Since there are no chemical treatments to control the pathogen, management strategies are focused on preventive measures. Along these lines, the use of biocontrol agents has been evaluated as an alternative to control *V. dahliae*. In the present study it was investigated the mode of action of the non pathogenic isolate *Fusarium oxysporum* F2 against *V.dahliae*. The non pathogenic isolate *Fusarium oxysporum* F2 has been isolated from a suppressive compost amendment and its suppressive action against *V.dahliae* has been reported in a previous study. It was shown that the ability of strain F2 to protect eggplants against *V. dahliae* is a dose-response effect. On the other hand, the strain F2 did not trigger the induction of systemic resistance in eggplants against *V.dahliae* in a split root system. Strain F2 was transformed with the *eGFP* gene and *V. dahliae* was transformed with the *DsRed2* fluorescent gene to facilitate monitoring of the simultaneous colonisation of the eggplants root system by both the antagonist and the pathogen. It was demonstrated that the reported efficacy of strain F2 against *V. dahliae* can be attributed to the phenomenon of competition for site on the root system.

## **Means and methods as an alternative to methyl bromide disinfection**

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Methyl bromide (MB) is considered as an important factor of reduction of ozone in atmosphere. Regulations EC2037/00 and EC3093/94, require reduction of use of methyl bromide and draw its progressive suppression in the EU up to 31/12/08. In the countries of EU, the non chemical and chemical alternative methods of methyl bromide (MB) are available and have been adopted for various uses in soil disinfections and in postharvest applications. Nevertheless, it appears that a lot of factors limit the wide distribution and application. For certain uses the extension of application of these alternative solutions is awkward. The aim of the program is to organize and to apply a framework of coordination that improves the promotion of viable alternative methods. In the working team participate 11 partners from 7 countries: France, Italy, Spain and Greece that used increased quantities of (MB), Belgium and Holland where the use of (MB) is decreased continuously as well as Romania, that represents all the different types of institutions for the critical mass that is required. The objectives are: 1) Determination of problems that prevent the adoption of alternative methods of methyl bromide, collection of elements with regard to the development and the scientific research for the use of successfully alternative methods and means. 2) Evaluation of application of successfully alternative solutions and choice of efficient practices in the effective alternative solutions. 3) Determination of tools of distribution. 4) Guarantee a dynamic and efficient management of program. 5) Internet dissemination: create an online list of electronic contacts of public administrations, chemical industries, academics representatives from EU member states that will be updated on the status of the project and the content of the knowledge base as it is updated by means of an e-newsletter.

The suitable alternative solutions that are enumerated by the consortium are:

A. Applications in the soil: 1. Fumigation: a. Existing chemical substances: Chloropicrine, Dazomet, Dichloropropene, Metam sodium and potassium alone or in combination with other methods. b. New chemical substances: Dimethyl disulphide, ethadinytrile, methyl iodide. 2. Biological alternative solutions. 3. Integrated pest management. 4. Solarization. 5. Steam. 6. Hydroponic cultures. 7. Biofumigation. 8. Grafted plants and resistant varieties.

B. Post harvest applications: 1. Existing chemical substances: Phosphine and contact insecticides. 2. New chemical substance: sulfuryl fluoride. 3. Heat.



## Use of essential oils and aromatic plants for the control of soilborne pathogens

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In the present study eight essential oils derived from aromatic plants of the greek flora were studied for their effectiveness against the soil-borne fungi *Fusarium oxysporum* f.sp. *radicis-lycopersici* and *Verticillium dahliae* *in vitro*. The essential oils were derived from the distillation of spearmint (*Mentha spicata*), coriander (*Coriander sativus*), rosemary (*Rosmarinus officinalis*), fennel (*Foeniculum vulgare*), dittany of Crete (*Origanum dictamnus*), hyssopus (*Hyssopus officinalis*), sage (*Salvia triloba*) και pennyroyal (*Mentha pulegium*). It was observed that all the essential oils used showed antifungal action and then the concentration that causes 50% inhibition on the mycelial growth was estimated. The most effective essential oil for the control of both pathogens was that of dittany of Crete which inhibited completely the mycelial growth of *Fusarium oxysporum* f.sp. *radicis-lycopersici* and *Verticillium dahliae* at concentrations of 260 and 34 ppm respectively, while from the rest oils higher concentrations were demanded. Furthermore, the effect of dried plant parts of spearmint, sage, coriander and fennel on the foot and root rot disease of tomato caused by the fungus *F. oxysporum* f.sp. *radicis-lycopersici* was studied *in planta*, after their incorporation in the soil mixture. The greatest reduction on the disease severity was caused by sage, followed by coriander and spearmint, while fennel had no influence on the disease severity.

## Biosynthesis of plant bioactive substances from yeast through metabolic engineering to control plant pathogens

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Compounds of phenylpropanoid metabolism belonging to the stilbenoid, isoflavonoid and flavonol subgroup are thought to possess diverse functions such that depict them as antimicrobial agents (phytoalexins), feeding repellants (insect and herbivore protectants), photoreceptors, visual attractors (flower pigments), UV protectants, signals in the early steps of rhizobia-legume symbiosis, regulators of auxin transport and stimulators of pollen germination [1,2]. Resveratrol, a stilbenoid, genistein, an isoflavone and kaempferol, a flavonol, have been shown to belong to substances with high prospects. Those compounds beyond the beneficial properties for human health, have been shown to act as phytoalexins, thus acting as antimicrobial agents following the pathogen attack (all types of biological activities are reviewed in [1,2]). To exploit such behaviour three genetically modified yeast strains harbouring plasmids that permit the biosynthesis of resveratrol, genistein and kaempferol have been constructed. The transcriptionally active yeast strains were used in suspensions for evaluating the production efficiency, from precursor compounds. The potential to use the enriched suspensions in crude or in purified form for controlling the populations of the pathogens is evaluated.

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